The Impact of Humic Substances as Feed Additive on Ruminal Fermentation

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Abstract
The rumen is settled with a complex microbial ecosystem where feeds consumed by animals are digested with the help of rumen microorganisms, which play an important role in contributing nutrients to the host animal. This study focused on investigating the effect of humic substances (HS) dietary inclusion on the protozoal community in the rumen and qualitative parameters of the ruminal fluid. In the presented research, three cannulated beef cows were housed in individual pens and were fed with the basal diet enriched with the organic feed additive Humac®Natur AFM (comprise: 65 % humic and 5 % fulvic acids, Ca 42.28, Mg 5.11, Fe 19.05 g/kg, Cu 15, Zn 37, Mn 142, Co 1.24, Se 1.67, Mo 2.7 mg/kg of DM) in 3 different concentrations: 60 g/day (4,8 g/kg of DM) and 110 g/day (8,9 g/kg of DM) and 200 g/day (16,1 g/kg of DM). The rumen liquor of each animal was collected two times per week via a rumen cannula with a probe connected to a vacuum pump for further laboratory analyses. Test of rumen fluid included: measurement of pH, physical characteristics, the concentration of nitrogenic compounds and ammonia, determination of rumen protozoa activity, and counting of the total number of protozoal ciliates; the motility of ruminal ciliates was also observed. The rumen pH level was affected by the sampling period, but diet treatment had no significant impact on rumen pH. The pH values in rumen fluid samples were within the normal range, the same as physical characteristics (color, odor, consistency, and sedimentation time). In conclusion, dietary HS supplementation significantly reduced protozoal activity in the rumen in all tested concentrations, which was also in correlation with lower protozoal motility and ammonia concentrations.

Keywords: *animal nutrition; humic substances; protozoa; ruminants*

1. Introduction

The use of humic substances (HS) as medicine has a long tradition due to their broad biomedical effects, such as antibacterial, antiviral, immuno-modulatory, antioxidant, diuretic, antihypertensive, and hypoglycemic effects [1]. HS (i.e., humates) are organic substances commonly present in drinking water, rivers, lakes, soil, and plants. Based on acid-base solubility and molecular weight, humates can be divided into three main categories: fulvic acid (FA), humic acid (HA), and humin. Fulvic acids are soluble at acidic or alkaline pH, humic acids are soluble at alkaline pH, and humins are insoluble at any pH [2]. Humic acids have an excellent ability to bind toxic substances such as heavy metals, mycotoxins, microbial toxins, ammonia, pesticides, and others. Besides, they have anti-inflammatory and antioxidant effects, making them a healthy alternative to antibiotics in cattle breeding and production [3]. Due to their high biological activity, their addition to animal feed has the potential to improve animals' performance and health status and thus positively affect the economics of livestock farming [4]. HS can be used as feed additives in almost all livestock species, e.g., poultry, cattle, pigs, sheep, goats,

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and horses. They can form a protective film on the mucous epithelia of the gastrointestinal tract against infections and toxins; their macro-colloidal structure ensures good shielding on the mucous membrane of the stomach and gut, the peripheral capillaries, and damaged mucous cells. As a result of this process, the resorption of toxic metabolites is reduced or fully prevented, especially after infections or in case of the presence of harmful substances in animal feed. Furthermore, HAs also help to prevent excessive loss of water via the intestine [5].

This study focused on investigating the effect of humic substances (HS) dietary inclusion on the protozoal community in the rumen and qualitative parameters of the ruminal fluid.

2. Materials and methods

In the presented study, three fistulated beef cows were used to evaluate the effect of enriched basal ration with humic acid (Humac®Natur AFM, HUMAC s.r.o., SK) on ruminal fermentation. The cows were housed in individual pens and fed once daily in the morning with a basic feed ration (BFR) consisting of 56.5% grass hay, 32% corn silage, and 10.5% cereals concentrate "Biostan". Total daily dry matter intake per feeding was 11.9 kg, and water was available free-choice. Before the start of the experiment, animals were divided following way: the first cow represented the control (CON) fed with BFR, and another two cows represented the experimental group supplemented with HUMAK.

The experimental setup involved 14 days of adaptation period to harmonize the microflora of the rumen and 21 days of dietary HUMAK supplementation repeated in 3 experimental regimes. Experimental diets consisted of BFR mixed with HUMAK. The first diet (H60) included 60g (4.8 g/kg of DM) of feed additive, in the second diet (H100) was added 110g (8.9 g/kg of DM), and in the third diet (H200) was added 200g (16.1 g/kg of DM) of HUMAK. Rumen liquor samples were collected two times per week, three hours after morning feeding via a rumen cannula with a probe connected to a vacuum pump. The samples were immediately transported in a water bath (39°C) to the laboratory for analysis. Samples were filtered through the synthetic cloth (119μm, Uhelon 59 S, Silk & Progress). Sample filtration helps to remove larger particles from the sample and makes the visualization of the ciliate's protozoa easier [6]. Immediately after collection, the ruminal fluid samples were examined for physical characteristics such as color, odor, and consistency. Also, sedimentation activity tests were carried out by putting the sample of rumen fluid into a test tube and allowing it to stand. The sedimentation time of fine particles in all samples ranged between 8-9 minutes throughout the study. The protozoal activity was examined by placing one drop of ruminal fluid on a pre-warmed microscope slide, and a coverslip was placed. The motility of the protozoa was tested in a fresh film of the rumen liquor under low magnification (40x) and was graded in four categories: 4- high motility and very crowded: >10 mobile protozoa per one field in the Bürker chamber; 3- medium motility: 6-9 mobile protozoa per chamber square; 2- subnormal: 3-5 mobile protozoa per square; 1- very low motility: <3 mobile protozoa per square.

Our results showed that the highest motility of the protozoa in the cow's rumen was observed during feeding animals with treatment included diet H60, which correlates with results achieved during microscopic counting of the total amount of ciliates (Table 1).

Table 1. Grading of protozoal motility and total protozoa count

Diet treatment	Motility score	Total protozoa count ($x10^4$/ml)
CON	+++	25
H60	++++	30.87
H110	++	24.41
H200	++	24.3

The fixing rumen content samples were preserved in 18.5% formaldehyde solution and stained with Brilliant Green Dye, then samples were mixed and allowed to stand overnight. The density of the rumen protozoa per mL was obtained using a Bürker counting chamber in an optical microscope at a magnification of 40x. The microscopic examination of protozoa is widely

accepted as a golden standard for analyzing ciliate community structure.
It can provide useful information about the physiological processes associated with animal nutrition in the rumen [7]. The density of the protozoa in samples was identified according to criteria described by Imai & Ogimoto [8], Baraka T.A. [9], and Burk A. Dehority [6]. The pH of the ruminal fluid was measured immediately after collecting the samples (EUTECH CyberScan PC510 pH/Conductivity Bench Meter, USA). A small amount (15 ml) of rumen liquor was kept for further measurement of ammonia and nitrogen concentrations.

3. Results and discussion

The concentration of ammonia (NH_3) in the rumen depends mainly on the pH value of the rumen fluid. The optimal ruminal pH ranges from 6.10 to 6.80 [10]. The pH values in the experimental period were close to the physiological value for both control and experimental animals in the range of 6.1 - 6.5 (Figure 1). The pH values of animals supplemented with HUMAK were higher compared to the control, reflecting HS's buffering capability. Buffering ability of HS can stabilize the ruminal acidity and support the optimal activity and efficiency of ruminal microbes, which can be beneficial for improving ruminal efficiency and animals' overall performance [10].

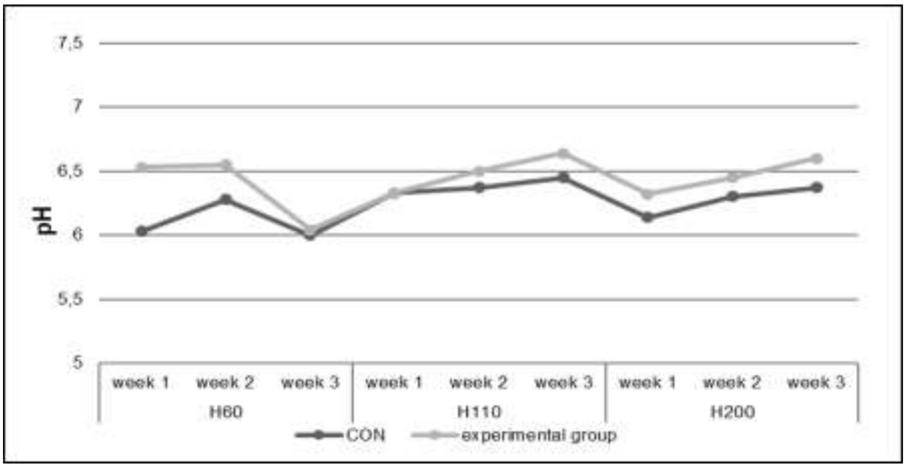

Figure 1. Influence of HUMAK dietary inclusion on pH values in the rumen

Mean ammonia concentrations ranged from 4.27 to 8.91 mmol/l during the experimental period in cows supplemented with HUMAK, and from 2.69 to 4.7 mmol/l for the control (Figure 2).

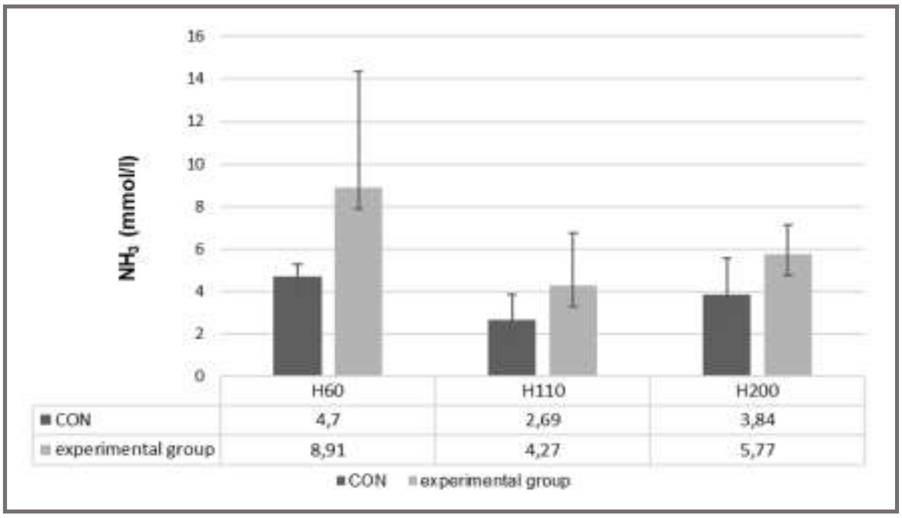

Figure 2. The concentration of ammonia depending on the HUMAK dietary inclusion

By comparing the concentrations of ammonium ions between the individual experimental regimes, it is possible to say that they decreased with the highest dosages of HUMAK in feed (8.9 and 16.1 g/kg DM) from an average value of 4.27 mmol/l (H60) to 2.69 mmol/L (H110) and 3.84 mmol/L (H200). Due to the properties of the ionic complex, humic substances bind to rumen nitrogen, thus reducing the NH_3-H content. During this experiment, the concentration of N-substances in the rumen fluid varied depending on the proportion of HUMAC as follows: 5.05 g/kg (H60), 3.36 g/kg (H110), and 4.96 g/kg (H200). These results revealed lower ruminal NH_3 concentrations in animals supplemented with HS. The concentration of ammonia ions was dose-dependent, and the lowest NH_3 was in animals supplemented with a higher amount of HUMAK additive.

It can be connected with the effectiveness of HS in reducing NH_3-H accumulation, which highlights the effectiveness of using HS as an organic feed additive. Strong N-binding properties of HS may induce extra reduction of NH_3-H in the rumen. Dietary HS can enhance crude protein utilization by concurrently decreasing NH_3-H loss owing to reduced solubility under the inhibitory effect of HS on urease activity [11]. The addition of HUMAC to the feed ration of cows should reduce the excretion of N-substances from the body due to reduced solubility [10], which was confirmed by our results as well.

Dietary HS can enhance ruminal fermentability by sequestering NH_3-H and slowly releasing it for microbial growth. A reduced number of protozoa in the rumen may increase microbial crude protein flow in the small intestine [12]. The decrease in ruminal NH_3 concentrations was accompanied by the reduction in protozoa number and their motility (Table 1). The total protozoal counts in rumen fluid samples from cows fed with a diet H110, H200 were lower than the control, showing the eliminative effect of HS supplementation. Similar results were reported by a number of other studies, e.g., [10, 13-15]. Interestingly, a lower dosage of HS (H60) increased the population of protozoa which was also correlated with higher ammonia concentration in the rumen (Table 1, Figure 2). Based on this, we can recommend higher doses of HS in ruminants' diets over the lower doses.

4. Conclusions

Dietary HS supplementation significantly reduced protozoal activity in the rumen, which was also in correlation with lower protozoal motility and reduced ammonia concentrations. Further in vivo studies are recommended to investigate the effects of HS on interactions of fermentative activity and overall performance of ruminants.

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