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Effects of Different Dietary Energy and Protein Levels on Rumen pH, Urea Levels and Rumen Protozoal Population in Sheep

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Abstract: The effects of different levels of energy and protein rations on rumen fluid, pH, ammonia, urea levels, kinds and numbers of species of protozoa in sheep are examined in the present experiment. Six male, Merino sheep were utilized in a 6 X 6 Latin square design. The rations were prepared as standard protein and standard energy level /(S-Group 1); low energy (LE-Group 2); low protein (LP-Group 3); high protein (HP-Group 4); high energy (HE-Group 5) and 10 g/day bicarbonate added to the high energy (HE+HCO_3-Group 6). The experimental period extended to 10 days feeding period for each ration. Rumen fluid samples were taken from animals 0 hours before feeding as well as in the 2nd and the 4th hours after feeding during the last two days of feeding period. The end of the experiment time and feed factor showed statistically significant differences in pH values ($P<0.05$). The lowest pH level was observed in sheep fed ration HE+HCO_3 before feeding time and pH levels were not statistically significant in the 2nd and 4th hours after feeding. It was found that the ammonia level in animals fed HP ration was the lowest ($P<0.05$) before feeding. Differences among time factors were found to be statistically significant ($P<0.05$) before feeding and 4th hours after feeding. Among the protozoa species *Entodinium minimum* was the most common species. Differences among feed factors for *Entodinium caudatum* level are found to be statistically significant ($P<0.05$).

Key Words: Energy, protein, protozoa, rumen parameters, sheep.

Rasyondaki Farklı Protein ve Enerji Seviyelerinin Koyunlarda Rumen pH'sı, Üre Düzeyi ve Rumen Protozoa Populasyonu Üzerine Etkisi

Özet: Araştırmada, enerji ve protein düzeyleri farklı rasyonların koyunlarda rumen sıvısı, pH, üre düzeyleri ve rumen protozoa sayıları ile türleri üzerine olan etkileri incelenmiştir. Araştırmada 6 erkek Merinos koç 6 X 6 latin kare deneme düzeninde kullanılmıştır. Rasyonlar sırasıyla standart protein ve enerji seviyeli (S-Grup 1); düşük enerji (LE-Grup 2); düşük protein (LP-Grup 3); yüksek protein (HP-Grup 4); yüksek enerji (HE-Grup 5) ve yüksek enerjili rasyona 10 g/gün bikarbonat ilavesi (HE+HCO_3-Grup 6) olarak hazırlanmıştır. Hayvanlardan yemlemeden önce ve yemlemeden sonra 2. ve 4. saatte rumen sıvısı örnekleri alınmıştır. Araştırma sonunda pH değeri için zaman ve yem faktörleri arasındaki farklılık istatistiksel olarak önemli bulunmuştur ($P<0.05$). En düşük rumen sıvısı pH değerlerinin yemlemeden önce 6.(HE+HCO_3) rasyonla beslenen koyunlarda belirlenirken yemlemeden sonraki 2. ve 4. saatlerde istatistiki açıdan önemli farklılık oluşmamıştır. Yemlemeden önce en düşük amonyak değerleri yüksek proteinli (HP) rasyon grubunda bulunmuştur ($P< 0.05$). Amonyak değerleri yemlemeden önce ve sonraki 4. saatte zaman faktörleri arasındaki farklılık istatistiksel olarak önemli bulunmuştur ($P<0.05$). Rumen protozoa türlerinden en sık olarak *Entodinium minimum* belirlenmiştir. *Entodinium caudatum* düzeyi için yem faktöründeki farklılıklar istatistiksel açıdan önemli bulunmuştur ($P<0.05$).

Anahtar Sözcükler: Enerji, protein, protozoon, rumen parametreleri, koyun.

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Introduction

The metabolism and gastrointestinal environment in domestic livestock are affected by many factors including diet and feeding regimen. Since rumen is the first and major site for digestion, adjusting its environment for optimal digestion has been the major goal for nutritionists. Substrate availability and pH in ruminal environment seem to be the most important items to be taken into account during the manipulation of nutrients in feeds. Additionally, total number of rumen microorganisms living symbiotically in ruminants and relative distribution of species vary depending on the composition of the feed. Although the number protozoa is numerically less than bacteria inside rumen microbial population, they form a volume almost as much as bacteria[23]. Digestibility of some rations may increase in the presence of protozoa. Moreover, it is known that they have an effective role on live weight gain and N-retention[20,4,10].

Anaerobic rumen protozoa are divided into two classes; *Holotrich* and O*ligotrich*[22]. In sheep's rumen, 10 species of both groups exist[16]. In fermentation of easily dissolvable carbohydrates, *Holotrich* species are more active than *Oligotrich* species[9]. Protozoa population is directly affected by pH value of rumen content: as the pH decreases, the number of protozoa decreases as well[18].

In sheep and goats living only on alfalfa, number of protozoa in rumen content was reported to be 1.12-1.93 x 10^5 /ml[8], 3.03-4.09 x 10^5 /ml[3] and in those fed with a concentrate / hay mixture, it was 3.64 x 10^5 /ml[17]. In a trial carried out with lamb fed with a ration containing sugar-beet bagasse with molasses, alfalfa hay, straw, starch, urea and a vitamin/mineral concentrate; number of protozoa was found to be 1.62 x 10^5 /ml prior to feeding and 1.44 x 10^5 /ml 3 hours after feeding[14]. It was reported that while feeds rich in starch decreased the number of protozoa species, they relatively increased *Entodinium* species[3,11] and feeds rich in alfalfa increased *Isotricha* species[12]. Roughage rich rations caused the number of protozoa species to decrease[1]. In sheep fed with alfalfa, it was observed that *Isotricha* ratio was 4.1%, *Dasytricha* was 3.8%, *Entodinium* was 67.1%, *Diplodinium* was 5.2% and *Epidinium ecaudatum* was 18.7%[3]. The aim of this trial was to investigate the effect of 6 rations in different energy and protein levels on rumen fluid parameters and rumen protozoa profile in sheep.

Materials and Methods

The present experiment was carried out using the sheep facility of the experimental units of Veterinary Faculty at Ankara University. Six Merino sheep (male), each about 60 kg body weights, 2.5 years old were fed in individual cages. The chemical compositions of the rations used in the study are given in Table 1. Basal ration consisted of alfalfa hay, and additionally, the experimental animals were fed with rations containing different energy and protein levels. The energy and protein contents included in the rations was as follows: Standard protein and standard energy levels (S-Group1); standard protein and low energy levels (LE-Group2); low protein and standard energy levels (LP-Group3); high protein and standard energy levels (HP-Group4); standard protein and high energy levels (HE-Group5). Finally, 10 g/day (%0.8) bicarbonate was added to ration 5 in order to form ration 6 (HE+HCO_3-Group6).

Feed was offered to the animals at 9^{00} and 16^{30} each day in accordance with 6x6 Latin square methods. Water was supplied *ad libitum*. Nutrient levels of feed ingredients and rations used in the trial were determined by the methods reported in AOAC[2] and metabolizable energy levels were calculated with the method suggested by TSE (Turkish Standards Institute) using the following formula:[21] (Table 1).

ME, (kcal/kg OM) = 3260+0.455CP-(4.037 CF+3.517 EE)

CP = crude protein, g/kg OM; CF = crude fiber, g/kg OM; EE = ether extract, g/kg OM.

In the trial, at the last two days of each feeding period, rumen fluid samples were taken before feeding as well as at 2 and 4 hours after feeding. The pH level was measured using pH meter in fresh rumen fluid. NH_3 was measured with an ammonia electrode (Orion^R) sensitive to gas in fresh rumen fluid. The ammonia data of experiments were determined with pH-meters like as mV, calibrated and read as ppm and then converted to mmol/L. Urea was determined according to Frazer's Neslerization method[7] in rumen fluid.

Number and identification of protozoa in rumen fluid: After an adaptation period followed by the feeding of the ration for ten days, rumen fluid was taken by rumen cannula. The

number of protozoa was counted and their identification in rumen fluid was performed using Fuchs-Rosenthal hematocytometer under light microscope according to the method described by Ogimoto and Imai[15]. A volume of 0.5 ml of rumen fluid was mixed with 4.5 ml MFS (methylgreen-formalin-salt) solution and kept at room temperature for counting. Then it was transferred into a counting chamber and the protozoa were counted in 4 microscopic fields. The counts were repeated for four times and thus 16 microscopic fields were counted for each sample.

Table 1. Amounts of feeds daily offered to each animal, kg

Tablo 1. Günlük hayvan başına verilen yem miktarları,kg

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Feedstuffs, kg	N	LE	LP	HP	HE	HE+HCO_3
Barley	1.01	0.52	0.17	0.72	0.30	0.30
Corn	-	-	0.65	0.20	0.80	0.80
Brain	0.18	0.52	-	-	-	-
Sunflower meal	-	-	-	0.27	0.10	0.10
Salt	0.02	0.02	0.02	0.02	0.02	0.02
Vit+Min premix	0.02	0.02	0.02	0.02	0.02	0.02
Bicarbonate	-	-	-	-	-	0.01
Total, kg	1.23	1.08	0.86	1.23	1.24	1.25
Straw	-	0.10	0.38	-	-	-
Alfalfa, dry	0.80	0.80	0.80	0.80	0.80	0.80
Nutrient values and metabolizable energy						
Dry Matter, %	91.98	92.25	92.55	92.06	91.51	91.44
Ash, %	6.40	7.38	6.51	7.04	5.80	5.70
Ether extract, %	2.20	2.25	2.19	1.66	2.42	2.36
Crude Fiber, %	19.90	25.03	19.38	20.80	16.03	15.88
Crude Protein, g	9.10	9.30	7.90	11.60	9.08	9.00
Metabolizable Energy, Mcal	2105	1878	2136	2043	2273	2278

Group 1: Standard protein and energy levels;
Group 2: Standard protein and low energy level;
Group 3: Low protein and standard energy level;
Group 4: High protein and standard energy level;
Group 5: Standard protein and high energy level;
Group 6: 0.01 kg/day bicarbonate was added to group 5 ration.
*: Metabolizable energy levels were calculated as described in TSE (1991).
ME, (kcal/kg OM) =3260+0.455CP-(4.037 CF+3.517 EE)
CP = crude protein. g/kg OM; CF =crude fiber, g/kg OM; EE = ether extract, g/kg OM.

The data of the experiment were tested by the Repeated Measure Anova technique. Feed and time factors have 6 (group N, LE, LP, HP, HE, HE+HCO_3) and 3 (0, 2, 4 hours) levels respectively. The count of observations was 6 for each group. Pairwise comparisons between factors were determined with the Duncan test. The protozoa data which were determined as percentage units were transformed with arcsine transformation technique first. Then it was evaluated with Repeated Measure Anova. SPSS 10.0 program was used for statistical analysis.

Results and Discussion

This trial in which the effects of 6 rations of different energy and protein levels on rumen fluid parameters and rumen protozoa in Merino male sheep were investigated. The mean pH value in rumen fluid (Table 2) after feeding was shown to be lower than that before feeding in all ration groups as was the case in trials with similar aspects[14,12,6,25]. It appears that, prior to feeding, there were significant differences ($P<0.05$) regarding the pH value between sheep fed with HE+HCO_3 ration and sheep that consumed rations 3, 4 and 5. Statistically significant differences in pH were also found between the group fed with ration 5 with normal protein and high energy levels and the groups which consumed rations 1 and 2. Another result of this experiment is that there were statistically significant differences ($P<0.05$) between group of animals that had ration 2 (LE) and the group that had ration 3 (LP) for pH values.

Table 2. The pH, NH_3 (mmol/L) and urea (mg/100 ml) values of rumen fluid (mean)

Tablo 2. Rumen içeriğinin pH, NH_3 (mmol/L) ve üre (mg/100 ml) değerleri

	Hour	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
		S	LE	LP	HP	HE	HE+HCO_3
		X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx
pH	0	6.32±0.13 Abcd	6.23±0.12 aCD	6.52±0.07 aABC	6.71±0.14 aA	6.63±0.07 aAB	6.06±0.26 aD
	2	6.00±0.07 Ba	5.96±0.09 bA	6.01±0.08 aA	6.04±0.11 bA	6.06±0.04 bA	5.84±0.25 bA
	4	5.87±0.09 bA	5.97±0.07 bA	5.84±0.08 aA	5.88±0.11 cA	5.68±0.08 cA	5.78±0.19 bA
NH_3	0	8.25±12.8 abA	7.29±15.7 bA	6.98±9.36 bA	3.88±14.20 bB	6.92±27.1 bA	7.91±11.8 a A
	2	9.25±10.3 Aa	9.66±24.7 aA	11.31±19.6 aA	10.43±32.3 aA	10.46±14.4 aA	9.08±11.4 aA
	4	7.08±9.2 bA	9.25±32.1 aA	9.56±29.5 aA	8.49±19.9 aA	6.66±18.2 bA	8.91±11.2 aA
Urea	0	14.00±3.73	16.14±6.01	11.53±1.42	15.81±2.02	14.88±5.08	22.41±4.01
	2	11.49±2.24	15.29±5.10	15.11±2.17	19.85±2.31	11.41±1.60	22.42±3.23
	4	11.53±3.54	10.19±4.87	11.73±3.48	20.37±2.46	15.96±2.37	17.46±3.41

a,b,c: Means on the same column (for time factor) with different letter significantly: $P < 0.05$
A,B,C,D: Means on the same line (for feed factor) with different letter significantly: $P < 0.05$.

In this study variance analysis of time and feed factor showed statistically significant results for NH_3 values ($P<0.05$). It was deter-

mined that the ammonia level in group 4 (high protein and standart energy level) was the lowest (P<0.05) before feeding. While ammonia values after feeding (Table 2) increased till the second hour, it decreased again in the fourth hour depending on the availability of protein and energy. These findings are similar to several comparable results[23,14,25].

In this study variance analysis of time and feed factor did not show statistically important results for urea (Table 2). Urea values in rumen fluid in the second hour after feeding were numerically higher in groups 6. The effect of bicarbonate on saliva secretion might have caused urea level to increase in group 6.

Total number of protozoa in rumen fluid (Table 3) depending on the feed types, decreased by folds ($x10^5$) of 1.93, 3.54, 2.40, 4.80, 3.22 and 2.96 in test groups, respectively, for the 2nd hour after feeding compared with the 4th hour and before feeding. But it started an increasing trend in the fourth hour. A variety of time factor's mean is found statistically important (P<0.01). Purser and Moir[17] reported that there could be a 3 fold in the number of protozoa after feeding compared to that before feeding. In present trial, the greatest decrease for the number of protozoa was 4.80 log units in group 4 (high protein level), group 2 (low energy level) followed this decrease by 3.54 log units.

Table 3. Total number of protozoa per millilitre of rumen fluid (n=6)

Tablo 3. Rumen içeriğinde (ml) toplam protozoa sayısı (n=6)

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	
	S	LE	LP	HP	HE	HE+HCO_3	Total
Hour	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx
0	823750.0 ± 240963.1	893291.7 ± 284989.0	1619583.3 ± 890914.9	1046583.3 ± 295051.6	1249750.0 ± 289080.7	1210833.3 ± 275537.9	1140631.9 ± 173403.5a
2	426250.0 ± 36995.2	252470.8 ± 100164.2	337083.3 ± 111097.6	325416.7 ± 102809.3	521666.7 ± 106124.3	409500.0 ± 87236.3	378731.3 ± 38426.6b
4	532083.3 ± 117656.7	437545.8 ± 125824.7	455833.3 ± 84226.1	405416.7 ± 200003.6	558333.3 ± 126474.1	417500.0 ± 76395.4	467785.4 ± 49416.6b

Group 1: Standard protein and energy levels;

Group 2: Standard protein and low energy level;

Group 3: Low protein and normal energy level;

Group 4: High protein and normal energy level;

Group 5: Standard protein and high energy level;

Group 6: 0.01 kg/day bicarbonate was added to group 5 ration.

a,b: Means on the same column (for time factor) with different letter significantly: P < 0.01.

No significant differences between ration groups for the number of protozoa in rumen fluid were observed. The present results can be interpreted as of rather constancy of protozoa mass depending on the improvement in ruminal N utilization[5,19].

The number of protozoa in group 6 which was subject to the same feeding conditions with group 5 but which has added bicarbonate showed lower values than that in group 5 (P>0.05). This can be explained with results[24] that there could be a possibility of change in the number of protozoa with feed content, saliva secretion and the amount of drinking water.

Mackie et al.[13] reported that a too high consumption of rations rich in easily fermentable carbohydrates results in an increase in protozoa death and decrease in pH value in rumen fluid. However in this trial, while the number of protozoa decreased after feeding; since there was not too much consumption of excessive carbohydrates, pH values and number of protozoon's after feeding did not show a difference between groups.

Among Oligotrich species in rumen fluid (Table 4), *Entodinium minimum* levels before feeding are found to be numerically lower in group 5 than those in other groups. *Entodinium caudatum* showed a low level in the presence of bicarbonate (group 6) and low energy (group 2), compared to group 5 and group 3 (P < 0.05). Variance analysis of time and feed interaction did not show statistically important results for *Entodinium caudatum* (P>0.05). But varieties of feed factor's mean were found statistically important (P<0.05) for 4 hours after feeding. As is the case in other studies[12,25] (Table 4), species of *Entodinium*, especially *Entodinium minimum* was predominant also in this trial.

At the end of the experiment, no important interaction was found between the groups for ruminal pH and protozoa accounts during the same time periods after feeding. When the protein level in ration increased the ruminal pH was also increased but the level of urea decreased. When the protein level decreased, contrarily the NH_3 levels were increased. Since the differences in groups were too much for NH_3 and urea we couldn't evaluate variations between the groups. The numbers of protozoa decreased after feeding. In the fifth experimental group (consumed high energy) the highest *Entodinium caudatum* numbers were found after feeding compared with those in other groups. *Entodinium spp*. became predominant in rumen

Table 4. Species and distribution (%) of rumen protozoa in rumen fluid (n=6)

Tablo 4. Rumen içeriği protozoa populasyonunu oluşturan türler ve dağılımı (%), (n=6)

		Groupn1	Group 2	Group 3	Group 4	Group 5	Group 6
Protozoon Species	Hour	S	LE	LP	HP	HE	HE+HCO_3
		X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx	X ± Sx
HOLOTRICH							
Isotricha Intestinalis	0	1.83 ± 0.98	1.00 ± 0.63	1.17 ± 0.75	0.17 ± 0.17	0.83 ± 0.65	0.17 ± 0.17
	2	0.67 ± 0.67	0.83 ± 0.48	0.50 ± 0.34	0.17 ± 0.17	0.50 ± 0.50	0.33 ± 0.33
	4	2.33 ± 2.33	0.67 ± 0.49	0.33 ± 0.33	0.00 ± 0.00	0.17 ± 0.17	0.67 ± 0.49
Isotricha Prostoma	0	1.00 ± 0.52	1.00 ± 0.37	0.33 ± 021	0.17 ± 0.17	0.33 ± 0.33	0.50 ± 0.34
	2	0.50 ± 0.34	0.67 ± 0.49	0.67 ± 0.42	0.17 ± 0.17	0.17 ± 0.17	0.33 ± 0.33
	4	1.00 ± 0.68	0.00 ± 0.00	0.17 ± 0.17	0.33 ± 0.33	0.67 ± 0.33	0.17 ± 0.17
Dasytricha Ruminantum	0	0.67 ± 0.49	0.67 ± 0.33	0.83 ± 0.48	0.17 ± 0.17	0.50 ± 0.22	0.83 ± 0.65
	2	0.33 ± 0.33	0.17 ± 0.17	0.67 ± 0.33	0.50 ± 0.34	0.33 ± 0.21	1.00 ±0.82
	4	0.33 ± 0.33	0.67 ± 0.21	0.83 ± 0.65	0.50 ± 0.34	0.67 ± 0.33	0.83 ±0.31
OLIGOTRICH							
Entodinium Minimum	0	57.00 ± 4.20	61.67 ± 2.49	48.68 ± 4.86	57.17 ± 6.02	47.67 ± 3.38	62.67 ± 5.18
	2	51.67 ± 9.17	69.17 ± 4.61	50.83 ± 8.35	57.17 ± 6.38	49.67 ± 5.68	59.67 ± 5.34
	4	60.33 ± 6.46	67.50 ± 3.82	55.83 ± 7.14	56.00 ± 5.86	54.00 ± 4.85	66.50 ± 4.37
Entodinium Caudatum	0	13.17 ± 3.25	11.00 ± 2.48	22.50 ± 5.19	15.00 ± 5.52	20.17 ± 2.21	10.33 ± 4.10
	2	22.50 ± 7.07	6.17 ± 1.89	23.33 ± 5.95	13.17 ± 6.01	25.83 ± 5.62	8.00 ± 4.01
	4	10.83 ± 2.77 AB	7.00 ± 1.75 B	16.83 ± 4.48 A	13.50 ± 5.48 AB	22.33 ± 4.00 A	6.33 ± 2.45 B
Entodinium Longinucleatum	0	3.50 ± 0.72	5.33 ± 1.17	2.33 ± 0.80	4.83 ± 2.14	3.33 ± 1.05	4.00 ± 1.29
	2	3.67 ± 1.05	4.33 ± 0.67	2.50 ± 0.99	5.50 ± 2.23	2.17 ± 0.60	5.00 ± 1.03
	4	3.67 ± 1.09	3.33 ± 1.12	3.17 ± 1.14	4.50 ± 1.12	2.83 ± 1.14	3.00 ± 0.58
Polyplastron Multivesiculatum	0	3.67 ± 1.65	1.33 ± 0.56	2.83 ± 0.83	4.00 ± 1.39	4.17 ± 1.42	3.00 ± 082
	2	1.33 ± 0.61	4.17 ± 1.56	1.17 ± 0.48	2.83 ± 1.22	1.50 ± 0.56	5.17 ± 1.22
	4	3.67 ± 1.52	3.83 ± 1.14	3.50 ± 1.26	4.33 ± 1.20	2.00 ± 1.00	3.33 ±1.05
Epidinium Caudatum	0	2.83 ± 1.05	2.17 ± 0.65	4.50 ± 1.28	2.33 ± 0.49	5.00 ± 2.31	1.33 ± 0.61
	2	2.00 ± 0.93	1.33 ± 0.56	4.33 ± 1.05	2.00 ± 0.58	3.67 ± 0.71	2.00 ± 1.26
	4	2.17 ± 1.17	1.83 ± 1.47	4.33 ± 1.52	2.83 ± 0.79	2.67 ± 1.20	3.17 ±0.91
Ostracodinium Gracile	0	2.17 ± 0.91	0.67 ± 0.33	1.00 ± 0.37	1.33 ± 0.42	0.83 ± 0.31	1.67 ± 0.71
	2	3.50 ± 2.94	1.00 ± 0.26	3.00 ± 1.26	3.00 ± 1.55	2.67 ± 1.15	2.67 ± 1.38
	4	2.67 ± 1.09	0.67 ± 0.49	2.67 ± 1.31	1.00 ± 0.37	1.83 ± 0.79	1.67 ± 0.76
Ophryoscol Excaudatum	0	0.83 ± 0.83	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.17	0.83 ± 0.54	0.00 ± 0.00
	2	0.50 ± 0.50	0.17 ± 0.17	0.17 ± 0.17	0.00 ± 0.00	0.33 ± 0.33	0.00 ±0.00
	4	0.33 ± 0.33	0.00 ± 0.00	0.50 ± 0.50	0.00 ± 0.00	0.67 ± 0.49	0.00 ±0.00
Diplodinium	0	1.50 ± 0.67	1.33 ± 0.61	2.00 ± 0.86	1.83 ± 0.48	1.83 ± 0.48	0.67 ± 0.42
	2	1.17 ± 0.65	1.00 ± 0.26	2.00 ± 0.89	2.50 ± 1.06	2.00 ± 1.06	1.33 ± 0.42
	4	1.67 ± 0.67	2.83 ± 0.75	1.83 ± 0.70	2.50 ± 0.62	1.67 ± 1.12	0.83 ± 0.65
Epidinium Ecaudatum	0	9.17 ± 2.40	11.17 ± 1.85	12.33 ± 2.53	11.67 ± 1.38	13.17 ± 2.40	12.17 ± 2.82
	2	9.00 ± 2.83	8.33 ± 1.98	9.00 ± 1.73	10.83 ± 2.43	8.67 ± 2.25	12.17 ± 3.48
	4	9.33 ± 2.11	10.67 ± 2.50	9.67 ± 1.84	11.33 ± 2.20	9.00 ± 2.07	11.33 ± 2.72
Eudiplodinium	0	2.67 ± 1.52	2.67 ± 0.99	1.50 ± 0.96	1.17 ± 0.79	1.33 ± 0.56	2.67 ± 1.12
	2	3.17 ± 1.42	2.67 ± 1.71	1.83 ± 0.91	2.17 ± 0.70	2.50 ± 1.36	2.33 ± 0.92
	4	1.67 ± 0.99	1.00 ± 0.82	0.33 ± 0.21	3.17 ± 1.14	1.50 ± 0.85	2.17 ±1.60

Group 1: Standard protein and energy levels; Group 2: Standard protein and low energy level;

Group 3: Low protein and standardenergy level; Group 4: High protein and standard energy level;

Group 5: Standard protein and high energy level; Group 6: 0.01 kg/day bicarbonate was added to group 5 ration.

A, B: Means on the same line (for feed factor) with different letter significantly: $P < 0.05$.

fluid. Animals which consumed normal energy and normal protein rations showed that *Isotrichia spp*. became dominant in their rumen fluids.

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