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**Research Article**

# Effects of Defaunation and Dietary Nitrogen Source on Sodium, Potassium, Iron and Zinc in the Rumen Fluid, Plasma and Wool of Lambs*

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**Abstract:** This study was conducted to investigate the effects of defaunation, and the addition of urea and sulfur in the replacement of plant protein (PP) sources on concentrations of Na, K, Zn and Fe in the ruminal fluid, blood plasma and wool of lambs. Forty male Ramlic lambs, 2.70 ± 0.30 months of age, and weighing approximately 16.44 ± 0.41 kg, were used. Lambs were divided into 4 groups as follows: faunate + control diet (FC), defaunate + control diet (DC), faunate + experimental diet (FE), and defaunate + experimental diet (DE). The control diet contained plant protein as a N source, whereas the experimental diet was supplemented with urea and sulfur, both at 3% of BW per day. The forage portion of the diet was 350 g of alfalfa hay per lamb per day. Diets were fed twice daily in equal amounts. Defaunation, or feeding a diet supplemented with urea and sulfur in place of plant protein sources, had no effect on ruminal fluid Na concentrations but there was an interaction on Na concentration in the rumen fluid. Ruminal K concentration was lower ($P < 0.021$) in the experimental diet versus the control diet, while it was not affected by defaunation. There was an interaction effect on Zn concentration in rumen fluid. Plasma Na, K and Zn concentrations were not affected by treatments, whereas defaunation significantly decreased ($P < 0.048$) plasma Fe concentration. Defaunation or N source had no effect on Na, K, Zn and Fe concentrations in wool. We concluded that both defaunation and feeding a diet supplemented with urea and sulfur in place of plant protein sources did not affect blood plasma, except for Fe, and wool Na, K, Zn and Fe concentrations despite differences in their ruminal concentration.

**Key Words:** Lamb, defaunation, feeding with urea, sulfur, minerals

## Defaunasyon ve Rasyon Azot Kaynağının Kuzularda Rumen Sıvısı, Plazma ve Yapağı Sodyum, Potasyum, Demir ve Çinko Düzeylerine Etkileri

**Özet:** Bu çalışma, defaunasyon işlemi ile rasyona bitkisel protein kaynağı yerine üre ve kükürt ilave edilmesinin kuzularda rumen sıvısı, plazma ve yapağılarındaki Na, K, Zn ve Fe düzeylerine etkilerini belirlemek amacıyla yapıldı. Çalışmada; ortalama 2,70 ± 0,30 aylık, 16,44 ± 0,41 kg canlı ağırlığa sahip 40 adet erkek Ramlıç kuzu kullanıldı. Kuzular faunalı kontrol rasyon (FK), defaunalı kontrol rasyon (DK), faunalı deneme rasyon (FD) ve defaunalı deneme rasyon (DD) olmak üzere dört gruba bölündü. Azot kaynağı olarak, kontrol rasyonu bitkisel protein içerirken deneme rasyonu üre ve kükürt içerdi ve her iki rasyon da hayvanlara canlı ağırlıklarının % 3'ü oranında verildi. Kaba yem olarak her kuzuya günlük 350 g yonca kuru otu verildi. Yemleme eşit miktarda günde iki kez yapıldı. Araştırmada; defaunasyonun veya bitkisel protein kaynağı yerine üre ve kükürt ilave edilen rasyonla beslemenin, rumen sıvısındaki Na düzeylerine etki etmediği bunun yanında, rumen sıvısı Na düzeyi bakımından uygulamalar arasında bir etkileşimin olduğu tespit edildi. Rasyondaki azot kaynağı rumen sıvısı K düzeyini etkilerken ($P < 0,05$), defaunasyonun ise söz konusu parametreyi etkilemediği bulundu. Rumen sıvısı Zn düzeyi bakımından uygulamalar arasında bir etkileşim olduğu tespit edildi. Araştırmada; plazma Na, K ve Zn düzeylerinin uygulamalardan etkilenmediği, defaunasyonun ise plazma Fe düzeylerini önemli oranda azalttığı tespit edildi ($P < 0,05$). Sonuç olarak; defaunasyonun ve rasyona bitkisel protein kaynakları yerine üre ve kükürt ilave edilmesinin, rumen düzeylerindeki farklılığa rağmen, plazma (Fe hariç) ve yün Na, K, Zn ve Fe düzeylerini etkilemediği tespit edildi.

**Anahtar Sözcükler:** Kuzu, defaunasyon, üre ile besleme, kükürt, mineraller

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## Introduction

Ruminants have a highly efficient anaerobic fermenter located at the beginning of their digestive tract. This allows them to digest fibrous feed and to use non-protein nitrogen (NPN) to synthesize microbial matter, thereby reducing their competition with humans for food. The rumen is essentially a fermentation chamber in which the resident microbial population helps to digest the diet and is inhabited by diverse and interdependent populations of bacteria, protozoa and fungi. The elimination of protozoa, defaunation, from the rumen microbial ecosystem is a means of overcoming the loss of available protein due to protozoal turnover (1). After defaunation, bacterial numbers in the rumen generally increase (2,3), leading to a positive effect on the efficiency of feed utilization (1). In contrast to ruminal bacteria, protozoa cannot convert NPN to protein because they have no ureases and so cannot utilize urea and ammonia to synthesize amino acids (4). Thus, the presence of protozoa may limit the productivity of ruminants, particularly when the diet contains urea. Studies on defaunated animals have shown that defaunation increased live weight gain and wool production in lambs fed a diet containing urea (5-7).

It is well known that moderate mineral deficiencies or improper proportions of minerals in the diet of ruminants often induce depressed food intake; this depression may be at least partly due to the impaired activity of rumen microorganisms. Minerals are necessary for microbial growth and for the various processes of fermentation in the rumen. Ivan et al. (8) suggested that the ruminal and abomasal solubility of some minerals could be affected by the presence of ruminal protozoa and dietary protein type. Eryavuz et al. (9) showed that defaunation did not change the ruminal fluid and plasma zinc concentrations, but increased its mohair concentration in Angora goats. In addition, Dayrell et al. (10) found that defaunation had no effect on plasma selenium concentration but increased the selenium concentration in the kidney cortex and spleen in Canadian Arcott rams. Levels of some microminerals in hair may be correlated with dietary intake or mineral status of ruminants (11). Thus hair analyses may help to detect severe deficiencies of some required minerals in ruminants fed with the same diet in the long term. This experiment was, therefore, conducted to study the effects of presence or absence of rumen protozoa and dietary nitrogen source on the ruminal fluid, plasma and wool concentrations of Zn, Fe, Na and K in lambs.

## Materials and Methods

### Animals and diet

Forty male Ramlic lambs (65% Rombouillet and 35% Daglic genotype), 2.70 ± 0.30 months of age, and weighing approximately 16.44 ± 0.41 kg, were used. The lambs were equally divided into 4 groups (faunate + control diet (FC), defaunate + control diet (DC), faunate + experimental diet (FE), and defaunate + experimental diet (DE)) as similar as possible with regard to live weight at the beginning of the experiment. The lambs in groups FC and DC were fed control rations, and the lambs in groups FE and DE were fed the experimental ration shown in Table 1. In addition, 350 g of alfalfa hay was given daily to each lamb. All diets were pelleted except for the alfalfa hay. All lambs in each group were housed in a pen isolated from the others. The lambs in each group were fed twice a day, their rations being divided into 2 equal parts as 3% of live weight. Water was available ad libitum during the experimental period. The lambs in groups FE and DE were adjusted to their diet with urea 2 weeks after the defaunation procedure. All lambs were treated in accordance with the regulations laid down by Turkish law. The experimental period was 105 days.

### Defaunation procedure

The method described by Ankrah et al. (12) was employed for defaunation. Briefly, 1 day prior to starting the defaunation procedure, all the lambs in groups DC and DE were fed half of their ration. In these groups, feeding was stopped for the first 3 days of the defaunation period. The lambs in groups DC and DE were defaunated with a solution (100 ml daily for each animal) of dioctyle sodiumsulphosuccinate (DSS: Sigma Co, Cat no: 4422) (2% W/V) delivered directly into the rumen through a polyethylene tube inserted down the esophagus. This was given for 3 days without any interval. On days 2 and 3, 200 ml of a substrate solution (20 g starch, 40 g sucrose, 20 g casein and 20 g electrolyte mix NaCl, 37; $KH_2PO_4$, 37; $(NH_4)_2SO_4$, 18.5; $MgSO_4$, 3.7; $CaCl_2$, 3.7% of total mix) was infused into the rumen of each lamb in groups DC and DE via the esophagus 2 h post-dosing with DSS. The substrate solution was used to sustain the bacterial population in the rumen when the animals were off feed. All lambs were fed their assigned diets starting from the last day of DSS dosage. Since omasal protozoa are extremely difficult to remove and are responsible for reinoculating

Table 1. Composition of control and experimental diets (%).

| | Control | Experimental | Alfalfa |
|---|---|---|---|
| Barley | 48.0 | 61.8 | |
| Oats | 21.5 | 24 | |
| Molasses | 2.0 | 6.5 | |
| Cottonseed meal | 17.4 | - | |
| Soybean meal | 5.0 | - | |
| Sunflower meal | 3.1 | - | |
| Urea | - | 2.9 | |
| Sodium sulfate | - | 1.8 | |
| Granite powder | 1.8 | 1.8 | |
| NaCl | 0.5 | 0.5 | |
| Dicalcium phosphate | 0.5 | 0.5 | |
| Mineral mix (Remineral 2) | 0.1 | 0.1 | |
| Vitamin mix (Rovimix 301-F) | 0.1 | 0.1 | |
| Chemical composition (% of DM) | | | |
| Crude protein | 13.7 | 15.7 | 10.3 |
| Ether extract | 3.7 | 2.4 | 1.0 |
| Neutral detergent fiber | 22.7 | 19.4 | 53.6 |
| Acid detergent fiber | 11.2 | 8.1 | 34.8 |
| Sodium (%) | 0.26 | 0.86 | 0.04 |
| Potassium (%) | 0.78 | 0.69 | 0.83 |
| Iron (%) | 0.02 | 0.01 | 0.01 |
| Zinc (ppm) | 92.4 | 84.1 | 6.8 |

Mineral mixture (Remineral 2, Roche, İstanbul, Turkey), contained 50 g manganase, 50 g iron, 50 g zinc, 10 g copper, 0.15 g selenium, 0.15 g cobalt and 0.8 g iodine per kg.

Vitamin supplement (Rovimix 301-F, Roche, İstanbul, Turkey), composed of 15,000,000 IU Vitamin A; 4,000,000 IU Vitamin D3; 20 g Vitamin E; 4 g Vitamin B1; 10 g Vitamin B2; 5 g Vitamin B6; 15 g calcium-D-pantptothenate; 20 g niacin; 20 mg Vitamin B12; 50 mg D-Biotin and 200 g choline chloride per kg.

transiently defaunated rumens, 2 weeks from the beginning of the defaunation period a 100 ml solution of DSS (5% W/V) was again infused into the rumen of each lamb in groups DC and DE without fasting the lambs. This procedure has been found to be very effective in completely removing protozoa from the rumens of lambs and allowing them to return to a normal appetite in a short period (12).

### Preparation of samples

Rumen contents were sampled from each lamb in all groups 4 h after the morning feeding on day 105 of the experimental period. Rumen contents collected via a stomach tube were strained through 4 layers of cheesecloth to yield rumen fluid for Na, K, Zn and Fe determinations. At the same time, the blood samples were collected into evacuated heparinized tubes from the jugular vein of animals and plasma was prepared by centrifugation (3000 x g, 20 min, 4°C) to measure plasma concentrations of Na, K, Zn and Fe.

Hair samples were collected from the right flank region of animals. This area was thoroughly groomed to remove foreign materials and the hair was clipped using a pair of stainless steel scissors. Approximately 1 g of each sample was weighed in a clean crucible. They were washed well with deionized water without loosing the samples. Then the samples were dried in a hot air oven

for 18 to 24 h at 60 °C. After determining the dry matter, complete ashing was done by placing them at 550 °C for 24 h in a muffle furnace. Thus the ash content of each sample was calculated. Concentrated hydrochloric acid (3 ml) was added to the ash and evaporated to dryness over a water bath. Nitric acid (5 ml, 10 p. 100) was then added and the solution filtered in a 50-ml volumetric flask. The filtrate was made up to the mark with deionized water. All minerals were estimated using appropriate dilutions. Precautions were taken to avoid mineral contamination (13). Analysis of sodium and potassium was carried out in a flame photometer using respective filters (Jenway PFPZ). Concentrations of iron and zinc were estimated by atomic absorption spectrophotometer (Buck Scientific Modem 200A).

### Statistical analysis

The experiment was a 2 x 2 factorial experiment and statistical models contained effects of microfauna, N source and their interaction. Data were analyzed by one- and two-way ANOVA statistical procedures of Harvey (14) and Systat. The multiple comparison of significant differences was applied using the contrast (14) and Bonferroni options of Systat, respectively.

## Results

One faunate lamb fed the experimental diet and one defaunate lamb fed the control diet suddenly started to lose BW for unknown reasons during the 2nd and 3rd week of the experimental period. These lambs were removed from the experiment and their data were excluded from the analysis. All other lambs were healthy throughout the experiment. Ruminal Na and Zn concentrations were not affected either by defaunation or adding urea and sulfur to the diet, but there was an interaction effect on Na and Zn concentrations in the rumen fluid (Table 2). Ruminal K concentration was not affected by defaunation, but lambs fed the experimental diet had lower ($P < 0.05$) ruminal K concentrations compared to lambs fed the control diet. Ruminal Fe concentration was not affected by treatments, but plasma Fe concentration was lower ($P < 0.05$) in defaunated lambs than in faunated animals (Table 2). However, defaunation and feeding with urea and sulfur did not affect plasma Na, K and Zn concentrations. Similarly, wool Na, K, Zn and Fe concentrations did not differ among the treatments (Table 2).

Table 2. Effects of defaunation and dietary nitrogen source on sodium, potassium, iron and zinc in rumen fluid, plasma and wool of lambs.

| | Faunate (F) | | Defaunate (D) | | S.E.M. | Probability | | |
|---|---|---|---|---|---|---|---|---|
| | PP[1] | NPN[2] | PP | NPN | | FD[3] | PN[4] | FD x PN[5] |
| Number of lambs | 10 | 9 | 9 | 10 | | | | |
| Rumen fluid Na (mg/dl) | 145.90 | 139.00 | 135.33[b] | 150.00[a] | 2.111 | 0.959 | 0.364 | <0.05 |
| Rumen fluid K (mg/dl) | 71.60 | 64.33 | 74.22 | 59.40 | 2.281 | 0.802 | 0.021 | 0.413 |
| Rumen fluid Zn (µg/ml) | 0.467[b] | 0.996[a] | 0.794 | 0.569 | 0.078 | 0.751 | 0.337 | <0.05 |
| Rumen fluid Fe (µg/ml) | 0.695 | 0.833 | 0.926 | 0.866 | 0.086 | 0.447 | 0.819 | 0.567 |
| Plasma Na (mmol/l) | 127.75 | 126.04 | 129.47 | 128.40 | 1.097 | 0.359 | 0.531 | 0.886 |
| Plasma K (mmol/l) | 4.76 | 4.27 | 4.47 | 4.69 | 0.156 | 0.830 | 0.665 | 0.267 |
| Plasma Zn (mmol/l) | 10.21 | 12.14 | 9.95 | 11.79 | 0.581 | 0.793 | 0.114 | 0.970 |
| Plasma Fe (mmol/l) | 47.63 | 32.03 | 29.24 | 31.33 | 2.328 | 0.048 | 0.155 | 0.066 |
| Wool Na (µg/g) | 317.60 | 272.22 | 301.22 | 266.80 | 14.413 | 0.708 | 0.175 | 0.850 |
| Wool K (µg/g) | 127.30 | 91.88 | 137.55 | 127.10 | 10.654 | 0.294 | 0.289 | 0.562 |
| Wool Zn(µg/g) | 66.660 | 66.086 | 65.122 | 81.717 | 4.046 | 0.390 | 0.329 | 0.296 |
| Wool Fe (µg/g) | 31.386 | 29.176 | 29.250 | 33.780 | 1.536 | 0.690 | 0.708 | 0.280 |

Same line with different superscript differ significantly.

[1] Plant protein.

[2] Non-protein nitrogen.

[3] Faunate vs. defaunate.

[4] Plant protein vs. non-protein nitrogen.

[5] Faunate and defaunate vs. plant protein and non-protein nitrogen.

## Discussion

Proteins vary widely in sulfur content, depending on their amino acid composition. Bacterial proteins have more the sulfur-containing amino acids than the protozoal proteins (15). A suitable supply of sulfur in the diet especially containing urea is required and this sulfur is used by the rumen bacteria for synthesis of sulfur-containing amino acids (methionine, cystine/cysteine) (16). Therefore, in this study, $Na_2SO_4$ was added to an experimental diet containing urea.

The functions of minerals in animal physiology are interrelated; seldom can they be considered as single minerals with independent and self-sufficient roles. Because protozoa engulf bacteria and can be selectively retained within the rumen, this may decrease the flow of bacterial biomass from the rumen and reduce the availability of protein and energy as well as minerals to the host animal (17). In addition, they appear to need more some macro- and microminerals than bacteria (15). Ivan et al. (18) suggested that insoluble complexes between feed Cu (Fe, Zn) and sulfide released from protein degradations should be formed. As protein degradation in the rumen is markedly reduced by defaunation (1), less sulfide should be released from dietary protein and thus trace mineral absorption could increase. Ivan (19) suggested that the degradation of dietary proteins in the rumen by protozoa could contribute to increased ruminal sulfide concentrations, and consequently to lower availability of Cu due to the formation of copper sulfide. This effect of protozoa on the dietary Cu metabolism has been found in sheep supplemented with soybean meal (18,19) but not in those supplemented with urea (20). However, Bonhomme et al. (21) demonstrated that, with zinc concentrations in the medium exceeding 2.5 mg/ml, the protozoa incorporated this metal even if it was toxic. Moreover, they found that some of the sheep fed a 1000 ppm zinc diet were defaunated during the 6 weeks.

In the present study, ruminal Na, Fe and Zn concentrations were not affected either by defaunation or adding urea and sulfur to the diet, but there was an interaction effect on Na and Zn concentrations in the rumen fluid (Table 2). However, this interaction was not observed in plasma or wool samples. This result for Na may be due to the experimental diet containing higher Na than the control diet (Table 1). The observation of a significant interaction between faunation type and nitrogen source for Zn in this study supported the results published by Ivan (19).

In vitro studies with sheep rumen microorganisms revealed that K, but not Na, was essential for the rumen microbial population (22). In addition, Khorasani and Armstrong (23) suggested that the effect of mineral salts on the apparent digestibility of N might be related to the content of the diets and the N status of the animals. On the other hand, the mineral content of rumen bacteria increased when the bacteria were exposed to an environment with a higher mineral content (23). Our results showed that K concentration in the ruminal fluid was affected by adding urea and sulfur to the diet and, lambs fed with diet containing urea had lower K in rumen contents than in lambs fed with a diet containing plant protein. This result may indicate that bacteria used more K for protein synthesis in defaunated animal fed with a diet containing urea because bacteria have amino acids synthesized from ammonia in the rumen in contrast to protozoa.

The effects of rumen protozoa on micromineral absorption, metabolism and retention were first investigated by Ivan et al. (8,18). Protozoal effects on micromineral absorption might occur because of both the higher feed protein degradation in the presence of rumen protozoa (1) and the well-documented interactions between protein and microminerals in small intestinal absorption (24). Ivan et al. (18) provided indirect evidence for an increased absorption of Fe, Zn and especially Cu in the absence of protozoa, as liver concentrations of these elements dramatically increased and both plasma Cu concentration and the activity of the Cu-dependent ferroxidase were higher. However, the effects of Cu, Fe and Zn accumulation were lower in the complete liver than the effects on the concentrations per gram of liver, obviously because of lower liver weights in protozoa-free animals. Subsequent experiments showed only lower effects of defaunation on Cu concentration in the liver (8,19), whereas Eryavuz et al. (25) reported that defaunation had no effect on plasma concentrations of Mg, Ca, K, Zn or Cu in Angora goats. In addition, Kreuzer and Kirchgessner (24) found that fecal losses of Cu, Zn, and Mn were not affected, while fecal excretion of Fe was slightly increased by defaunation. In the present study, plasma concentrations of Na, K and Zn were not affected by defaunation, whereas defaunation decreased the plasma concentration of Fe.

Microminerals play critical roles in the normal development of wool (26). Therefore, the analysis of hair has been shown to be a useful diagnostic aid in determining the mineral status of animals (13) because growing hair is metabolically active and functions as a sequestering tissue. Hair zinc content was found to reflect dietary zinc levels more consistently than any other tissue (27). In the present study, wool mineral concentrations of lambs did not differ among the treatments. This result contradicts the results reported by Eryavuz et al. (9), who found that defaunation did not affect rumen or plasma Zn concentrations, but increased mohair Zn at the end of about 4.5-month periods in Angora goats. This discrepancy between our result and those researchers for Zn (9) may be due to diet and animal species. The lambs in this study were fed more concentrate diet than diet reported by researchers (9). This may cause more intake of minerals with the diet by animals. The dietary concentrations of Na, K, Fe and Zn in this study were sufficient and non-toxic regarding the recommendations of NRC (28) for sheep (Table 1).

In conclusion, both defaunation and feeding a diet supplemented with urea and sulfur in place of plant protein sources did not affect plasma, except for Fe, and wool Na, K, Zn and Fe concentrations. However, rumen mineral concentrations were affected by both defaunation and nitrogen sources in the diet.

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**References**

1. Jouany, J.P.: Effect of rumen protozoa on nitrogen utilization by ruminants. J. Nutr., 1996; 126: 1335-1346.
2. Hsu, J.T., Fahey, G.C.Jr., Merchen, N.R., Mackie, R.I.: Effects of defaunation and various nitrogen supplementation regimens on microbial numbers and activity in the rumen of sheep. J. Anim. Sci., 1991; 69: 1279-1289.
3. Koenig, K.M., Newbold, C.J., McIntosh, F.M., Rode, L.M.: Effects of protozoa on bacterial nitrogen recycling in the rumen. J. Anim. Sci., 2000; 78: 2431-2445.
4. Jouany, J.P., Demeyer, D.I., Grain, J.: Effect of defaunating the rumen. Anim. Feed Sci. Technol., 1988; 21: 229-265.
5. Bird, S.H., Hill, M.K., Leng, R.A.: The effects of defaunation of the rumen on the growth of lambs on low-protein-high-energy diets. Br. J. Nutr., 1979; 42: 81-87.
6. Demeyer, D.I., van Nevel, C.J., van de Voorde, G.: The effect of defaunation on the growth of lambs fed three urea containing diets. Arch. Tierernaehrung Berlin, 1982; 32: 595-604.
7. van Nevel, C.J., Demeyer, D.I., van de Voorde, G.: Effect of defaunating the rumen on growth and carcass composition of lambs. Arch. Tierernaehrung Berlin, 1985; 35: 331-337.
8. Ivan, M., Dayrell, M.S., Hidiroglou, M.: Effects of bentonite and monensin on selected elements in the stomach and liver of fauna-free and faunated sheep. J. Dairy Sci., 1992; 75: 201-208.
9. Eryavuz, A., Durgun, Z., Keskin, E.: Effects of ration supplemented with zinc on some rumen and blood parameters, mohair production and quality in faunated and defaunated Angora goats. Turk. J. Vet. Anim. Sci., 2002; 26: 753-760.
10. Dayrell, M.S., Ivan, M., Hidiroglou, M.: The effects of ruminal protozoa on selenium status in sheep. Can. J. Anim. Sci., 1991; 71: 1269-1270.
11. Combs, D.K., Goodrich, R.D., Meiske, J.C.: Mineral concentrations in hair as indicators of mineral status: A review. J. Anim. Sci., 1982; 54: 391-398.
12. Ankrah, P., Loerch, S.C., Kampman, K.A., Dehority B.A.: Effects of defaunation on in situ dry matter and nitrogen disappearance in steers and growth of lambs. J. Anim. Sci., 1990; 68: 3330-3336.
13. Kumaresan, A., Kapioh, M.A.: Hair as indicator of mineral status in Yankassa sheep. Rev. Elev. Med. Vet. Pays. Trop., 1984; 37: 61-64.
14. Harvey W.R.: Users Guide for LSMLMW PC-1 Version. Mixed Model Least-Squares and Maximum Likelihood Computer Program, Mimeo. Ohio State University, Columbus, OH. 1987.
15. Bonhomme, A.: Rumen ciliates: their metabolism and relationships with bacteria and their hosts. Anim. Feed Sci. Technol., 1990; 30: 203-266.
16. Hays, V.W., Swenson, M.J.: Dukes' Physiology of Domestic Animals, Edited by Melvin J. Swenson, William O. Reece, Eleventh Edition, Cornell University Press, Ithaca and London, 523 pp. 1993.
17. Ushida, K., Jouany, J.P., Demeyer, D.I.: Physiological Aspects of Digestion and Metabolism in Ruminants; 1st Edition; London Academic Press; p.626-641, 1991.
18. Ivan, M., Veira, D.M., Kelleher, C.A.: The alleviation of chronic copper toxicity in sheep by ciliate protozoa. Br. J. Nutr., 1986; 55: 361-367.
19. Ivan, M.: Effects of faunation and type of dietary protein on gastric solubility and liver content of copper in sheep. J. Anim. Sci., 1989; 67: 3028-3035.

20. Ivan, M., Veira, D.M.: Duodenal flow and soluble proportions of zinc, manganese, copper and iron in the rumen fluid and duodenal digesta of faunated and defaunated sheep. Can. J. Anim. Sci., 1982; 62: 979-982.

21. Bonhomme, A., Quintana, C., Durand, M.: Electron microprobe analysis of zinc incorporation into rumen protozoa. J. Protozool., 1980 ; 27: 491-497.

22. Hubbert, F., Cheng, E., Burroughs, W.: The influence of potassium, sodium, rubidium, lithium and cesium on in vitro cellulose digestion by rumen microorganism with observation upon sodium and potassium influences in lambs fattening rations. J. Anim. Sci., 1958; 17: 576-585.

23. Khorasani, G.R., Armstrong, D.G.: Effect of sodium and potassium on overall digestibility of a semi-purified diet and microbial protein production in the rumen of sheep. Livest. Prod. Sci., 1990; 24: 347-357.

24. Kreuzer, M., Kirchgessner, M.: Faecal and urinary excretion of iron, copper, zinc and manganese in faunated and defaunated wethers fed semi-purified diets of different carbohydrate composition. Anim. Feed Sci. Technol., 1990; 28: 135-143.

25. Eryavuz, A., Altunok, V., Keskin, E., Haliloğlu, S.: Effects of defaunation and ration supplemented with zinc on some plasma mineral levels in Angora goats. Hayv. Araş. Derg., 2001; 11: 39-43.

26. McDowell, L.R.: Minerals in Animal and Human Nutrition. Ed. By T.J. Cunha. Academic Press Inc. San Diego. 1992.

27. Beeson, W.M., Perry, T.W., Zurcher, T.D.: Effect of supplemental zinc on growth and on hair and blood serum levels of beef cattle. J. Anim. Sci., 1977; 45:160-165.

28. National Research Council, (NRC): Nutrient Requirements of Sheep, $6^{th}$ rev. edn. Natl. Acad. Sci., National Academy Press, Washington, DC. 1985.