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Effects of subacute ruminal acidosis challenges on fermentation and biogenic amines in the rumen of dairy cows

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ABSTRACT

This study was conducted to investigate if subacute ruminal acidosis (SARA) challenges result in an increase of biogenic amines in the rumen fluid and peripheral blood. Four rumen cannulated primiparous (60–140 d in milk) Holstein dairy cows were assigned to two diets in a crossover experimental design. Each experimental period lasted for 21 d. The diets contained (dry matter basis): 40% (COD; control) and 70% (SAID) concentrate feeds. Rumen fluid samples were collected on days 12, 15, 17 and 21 of the experimental period for measurement of pH, volatile fatty acid, ammonia-nitrogen and biogenic amine contents. Peripheral blood was also collected on days 12, 15, 17 and 21 to measure biogenic amine content. Based on adopted threshold of SARA of at least 180 min/d below pH 5.8, SARA was induced by the SAID feeding. The average concentrations of tyramine (147.98 vs. 99.94 μmol/L); putrescine (39.44 vs. 17.52 μmol/L); histamine (161.19 vs. 46.43 μmol/L); methylamine (31.81 vs. 28.09 μmol/L) and tryptamine (72.25 vs. 48.77 μmol/L) were higher ($P<0.05$) in the SAID group than in the COD in the rumen, and similar results were also observed in the peripheral blood. SAID feeding increased ($P<0.05$) the averages of total volatile fatty acids (from 110.83 to 125.21 mmol/L), butyrate (from 11.29 to 14.53 mmol/L), valerate (from 4.76 to 7.05 mmol/L), and total branched-chain VFA (from 3.80 to 4.93 mmol/L), and decreased ($P<0.05$) the ruminal pH (from 6.38 to 6.05) and acetate to propionate ratio (from 3.63 to 3.29) in the rumen. Correlation analysis showed that there were negative correlations ($P<0.05$) between ruminal pH and the levels of tyramine, putrescine, histamine, methylamine or tryptamine in rumen fluid or in peripheral blood. Positive correlations ($P<0.05$) were observed between the concentration of tyramine, putrescine, histamine and methylamine in rumen fluid and the corresponding biogenic amine in the peripheral blood during SAID feeding. In addition, the 16S rRNA gene copy number of *Lactobacillus* spp. was higher ($P<0.05$) in the SAID group than in the COD, whereas no effect was observed for the gene copy number for *Streptococcus bovis*. Significant positive correlations ($P<0.05$) were detected between the levels of ruminal tyramine, putrescine, histamine or methylamine and the number of *Lactobacillus* spp. In conclusion, this study showed that SARA challenges affected rumen pH, rumen fluid concentrations of total volatile fatty acids, lactate and biogenic amines, and revealed that a low rumen pH is associated with increases in rumen biogenic amine concentrations, which is accompanied by increases in plasma biogenic amine concentration.

1. Introduction

Diets containing large proportions of readily fermentable carbohydrates have become economically important in

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the production of meat and milk from ruminants. However, feeding diets high in cereals is often associated with multiple metabolic disorders like rumen acidosis (Nagaraja and Titgemeyer, 2007), laminitis (Nagaraja and Titgemeyer, 2007), fatty liver (Ametaj et al., 2010), displaced abomasum (Shaver, 1997), and bloat (Cheng et al., 1998). Among these diseases, subacute rumen acidosis (SARA) represents one of the most important metabolic disorders in intensive dairy farms that affects rumen fermentations, animal welfare, productivity and farm profitability, and it is characterized by reduced pH-values (5.8–5.0) in the rumen content, occurring either for longer periods or repeatedly (Morgante et al., 2007). Although ruminal acids are considered the main contributors to the pathophysiology of SARA, other toxic factors of microbial origin, including ethanol, amines, bacterial endotoxins, and possibly other unidentified toxins have been implicated to play a role (Dunlop, 1972; Owens et al., 1998). Among these toxic factors, amines, such as histamine, tyramine and tryptamine, are produced in the rumen by decarboxylation of precursor amino acids under the degradation of ruminal microbes. Among these particular 3 amines, histamine has received considerable attention because of its putative role in laminitis (Nocek, 1997). However, little is known about the origin, production and fate of other amines including tyramine, tryptamine, putrescine and methylamine in the rumen (Nagaraja and Titgemeyer, 2007).

Biogenic amines, such as histamine, are found at low levels in the rumen fluid of healthy animals (Sjaastad, 1967). In normal rumen fluid, concentrations of histamine are low, ranging from 0 to 0.3 μg/mL (Dain et al., 1955), whereas the ruminal levels of histamine can rise as high as 3.0 to 70.0 μg/mL during an acidic condition at a pH level of 4.5 (Suber et al., 1979). However, the association of amine accumulation with low pH is not very clear. Irwin et al. (1979b) showed that the ruminal tyramine and tryptamine concentrations increased with decreased ruminal pH, but histamine concentration did not change significantly in sheep dosed via a stomach tube with a mixture of 90% glucose and 10% casein. On the contrary, other researchers showed a direct relationship between ruminal pH and histamine concentration (Dain et al., 1955). The association between the biogenic amines in rumen and in blood is also questioned. Motoi et al. (1984) reported a positive relationship between rumen and plasma histamine concentrations in concentrate-fed cattle, whereas Golder et al. (2012) reported that there was no significant correlation between ruminal and plasma histamine, and suggested that ruminal histamine is not absorbed across the rumen epithelial wall. Therefore, the correlation between the decreases in ruminal pH and biogenic amine formation is still not very clear, and the question whether absorption of histamine occurs from the rumen or from the other components of the gastrointestinal tract needs to be clarified.

The bovine rumen constitutes a classical host–microbial symbiosis and disturbances in the balanced rumen ecosystem may lead to development of disease in the host. Studies monitoring changes in rumen and hindgut microbiota by quantitative culture have implicated a number of bacterial species, including *Streptococcus bovis* (Bailey et al., 2003a), various species of *Lactobacillus* (Bailey et al., 2003a), *Allisonella histaminiformis* (Garner et al., 2002) and *Mitsuokella jalaludinii* (Al Jassim et al., 2005) in biogenic amine production in cattle or horses. However, these studies were mainly based on culture methods, and culture dependent methods for enumerating bacterial numbers have a known bias, since bacteria can only be cultivated if their metabolic and physiological requirements can be reproduced in vitro. Thus, further understanding is needed regarding the microbial communities that contribute to biogenic amine formation, especially the biogenic amine producing bacteria such as *Streptococcus* and *Lactobacillus* spp., and their relationship with biogenic amine production.

The objectives of this study were to investigate biogenic amine production in the rumen fluid and plasma and the changes in the biogenic amine producing bacteria during SARA. Correlations were evaluated between ruminal pH and the concentration of amines in the rumen fluid and blood, the relationship between biogenic amines in the rumen fluid and in plasma, and their link with the biogenic amine producing bacteria in the rumen.

2. Materials and methods

All procedures in this study were approved by the China Agricultural Research Center Animal Care and Use Committee.

2.1. Experimental design

Four primiparous Holstein cows (474 ± 50 kg of body weight; 18 ± 2.3 kg/d of dry matter intake (DMI); 136 ± 7 d in lactation and 20.5 ± 3.5 kg milk/d at the beginning of the trial) fitted with 10 cm ruminal cannulas (Bar Diamond, Parma, ID) were used in this experiment. Cows were randomly assigned to experimental treatments in a 2×2 crossover design trial. Treatments were a control or low concentrate diet (COD; 40% concentrate feeds, dry matter (DM) basis) and a SARA induction or high concentrate diet (SAID; 70% concentrate feeds, DM basis) (Table 1). The diets were formulated (NRC, 2001) to meet or exceed the energy requirements (at 18 kg/d DMI) of a Holstein cow yielding 20 kg of milk/d with 3.50% milk fat and 3.10% true protein. Diets were fed ad libitum as a TMR to avoid selection of dietary components. Cows were fed at 0700 and 1800 h (one-half of the daily allowed feed at each feeding). Each experimental period consisted of 21 d. For the SAID diet, the concentrate level was gradually increased during the first 4 d (by approximately 10% units/d compared with COD). This period was followed by a 7 d adaptation period to the diet and then by 10 d of sampling. The COD diet had 11 d of adaptation and 10 d of sampling. Throughout the 21 d experimental period, the cows were housed in tie stalls and fed ad libitum to about 5% orts, with free access to fresh water during the trial.

2.2. Ruminal fluid and blood sampling

Samples of ruminal contents were collected from the cows at 0 and 4 h following the a.m. feeding on days 12, 15,