

EFFECT OF SULFUR SUPPLEMENTATION ON NUTRIENTS UTILIZATION AND RUMEN MICROBIAL ACTIVITY IN OSSIMI SHEEP

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ABSTRACT: *The present experiment was conducted to study the effect of sulfur supplementation on nutrients digestibility, feeding values and rumen microbial activity using four Ossimi rams surgically fitted with ruminal fistulae. The experimental design was 4X4 Latin square design. The S levels were 0, 2, 4 and 6g/head/d (R0, R2, R3 and R4, respectively)*

The results obtained showed that S-supplementation led to increase in the digestion coefficients. Ration 3 showed highly significance effect in digestion coefficient of DM, OM, CP, NFE and EE than the other studied rations. Nutritive value (TDN and DCP) was highly significant for ration 3 (N:S ratio 10:1). VFA production and ammonia-N in the rumen of sheep fed ration 3 was significantly higher than the other rations.

Key words: *dietary sulfur, digestibility, microbial activity, Ossimi sheep.*

INTRODUCTION

Sulfur (S) is an essential element for ruminants; sulfide, the reduce form of sulfur, is needed for the synthesis of the amino acids cysteine and methionine (Block *et al.*, 1951; Thomas *et al.*, 1951), a diet deficient in sulfur results in decreased microbial protein synthesis. Sulfur also increases the efficiency of N utilization and decreases the N loss and reduces the environmental pollution. Numerous papers have been published on the effect of S-supplementation on nutrient utilization, digestibility, nutritive value, N and S balances (Chichaeva, 1980; Ben-Ghedalia and Miron, 1984; Morrison *et al.*, 1985; Walli and Mudgal, 1985; Gangwar and Sharma, 2001; Rakesh and Sharma, 2001) indicating that almost all nutrient digestibility was significantly higher with ration supplemented with S than the control. Oshea and Baldwin (1986) reported an improvement in straw *in vivo* digestibility of the order of 11%. Zinn *et al* (1997) showed that rumen digestion of ADF and starch was slightly lower and post-rumen digestion of ADF and starch was correspondingly greater (quadratic effect, $P<0.05$) with supplemental S. It is obvious that S-supplementation improved the microbial activity in the rumen of the experimental sheep. Judkins, *et al.* (1994) reported that added S in the form of methionine to the ration of 8 rumen-cannulated Suffolk ewes did not affect rumen pH. Zinn *et al.* (1997) came to the same conclusion. Al-Dobeab

(2004) used 4 mature fistulated Naeimi rams in a 4x4 and fed a control diet without or supplemented with urea or sulfur or both. The pH values of rumen liquor (before feeding) did not differ between the 4 diets used. At 2 h after feeding, a sharp drop in pH values was observed in urea-sulfur-diet. Saini et al. (2005) evaluated the effect of sulfur supplemented wheat straw diet on rumen metabolic profile. They reported that ruminal pH was higher with control group in comparison to treatment groups. Quispe et al. (1991) found higher acetate and iso-acids in the rumen of S-fed sheep. Chamberlain and Thomas (1983) reported that ruminal infusion of 7g methionine and 6.7g sodium sulfate had no effect on the concentration of total or individual volatile fatty acids (VFA) in the rumen fluid. Kumar and Bhatia (1984) and Judkins, et al. (1994) came to the same conclusion.

The present study was carried out in order to test the effect of S-supplementation at different levels to correct N:S ratios on digestibility and nutritive values. Rumen microbial activity was also studied.

MATERIALS AND METHODS

This study was carried out at the Animal Production Farm of the Faculty of Agriculture, Menofiya University in order to study the effect of feeding sulfur (S) supplemented ration to sheep. This experiment lasted for 12 weeks. Four male Ossimi rams (average live body weight of 62.81kg) surgically fitted with ruminal fistulae were used in a 4x4 Latin square experiment. Fistulation took place at least 4 weeks prior to the experiment. Animals were housed in individual pens with slatted floors. Animals were fed four different rations containing graded levels of potassium sulfate. Animals received their nutrients requirements according to NRC (1985). All animals were fed a basal ration containing concentrate feed mixture (CFM) and Berseem hay (*Trifolium alexandrinum*). Ratio of N: S of the control ration (R0) was 11.58: 1. Animals in the treated group were fed the same ration supplemented with 2, 4 or 6g potassium sulfate/ head/ day (rations 2, 3 and 4, respectively). These three levels were chosen to correct the N: S ratio to be 10.48: 1, 10: 1 and 9.17:1, in R2, R3 and R4, respectively. Potassium sulfate was well mixed with the concentrate part of the ration. Animals were fed twice daily at 8.00 and 14.00 hr. Water was available all the time. Feed ingredients were mixed to keep the roughage: concentrate ratio at 40: 60. The complete chemical composition of the experimental rations is shown in Table 1.

During the experimental period, animals of each group were placed in the metabolic cages as described by Maynard et al. (1979) allowing quantitative fecal collection. Animals were adapted to the cages for 14 days followed by 5-day collection period. Fecal samples were dried at 60°C to 70°C for 24 h in a forced air oven. Dried samples of tested feeds and feces were ground through 1mm screen sieve and kept until chemical analysis for crude protein, crude fiber, ether extract and ash according to the methods of AOAC (2000).

Effect of sulfur supplementation on nutrients utilization and.....

Table 1: Chemical composition of the experimental rations

Items	Treatments			
	Ration 1	Ration 2	Ration 3	Ration 4
DM	88.67	88.67	88.67	88.67
On DM basis				
OM	88.86	88.86	88.86	88.86
CP	13.75	13.75	13.75	13.75
EE	2.86	2.86	2.86	2.86
CF	22.95	22.95	22.95	22.95
NFE	49.43	49.43	49.43	49.43
Ash	11.14	11.14	11.14	11.14
S	0.19	0.21	0.22	0.24
N	2.2	2.2	2.2	2.2
N : S	11.58 : 1	10.48 : 1	10 : 1	9.17 : 1

⁽¹⁾ CFM, concentrate feed mixture;

⁽²⁾ DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen-free extract; N, nitrogen; S, sulfur.

Rumen fluid samples were collected for two successive days from different locations and at different depths in a glass container via a small rubber tube using gentle mouth suction. Rumen fluid was sampled before and at 2, 4, and 6 hours after feeding. The samples were filtered through four layers of cheesecloth and homogenized. Samples were preserved by the addition of 0.1 ml of concentrated hydrochloric acid to 5 ml of rumen liquor for later analysis of ammonia-N. Rumen fluid used for analysis of volatile fatty acids (VFA) was prepared by combining 5 ml of rumen fluid with 1 ml orthophosphoric acid.

The ruminal pH was measured immediately after sampling using digital pH meter. Free ammonia-N and total VFA were determined in the rumen fluids as described by Ahmed (1976). Percentage of S in the samples of feeds and feces was determined as Barium sulfate percentage according to Winton and Winton (1985). The obtained results were statistically analyzed according to SPSS, (1997). The following models were used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = The parameter under analysis

μ = general mean.

T_i = The fixed effect of the i th sulfur supplementation, ($i=1,2,3,4$). Experiment 1.

T_i = The fixed effect of the i th sulfur supplementation, ($i=1, 2$). Experiment 2.

e_{ij} = Random error assumed to be independent normally distributed with mean and variance σ^2 .

RESULTS AND DISCUSSION

Data in Table (2) and Fig (1) present the effect of feeding different sulfur levels on nutrient digestibility of the experimental rations. Ration 3 showed highly significant ($P < 0.01$) effect on digestion coefficient of DM, OM, CP, NFE and EE being 64.22, 69.12, 70.75, 71.54, and 72.12, respectively than the other studied rations. Ration 4 (1.1g S), however, had non-significantly higher CF digestion coefficient than Ration 3.

Table 2: Nutrient digestion coefficients as affected by the dietary treatments

Item	N	Treatments	Mean	Std. Error	Sig.
DM %	12	1	59.72 ^a	0.222	0.01
	12	2	60.87 ^b	0.379	
	12	3	64.22 ^c	0.448	
	12	4	61.73 ^b	0.353	
OM %	12	1	62.96 ^a	0.134	0.01
	12	2	63.07 ^a	0.145	
	12	3	69.12 ^c	0.300	
	12	4	66.27 ^b	0.124	
CP %	12	1	63.88 ^a	0.570	0.01
	12	2	65.23 ^a	0.644	
	12	3	70.75 ^b	0.636	
	12	4	65.09 ^a	0.421	
CF %	12	1	51.45 ^a	0.729	0.01
	12	2	51.49 ^a	0.457	
	12	3	53.35 ^{ab}	0.734	
	12	4	54.46 ^b	0.619	
NFE %	12	1	68.56 ^a	0.492	0.01
	12	2	68.81 ^a	0.715	
	12	3	71.54 ^b	0.499	
	12	4	69.71 ^a	0.348	
EE %	12	1	68.57 ^a	0.380	0.01
	12	2	69.58 ^{ab}	0.641	
	12	3	72.12 ^c	0.795	
	12	4	71.10 ^{bc}	0.642	

^{a,b,c} values having different superscript within each item in the same column differ.

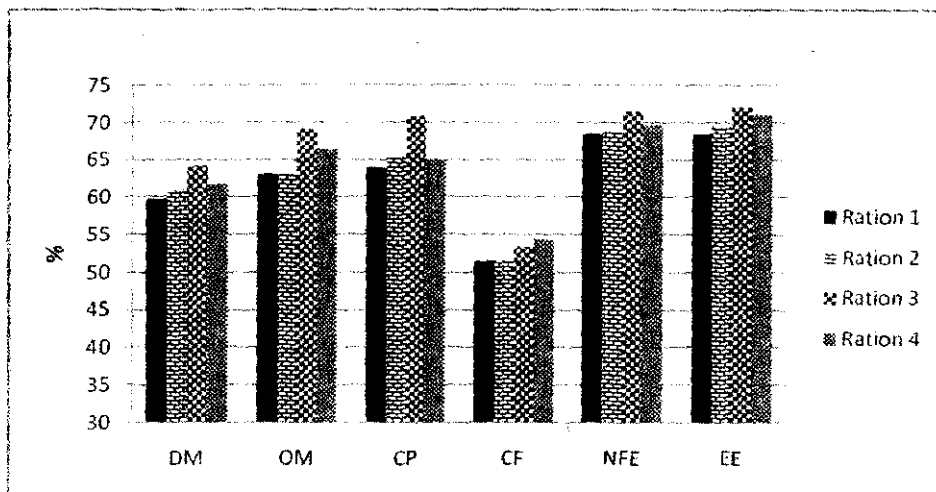


Fig. 1: Nutrient digestibility as affected by the dietary treatments.

Numerous papers have been published on the effect of S-supplementation on nutrient digestibility. Chichaeva (1980) reported that supplementing the control diet with 3g or 5g synthetic DL-methionine or with 1g crystalline S daily per ewe lead to an improvement in the digestibility of DM, OM, EE, NFE and CP. Spears *et al.* (1985) studied the effect sulfur fertilization of tall fescue and cocksfoot grass on nutrient digestibility. They found that fertilization increased apparent digestibility of DM, NDF and ADF but decreased protein digestibility by 5.7%.

Walli and Mudgal (1985) used three iso-nitrogenous diets but varied only in S content to give N:S ratios of 20:1 (T1), 10:1 (T2) and 5:1 (T3). Results indicated that the CP digestibility coefficient for treatments 2 and 3 were significantly higher than treatment 1. The cellulose digestibility was highest with N:S ratio 10:1, and significantly higher than with 20:1. Zinn *et al.* (1997) showed that post-rumen digestion of ADF and starch was correspondingly greater (quadratic effect, $P < 0.05$) with supplemental S. Rakesh and Sharma (2001) found a significant improvement in digestibility of CP and gross energy as a result of sulfur supplementation in urea-treated as well as urea-supplemented stover diets. Al-Dobeab (2004) obtained a significant improvement in the digestibility of CP and CF. Urea-sulfur-diet improved the digestibility of NFE, whereas the digestibility of EE was unaffected. Supplementing urea-ammoniated wheat straw with sodium sulfate improved the digestibility of DM, OM, CP and NDF (Nair *et al.*, 2005). Lokesh and Murdia (2006) found that urea-treated wheat straw with sulfur improved the digestibility coefficient of all nutrients. Feeding casein (as a high-S-protein) to Ossimi lambs improved the nutrients digestibility (Saddick and Ahmed, 1991).

Data in Table (3) and illustrated in Fig (2) show that S-supplementation significantly increased the values of TDN at ($P<0.05$) and DCP at ($P<0.01$); the preference was to R3 (61.83 and 9.73% in both of TDN and DCP, respectively); however, TDN did not differ between rations 3 and 4 being 61.83 and 60.42 %, respectively. This could be attributed to the higher digestion coefficient (Table 2) of the treated groups.

Al-Dobeeb (2004) found that urea (U) and sulfur (S) treatments increased TDN value by 4% in the U-diet and 8.5% in the US-diet relative to the C-diet. S-diet did not lead to any improvement in the value of TDN or digestible CP. Lokesh and Murdia (2006) determined the effect of feeding urea-treated wheat straw with or without sulfur on the nutritive values using 25 crossbred heifers. The TDN, DE and ME contents were significantly higher in the treated groups.

Table 3: Nutritive value of the experimental rations as affected by the dietary treatments

Item	N	Treatments	Mean	Std. Error	Sig.
Nutritive value					
TDN	4	1	58.84 ^b	0.577	0.05
	4	2	59.21 ^b	0.693	
	4	3	61.83 ^a	0.588	
	4	4	60.42 ^{ab}	0.433	
DCP	4	1	8.79 ^b	0.139	0.01
	4	2	8.97 ^b	0.164	
	4	3	9.73 ^a	0.165	
	4	4	8.95 ^b	0.111	

^{a,b}, values having different superscript within each item are significantly different

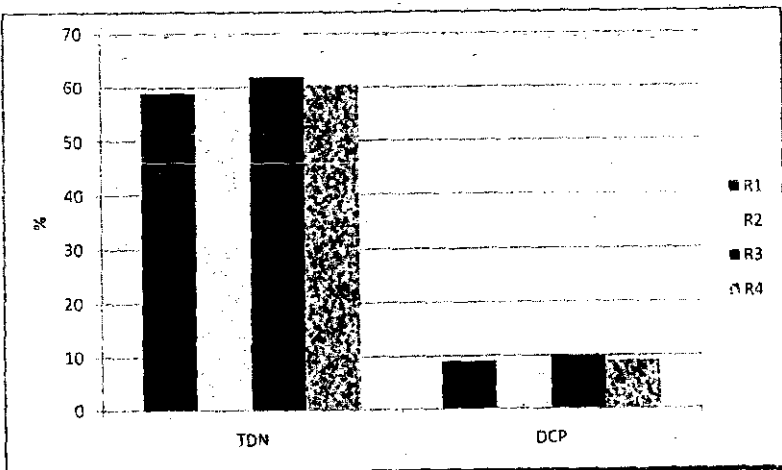


Fig. 2: Nutritive value as affected by dietary treatments.

Effect of sulfur supplementation on nutrients utilization and.....

Table (4) and Fig (3) reveals that there is significant effect ($P < 0.01$) among studied rations on rumen pH at different sampling time (0, 2, 4, and 6 hr); in case of pH at 0 and 2 hr, R0 as a control revealed the highest values being 6.86 and 6.42, respectively; at 4 hr post feeding, R3 showed lower value (6.08) than the other studied rations which were almost equal ; pH at 6 hr were different in the preference of R2 (6.42). It is obvious that all pH values within all the treatment decreased post feeding to reach the lowest level at 4h and increased thereafter (Fig 3).

Judkins *et al.* (1994) reported that added S in the form of methionine to the ration of 8 rumen-cannulated Suffolk ewes did not affect rumen pH. Zinn *et al.* (1997) came to the same conclusion. Al-Dobeib (2004) used 4 mature fistulated Naeimi rams in a 4x4 and fed a control diet without or supplemented with urea or sulfur or both. The pH values of rumen liquor (before feeding) did not differ between the 4 diets used. At 2 h after feeding, a sharp drop in pH values was observed in urea-sulfur-diet. Saini *et al.* (2005) evaluated the effect sulfur supplemented wheat straw diet on rumen metabolic profile. They reported that ruminal pH was high with control group in comparison to treatment groups.

Table 4: Rumen pH in sheep as affected by dietary treatments

Sampling Time	N	Treatment	Mean	Std. Error	Sig.
0 hr	20	1	6.86 ^a	0.024	0.01
	20	2	6.79 ^b	0.014	
	20	3	6.81 ^b	0.016	
	20	4	6.78 ^b	0.007	
2 hr	20	1	6.42 ^a	0.005	0.01
	20	2	6.35 ^c	0.005	
	20	3	6.31 ^d	0.007	
	20	4	6.39 ^b	0.007	
4 hr	20	1	6.29 ^a	0.006	0.01
	20	2	6.30 ^a	0.006	
	20	3	6.08 ^b	0.008	
	20	4	6.29 ^a	0.011	
6 hr	20	1	6.40 ^b	0.005	0.01
	20	2	6.42 ^a	0.008	
	20	3	6.20 ^d	0.006	
	20	4	6.36 ^c	0.013	

^{a, b, c, d} values having different superscripts within each column are significantly different.

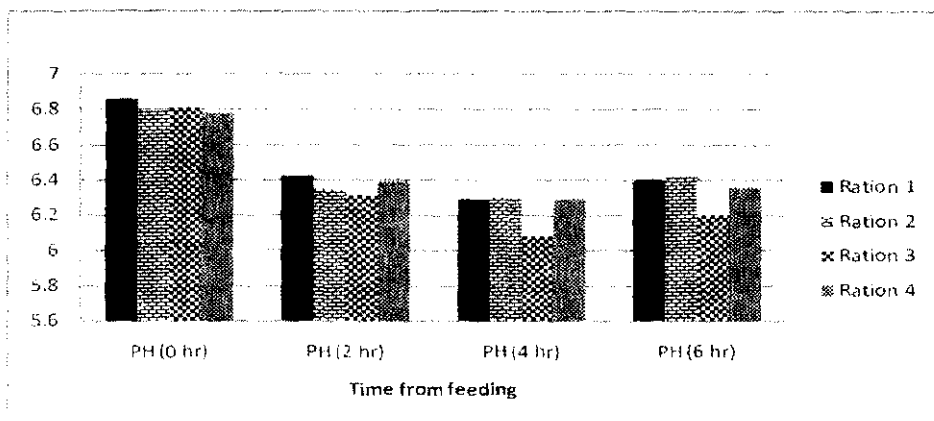


Fig. 3: Rumen pH in sheep as affected by dietary treatments.

Data in Table (5) and illustrated in Fig (4) present the ruminal total VFA concentration as affected by the sulfur supplementation. Total VFA was significantly higher ($P < 0.01$) at all times (0, 2, 4, and 6 hr) for R3 being 6.33, 10.47, 14.40 and 9.65, respectively. The lowest values were obtained with the control ration being 6.07, 8.07, 10.47 and 7.77 meq/dl at 0, 2, 4 and 6h post-feeding. Values for the other treatments (R2 and R4) were intermediates.

Table 5: Rumen VFA (meq/dl) in sheep as affected by dietary treatments

Time	N	Time	Mean	Std. Error	Sig.
0 hr	20	1	6.07 ^c	0.013	0.01
	20	2	6.19 ^d	0.010	
	20	3	6.33 ^a	0.024	
	20	4	6.22 ^d	0.008	
2 hr	20	1	8.07 ^c	0.018	0.01
	20	2	8.12 ^c	0.010	
	20	3	10.47 ^a	0.108	
	20	4	8.46 ^b	0.026	
4 hr	20	1	10.47 ^c	0.033	0.01
	20	2	10.53 ^c	0.027	
	20	3	14.40 ^a	0.106	
	20	4	11.91 ^b	0.107	
6 hr	20	1	7.77 ^b	0.090	0.01
	20	2	7.86 ^b	0.083	
	20	3	9.65 ^a	0.136	
	20	4	8.06 ^d	0.064	

^{a, b, c,} values having different superscripts within each column are significantly different.

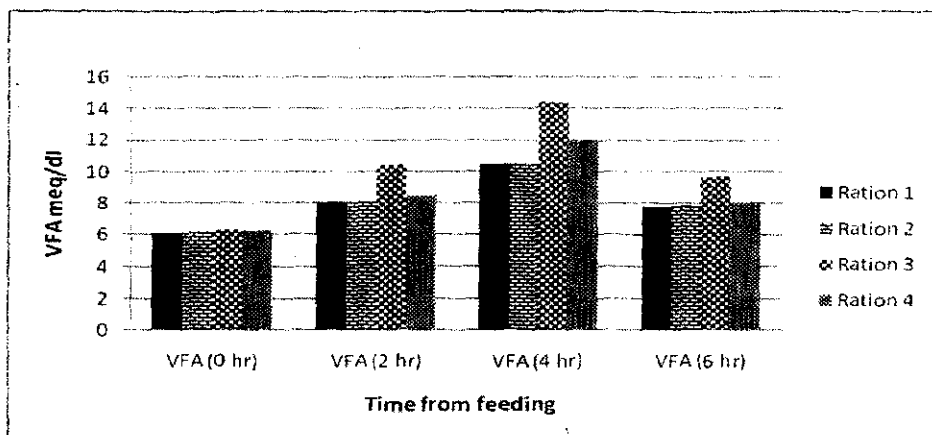


Fig. 4: Rumen VFA in sheep as affected by dietary treatments.

Generally with all dietary treatment, the highest values of VFA were obtained at 4h post feeding. It is obvious that S-supplementation improved the microbial activity in the rumen of the experimental sheep. Lundquist *et al.* (1985) reported high production of butyric, isobutyric and isovaleric acids in the rumen as a result of feeding methionine. Quispe *et al.* (1991) determined higher acetate and iso-acids levels in the rumen of S-fed sheep. Al-Dobeab (2004) found that 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. Total VFA was reported to increase as a result of feeding S-supplemented ration to sheep (Saini *et al.*, 2005). On the other hand, Chamberlain and Thomas (1983) reported that ruminal infusion of 7g methionine and 6.7g sodium sulfate had no effect on the concentration of total or individual volatile fatty acids (VFA) in the rumen fluid. Kumar and Bhatia (1984) and Judkins *et al.*, (1994) came to the same conclusion.

Table (6) and Fig (5) shows that there is a significant effect ($P<0.01$) among studied rations on rumen $\text{NH}_3\text{-N}$ at different studied time (0, 2, 4, and 6 hr); $\text{NH}_3\text{-N}$ concentration at 0 hr for rations 3 and 4 were 15.27 and 15.31, respectively with no significant difference between them, but significantly higher than that of rations 1 and 2. Concentration of $\text{NH}_3\text{-N}$ at 2, 4 and 6 hr for R3 was 18.69, 23.50 and 17.20, respectively, and being higher than the other studied rations.

Al-Dobeab (2004) determined the effect of urea (U) and sulfur (S) supplementation (in the form of potassium sulfate) on $\text{NH}_3\text{-N}$ concentrations. U-diet and US-diet showed a significant increase in rumen ammonia-N before and after feeding, whereas S-diet did not show such increments. Saini *et al.* (2005) evaluated the effect sulfur supplemented wheat straw diet on rumen

ammonia nitrogen which was found to be significantly ($P<0.01$) higher in sulfur-supplemented groups than the control group.

Table 6: Rumen ammonia-N (mg/dl) in sheep as affected by dietary treatments

Time	N	Time	Mean	Std. Error	Sig.
0 hr	20	1	14.71 ^b	0.072	0.01
	20	2	14.77 ^b	0.068	
	20	3	15.27 ^a	0.028	
	20	4	15.31 ^a	0.053	
2 hr	20	1	17.68 ^b	0.046	0.01
	20	2	17.85 ^b	0.054	
	20	3	18.69 ^a	0.177	
	20	4	17.68 ^b	0.055	
4 hr	20	1	19.21 ^c	0.071	0.01
	20	2	19.56 ^c	0.119	
	20	3	23.50 ^a	0.133	
	20	4	21.09 ^b	0.168	
6 hr	20	1	16.21 ^c	0.069	0.01
	20	2	16.44 ^{bc}	0.077	
	20	3	17.20 ^a	0.118	
	20	4	16.67 ^b	0.089	

^{a, b, c,} values having different superscripts within each column are significantly different.

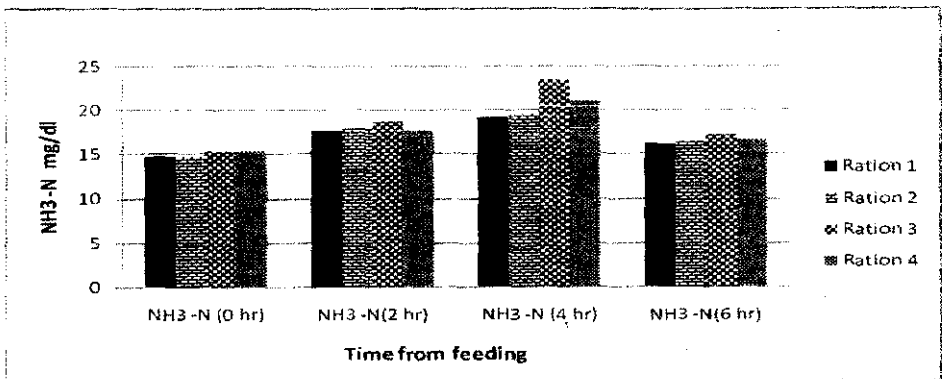


Fig. 5: Rumen ammonia-N in sheep as affected by dietary treatments.

Effect of sulfur supplementation on nutrients utilization and.....

From the results of the present study, it could be concluded that the sulfur content of the conventional rations usually used in the small holder farms is not sufficient to cover the S requirements for Ossimi sheep. The present study indicates that the best N:S ratio for Ossimi sheep should be in the range of 10:1. This level leads to better nutrient utilization and better microbial activity which in turn well lead to more retained nutrients and lesser output leading to cleaner environment. The findings of the present study highlight the need to evaluate feed sources in terms of protein degradability and sulfur amino acid composition.

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تأثير الكبريت على الاستفادة من الغذاء والنشاط الميكروبي بكرش الأغنام الأوسيمي

بركات محمد أحمد ، إبراهيم محمد صديق ، حمدي توفيق طابع ،

كمال محمد عبد الرحمن ، ماجد مروان محمد علي

قسم الإنتاج الحيواني - كلية الزراعة - جامعة المنوفية

الملخص العربي

أجريت التجربة بهدف دراسة تأثير إضافة عنصر الكبريت على معاملات الهضم والقيمة الغذائية والنشاط الميكروبي بكرش أربعة من كباش الأوسيمي المزودة بناسور بالكركش. في تصميم تجريبي 4×4 المربع اللاتيني استخدم أربعة مستويات من الكبريت المضاف بمعدلات صفر، 2، 4، 6 جم للرأس يوميا للعلاقى التجريبية المقارنة والمعاملة 2، 3، 4 على التوالي. أشارت نتائج التجربة إلى تحسن معاملات هضم المادة الجافة والعضوية والبروتين الخام والدهن الخام الألياف الخام والكربوهيدرات الذائبة نتيجة إضافة الكبريت وكانت أفضل النتائج بالعليقة الثالثة. أدى التحسن في معاملات الهضم إلى تحسن معنوي في القيمة الغذائية مقدرة في صورة مركبات كلية مهضومة وبروتين خام مهضوم وكانت أفضل النتائج في العليقة الثالثة والتي كانت نسبة النيتروجين إلى الكبريت بها 10:1. أدت التغذية على مستويات الكبريت إلى تحسن النشاط الميكروبي بالكركش مقدرا في صورة أحماض دهنية طيارة وإنتاج أمونيا - حيث بلغت أقصاها عند التغذية على العليقة الثالثة أيضا.