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The Effect of use of Humic Acid in Some Blood Parameters and Rumen Protozoa in Norduz Lambs

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ABSTRACT: This study investigated the effect of different levels of humic acid on some rumen and blood parameters and rumen protozoa in Norduz sheep. The study was designed and conducted in four-factor repeated measures analysis of variance. Totally 24, approximately 16-week old, female Norduz sheep were used in the study. Four feeding groups were designed. The first group was not given humic acid (C, control GI); the second group was given humic acid of 0.015% of the live weights of the sheep (GII); the third group was given humic acid of 0.030% of the live weights of the sheep (GIII); the fourth group was given humic acid of 0.060% of the live weights of the sheep (GIV). A total of eight sheep having similar ages and live weights were randomly distributed to each group (live weights of 40.230±1.25). The feed consumed by the sheep was limited to 2.5% of their live weights. Blood and rumen fluid samples were collected 2 times (before the test and on 25th day of the test). Comparison of some blood parameters were collected from the animals before and after the test revealed no significant difference in serum triglyceride and rumen pH levels. It was observed that while live weight, blood serum total protein, blood serum potassium, chloride, and sodium levels decreased ($P< 0.05$), blood urea and number of rumen protozoa increased ($P< 0.05$). Considering the values obtained before and after the test, the use of humic acid increased the number of rumen protozoa without negatively affecting blood parameters.

Keywords: Blood metabolites, humic acid, lamps, protozoa

Humik Asit Kullanımının Norduz Kuzularında Bazı Kan Parametreleri ile Rumen Protozoaları Üzerine Etkisi

ÖZET: Bu araştırmada, humik asidin farklı düzeylerinin Norduz kuzularının bazı rumen ve kan parametreleri ile rumen protozoonları üzerindeki etkileri incelendi. Deneme üç faktörlü olarak ölçümlü varyans analizi yapılacak şekilde planlandı ve yürütüldü. Denemede sütten kesilmiş, yaklaşık 16 haftalık yaşta 4 adet Norduz dişi kuzu kullanıldı. Kontrol gribini oluşturan birinci gruptaki kuzulara humik asit verilmedi (GI kontrol), ikinci grupta CA'ın % 0.015 düzeyinde humik asit (GII), üçüncü grupta CA'ın % 0.030 düzeyinde humik asit, (GIII), dördüncü gruba ise % 0.060'i düzeyinde humik asit (GIV) verilecek şeklinde dört grubu yemleme oluşturuldu. Her grupta yaklaşık aynı yaşta ve canlı ağırlıkta (40.230±1.25) sekiz hayvan olacak şekilde kuzular gruplara rasgele dağıtılmıştır. Kuzuların tükettikleri yem miktarı canlı ağırlıklarının % 2.5i olarak sınırlanmıştır. Kan ve rumen sıvısı örnekleri, biri deneme başlangıcında biri de denemenin 25. gününde olmak üzere 2 defa alınmıştır. Hayvanlardan deneme öncesinde elde edilen kan parametrelerinin sonuçları deneme sonradaki değerlerle karşılaştırıldığında kan serum trigliserit ve rumen pH düzeylerinde gözlenmemiştir. Hayvanlarda canlı ağırlık, kan serumu total protein, kan serum potasyum, klor ve sodyum düzeyinde azalma gözlenirken ($P< 0.05$), kan üre ve rumen protozoa sayısında artma gözlenmiştir ($P< 0.05$). Araştırmanın sonucunda dönemler dikkate alındığında humik asit kullanımının kan parametreleri üzerinde olumsuz bir etki yapmada rumen protozoa sayısını artırdığı gözlenmiştir.

Anahtar Kelimeler: Humik asit, kan parametreleri, kuzu rumen, protozoonları

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INTRODUCTION

Humic acids increased absorption of minerals in intestinal wall. As a result, bone mineralization is reported to increase in animals (Mosley, 1996; Tunç, 2007). Humates are known to prevent the development of some hazardous bacteria and stimulate bacterial growth (Riede et al., 1991). The use of humate in dairy goat decreased mastitis cases (Mosley, 1996). Humates have positive effects on immune system of animals. In addition to strengthening the resistance of the animals against pathogens like E.coli, they significantly reduce digestive disorders like diarrhea and others (Humin and Laub, 1998). Lotosh (1991) reported that humates were the drugs which increased resistance to diseases by positively enhancing general health system (Tunç, 2007). Findings of various studies using humates revealed that use of humate promoted growth, positively affected carcass efficiency, enhanced feed efficiency, and reduced instantaneous death rate (Stepchenko et al.. 1991; Yörük et al., 2004; Karaoğlu et al., 2005). It was gointed out that humates increased milk and milk fat amount in dairy cattle, improved live weight increase in livestock, and also decreased temperature stress (Livestock, 2003). Humic acid also helps to prevent excessive loss of water by enhancing water absorption from intestinal lumen (Humin and Eaub, 1998). Similarly, the use of humates was observed to increase live weight gain both in male and female lambs (Teravita, 2004). Adding humate to ratios positively affects feed efficiency and digestive system (Humin and Eaub, 1998). Studies using humic acid reported that humic acids prevented the growth of pathogen bacteria and decreased mycotoxin level (Humin and Laub, 1998; Tunç 2007). Humic acid stimulates neutrophil activity and protects against bacterial agents (Dabovich et al., 2003). Supporting protective epithelial tissue in digestive tract, humates increase resistance to toxins and infections (Kühnert et al., 1991). Previous studies reported that humates had positive effects on lipid metabolism (Stepchenko et al., 1991; Bailey et al., 1996) and might be used in treatment of lipid metabolism disorders (Banaszkiewicz and Drohnik, 1994). It was reported that blood serum total cholesterol, total lipid and glucose levels decreased in rats fed with humate-added feed; whereas, lipoprotein, globulin, hemoglobin, hematocrit value and erythrocyte number increased (Banaszkievicz and Drobnik, 1994; Tunç, 2007). A literature review showed that there was only a limited body of research in Turkish or in foreign languages on effect of humic acid added into ruminant ratios on number of rumen protozoa. Only one study conducted in Turkey was found in literature (Tunç, 2007). The study showed that protozoa were not compulsory for rumen fermentation. However, many studies reported that protozoa increased digestibility capacities of some ratios, daily live weight gain of animals and retention of nitrogen in the body for a longer time and were effective in increasing ammoniac and volatile fatty acids in the rumen. Having proteolytic characteristics, protozoa are known to break down bacteria in addition to feed proteins. In the presence of ciliata in the rumen, ammoniac and volatile fatty acids increase and protozoa with high biological value is synthesized (Coleman, 1986).

Sodium is major intracellular cation and its intra-erythrocyte density is approximately 23 times higher than plasma (Turgut, 2000; Vakit, 2008). While totally 80% of phosphor in the body is found in bones and teeth in the form of inorganic salts, 20% is found in soft tissues. Normal serum phosphorus concentration is 1.6—2.4 mmol-L. 75% of potassium is found in muscles and less than 2% found in extracellular fluids. In sheep, normal serum potassium concentration is 4.0–6.0 mEq-L (Turgut, 2000; Vakit, 2008). Chloride is the main anion in intercellular fluid and blood plasma. Chloride particularly functions in the formation of osmotic pressure as an electrolyte. The normal serum Cl level in plasma Cl concentration is 98–115 mEq-L (Turgut, 2000; Vakit, 2008). Sodium is the largest cation of extracellular fluid. It constitutes 90% of 154 mols of inorganic cation in each liter of plasma fluid. For this reason, it is responsible for the half of plasma osmolality. In cases of diarrhea, excessive perspiration, and excessive loss of blood and renal infections, Na concentrations might increase. Normal serum Na density in sheep is 136–154 mEq-L (Turgut, 2000). The use of humic acid is known to increase oxygen carrying capacity by increasing red blood cells and hemoglobin count, thus arousing the feeling of liveliness to the organism (Malinowska et al., 1993).

MATERIAL AND METHOD

Material

Animal Material: Totally 24, 0.5 year old Norduz sheep were used in the experiment. The data used in statistical analyses were collected from these 24 animals before and after the experiment. The sheep were supplied from sheep raising unit of Yüzüncü Yıl University research and application farm. Feed material: Dry meadow was used as the main feed material.

Table 1. Daily ration feed of the sheep in the trial

Feedstuff	%
Barley	40.000
Wheat Bran	10.000
Soybean meal	20.000
Sunflower seed meal	20.000
Salt	1.000
Marble dust	2.500
Vitamins + Minerals	0.500
Energy kcal/kg ME*	2690

Table 2. The concentrate in the ration

Feedstuff	Dry matter %
Meadow	80
Concentrate	20

A commercially available humic acid called as AGROHUM was used as humic acid. Tables 2.3. and 2.4 illustrate the content of the used humic acid.

Table 3. Chemical composition and properties of the humic acid

Properties	Rates
Humidity	75±10%
Total organic matter	71±5%
Trace elements	*85*%
Soil minerals (SiO2, Al)	3%
pH	7
Electron Capacity.	2.5dS/m±0.5
C/N	17±1
Size	0–3 mm
Resolution (in 1% KOH solution)	very high

Table 4. Elemental composition of the humic

Element	Amount%
Hydrogen (H)	31.0
Carbon (C)	3.2
Oxygen (0)	33.0
Sulfur (S)	1.7
Nitrogen (N)	1.8
Phosphorus (P)	0.5
Potassium (K)	0.7
Iron (Fe)	0.9
Calcium (Ca)	0.5
Magnesium (Mg)	0.1

Method

Feeding method: The first group was not given humic acid (control GI); the second group was given humic acid of 0.015% of the live weights of the sheep (G II); the third group was given humic acid of 0.030% of the live weights of the sheep (G III); the fourth group was given humic acid of 0.060% of the live weights of the sheep (GIV). Humic acid was given to the animals by being mixed in 0.5 l water for 25 days at the same hour each day. The feed consumed by the animals was restricted to 2.5% of their live weights.

Table 5. Humic acid administered to the animals (g)

GI	GII	GIII	GIV
0.0	6.5 g	11.7 g	26 g

Blood analysis

For blood analyses, 10 ml blood was collected from vena jugularis of each animal for two times (before and after the test) by using blood collection cannula. Blood samples were centrifuged at 4000 rev/min and after collecting serum, they were sent to Yüzüncü Yıl University Faculty of Medicine, Biochemistry and Physiology Laboratory. Urea, BUN, total protein, triglyceride, VLDL, Na, K, P, CL, HDL and LDL amounts were analyzed in serum samples. Tokyo/Japan origin modular type Hitachi Automatic Analyzer device and Roche kits were used for blood analyses (Henry, 1965; Ersoy and Bayşu, 1981; Christian and Feldman, 1982).

Rumen pH analysis

50 ml rumen content was collected from each animal using rumen sound two times (before and after the test). pH of the collected rumen contents was immediately measured by using a digital pH meter (Dado and Alien, 1993; Vakit, 2008).

Protozoa counting method

For protozoa counting, rumen content was put into a plastic bottle by using rumen sound. Like in blood sample collection, rumen fluid was collected at the same hour of the day before and after the test. 5 ml of rumen content was separated to achieve stability of the collected rumen content. 15 ml fixing solution (1 lt ethyl alcohol, 5 g pure NaCI, 0.3 g methyl grin) was added to the sample. The samples were kept at a cold and dark place until counting. For protozoa counting, 0.05 ml of the sample was placed in Thoma slide following homogenization. Lamella was placed on the slide in such a way to create no air bubble. Counting was conducted by using a standard microscope, with 104x40 magnification with the help of a camera and screen. Count of protozoa was calculated by using the following formula. Density (mm3) number of counted protozoa, number of counted small squares volume of one small square dilution ratio (Ülker, 2007).

Statistical analyses

Descriptive statistics of the properties were expressed as means and standard error. To determine if there were differences between the application groups (GI, GII, GIII, GIV) and times (before the test-after the test), Two-Way ANOVA with repeated measurement at one factor level was used. Tukey multiple comparison test was used to determine means of different groups after the analysis of variance.

In factors for which the difference between the means was significant, Group x Time interaction was found to be statistically significant; therefore, Tukey multiple comparison test was used in the level of sub-groups. All statistical analyses were performed by using STATISTICA statistical package program (Winer 1971).

RESULTS AND DISCUSSION

This study was conducted to determine the effects of humic acid on rumen protozoa and blood parameters. Descriptive data obtained from statistical analysis of the collected data are summarized in the following tables. Data obtained from the tests are shown in Tables 6, 7 and 8.

Table 6. Mean values of live body weights (kg) of the groups before and after the test and standard error (Mean ± SE).

		GI	GII	GIII	GIV
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
Live body weight	BT*	39.40±1.88#	41.73±1.00#	38.43±1.28#	41.37±0.84#
	AT*	34.88±1. 82	36.73±1.21	33.90±0.91	35.57±1.16

Shows the difference between the same group (in the same column) before and after the test (p>0.05)
*BT, AT before and after the test

Table 7. Mean values of total protein, triglyceride and urea of the groups before and after the test and standard error (Mean ± SE).

		GI	GII	GIII	GIV
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
Total Protein (g dL-1)	BT	8.10±0.36 #	6.58±0.70	8.62±0.499 #	8.28±0.28 #
	AT	5.83±0.53 b	6.89±0.16	6.39±0.26ab	5.89±0.39b
Triglyceride (mg dL-1)	BT	29.5±0.53	21.35±0.69	30.34±0.70	29.91±0.65
	AT	25.67±0.08	23.43±0.07	30.20±0.87	35.71±0.17
Urea (mg dL-1)	BT	20.20±0.75	16.86±0.60	20.73±0.75	15.33±1.89 #
	AT	24.78±0.83	19.43±0.88	22.58±0.78	24.55±0.83

Shows the difference between the same group (in the same column) before and after the test (p>0.05)
ab Shows the difference between the same time groups (in the same line) in the same time (p>0.05)

Table 8. Phosphorus, potassium, chloride and sodium mean values of the groups before and after the test and standard error (Mean ± SE)

		GI	GII	GIII	GIV
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
Phosphorus (mg dL-1)	BT	6.77±0.37 #	6.70±0.28	6.24±0.33	6.95±0.48
	AT	5.61±0.40	24.44±0.91	5.30±0.43	5.89±0.42
Potassium (mmol dL-1)	BT	5.90±0.40 ab	4.03±0.76b	5.93±0.25 ab#	6.52±0.54 a
	AT	5.06±0.78	3.21±0.39	3.70±0.87	6.33±0.31
Chloride (mmol dL-1)	BT	121.67±5.19 ab#	105.80±17.97 b	136.50±4.75 a#	138.67±8.12 a#
	AT	96.00±11.90 b	96.97±18.14 b	136.50±4.75 b	138.67±8.12 ab
Calcium (mmol dL-1)	BT	165.18±0.44	147.40±4.27	182.±6.11 #	184.50±8.84 #
	AT	132.33±0.45	131.67±5.06	111.67±5.72	157.67±4.37

Shows the difference between the same group (in the same column) before and after the test (p>0.05)
ab Shows the difference between the same time groups (in the same line) in the same time (p>0.05)

Table 9. Phosphorus, potassium, chloride and sodium mean values of the group before and after the test and standard error (Mean ± SE).

		GI	GII	GIII	GIV
		Mean±SE	Mean±SE	Mean±SE	Mean±SE
pH	BT	7.01±0.08 #	6.94±0.21	6.98±0.01	7.01±0.01
	AT	6.38±0.20	7.18±0.18	6.49±0.27	6.42±0.15
Number of protozoa	BT	2692.5±223.5 b	2984.38±140.9 ab	2620.0±67.4 #b	3333.5±223.5 #a
	AT	3312.5±152.7 c	6286.3±403.4 a	6081.3±202.4 a	5270.6±152.7b

Shows the difference between the same group (in the same column) before and after the test (p>0.05)
ab Shows the difference between the same time groups (in the same line) in the same time (p>0.05)

It was found that live weights decreased in all groups at the end of the test (p<0.05). It was thought that this weight loss may be explained from the low-quality meadow hay used as experimental feed and the restriction of consumed feed by each animal with 3% of their live weights.

It was considered that the decrease in total protein level observed in blood serum before and after the test was associated with the decrease of live weight. However, it was observed that in the second and third groups, blood total protein decreased due to use of humic acid (p<0.05). Blood serum urea level increased in the fourth group compared to the other groups (p<0.05). It was thought that this resulted from the breakdown of tissue proteins as a result of live weight loss. A statistically insignificant increase was observed in blood urea level in all groups. As a consequence these results like to findings to Tunç and Yörük's study (2012).

Analysis of blood phosphor, potassium, chloride and sodium values showed that the use of humic acid decreased sodium, chloride, and potassium levels in different groups (p<0.05). The use of humic acid decreased sodium value to normal levels which was above normal limits before the test. However, Tunç and Yörük (2012) found no difference in these parameters. No salt was given to the sheep during the test. Despite the insignificant decrease in the control group, a sharp decrease of sodium observed in humic acid groups might show that humic acid functioned to stabilize sodium. Decreased chloride level observed in blood levels of the animals which were not given salt (p<0.05) could be interpreted like the case in sodium (GIV). There was no difference between the groups in terms of blood phosphorus level and the values were within normal limits (Tunç and Yörük, 2012). The fourth group had an increase in blood serum urea levels in question compared to the other groups (p <0.05).

It was observed that the use of humic acid, which did not have any effect on rumen pH, increased number of rumen protozoa in all three doses unlike Tunç and Yörük's (2012) results ($p<0.05$). Considering the used humic acid doses, it was found that number of protozoa was the highest in the first group (the lowest dose level).

In this test during which low-quality meadow hay was used, it was observed that different doses of humic acid did not have a negative effect on blood parameters and similarly did not create a negative effect (Tunç and Yörük, 2012) on rumen pH ($p<0.05$). However, it was found that the use of humic acid significantly increased number of rumen protozoa ($p<0.05$). It is known that the increase in number of protozoa is important and it enhances nitrogen retention in the body of the animals. In conclusion, it can be asserted that the use of humic acid increases the number of protozoa. However, effects of humic acid on serum can be analyzed by future studies.

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