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Short communication

Effects of hainanmycin or monensin supplementation on ruminal protein metabolism and populations of proteolytic bacteria in Holstein heifers

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ABSTRACT

Three ruminally cannulated cows were used in a 3 × 3 Latin square design (21-d periods) to evaluate effects of hainanmycin (HAI) or monensin (MON) supplementation on ruminal protein metabolism and populations of proteolytic bacteria in Holstein heifers. The three dietary treatments were: (1) basal diet (control); (2) basal plus 20 mg/heifer per day HAI and (3) basal plus 350 mg/heifer per day MON. The supplementation with HAI and MON had minor effects on ruminal pH and total VFA. In general, HAI and MON decreased acetate, butyrate concentrations, and acetate to propionate ratio, and increased propionate concentration. Supplementation of HAI and Mon resulted in higher relative population sizes of the genus *Prevotella* and *Prevotella ruminicola*, and fewer *Butyrivibrio fibrisolvens* and hyper-ammonia-producing bacteria (P<0.05). HAI supplementation increased concentrations of peptide nitrogen and amino acid nitrogen, and reduced ammonia concentration (P<0.05), and decreased peptidase and deaminase activities (P<0.05). Overall, HAI was as effective as monensin as ionophore in regulation of ruminal protein metabolism and populations of proteolytic bacteria for dairy cows.

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1. Introduction

The breakdown of protein to ammonia in the rumen may lead to an appreciable loss of nitrogen to the animal (Wallace, 1996). Bacteria are thought to be responsible for the majority of the breakdown in the rumen (Wallace et al., 1987). Many of ruminal bacteria have peptidase and deaminase activities, for instance *Prevotella* sp. and hyper-ammonia-producing (HAP) bacteria (Wallace et al., 1997).

Monensin caused an accumulation in amino acids N and peptide N by mixed ruminal microorganisms *in vitro*, and decrease rumen ammonia production (Chen and Russell, 1991; Ghorbani et al., 2008). Much of the decrease in ruminal ammonia production caused by monensin addition can be specifically attributed to the inhibitory effects on HAP bacteria (Eschenlauer et al., 2002). Hainanmycin is a polyether monocarboxylic acid ionophore from one of fermentation products of a

Abbreviations: ADF, acid detergent fiber; A:P, acetate to propionate ratio; BW, body weight; CP, crude protein; DM, dry matter; HAI, hainanmycin; HAP, hyper-ammonia-producing; ME, metabolisable energy; MNA, methoxynapthylamide; MON, monensin; N, nitrogen; NDF, neutral detergent fiber; RPS, relative population sizes; VFA, volatile fatty acid.

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rare streptomycete, *Streptomyces padanus* var *dangfangeus*, which was isolated from soil sample collected in Hainan province of China. The chemical formula of hainanmycin is $C_{47}H_{79}O_{15}N$, which capable of forming stable complexes to monovalent cations with a specific affinity for Na^+. Hainanmycin lowers ruminal ammonia production in the sheep, and increase ruminal concentrations of peptide N and amino acid N, and affects growth of ammonia-producing bacteria *in vitro* (Wang et al., 2013). However, there is little information on the effects of hainanmycin on ruminal protein metabolism and populations of proteolytic bacteria in cattle. The objective of the present study was to evaluate the effects of the supplementation of hainanmycin on ruminal protein metabolism and populations of proteolytic bacteria in Holstein heifers.

2. Material and methods

The protocol used in this experiment was approved by the Northeast Agricultural University Animal Science and Technology College Animal Care and Use Committee.

2.1. Feed additives

The hainanmycin was obtained from Sheng Li Inc. Shandong province, China.

2.2. Animals and treatments

Three Holstein heifers fitted with ruminal cannulas were used in a 3 × 3 Latin square design to evaluate effects of hainanmycin (HAI) or monensin (MON) supplementation on ruminal protein metabolism and populations of proteolytic bacteria in Holstein heifers. The heifers were fed a diet which consisted of dry-rolled corn (99.7 g/kg), soybean meal (109.7 g/kg), Chinese wild rye grass (378.9 g/kg), and corn silage (358.9 g/kg). The CP, NDF, ADF, and ME contents of the diet (DM basis) were 128.8 g/kg, 454.2 g/kg, 319.4 g/kg, and 9.17 MJ/kg, respectively. Treatments consisted of: (1) basal diet (no ionophore); (2) basal plus 20 mg/heifer per day HAI; and (3) basal plus 350 mg/heifer per day MON. The HAI dose was calculated according to the heifer's body weight (0.05 mg/kg BW per day) (Ren et al., 1998). Each period lasted 21 with 19 d of adaptation and 2 d of sample collection. Diet was offered twice daily at 08:00 and 18:00 h and 10.5 kg (DM basis)/day per heifer.

2.3. Sampling and measurements

On d 20 and 21 of each period, ruminal fluid was collected immediately at 0, 2, 4, 6, 8, and 10 h for ruminal pH, VFA concentration, protein fractions (peptide N, amino acid N, and NH_3), and total DNA extraction. Strained ruminal fluid at 4 h after feeding was used to determine *in vitro* protease and deaminase activities (Siddons and Paradine 1981). The peptidase activity was assayed using the substrate Glycine-Arginine methoxynapthylamide (MNA) (Wallace and McKain 1989). Concentrations of peptide N plus amino acid N in ruminal fluid were determined as described by Winter et al. (1964). In this method, the tungstic acid precipitates small peptides only partially (Raacke 1957), therefore the values for peptides N as determined by tungstic acid would be low in present experiment. PCR assays for enumeration of selected bacterial species were performed according to the methods described by Stevenson and Weimer (2007). Enumeration for HAP were determined according to the methods described by Russell et al. (1988).

2.4. Statistical analysis

Data were statistically analyzed as a 3 × 3 Latin square design using the PROC MIXED procedure of SAS (SAS Institute, version 9.1) according to the following model. $Y_{ijk} = \mu + T_i + P_j + C_k + E_{ijk}$. Where Y_{ijk} is observation, μ is overall mean, T_i is treatment (i = 1–3), P_j is period (j = 1–3), C_k is heifer (k = 1–3), and E_{ijk} is residual error. Differences were declared significant at $P<0.05$. Results are reported as least squares means ± standard error of the means.

3. Results and discussion

3.1. Ruminal fermentation characteristics

Ruminal fermentation characteristics are shown in Fig. 1 and Table 1. In general, the supplementation with HAI and MON had minor effects on ruminal pH and total VFA. Similar to the results in the present trial with HAI, HAI also had no changed pH and total VFA in our previous study *in vitro*, (Wang et al., 2013). Two ionophores caused lower ($P<0.05$) acetate concentrations at 6 h after feeding, and higher ($P<0.05$) concentrates of propionate at 0, 2, 4, and 8 h after feeding. Supplementation of HAI or MON decreased ($P<0.05$) acetate: propionate ratio at 0, 2, 4, and 6 h after feeding. Changes in acetate, propionate and their ratio in the present study were in agreement with the observations of Ren et al. (1998). HAI and MON caused lower ($P<0.05$) concentrations of butyrate at 0 and 2 h after feeding, respectively. Concentrations of valerate or isobutyrate were not affected by HAI and MON (data not shown). MON decreased ($P<0.05$) isovalerate concentrations at 2 and 6 h after feeding compare with control, and HAI also caused lower concentrations of isovalerate ($P<0.05$) at 6 h after feeding (data not shown).

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