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Evaluation of Digestibility, Nitrogen and Sulfur Balances and Rumen Fermentation of Diets Supplemented with Urea and/or Potassium Sulfate in Naeimi Sheep

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Abstract: Four mature fistulated Naeimi rams were used in a 4×4 Latin square design to determine the effect of urea (U) and/or sulfur (S) supplementation (in the form of potassium sulfate) on nutrient digestibility, balances of nitrogen (N) and S and rumen fermentation (in terms of pH, total VFA and NH_3-N concentrations). Animals were fed individually in metabolism cages and offered four experimental diets. The basal control diet (C-diet) consisted of ground Rhodes grass hay, ground barley grain and wheat bran, while the other three diets were supplemented with urea (U-diet), potassium sulfate (S-diet) or both U+S (US-diet). U-diet and US-diet gave a significant improvement in the digestibility of CP and Crude Fiber (CF). US-diet had improved the digestibility of Nitrogen Free Extract (NFE), while the digestibility of Ether Extract (EE) was not affected. TDN value of digestible portions was increased by 4% in U-diet and 8.5% in US-diet relative to the C-diet. S-diet did not lead to any improvement in the value of TDN or digestible CP. U-diet and US-diet were associated with significant increase in the N excreted in the feces and urine relative to the C-diet. N retained in U-diet and US-diet was also increased significantly by about 35.3 and 72.3% comparable to the C-diet, respectively. More than 82.7% of the N intake in U-diet was excreted, while 78.3% of the N intake in US-diet was excreted. Percentages of N retained relative to N intakes in C-diet, U-diet, S-diet and US-diet were 16.8, 17.3, 19.8 and 21.7%, respectively. US-diet showed insignificant increase in N and S retained by about 15.5 and 50% over that observed in the C-diet. US-diet gave an improvement in N retention over that in U-diet by about 27.4%. Excretions of S in feces and urine were approximately doubled in U-diet and US-diet comparable to the C-diet. S retained as percentages to S intakes in C-diet, U-diet, S-diet and US-diet were 25.1, 25.0, 18.2 and 18.0%, respectively. The pH values of rumen liquor (before feeding) did not differ between the four diets used. For 2 h after feeding, a sharp drop in pH values was observed in US-diet only. U-diet and US-diet showed significant increase in rumen ammonia-N before and after feeding, while S-diet did not show such increments. Concentrations of VFA 2 h after feeding were doubled in all treatments relative to the concentration before feeding. Diets supplemented with both U and S (but not any of them alone) were accompanied with an increase in VFA in rumen before or 2 h after feeding.

Key words: Digestibility, rumen fermentation, urea, nitrogen, sulfur balances, Naeimi sheep

INTRODUCTION

The maximum amounts of non-protein nitrogen to be supplemented in diets of ruminants to provide new protein as milk and meat for humans were earlier recognized[1]. Replacing different levels of protein in ruminant rations by urea (as a nitrogen supplement) was attempted safely and efficiently for more than thirty years ago[2-5]. However, sheep diets containing high level of urea require more attention to supply these diets with adequate levels of minerals such as sulfur which is being important especially for microbial synthesis of sulfur-containing amino acids and could possibly involved in stimulating cellulose digestion[6-8]. Using urea alone in feeding of dairy cows lead to low content of essential amino acids in blood plasma, while adding minerals in diets supplemented with urea were associated significantly with an increase in essential amino acids in plasma[1].

The traditional diet used for feeding local sheep in Saudi Arabia (Al-Qassim region) consists of barley grain, wheat straw and alfalfa or Rhodes hay. This diet was thought to have less protein than the requirements recommended for sheep by National Research Council[9]. In this region, wheat straw and Rhodes hay are less in protein, while alfalfa is rich in protein but expensive and not available for many sheep breeders. Therefore, urea supplementation may improve the nutritive value of this diet to be used efficiently in our Arabian Gulf area. As a result of adding urea in diets, nitrogen to sulfur ratio in the sheep diet will be disturbed. Urea-supplemented diet should be also supplemented with sulfur in order to keep the N:S ratio within the recommended levels[7,10-12]. The

N:S ratio was reported to be an essential factor affecting the ruminal microbial activity in sheep[7,13-15]. Early report[16] suggested the desirable N: S ratio for sheep to be 13.5:1, while other studies in the Arabian area on Ossimi sheep reported narrower ratio to be 10:1[10-12].

The present study was conducted to fulfill the concepts of digestibility of nutrients, balances of nitrogen and sulfur and rumen fermentation for diets supplemented with urea and/or potassium sulfate to be used in feeding routine of Naeimi sheep in Saudi Arabia.

MATERIALS AND METHODS

Digestibility and nitrogen and sulfur balances trials were executed in year 2001 at Animal Production Research Station, College of Agriculture and Veterinary Medicine, Al-Qassim, King Saud University, Saudi Arabia.

Design and dietary treatments: Four mature Naeimi rams of about 9 month-old and average body weight of 45 kg were used in a 4×4 Latin square design (4 rams x 4 periods x 4 dietary treatments). Animals were housed and fed individually in metabolism cages and offered four experimental diets as presented in Table 1. The basal control diet (C-diet) consisted of ground Rhodes grass hay, ground barley grain and wheat bran. The other three diets were supplemented with urea (U-diet, with 1% urea of the total ingredients), potassium sulfate (S-diet, with 0.6% potassium sulfate of the total ingredients) or both urea+ potassium sulfates (US-diet, with 1% urea plus 0.6% potassium sulfate of the total ingredients used). The approximate chemical composition of the four experimental diets and the ingredients used in formulating these diets are shown in Table 1 and 2. The chemical composition of the diets containing urea and/or sulfur (U-diet and US-diet) was corrected according to the suggestion of Kowalczyk[17].

Animals were placed in metabolism cages as described by Maynard *et al.*[18] to collect urine and feces separately. Animals were adapted to the cages for 14 day followed by 6 day collection period. According to Church[19], rams were fed the experimental diets at the levels to cover their nutrient requirements. Feed ingredients were mixed to keep the roughage: concentrate ratio to be 30:70. Supplementation of urea, potassium sulfate, or both in the diet was performed by simple mixing just before feeding. Feed was offered twice daily at 8:00 and 14:00 h. Every morning, feed residue, if any, was weighed and subtracted from the amount offered to calculate the feed intake. Water was available all the time. Daily fecal collections were weighed, sampled (10% of the daily collection) and stored at -20°C to be analyzed later.

Table 1: The ingredients used in formulating the experimental diets associated with approximate chemical composition for these diets

		Diet supplemented with		
Items	Control diet	Urea	Sulfur	Urea+Sulfur
Ingredients (%)				
Rhodes grass hay	31.8	30.8	31.2	30.2
Barley grain (ground)	40.9	40.9	40.9	40.9
Wheat bran	27.3	27.3	27.3	27.3
Urea	-	01.0	-	01.0
Potassium sulfate	-	-	00.6	00.6
Chemical composition on dry matter basis (%)				
CP	11.23	14.47	11.04	14.58
EE	02.25	02.24	02.32	02.18
NFE	66.20	65.67	67.71	65.28
CF	14.01	13.21	12.38	13.07
Ash	06.31	06.22	06.55	06.70
N	01.796	02.315	01.766	02.333
S	00.114	00.110	00.232	00.233
N:S ratio	15.8:1	21.1:1	07.6:1	10.0:1

CP= Crude protein; EE= Ether extract; NFE= Nitrogen free extract; CF= Crude fiber; N= Nitrogen; S= Sulfur

Table 2: The proximate chemical analysis of the feed ingredients used in formulating the experimental diets on fresh and dry matter basis

	Ingredients used		
Items	Barley	Wheat bran	Rhodes grass hay
DM	93.20 (100)	90.41 (100)	91.10 (100)
OM	90.35 (96.94)	84.89 (93.89)	77.90 (85.51)
CP	10.66 (11.44)	11.79 (13.04)	07.35 (8.07)
EE	02.06 (2.21)	02.71 (3.00)	01.12 (1.23)
NFE	73.41 (78.76)	59.58 (65.90)	40.28 (44.22)
CF	04.22 (4.53)	10.82 (11.97)	29.15 (32.00)
Ash	02.85 (3.06)	05.51 (6.09)	13.20 (14.48)
N	01.71 (1.83)	01.89 (2.09)	01.18 (1.29)
S	00.131 (0.141)	00.165 (0.183)	00.151 (0.165)
N : S ratio	13.1:1	11.5:1	08.7:1

DM= dry matter; OM= Organic matter; CP= Crude protein; EE= Ether extract; NFE= Nitrogen free extract; CF= Crude fiber; N= Nitrogen; S= Sulfur, Values given in parenthesis were calculated on dry matter basis

They were then thawed and dried in a forced air oven at 70°C for 24 h to determine the dry matter content. All dried samples of each animal were carefully mixed and a representative sample for these dried samples was taken for chemical analysis.

Data collected: Data on nutrient intakes and outputs were taken and coefficients of digestion of DM, CP, CF, EE and ash were analyzed using BIDER drying oven (German), Kjeltec 2300 Analyzer Unit (Foss, Swedish), 2010 Fibertec (Foss, Swedish), Goldfish method (Labconco, USA) and Muffle furnace (Fisher Scientific, USA), respectively. Feeding values of digestible portions (in terms of TDN and digestible CP) and digestion coefficients (in terms of OM and NFE) were also calculated. Chemical analyses were conducted according to AOAC[20].

To measure N and S balances, urine was collected daily and 10% aliquot of urine excretion was taken and frozen at -20°C for nitrogen and sulfur analysis. Nitrogen

in urine samples was determined by Micro-Kjeldahl method. Sulfur concentration in urine samples was determined by ICP-ES Spectrometry (GBC Integra XL, Australia) at a wave length of 182.04 nm. Data of N intake (NI), fecal (FN), urinary (UN) and retained (NR) were recorded as parameters of the N balance along with percentages of NR/NI. The same respective parameters were determined for S balance.

For the rumen fermentation study, animals were fistulated prior to the experiment. Rumen samples were collected during the three days following the collection period of the digestibility trial just before feeding and 2 h post-morning feeding to determine pH and total VFA and ammonia-nitrogen concentrations. Rumen fluid was strained through four-layers cheesecloth; pH was immediately measured using a hand pH-meter (pH meter 315i, German) with glass electrode followed by the addition of 2 mL H_2SO_4 (50%vol/vol) to retard ammonia loss. Samples were frozen for subsequent chemical analyses to determine ammonia and total VFA. Total VFA's concentration was determined by steam distillation method as described by Warner[21]. Ammonia-nitrogen (NH_3-N) concentration in rumen samples was determined by steam distillation method with MgO in which the NH_3 librated is collected in H_3BO_3-indicator solution and was determined by titration with standard H_2SO_4 (0.005 N) according to Al-Rabbat *et al.*[22].

Model of analysis: Using GLM procedure of SAS program[23], data were analyzed by adopting the following linear model:

$$Y_{ijk} = \mu + A_i + B_j + T_k + e_{ijk}$$

where, Y_{ijk} is the observation on ijkth trait, μ is the overall mean, A_i is the effect of ith ram, B_j is the effect of jth period, T_k is the effect of the kth dietary treatment and e_{ijk} is the residual error assumed to be normally and independently distributed. Duncan's test[24] was used to compare the treatment means.

RESULTS AND DISCUSSION

Digestibility of nutrients (%): Results of digestibility trial revealed that the diet supplemented with urea (U-diet) or urea + potassium sulfate (US-diet) led to significant improvement in the digestibility of CP and CF (Table 3). US-diet had improved the digestibility of NFE, while the digestibility of EE was not affected. *In vitro* trial, Bull and Vandersall[25] reported that sulfur supplementation in diets leads to an increase in DM and ADF digestibility. Also, Spears *et al.*[26] reported that addition of sulfate to diets

Table 3: Means±SE for digestibility of all nutrients (%) and feeding value of the digestible portions (%) in the control diet and other diets affected by urea and/or sulfur supplementation

		Diet supplemented with			
Items	Control diet	Urea	Sulfur	Urea+Sulfur	SE
Digestion coefficients (%)					
CP	61.3[a]	65.7[b]	62.5[a]	68.3[c]	2.3
EE	70.8[a]	68.7[a]	69.2[a]	71.2[a]	3.1
NFE	70.9[a]	71.2[a]	69.8[a]	75.6[b]	2.1
CF	51.1[a]	53.9[b]	51.9[a]	55.6[b]	3.4
Feeding value of digestible portions (%)					
TDN	64.56[a]	67.14[b]	64.20[a]	70.07[c]	3.4
DCP	06.88[a]	09.51[b]	06.90[a]	09.96[b]	0.77

CP= Crude protein; EE= Ether extract; NFE= Nitrogen free extract; CF= Crude fiber; TDN= Total digestible nutrients; DCP = Digestible crude protein

[a,b,c]Values having different superscripts within each row are significantly different ($p<0.5$)

was associated with an increase in cellulose digestion. Pendlum *et al.*[2] gave evidence to that adding sulfur to diets containing urea gave an improvement of cellulose digestion in steers. Ahmed and Saddick[12] found that sulfur supplementation in the form of methionine amino acid in diets of sheep showed an improvement in the digestibility of DM, OM, CP and NFE. Ahmed *et al.*[7] reported that adding sulfur at a level of 2.4 g potassium sulfate in diets of sheep was safely effective to improve the digestibility of all nutrients. Puoli *et al.*[8] reported that addition of urea to sheep diets was associated with an improvement in digestible DM and NDF, while supplementation of diets with S had no effect. Also, Nyarko-Badohu *et al.*[27] and Nianogo *et al.*[28] reported that urea supplemented diets gave an increase in DM digestibility. In contrast, Nianogo *et al.*[29] found that urea supplementation had no effect on digestion coefficients of DM, OM, CP, NDF and ADF. Recently, Ferrell *et al.*[30] found that supplementing N in sheep diets has no effect on digestibility of DM, OM and N.

Feeding values of digestible portions: The increases in digestibility due to urea supplementation led to a significant increase in the feeding values of digestible portions ($p<0.05$) expressed as TDN or digestible CP (Table 3). TDN value was increased by 2.58 units (4%) in U-diet and by 5.51 units (8.5%) in US-diet comparable to the control diet. Sulfur supplementation alone in the diet (S-diet) did not lead to any improvement in the value of TDN or digestible CP. Nutritive Ratio (NR) was also improved due to urea supplementation either with or without sulfur. Ahmed[3] reported that non-traditional diet for sheep supplied with urea gave an improvement in the feeding value of digestible portions (expressed as TDN and digestible CP).

Nitrogen and sulfur balances: In nitrogen balance, adding urea in U-diet and US-diet was associated with significant increase in the N excreted by about 16.4 and

Table 4: Means±SE for nitrogen and sulfur balances (g/head/day) in the control diet and other diets affected by urea and/or sulfur supplementation

Items	Control diet	Diet supplemented with Urea	Sulfur	Urea+Sulfur
Nitrogen balance				
Nitrogen intake (NI)	27.50±2.16[a]	36.11±2.22[b]	26.91±2.81[a]	36.65±2.41[b]
Nitrogen in feces (FN)	10.64±1.04[a]	12.39±1.00[b]	10.09±0.91[a]	13.56±1.03[b]
Nitrogen in urine (UN)	12.24±0.91[a]	17.47±1.11[b]	11.48±1.20[a]	15.13±0.99[b]
Total N excretion	22.88±2.14[a]	29.86±2.25[b]	21.57±1.99[a]	28.69±2.56[b]
Nitrogen retention (NR)	04.62±0.51[a]	06.25±0.55[b]	05.34±0.63[ab]	07.96±0.75[b]
NR/NI (%)	16.80±1.04[a]	17.31±1.18[ab]	19.84±2.00[bc]	21.72±1.47[c]
Sulfur balance				
Sulfur intake (SI)	01.75±0.22[a]	01.72±0.16[a]	03.35±0.42[b]	03.66±0.27[b]
Sulfur in feces (FS)	00.67±0.07[a]	00.59±0.04[a]	01.24±0.11[b]	01.32±0.08[b]
Sulfur in urine (US)	00.64±0.06[a]	00.70±0.08[a]	01.50±0.12[b]	01.68±0.09[b]
Total Sulfur excretion	01.31±0.11[a]	01.29±0.13[a]	02.74±0.21[b]	03.00±0.19[b]
Sulfur retention (SR)	00.44±0.03[a]	00.43±0.04[a]	00.61±0.04[b]	00.66±0.03[b]
SR/SI (%)	25.14±2.15[b]	25.00±2.41[b]	18.20±1.65[a]	18.03±1.48[a]
NR:SR ratio	10.50:1	14.53:1	08.75:1	12.06:1

[a,b,c] Values having different superscripts within each row are significantly different ($p<0.5$)

27.4% in feces and 42.7 and 23.6% in urine comparable to the control diet, respectively (Table 4). The retentions of N in U-diet and US-diet were also increased significantly by about 35.3 and 72.3% relative to the control diet, respectively. The total N excreted in U-diet (82.7% of the N intakes) was more than that excreted in US-diet (78.3% of the N intakes). Ortigues *et al.*[31] reported that N retention in sheep was improved when they fed poor quality hay supplemented with urea. Ahmed[3] found that N balance in Ossimi sheep was improved in non-traditional diet supplemented with urea.

N retained as percentages of nitrogen intakes in C-diet, U-diet, S-diet and US-diet were 16.8, 17.31, 19.84 and 21.72%, respectively. The addition of potassium sulfate in S-diet showed insignificant increase in the N retained by about 15.5% over that observed in the control diet (Table 4). Adding sulfur with urea in the diet gave also an improvement in N retention of US-diet over that in U-diet by about 27.4% although the difference was not significant. However, improvement in N retention relative to N intakes in S-diet was mainly due to that N intake was lesser rather than N retention was higher in this diet.

In sulfur balance, S excreted in feces and urine was approximately doubled in diets supplemented with potassium sulfate without or with urea (S-diet and US-diet) comparable to the control diet (Table 4). The addition of potassium sulfate in S-diet and US-diet showed significant increase in S retention by 38.6 and 50% over that observed in the control diet, respectively. Such increase in S retention in S-diet and US-diet in comparison to C-diet and U-diet led to a decrease in percentage of SR/SI. S retentions as percentage of S intakes in C-diet, U-diet, S-diet and US-diet were 25.1, 25.0, 18.2 and 18.0%, respectively. *In vitro* experiment, Bull and Vandersall[25] found that sulfur supplementation in diets led to an increase in S and N balances with the concept of that sulfate form being superior to other forms of sulfur. Ahmed *et al.*[7] found that adding sulfur gradually in diets of sheep using upgraded levels of potassium sulfate lead to an increase in the S retention.

The ratios between NR and SR were calculated to be 10.5: 1, 14.5: 1, 8.8: 1 and 12.1: 1 for C-diet, U-diet, S-diet and US-diet, respectively (Table 4). This N:S ratio was reported to be an essential factor affecting the ruminal microbial activity in sheep[7,13-15]. For this reason, urea-supplemented diet for sheep must be also supplemented with sulfur in order to keep the N:S ratio within the recommended levels in our Arabian area[7,10-12]. Early report[16] suggested the desirable N: S ratio for sheep to be 13.5:1, while other studies in the Arabian area on Ossimi sheep reported narrower ratio to be 10:1[10-12].

Evaluation of rumen liquor: Rumen microbial activities evaluated as pH and concentrations of ammonia-N and VFA showed that pH values of rumen liquor (before feeding) did not differ between the four diets used (Table 5). For 2 h after feeding, a sharp drop in pH value was observed in US-diet only ($p<0.05$). Diets supplemented with urea alone or supplemented with urea+sulfur was companied with significant increase in rumen ammonia-N (before and after feeding) while sulfur supplemented diet did not show such increment (Table 5). For an experiment in steers, Pendlum *et al.*[2] reported that animals fed different levels of urea and sulfur showed an increase in ruminal ammonia-N concentration relative to those fed soybean meal as a protein source. Concentrations of VFA 2 h after feeding were doubled in all treatments relative to the concentration before feeding (Table 5). Diets supplemented with both urea and sulfur (but not any of them alone) were associated with an increase in VFA produced in the rumen before or 2 h after feeding (Table 5). In an early *in vitro* study, Kahlon *et al.*[32] reported that the amount of microbial protein synthesis was doubled in diets supplemented with urea in sulfate form than diets supplemented from other sulfur forms. Ortigues *et al.*[31] found that poor quality hay

Table 5: Means±SE for pH values of rumen liquor, ammonia-N concentration (NH_3-N) and total volatile fatty acids (VFA) in the control diet and other diets affected by urea and/or sulfur supplementation

		Diet supplemented with			
Items	Control diet	Urea	Sulfur	Urea+Sulfur	SE
pH value					
Before feeding	6.8[a]	06.5[a]	06.7[a]	06.9[a]	0.04
2 h post feeding	5.7[a]	05.9[a]	05.7[a]	05.2[b]	0.02
Ammonia-N (mg/100 mL)					
Before feeding	27.6[a]	35.1[b]	28.4[a]	36.3[b]	3.1
2 h post feeding	40.3[a]	55.7[b]	43.1[a]	59.8[b]	5.0
VFA (mmol/100 mL)					
Before feeding	6.1[a]	06.8[a]	06.7[a]	08.2[b]	0.5
2 h post feeding	12.5[a]	13.9[a]	13.2[a]	17.4[b]	1.2

[a,b]Values having different superscripts within each row are significantly different ($p<0.5$)

supplemented with urea gave an increase in VFA and propionate molar proportion. Ahmed *et al.*[10] reported that addition of potassium sulfate in diets of sheep was accompanied with an increase in VFA produced in the rumen. Huntington and Archibeque[33] stated that addition of urea in diets was associated with improvements in fiber digestion and microbial protein synthesis along with a decrease in absorption of ammonia. For an experiment in Holstein steers, Knaus *et al.*[5] reported that using urea as the only protein supplement in the diet could achieve maximum efficiency in N utilization and consequently allowing microbial protein synthesis in the rumen to be maximized.

Using urea (with 1% of the total ingredients used) or urea plus sulfur (with 1% urea plus 0.6% potassium sulfate of the total ingredients) in formulating diets of Naeimi sheep in the Arabian Gulf countries could be safely recommended without harmful effect. This is because diets supplemented with urea and sulfur could be associated with considerable improvements in: (1) digestibility of nutrients, (2) nutritive values of digestible portions, (3) nitrogen and sulfur utilization and (4) microbial protein bio-synthesis through the maximization of microbial activities in the rumen (in concentration of ammonia-N and volatile fatty acids).

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