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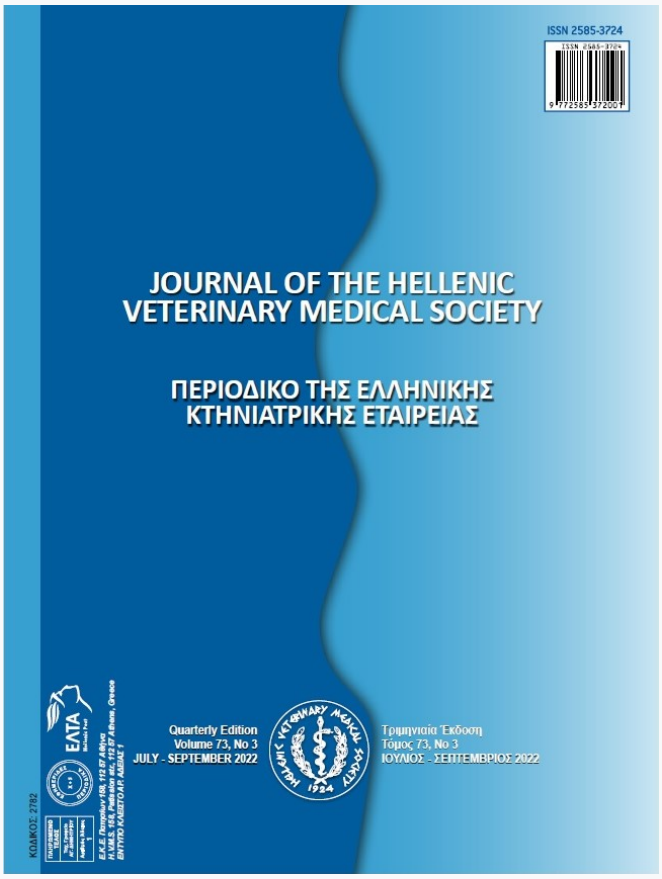
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 3 (2022)



Does supplementation of boron with yeast or humate substances have a better chance at modulating rumen fermentation in yearling rams?

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doi: 10.12681/jhvms.26454

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To cite this article:

Sizmaz, O., Koksal, B., Ramay, M., & Yildiz, G. (2022). Does supplementation of boron with yeast or humate substances have a better chance at modulating rumen fermentation in yearling rams?. *Journal of the Hellenic Veterinary Medical Society*, *73*(3), 4317–4324. https://doi.org/10.12681/jhvms.26454

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J HELLENIC VET MED SOC 2022, 73 (3): 4317- 4324
ΠΕΚΕ 2022, 73 (3): 4317- 4324

Research article
Ερευνητικό άρθρο

Does supplementation of boron with yeast or humate substances have a better chance at modulating rumen fermentation in yearling rams?

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ABSTRACT: This study aimed to determine the effects of boron fed alone or with yeast culture or liquid humate on ruminal pH, ammonia-N, short-chain fatty acid (SCFA) concentration, protozoa counts and some nutrients' apparent digestibility in yearling rams. Therefore, four Merino rams were allocated to 4×4 Latin square design and were randomly assigned to one of four treatments to basal diet: negative control with no supplements (NC), positive control (PC) with 30 ppm (Dry matter basis) boric acid alone, combinations of 30 ppm boric acid and 3 g/kg yeast culture (YC) and 30 ppm boric acid and 5 ml/kg liquid humate (LH). There were no negative effects of additives' supplementation on animals' daily feed consumption. No significant differences were observed among treatments for rumen parameters investigated, however, numerically lowest values for pH & ammonia-N in LH while highest SCFA concentrations in YC and LH groups were observed. Apparent nutrient digestibility was altered with boron addition to diets; however, the effect was not statistically significant. Boron analysis of blood serum and fecal samples also showed non-significant changes, except significantly higher serum concentration of boron in YC compared to PC. It can be concluded that tested dosage of boron was not enough to considerably modifyrumen fermentation characteristics in yearling rams. Better modulation of rumen fermentative characteristics might be possible when boron is supplemented with yeast or liquid humate, however, there was no definite effect observed in the present study, thus,this possibility should be verified in the large-scale future experiment.

Key words: Merino rams; rumen fermentation; boron; *Saccharomyces cerevisiae*;humate

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Date of initial submission: 01-07-2021
Date of acceptance: 28-03-2022

INTRODUCTION

In ruminant nutrition investigations, a wide range of organic and inorganic materials has been evaluated for their role in changing the availability of essential nutrients, especially to modify the rumen fermentation characteristics (Guedes et al., 2008; Seo et al., 2010).There has been lots of research to manipulate the rumen microbial ecosystem by microbial feed additives in order to improve animal productivity. Among these microbial additives, yeast cultures (YC) especially *Saccharomyces cerevisiae* have gained increasing attention in ruminant nutrition (Denev et al., 2007). *Saccharomyces cerevisiae*is used frequently in ruminant diets to increase the activity of cellulolytic bacteria and protozoa population, thereby decreasing ruminal ammonia-N and stabilizing rumen pH (Denev et al., 2007; Chaucheyras-Durand and Durand, 2010).

Additionally, humate substances which are the major organic constituents of soil (humus) and are produced by the activity of different microorganisms on dead plant and animal matter (Stevenson, 2010), have also been investigated for their potential to affect ruminal fermentation. Humate substances have been demonstrated to be effective in reducing ammonia concentrations from open feedlots (Shi et al., 2001). Galipet al.(2010) reported no negative effects of humic acid supplementation on ruminal protozoa populations in Kivircik rams. However, research regarding the effects of humic acids on rumen fermentation parameters, especially in rams, is scarce.

Several studies have revealed biological importance and essentiality of boron (B) for different metabolic and physiological processes both in humans as well as in animals (Devirian and Volpe, 2003; Yildiz et al., 2011; Kabu and Civelek, 2012; Yildiz et al., 2013). Bennett et al.(1999) reported that B provision can stimulate the growth of yeast, thus, indicating a plausible indirect effect of B on animal growth through yeast stimulation. In nature B rarely occurs alone, and is commonly present in combination with other elements, i.e. boric acid and borates (Cox, 2004). Majority of the boric acid movement in the environment is controlled by its adsorption reactions with organic and inorganic soil matter by forming the surface complexes (Xu and Peak, 2007). To the best of the authors' knowledge, the literature regarding effects of B supplementation on rumen fermentation is limited. Therefore, this study was carried out to evaluate the *in vivo* effects of B on rumen fermentation characteristics and protozoa counts in yearling rams as well as to examine the B absorption and apparent digestibility of some nutrients. Moreover, considering the boron stimulative effect on yeast growth and its adsorption reactions with soil matter, we also hypothesized that combined addition of B with yeast or humate substances might have a greater impact on rumen fermentative characteristics.

MATERIALS AND METHODS

Animals, Diets and Experimental Design

All procedures involving animal care, handling and sampling were approved by the Animal Experiments Local Ethics Committee of the Ankara University, Veterinary Faculty, Ankara, Turkey (20071557).

Four Merino rams (12 months age; ~60 kg) were randomly assigned to one of four treatments to the basal diet (dry matter basis) following a 4 x 4 Latin square design: a negative control with no supplements (NC), a positive control (PC) with 30 ppm boric acid alone (containing 17.5% B, Merck), a combination of 30 ppm boric acid and 3 g/kg yeast culture(YC, Farmavet International, Turkey), and 30 ppm boric acid with 5 ml/kg liquid humate (LH; consisted of a mixture of humic, fulvic, ulmic and humatomelanic acids, DURAFARM®, Farmavet International, Turkey). The feeding trial consisted of a total of 56 days equally divided into four experimental periods. Each experimental period lasted for 14 days, with the first 12d used to avoid earlier treatment carry over effect as well as adaptation to the new treatment and the last 2d for sample collection.

The diets were fed twice daily (morning & evening), and water was provided ad libitum. The basal diet provided a daily intake of 1400 g per animal comprising (dry matter basis) 65% forage as alfalfa pellets (500 g/day)&barley straw (400 g/day), and 35% concentrate (500 g/day), and on average it contained 14% crude protein and 2.6 Mcal/kg energy. The chemical composition of basal and experimental diets (Table 1) was determined according to AOAC (2000) and metabolizable energy levels were calculated according to the Turkish standards Institution (TSE,1994).

Rumen, Blood and Fecal Sampling

The rumen samples were taken 3h after the morning feeding using a rumen probe. The pH was analyzed immediately using a Nel pH/mV meter (Model 890, Nel Instruments Inc., Ankara, Turkey). Subsequently, ammonia-N (NH_3-N) concentration (mmol/L) was determined using the Orion Ammo-

Table 1: Analyzed chemical composition of forages and concentrates fed either alone as a negative control (NC), or supplemented with 30 ppm boric acid (PC), or with 30 ppm boric acid and 3 g/kg yeast culture (YC), or with 30 ppm boric acid and 5 ml/kg liquid humate (LH).

	Forages		Concentrate[1]			
Nutrients, (As fed)	Alfalfa	Straw	NC	PC	YC	LH
DM, %	92.80	89.00	90.80	91.00	91.35	91.20
OM, %	92.00	94.70	82.50	84.19	84.75	84.30
CP, %	9.51	2.70	15.00	14.20	14.40	15.40
CF, %	30.80	39.60	6.40	6.70	6.65	6.85
ADF, %	46.25	50.75	ND[2]	ND[2]	ND[2]	ND[2]
EE, %	1.10	2.18	4.20	4.90	4.10	4.55
ME, Kcal/kg	1180.00	1492.00	2647.00	2711.00	2704.10	2701.73
B, ppm	17.20	5.60	9.57	15.03	14.98	15.54

[1]The concentrate contained 25% corn, 24% barley, 3% soybean meal, 12% sunflower meal, 25% fine wheat bran, 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).
[2] ND = Not determined
DM, dry matter; OM, organic matter; CP, crude protein; CF, crude fiber; ADF, acid detergent fiber; EE, ether extract; ME, metabolizable energy; B, boron; NC, negative control (free of additives); PC, basal diet containing 30 ppm boric acid; YC, basal diet containing 30 ppm boric acid and 3 g/kg yeast culture; LH, basal diet containing 30 ppm boric acid and 5 ml/kg liquid humate.

nia Electrode (Model 9512) connected to Nel pH/mV meter. The numbers of protozoa were counted using Fuchs Rosenthal counting chambers (depth 0.1 mm) and a microscope, according to the method described by Ogimoto and Imai(1981). B concentration in samples, both feed and rumen, was determined using Ion Chromatography Spectrometry (ICS; Dionex-3000, USA) according to Vanatta et al. (1999). Rumen samples were also analyzed for SCFAs concentration using gas chromatography (GC; Shimadzu GC-2010, Shimadzu Co., Kyoto, Japan) (Geiissler et al., 1976).

Blood samples were collected shortly before the morning feeding on the 14th day of each experimental run from the jugular vein. Serum was obtained and immediately stored at -20 °C for subsequent B analysis.

Total 1-day fecal collection technique was performed on the last day of each experimental period and the samples were bulked (24 h) and stored in suitable containers at -20°C for further analyses. The dry matter (DM), organic matter (OM), crude fiber (CF), acid detergent fiber (ADF) and ether extract (EE) in fecal samples were determined according to AOAC (2000) methods. Fecal B concentrations were also analyzed, and B absorption (%) was calculated by dividing the absorbed amount (total B intake from feed - fecal excretion) with total B intake from feed (Bharti et al., 2008). Apparent nutrient digestibilities of DM, OM, CF, ADF and EE were calculated according to the following formula (Sena et al., 2015):

Apparent Nutrient digestibility, % = [(Nutrient intake from feed - Fecal excretion)/Nutrient intake from feed] × 100

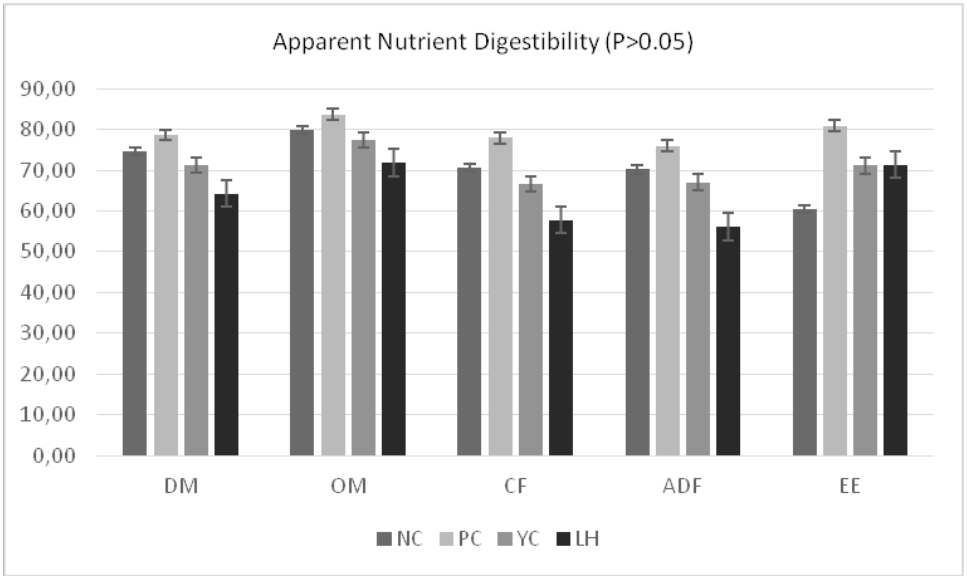
Statistical Analysis

All statistical analyses were performed bySPSS software, version 14.01 (SPSS Inc., Chicago, IL). The Kolmogorov-Smirnov test was used for testing normality. One-way ANOVA with Tukey's post hoc tests was used for multiple comparisons among meansof different experimental groups. Differences were declared statistically significant when $P<0.05$.

RESULTS

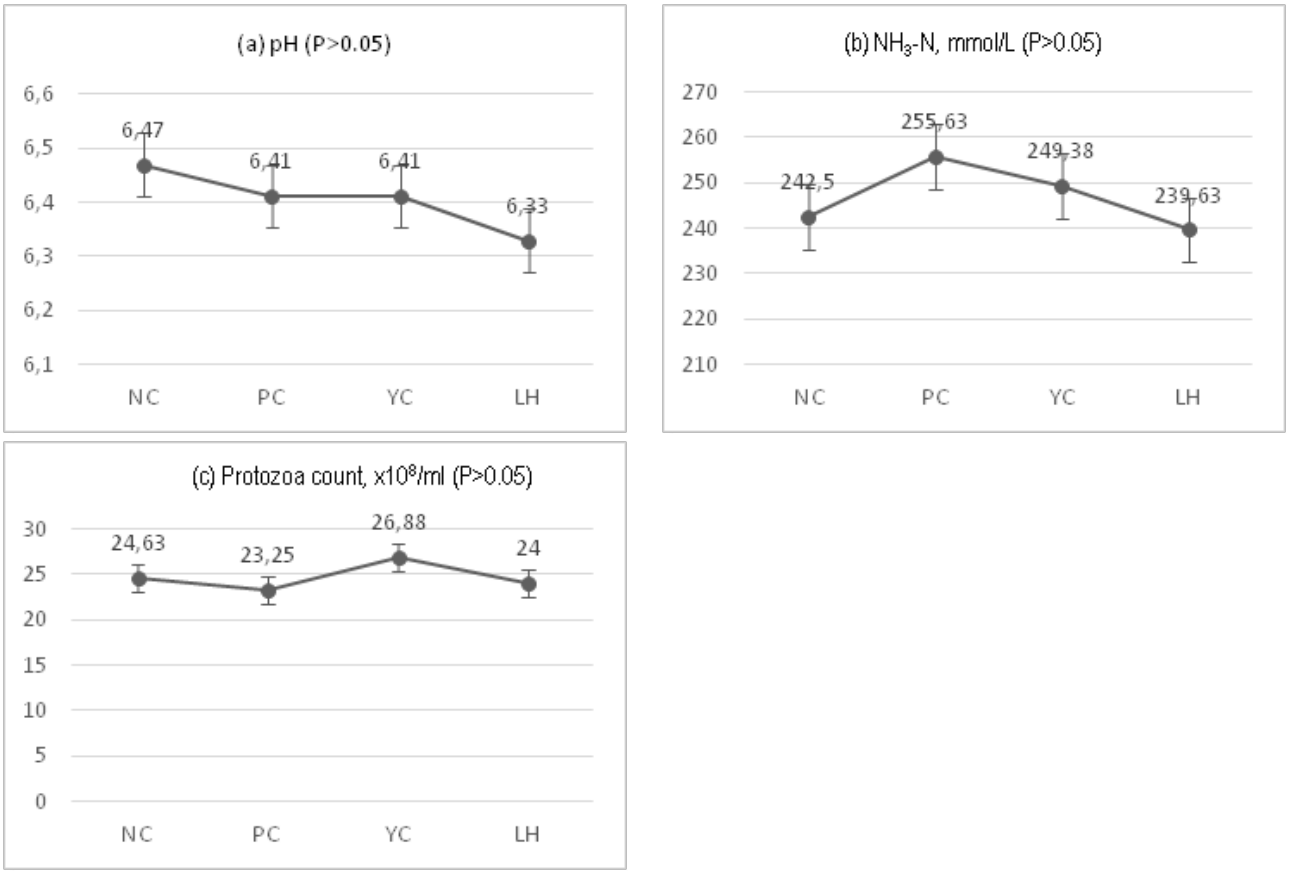
During the experiment, there were no feed refusals between daily meals showing that all the animals readily consumed the daily allowances provided to them. Data on apparent nutrient digestibility of the experimental diets are presented in Figure 1. Apparent digestibility values of observed nutrients (DM, OM, CF, ADF, EE) did not differ statistically ($P>0.05$) in all supplemental groups, though the group with added boric acid alone (PC) had highest digestibility values among all groups.

The addition of B to the diets either alone or with yeast or humate had no statistically significant ($P>0.05$) effect on ruminal pH, NH_3-N, protozoa counts (Figure 2) and SCFA (either total or individual basis) (Table 2). B analysis of rumen fluid showed no detectable level of this trace mineral. Furthermore,



NC = negative control (free of additives); PC = basal diet containing 30 ppm boric acid; YC = basal diet containing 30 ppm boric acid and 3 g/kg yeast culture; LH = basal diet containing 30 ppm boric acid and 5 ml/kg liquid humate.

Figure 1: Effects of dietary B supplementation either alone or in combination with yeast culture or liquid humate on nutrients' apparent digestibility in yearling rams



NC = negative control (free of additives); PC = basal diet containing 30 ppm boric acid; YC = basal diet containing 30 ppm boric acid and 3 g/kg yeast culture; LH = basal diet containing 30 ppm boric acid and 5 ml/kg liquid humate.

Figure 2: Effects of dietary B supplementation either alone or in combination with yeast culture or liquid humate on some ruminal parameters (a) pH, (b) NH_3-N, (c) protozoa count in yearling rams

Table 2: Effects of dietary B supplementation either alone or in combination with yeast culture or liquid humate on rumen SCFA in yearling rams

	Dietary treatments[1]					
Parameters	NC	PC	YC	LH	SEM	P-value
TSCFA[2]	82.24	84.31	86.33	87.15	2.06	0.867
Acetate	44.90	44.06	43.99	43.93	0.38	0.817
Propionate	23.96	24.51	25.49	26.27	0.44	0.266
Iso-butyrate	1.89	1.88	1.86	1.68	0.07	0.406
n-butyrate	20.75	21.68	20.88	20.17	0.40	0.535
Iso-valerate	2.24	2.33	2.26	2.03	0.10	0.378
n-valerate	6.04	5.29	5.23	5.60	0.21	0.723
Caproate	0.23	0.26	0.25	0.32	0.03	0.307
Acetate/Propionate	1.88	1.82	1.73	1.68	0.04	0.297

[1]NC = negative control (free of additives); PC = basal diet containing 30 ppm boric acid (B); YC = basal diet containing 30 ppm B and 3 g/kg yeast culture; LH = basal diet containing 30 ppm B and 5 ml/kg liquid humate
[2]TSCFA = Total Short Chain Fatty Acids, mmol/l

Table 3: Effects of dietary B supplementation either alone or in combination with yeast culture or liquid humate on B absorption and serum concentration

Variables	Dietary treatment[1]					P-values		
	NC	PC	YC	LH	SEM	NC vs PC	PC vs YC	PC vs LH
B absorption, %	99.48	99.33	99.29	99.21	0.28	0.882	0.997	0.932
Concentration of B, PPM								
Feces	0.17	0.25	0.27	0.30	0.03	0.668	0.998	0.920
Serum	0.31	0.64	1.31	0.73	0.10	0.059	0.001	0.893

[1]NC = negative control (free of additives); PC = basal diet containing 30 ppm boric acid (B); YC = basal diet containing 30 ppm B and 3 g/kg yeast culture; LH = basal diet containing 30 ppm B and 5 ml/kg liquid humate

no significant differences were observed among treatments for B absorption (Table 3). Mean serum concentrations of B were greater ($P<0.05$) in YC group compared to the PC group while PC and LH groups were statistically unvaried ($P>0.05$).

DISCUSSION

The main purpose of this study was to determine the effects of B on rumen fermentation in Merino rams and to examine the B absorption and some nutrients' apparent digestibilities. We also tested if combined supplementation of B with yeast or humate substances have a better chance at modulating rumen fermentation in yearling rams.

The results showed that addition of boric acid alone or with other additives had no negative effect on the feed consumption as all animals readily consumed their diets throughout the experiment and there was no residual feed observed between the meals. This was in agreement with the previous study conducted by Sizmaz et al.(2017). In accordance, Fry et al.(2010) also reported no effect on dry matter intake in beef steers when fed diets supplemented with B (5 and 50 ppm).

Overall, no statistically significant differences were observed between treatments regarding rumen fermentative parameters investigated. Nonetheless, a probabilistic perspective is presented based on the numerical differences ($P>0.05$) between treatments, so extra caution must be taken in the reading of the results for the future work and must be verified in the large-scale experiment.

In the present trial, numerically lowest values for pH and NH_3-N were observed in the LH group (6.33 and 239.63 mmol/L), which might be caused by the nitrogen binding effects of liquid humate, but it requires further testing. In an *in vitro*(rumen simulation technique) experiment conducted with rumen inoculum from sheep fed a high forage diet supplemented with humic acid, a decrease in NH_3-N was stated (Varadyova et al., 2009). A similar trend was also reported for NH_3-N in beef steers when humic acid was included in the diet (McMurphy et al., 2011). Humic acid can alter the ruminal fermentability by sequestering NH_3-N and then slowly releasing it for microbial growth, eventually enhancing the protein utilization by decreasing NH_3-N loss (El-Zaiat et al., 2018).Compared to NC, a nominal decrease in pH of

PC was observed, however, NH_3-N was higher (numerically) in PC group. This might reflect the ability of B to decrease the rumen pH which may as well be enhanced upon its supplementation with humate substance. In our previous study (Sizmaz et al., 2017), supplementation of B up to 70 ppm into yearling ram diets tended to decreaseNH_3-N concentration in rumen. Due to unavailability of more studies on this aspect, it is difficult to assess any definite effect and demands further research. Data on protozoa counts in rumen fluid showed no significant alterations between treatments. However, in our previous study (Sizmaz et al., 2017) boric acid supplementation increased the ruminal protozoa, which might be attributed to the different dosages of boric acid used.

The supplemental boron in PC group numerically lowered the acetic acid concentration while the propionic acid concentration showed an increment when compared to the NC group. The importance of pH in regulating acetate to propionate ratio in the rumen was reported in several *in vivo* and *in vitro* experiments (Lana et al., 1998; Bhatta et al., 2006). Although numerically, but lesser pH in the PC group might be the contributing factor in lower acetate and higher propionate values when compared to NC group. Propionic acid is the most important glucogenic precursor in ruminants (Herdt, 1988),thus, an enhanced concentration of propionate may beneficially contribute to the energy status of the animal. A similar but more obvious response was observed for acetate and propionate values when comparing the PC with the LH group having the lowest pH. In a trial on rams fed diets supplemented with humic acid, a decreased cellulolytic activity has been observed (Galip et al., 2010), which might be the underlying reason for this downshifting in acetate to propionate ratio in the LH group. However, the role of B and humate substances in altering the acetate to propionate ratio requires further investigation.The values of branched-chain fatty acids in PC and LH groups might suggest a linkage with NH_3-N production, as the higher NH_3-N(numerically) went along with a higher production of iso-butyric and iso-valeric acid(numerically) in this study. El-Shazly (1952) suggested a positive correlation between ammonia levels and concentrations of iso-butyric and C5 acids in the rumen. Furthermore, the addition of B in the PC group tended to enhance the total SCFA concentration as compared to NC. Total SCFA values were also numerically higher for YC and LH groups as compared to the PC suggesting their ameliorative effects. Variations in total SCFA concentration likely also contributed to the different pH values among different treatments, as the LH group with the highest SCFA value also had the lowest pH. More research is required to properly evaluate the effects of B on SCFA.

Sizmaz et al.(2019) reported that 200 ppm boric acid addition to the yearling ram rations increased the nutrients' apparent digestibility. On the contrary, in a trial with buffalo calves fed diets supplemented with boron and fluorine, no effect on the apparent nutrients' digestibility was reported(Bharti et al., 2007).Fluorine binding with born might be the underlying reason. Research shows that boron attends to the pathways of many enzymes and minerals in different animals, however, lack of dataon digestibility necessitates further studies especially analyzingthe ruminal bacterial community thereby allowing a better comprehension on the effects of boron on digestibility mechanism.

The present study also showed that between meals the B blood serum concentration was significantly higher ($P<0.05$) when fed with yeast than alone. Also, numerically higher serum concentration of B was observed for the LH group when compared to the PC. With almost equal B absorption in all treatments but elevated B serum levels in YC and LH groups compared to PC might suggest higher availability of the mineral, however, the actual B retained in the body to be utilized necessitates urine B analysis.

CONCLUSION

Results from the present study suggested that the tested dosage of B was not enough to induce any significant changes in rumen fermentation characteristics including pH, NH_3-N, protozoa count, SCFA concentration. Better modulation of rumen fermentative characteristics might be possible when boron is supplemented with yeast or liquid humate,however, data from present study were not statistically different, thus, this possibility should be verified in the large-scale future experiment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AOAC (2000) Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

Bennett A, Rowe RI, Soch N, Eckhert CD (1999) Boron stimulates yeast (*Saccharomycescerevisiae*) growth. J Nutr129:2236-2238.

Bharti VK, Gupta M, Lall D (2007) Effects of boron on haemogram and biochemical profile of urine in buffalo calves fed a high fluoride ration. Fluoride40:238.

Bharti VK, Gupta M, Lall D (2008) Effect of boron as an antidote on dry matter intake, nutrient utilization and fluorine balance in buffalo (*Bubalus bubalis*) exposed to high fluoride ration. Biol Trace Elem Res 126(1):31-43.

Bhatta R, Tajima K, Kurihara M (2006) Influence of temperature and pH on fermentation pattern and methane production in the rumen simulating fermenter (RUSITEC). Asian-Australas J Anim Sci 19:376-380.

Chaucheyras-Durand F, Durand H (2010) Probiotics in animal nutrition and health. Benef microbes 1:3-9.

Cox C. Boric acids and borates (2004) Journal of Pesticide Reform 24:10-15.

Denev SA, Peeva TZ, Radulova P, et al. (2007) Yeast cultures in ruminant nutrition. Bulg J Agric Sci 13:357-374.

Devirian TA, Volpe SL (2003) The physiological effects of dietary boron. Crit Rev Food Sci Nutr 43:219-231.

El-Shazly K (1952) Degradation of protein in the rumen of the sheep. 1. Some volatile fatty acids, including branched-chain isomers, found in vivo. Biochem J 51:640-647.

El-Zaiat HM, Morsy AS, El-Wakeel EA, Anwer MM, Sallam SM (2018) Impact of humic acid as an organic additive on ruminal fermentation constituents, blood parameters and milk production in goats and their kids growth rate. J Anim Feed Sci 27:105-113.

Fry RS, Lloyd KE, Jacobi SK, Siciliano PD, Robarge WP, Spears JW (2010) Effect of dietary boron on immune function in growing beef steers. J AnimPhysiolAnimNutr 94:273-279.

Galip N, Polat U, Biricik H (2010) Effects of supplemental humic acid on ruminal fermentation and blood variables in rams. Ital J Anim Sci 9:74.

Geiissler CH, Hoffmann M, Hickel B (1976) A contribution to the gas chromatographic determination of volatile fatty acids. Arch AnimNutr 26:123-129.

Guedes CM, Goncalves D, Rodrigues MAM,Dias-da-Silva A (2008) Effects of a *Saccharomycescerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Anim Feed Sci Technol145:27-40.

Herdt TH (1988) Fuel homeostasis in the ruminant. Vet Clin N Am Food A 4:213-231.

Kabu M, Civelek T (2012) Effects of propylene glycol, methionine and sodium borate on metabolic profile in dairy cattle during periparturient period. Revue Med Vet 163:419-430.

Lana RP, Russell JB, Van Amburgh ME (1998) The role of pH in regulating ruminal methane and ammonia production. J Anim Sci 76:2190-2196.

McMurphy CP, Duff GC, Sanders SR, Cuneo SP, Chirase NK (2011) Effects of supplementing humates on rumen fermentation in Holstein steers. S Afr J Anim Sci41:134-140.

Ogimoto K, Imai S (1981) Atlas of Rumen Microbiology. Japan Scientific Societies Press. Tokyo (Japan) 158.

Sena JA, Villela SD, Santos RA, et al. (2015) Intake, digestibility, performance, and carcass traits of rams provided with dehydrated passion fruit (*Passiflora edulis f. flavicarpa*) peel, as a substitute of Tifton 85 (*Cynodon spp.*). Small Ruminant Res 129:18-24.

Seo JK, Kim SW, Kim MH,Upadhaya SD, Kam DK, Ha JK (2010) Direct-fed microbials for ruminant animals. Asian-Australas J Anim Sci 23:1657-1667.

Shi Y, Parker DB, Cole NA, Auvermann BW, Mehlhorn JE (2001) Surface amendments to minimize ammonia emissions from beef cattle feedlots. Transactions of the ASAE 44:677-682.

Sizmaz O, Koksal BH, Yildiz G (2017) Rumen microbial fermentation, protozoan abundance, and boron availability in yearling rams fed diets with different boron concentrations. J Anim Feed Sci 26:59-64.

Sizmaz O, Koksal BH, Yildiz G (2019) Investigating of usage of boric acid in yearling rams. In: Proceedings of 1st Livestock Science Congress, Antalya, Turkey.

Stevenson FJ (2010) Humus Chemistry: Genesis, Composition, Reactions. John Wiley & Sons, New York.

TSE 1994 Animal Feeds. Metabolic Energy Determination (Chemical method). Turkish Standards Institute No: 9610, Ankara.

Vanatta LE, Coleman DE, Slingsby RW (1999) Low-level calibration study for a new ion chromatographic column to determine borate in deionized water. J Chromotogr A 850:107-117.

Varadyova Z, Kisidayova S, Jalč D (2009) Effect of humic acid on fermentation and ciliate protozoan population in rumen fluid of sheep in vitro. J Sci Food Agr 89:1936-1941.

Xu D, Peak D (2007) Adsorption of boric acid on pure and humic acid coated am-Al (OH) 3: a boron K-edge XANES study. Environ Sci Technol 41:903-908.

Yildiz G, Koksal BH, Sizmaz O (2011) Effects of dietary boric acid and yeast (*Saccharomyces cerevisiae*) supplementation on performance, carcass traits and some blood parameters of broilers. Kafkas Univ Vet Fak 17:429-434.

Yildiz G, Koksal BH, Sizmaz O (2013) Effects of dietary boric acid addition on growth performance, cholesterolemia, some carcass and tibia characteristics in different rearing periods in broiler chickens. Revue Med Vet 164:219-224.