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Short Communication / *Kısa Bilimsel Çalışma*

Rumen fermentation characteristics of rams fed supplemental boric acid and humic acid diets

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Abstract: The aim of the study is to investigate the effects of humic acid and boric acid as feed additives on rumen fermentation parameters in rams. For this purpose, 3 yearling rams were used in this experiment. Rams in each treatment; a control with no supplements (C), 180 ppm boric acid (B) and 5 ml/kg humic acid (H) with 65:35 forage to concentrate ratio. Each experimental period lasted 14 days, with 12 first days of diet adaptation. Totally, the experimental period lasted 42 days. In both time periods (0 and 3h after feeding), there were no significant differences (P>0.05) on rumen pH, ammonia, protozoa count, estimated methane production and volatile fatty acid composition. However, butyric acid concentration tended to be higher in experimental groups (P=0.08) compared with control. As a result of this study, these feed additives did not modify the rumen milieu and showed no negative effect in rams.
Keywords: Boron, humate, methane, volatile fatty acids.

Borik asit ve humik asit ilave edilen yemlerle beslenen koçların rumen fermentasyon karakteristikleri

Özet: Bu çalışmanın amacı, yem katkı maddesi olarak kullanılan humik asit ve borik asidin koçlarda rumen fermantasyon parametreleri üzerine etkilerini araştırmaktır. Bu amaçla, söz konusu denemede 3 adet damızlık koç kullanılmıştır. Her bir deneme grubu için gruplar; herhangi bir katkı maddesi içermeyen kontrol (C), 180 ppm borik asit (B) ve 5 ml/kg humik asit (H) içeren deneme grupları şeklinde oluşturulmuştur ve 65:35 kaba yem/konsantre oranı ile besleme gerçekleştirilmiştir. Her bir deneme periyodu 12 günlük adaptasyon ile beraber 14 gün olmak üzere deneme toplamda 42 gün sürmüştür. Denemenin sonunda elde edilen verilere göre, her iki zaman periyodunda da (beslenmeden 0 ve 3 saat sonra), rumen pH'sı, amonyak konsantrasyonu, protozoa sayısı, tahmini metan üretimi ve uçucu yağ asidi bileşimi üzerinde önemli bir fark (P>0,05) gözlemlenmemiştir. Ancak bütirik asit konsantrasyonun kontrol grubuna göre deneme gruplarında daha yüksek olma eğiliminde olduğu belirtilmiştir (P=0,08). Bu çalışmanın sonucunda, söz konusu yem katkı maddelerinin koçlarda rumen ortamını değiştirmediği ve herhangi bir olumsuz etki göstermediği ortaya konmuştur.
Anahtar sözcükler: Bor, humat, metan, uçucu yağ asitleri.

Efficiency of rumen fermentation can be regulated by manipulating the rumen milieu with chemical agents, feed additives, which modulate selected pathways of metabolism such as volatile fatty acids (VFA) and methane production (CH_4), by regulating ruminal pH (5). Fermentation provides not only the ruminant with VFA but also methane production that is a potential greenhouse gas, resulting in environmental pollution, and also energy loss for ruminants (6, 19). Recently, the use of feed additives has gained importance, modified rumen fermentation, especially after the prohibition of the use of synthetic additives such as antibiotics (10, 16, 17). Humic acid and boric acid are some of such feed additives.

Humic acid arises from the organic materials such as plant and animal matters that are naturally decomposed and modulates the rumen fermentation by changing the rumen fluid ammonia concentration (1, 8) as well as boric acid (16). It has been demonstrated that dietary boron had an influence on rumen microbial fermentation in yearling rams (16).

Little information is present on the effects of boric acid and humic acid supplementation to ram diets on rumen fermentation and methane production. Therefore, the current experiment aimed to evaluate the effect of humic acid and boric acid on rumen fermentation, and estimated methane production of rams. We hypothesized that these feed additives would alter ruminal fermentation, reduce potent methane production of rams.

Three 11-12 months' age Merinos rams, which weigh approximately 60 kg and are kept in individual pens. Feed and water were provided separately for each animal in plastic buckets which are installed onto the pens. Rams were fed alfalfa pellets (500 g/day), barley straw (400 g/day) and concentrates (500 g/day) (% 14 CP and 2.6 Mcal/kg ME). Forage concentrate ratio was 65:35 for all treatment groups. The chemical composition of the diet used in this experiment is given in Table 1.

The daily diet of animals was fed in two meals (9.00 am-16.30 pm), and water was provided *ad libitum*. Animals in each treatment; a control with no supplements (C), 180 ppm boric acid (B) and 5 ml/kg humic acid (H) were fed according to Bialek et al. (3) and Varadyova et al. (20). Each 180 mg of boric acid represents approximately 10 mg boron. The experiment was designed 3x3 Latin square in order to eliminate environmental differences. Each period was lasted 14 days resulted in 42 days of the experimental period.

Samples of rumen fluid were taken for 2 days following the 12-day adaptation period, before feeding (0 h) and 3 hours after feeding by using rumen catheter. Ruminal pH was measured using pH meter and NH_3 levels were measured using gas-sensitive ammonia electrode (Orion^R). Protozoan counts were performed in fresh rumen fluid by using Fuchs Rosenthal Lam (depth 0.1 mm) and a microscope (15). The VFA were determined using Gas Chromatography (GC; Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan). To calculate the estimated CO_2 and CH_4 based on the stoichiometry, CO_2 = (Acetic acid/2) + (Propionic acid/4 + 1.5 × Butyric acid) and CH_4 = (Acetic acid + 2 × Butyric acid) – CO_2 formula was used (4).

The data were analyzed with SPSS 14.01 program (SPSS Inc. Chicago, IL, USA) using the GLM procedures. The analysis included 3x3 between subject's factorial design and the main factors were different time sampling and humic acid, and boric acid supplementation. The Duncan's Multiple Range Test was used for the comparison of means. Statements of significance were based on P-value of equal to or less than 0.05.

The effects of additional humic acid or boric acid did not affect the dry matter intake (DMI), because of no residual feed remained between meals in any group. Fermentation parameters of the experimental rams' rumen fluid, pH value, ammonia concentration and protozoan numbers at 0h and 3 h post feeding were determined and summarized in Table 2. There were no statistically significant differences between the experimental groups ($P>0.05$), interaction of group and the sampling time, while the time effect altered the fermentation parameter ($P\leq0.001$), as expected. After 3 h of feeding, ruminal microbial fermentation patterns increased except for pH, isobutyrate, isovalerate, and protozoa counts.

In recent years, the use of boric acid and humic acid, which are thought to contribute economically, that is sourced from the country, has increased. Studies on the use of these feed additives in ruminant nutrition are being carried out and especially their effect on rumen microbial fermentation is tried to be revealed (9, 16). This study investigated the effect of supplemented humic acid and boric acid on microbial fermentation in rams. In the light of our previous study (16), which examined the effect of boric acid on the rumen environment, what kind of results would emerge in comparison with humic acid has been discussed. In the current study, additional humic acid and boric acid did not alter the rumen milieu in terms of fermentation. However, time sampling did significantly affect the pH, ammonia concentration, VFA, methane production and protozoa counts, as expected. It's well known that the fermentation parameters, in the expense of VFA, increased after feeding (14). The VFA concentration was not affected by using dietary used feed additives, whilst butyrate concentration tended to be higher both before and 3 h after feeding, albeit for total VFA and individual concentrations with ammonia level were exhibited relatively numerical increasing at 3h after feeding. In ruminants, persistent increase of butyric acid concentration in the digestive system have positive effects on gut development, nutrient utilization efficiency and the animal production (11). Conversely, Sizmaz et al. (16) reported that the higher level of boric acid increased the butyric acid concentration in rams. Our best knowledge is there is no other reporting on the effects of boric acid on rumen fermentation patterns. Similarly, some studies showed no effect (8; 20) on VFA with the using of humic acid in ruminant diets. Source of the ruminal proteins recycling by reactions of amino acids such as isoleucine and valine (13). Theoretically, increasing in ammonia level, which is formed by the breakdown of proteins as well as amino acids, manage to higher concentrations of iso-VFA in the milieu of rumen (12). This outcome coincides with the observed higher ammonia concentration in the treatment groups, which is similar to the previous study (16).

Table 1. Analyzed chemical composition of forages and concentrates used in the experiment (2).

	Forages		Concentrate[1]		
Nutrients	Alfalfa	Straw	C	B	H
DM, %	92.80	89.00	90.80	92.70	92.80
OM, %	92.00	94.70	82.50	84.94	85.78
CP, %	9.51	2.70	15.00	15.50	15.30
CF, %	30.80	39.60	6.40	6.60	5.97
EE, %	1.10	2.18	4.20	3.75	3.75

[1] The concentrate contained 25% corn, 24% barley, 3% soybean meal, 12% sunflower meal, 25% rasmol, 2% full fat soy, 4% molasses, 3% $CaCO_3$, 1% salt and 1% mineral-vitamin premix for yearling rams (contained per kg: retinol 3000 mg, cholecalciferol 75000 mg, tocopherol 30000 mg, thiamin 980 mg, niacin 99500 mg, biotin 20 mg, manganese 50000 mg, zinc 50000 mg, iron 50000 mg, copper 10000 mg, iodine 800 mg, cobalt 200 mg, selenium 300 mg, magnesium 250 mg).

Table 2. Effects of using boric acid and humic acid on rumen pH, NH_3 (ppm), VFA concentration (nM), estimated CO_2 and CH_4 (mmol/l) and protozoan counts (x10^4 number/ml) (Mean ± SD).

		Groups				**P values**		
Items	Time, h[1]	**C**	**H**	**B**	**Main effect**	**G**	**T**	**GxT**
pH	0	6.90±0.25	6.83±0.14	6.78±0.28	6.61	0.316	≤0.001	0.996
	3	6.44±0.16	6.37±0.12	6.33±0.24				
Ammonia	0	168.75±48.01	164.29±56.89	168.29±56.50	197.20	0.615	0.003	0.643
	3	210.63±70.73	218.75±78.32	252.50±64.81				
Acetate	0	34.05±11.29	37.58±7.64	40.61±11.95	45.42	0.250	≤0.001	0.979
	3	50.89±10.49	53.05±8.43	56.33±8.53				
Propionate	0	7.55±2.44	8.46±2.70	9.25±3.38	10.69	0.175	≤0.001	0.962
	3	12.13±2.26	12.71±2.64	14.04±2.42				
Isobutyrate	0	0.88±0.15	0.80±0.10	0.85±0.08	0.78	0.973	≤0.001	0.166
	3	0.69±0.10	0.76±0.11	0.71±0.05				
Butyrate	0	9.15±3.08	11.24±2.02	10.96±3.31	12.32	0.082	≤0.001	0.994
	3	12.85±2.98	14.90±2.36	14.83±2.71				
Isovalerate	0	1.03±0.18	0.88±0.17	1.02±0.12	0.81	0.780	≤0.001	0.092
	3	0.61±0.11	0.70±0.19	0.64±0.11				
Valerate	0	0.58±0.11	0.62±0.15	0.64±0.16	0.87	0.338	≤0.001	0.878
	3	1.04±0.13	1.14±0.28	1.17±0.22				
Total VFA	0	53.47±16.13	59.83±11.52	63.57±18.65	71.13	0.196	≤0.001	0.993
	3	78.46±15.31	83.51±12.88	87.96±13.81				
CO_2	0	42.64±10.27	37.77±6.92	39.06±11.70	43.86	0.163	≤0.001	0.992
	3	47.76±9.72	52.05±8.11	53.92±8.84				
CH_4	0	19.72±6.35	22.30±4.08	23.48±6.80	26.20	0.221	≤0.001	0.986
	3	28.84±5.92	30.80±4.80	32.07±5.03				
Protozoa	0	42.13±16.54	49.00±21.19	43.93±18.07	36.95	0.812	≤0.001	0.463
	3	31.19±16.64	27.8111.49	27.8111.49				

[a,b] Values within a row with different superscripts differ significantly at P<0.05
[1]0; before feeding, 3; 3h after feeding
G; group, T; time, GxT; group and time interactions.

Conversely, there are some studies with different results of using of different derivatives of humic acid on rumen fermentation in goats (7), lactating dairy cows (10), beef heifers (18) in vivo and in vitro (9) and effect of boric acid in previous study on protozoa number (16). This might be originated from the different type of diets, form of the feed additives and ruminants, and conditions in terms of in vitro and in vivo.

In conclusion, the current trial demonstrated that addition of humic and boric acid did not significantly affect the ruminal fermentation patterns before and 3 h after feeding. However, tended to increase the parameters compared with control groups. It should be noted that the feed additive supplementation level, in vitro or in vivo conditions and using in big or small ruminants are significant when feed additives are applied. It would be useful to further assess the effects of these additives on detailed and molecular analysis under both conditions.

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Ethical Statement

This study was approved by the Animal Experiments Local Ethics Committee of the Ankara University (Ethical Approve Number: 200515).

Conflict of Interest

The authors declare that they have no conflict of interest.

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