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The Effects of Peroral Intake of Humic Substances Preparation Combined with Urea on Ruminal Fermentation in Sheep

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Abstract: *Humate substances (HS) are naturally occurring organic molecules found in soil as a result of the humification of dead organic components, which are relatively easy to get as raw materials. The aim of this study was to investigate the effects of humic substances combined with urea as feed additives on the rumen fermentation indicators (pH, concentration of volatile fatty acids VFAs, and ammonia concentration) in the rumen fluid of 12 female crossbred merino sheep (n = 6 in each group; test and control). The daily ration for both groups consisted of grass hay and a cereal grain mixture. Urea was fed to both groups at a dose of 10 g/day/animal and mixed into a cereal grain mixture. The humic substances were mixed into a daily grain mixture at a dose of 20 g/day in the test group. Rumen samples were taken on days 4 and 18 at 3, 6, and 9 hours post-morning feeding. The results revealed that HS increased ammonia concentration on day 4, while the total VFA concentration as well as pH values of the rumen fluid were not changed. Ammonia was higher ($P<0.05$) in the test group at 6 hours after feeding on day 4. The differences in ammonia concentration on day 18 were not statistically significant among the groups. Results suggest humic substance addition with urea as a non-protein dietary nitrogen source may improve nitrogen retention in the rumen early after their supplementation to diet.*

I. INTRODUCTION

Humate compounds are easily obtainable raw materials used in agriculture and animal husbandry as feed or drinking water supplements [1]. Humates, based on solubility in acids and bases and by molecular weight, can be fractionated into three categories: fulvic acid, humic acid, and humin [2]. Humic and fulvic acids are the major extractable components of soil humates and are predominantly used to improve soil fertility and enhance nutrient uptake by plants [3]. Humic subsances are known to exhibit a high affinity for nitrogen (N), a property that has been postulated to improve rumen microbial synthesis and decrease N excretion into the environment [4].

These nitrogen-binding qualities could prove beneficial in the retention of ammonia nitrogen (NH3-N) in the rumen. Urea has commonly become an accepted non-protein nitrogen ingredient in the diets of ruminants. It is rapidly hydrolysed by rumen bacterial urease to ammonia, and the ammonia is utilized for the synthesis of microbial proteins [5]. According to Ampong et al. [6], the efficacy of HS depends on soil type, HS source, and application rate. Organic compounds known as humic acids have a complicated molecular structure and a large molecular weight and are generated in nature via the use of chemical, biological, and other procedures using components made from plants and animals that decay and appear as a result of the biological activity of microbes. Humic acids, as a products of the decomposition of animal and plant tissue, has been proposed as a feed supplement to stimulate the growth of animals and as a potential replacement for antibiotic growth stimulators [7 – 10].

Humates have been shown to reduce ammonia emissions when utilized as an amendment to soil, faeces, and urine [11] or when used as a feed supplement in swine [12]. The aim of this study was to investigate the effects of peroral intake (mixed into feed) of humic substance preparation combined with urea, a non-protein nitrogen, on the pH values, the concentration of ammonia, and the volatile fatty acids concentrations in the rumen of sheep in terms of time dependence.

II. Materials and Methods

Twelve crossbred merino ewes (two years of age, no pregnant, not lactating) were used in an 18-day experiment. The animals were housed in two groups: test and control. Sheep were fed a daily basal diet consisting of 1.25 kg of grass hay and 0.25 kg of a cereal grain mixture divided into two equal parts. The experimental dietary ingredients and chemical composition are presented in Table 1. Urea was fed to both groups at a dose of 10 g/day/animal and mixed into a grain mixture. Water was available ad libitum. All procedures were performed with animals approved by the Animal Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice according to Directive 2010/63/EU [13].

The experiment lasted 18 days. Urea was used as a non-protein nitrogen feed source due to its fast transformation into ammonia. We tested our hypotheses in order to assess the main effects of HS additives after peroral intake in the diet with urea. Sheep received a basal diet either without humic substance (HS) (control group) or HS mixed into the grain mixture at a dose of 20 g/day (test group). The characteristics of the applied natural HS preparation (HUMAC®Natur AFM; Humac s.r.o., Slovak Republic) were the following: the size of particles up to 100 µm, max. moisture 15%, the content of humic acids min. 650 g/kg, and fulvic acids min. 50 g/kg. The chemical analyses of diets were performed according to Commission Regulation (EC) No. 152/2009 [14].

The rumen contents were taken from the rumen using a tube at 3, 6, and 9 hours after morning feeding on days 4 and 18. The level of rumen fermentation was evaluated by analysing rumen pH, ammonia content (NH3) (mg/100 ml), the volatile fatty acids (VFA) concentrations (mmol/l), and the acetate: propionate ratio. Rumen fluid was collected, filtered through two layers of gauze, and analysed for pH with a pH meter (Consort C830, Belgium). The analysis of VFA was carried out in a two-capillary isotachophoretic analyser (EA100, Villa-Labeco, Slovak Republic). The quantification of NH3 was performed by direct distillation and titration of 10.0 ml of ruminal fluid with an automatic N-analyser (Foss Tecator 2300).

The data were expressed as a mean ± standard error (SEM). The differences between means were determined according to the unpaired t-test using the Graph-Pad Prism statistical program (Graph Prism software, USA). Differences ($P<0.05$) were considered statistically significant using conventional criteria.

Table 1: Chemical composition of the diet and formulation of cereal grain mixture

Parameters	Grain mixture	Grass hay
DM[1](g/kg)	880.70	868.80
Crude fibre (g/kg DM)	34.40	356.0
Crude fat (g/kg DM)	23.20	11.20
Crude protein (g/kg DM)	135.0	77.10
Total ash (g/kg DM)	15.10	63.10
Formulation of grain mixture, %		
Barley	50	
Wheat	50	

1 - dry matter

III. Results and Discussion

The effects of supplementing sheep with urea and HS on the ruminal fermentation parameters and the ruminal pH are presented in Table 2. The supplementation of humic substances with urea combination showed the following in hourly dynamics in the test group: significantly higher level of the ammonia content on day 4 at 6 h ($P<0.05$) after administration in comparison with the control group. The slower decrease of ammonia in the rumen between the third and sixth hours after feeding was observed in the test group, especially on day 4 after peroral addition of HS. The tendency for a slower decrease of ammonia was also detected between the third and sixth hours and the sixth and ninth hours on day 18 after peroral intake of HS (mixed into a concentrate mixture) in the test group. But there were no statistically significant differences in the ammonia concentration between groups on day 18.

Humates have nitrogen-binding qualities that could prove beneficial in the retention of ammonia nitrogen in the rumen [4]. The N-binding ability could improve the retention time and slow down the release of ammonia and nitrogen in the rumen. In the rumen, the ammonia can be assimilated by many rumen bacteria for the synthesis of microbial proteins [15 - 16].

The supplementation of humic substances with urea combination in our study showed a slower decrease of ammonia in the rumen in the time dependence after feeding (in hourly dynamics) in the test group early (day 4) after peroral HS administration. No differences between groups were observed for the total and particular (acetate and propionate) VFA concentrations (mmol/l) in all hourly collections during days 4 and 18, as well as in the pH parameter and the acetate: propionate ratio.

A number of studies have examined the value of humic substances as a feed additive for ruminants [1; 4; 17 – 20]. McMurphy et al. [4] determined that humate products high in humic acid content did not dramatically impact some aspects of rumen fermentation (pH value and VFA concentration). Galip et al. [1] observed no significant effect on rumen variables. Humic acids can improve rumen digestion and livestock performance by modulating gut microbiota and improving digestive enzymes [21]. According to their study, adding humic acid to the diet enhanced rumen acetate concentrations while decreasing rumen pH, ammonia, N, and butyrate levels [21].

The results from El-Zaiat et al. [19] revealed that humic acid increased ruminal pH, acetate, and propionate proportions, while ammonia concentrations decreased. The high rumen pH in HA-treated goats represents the buffering capacity of humic substances, which may lead to rumen acid stabilization and manipulation of rumen alterations to improve rumen microbial function efficiency [19]. The buffering capacity of sodium humate may explain why it raises the pH of goat urine. This stabilizes ruminal acidity, leading to changes that improve the efficiency of microbial rumen processes [22].

Table 2: Ruminal fermentation parameters and rumen pH in sheep after peroral intake of humic substances preparation combined with dietary urea (n = 12; two groups test and control with 6 sheep each). Values are presented as mean±SEM.

Item	ST[2] (h[3])	Day 4		Day 18	
		TEG[4]	CG[5]	TEG	CG
Ruminal pH	3	6.52±0.05	6.60±0.06	6.70±0,07	6.69±0.10
	6	6.44±0.03	6.47±0.05	6.57±0.09	6,49±0.12
	9	6.68±0.11	6,72±0.20	6.88±0.10	6.77±0.06
tVFA[1] (mmol/l)	3	92.9±4.4	90.9±1.6	90.3±2.0	90.5±1.1
	6	98.3±2.4	97.7±4.6	93.8±1.8	93.8±1.4
	9	93.3±3.0	88.7±2.4	80.8±3.2	82.2±1.9
Acetate (mmol/l)	3	59.7±3.0	58.5±1.3	57.4±1.1	58.2±0.7
	6	61.5±1.5	61.3±3.0	60.8±1.2	60.4±0.9
	9	61.6±2.1	58.15±1.1	54.6±1.4	54.8±1.6
Propionate (mmol/l)	3	20.2±0.8	19.65±0.5	18.3±0.5	18.6±0.3
	6	21.4±0.7	20.9±1.0	19.0±0.6	19.2±0.4
	9	17.7±0.6	16.6±0.5	16.0±0.4	15.9±0.5
acetate: propionate ratio	3	2.94±0.17	2.98±0.08	3.13±0.08	3.12±0.04
	6	2.86±0.08	2.92±0.18	3.19±0.07	3.13±0.05
	9	3.47±0.12	3.49±0.07	3.41±0.10	3.43±0.08
Ammonia (mg/100ml)	3	45.3±1.8	42.7±2.8	43.7±1.9	45.2±1,8
	6	39.9[a]±2.5	32.3[b]±2.1	34.6±0.8	32.8±1.9
	9	26.6±1.3	23.4±1.9	23.6±0.9	19.9±1.5

[1] - total volatile fatty acids; [2] - sampling time; [3] - hour; [4] - test group; [5]- control group; a, b – significant differences ($P<0.05$)

In the study of Terry et al. [20], humic substances increased ammonia nitrogen (NH3-N) concentration. This study indicated that total VFA and their individual concentrations were not affected by the addition of humic substances. The concentration of NH3-N and total protozoa count responded quadratically ($P = 0.03$) to increasing concentrations of HS. The quadratic response of protozoa numbers and NH3-N concentration to HS dose is consistent with the concept that rumen protozoa engulf rumen bacteria that use NH3-N to help meet their N requirements [23 – 24]. Thus, increased protozoa numbers may have led to less ruminal NH3 incorporated into microbial protein and increased NH3 concentrations [20].

IV. Conclusion

The addition of humic substances with urea has confirmed nitrogen-binding capacity in the rumen with longer retention time for potential releasing of ammonia for future synthesis of microbial proteins with significantly increased ammonia concentration in ruminal fluid, but only early after HS administration to diet. It had no significant effect on the ruminal pH, the VFA content, or the acetate-to-propionate ratio.

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