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Ammonia and amino acids modulates enzymes associated with ammonia assimilation pathway by ruminal microbiota in vitro

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ABSTRACT

The objective of this study was to evaluate the effects of ammonia and amino acids (AA) supplementation on activities of ruminal enzymes involved in ammonia assimilation. In addition, the temporal changes of ruminal bacterial populations and enzyme activities during *in vitro* incubation were investigated. Rumen fluid from four ruminally fistulated goats was used in a 3 × 3 factorial arrangement of treatments with ammonia equivalent to 1, 4, and 15 mM ammonium chloride (NH_4Cl), and with an added AA mixture (containing 992 g casein acid hydrolysate plus 1.4 g L-cysteine plus 8.68 g L-tryptophan) at 0, 1, and 15.5 g/L in the *in vitro* culture solution. Both ammonia and AA supplementation increased ($P < 0.01$) ammonia–nitrogen and volatile fatty acids concentrations. There was an interaction ($P = 0.04$) between NH_4Cl and AA concentrations on the yield of microbial crude protein. The population of total bacteria was dose-dependent with ammonia concentration ($P = 0.01$), but was increased following AA increment ($P < 0.01$). Supplement of AA activated ($P < 0.01$) enzymes of glutamine synthetase (GS), glutamate synthetase (GOGAT), and glutamate dehydrogenase (GDH), but inhibited ($P < 0.01$) enzyme of alanine dehydrogenase (ADH), all of which were important in ammonia assimilation for ruminal microbiota. And all these four enzymes were more active at the later period of process. The GDH activity was significantly associated with the population of *Prevotella ruminicola* ($r = 0.66$; $P < 0.01$), while the correlations between ADH activity and *Fibrobacter succinogenes* ($r = 0.41$, $P = 0.04$) or *Butyrivibrio fibrisolvens* ($r = 0.45$, $P = 0.02$) were not strong. Enzymes of GS, GOGAT and GDH had strong correlations ($P < 0.01$) with yield of MCP. These results suggest that AA supplementation alters fermentation pattern, and stimulates the GS–GOGAT and GDH pathways of ammonia assimilation.

1. Introduction

Ruminants have the unique ability to transform relatively low quality dietary protein into high quality meat and milk protein due to the symbiotic relationship with ruminal microbiota. However, ruminants produce meat

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and milk at the expense of a lower efficiency (10–20%) of nitrogen (N) utilization, and the majority (80–90%) of feed N consumed is excreted in manure of cattle (Koenig and Beauchemin, 2013). Thus, It is meaningful to improve the efficiency of N utilization and address the environmental issues related to N loss from ruminant production system.

Ammonia is usually produced in the course of dietary protein and non-protein nitrogen (such as urea and amino acids) degradation in the rumen, and is the major N source for ruminal microbial protein synthesis. Ruminal microbiota have the ability to synthesize amino acids (AA) *de novo* using ammonia and carbon skeleton, derived from various pathways in the rumen (Wang and Tan, 2013). The end-products of ammonia-assimilation pathways are AA, such as glutamate which is usually the most abundant AA in the ruminal fluid and the major nitrogen-donor for all other nitrogenous compounds biosynthesis in the cells (Wallace and Cotta, 1988). Ammonia concentrations in the ruminal fluid required for maximum microbial growth vary over a wide range, and ammonia is always the preferred nitrogen source for most ruminal bacteria (Schaefer et al., 1980). Thus, the improvement of ammonia–nitrogen (NH_3–N) utilization efficiency by enhancing ammonia assimilation or increasing quantities of ruminal microbiota is meaningful in practice for ruminant production.

Catabolism of AA by ruminal microbiota influences their bioavailability to ruminants. Previous results have reported the ruminal bacterial growth and microbial yield can be efficiently promoted when AA is added to either the diets of sheep or *in vitro* cultures (Chikunya et al., 1996; Guliye et al., 2005). Interestingly, parts of those affected ruminal bacteria, like *Ruminococcus flavefaciens*, instinctively own the assimilating-ammonia pathways (Duncan et al., 1992). In addition, parts of AA in the ruminal fluid are deaminized to ammonia and volatile fatty acids (VFA), instead of direct incorporation into microbial proteins (EL-Shazly, 1952), which possibly alter the fermentation pattern. Therefore, AA metabolism by rumen microboita is directly or indirectly associated with some rumen functions.

Since ammonia and AA are two important nutritional factors for the growth of ruminal microbiota, we hypothesized that both ammonia and AA would influence the activities of enzymes involved in several ammonia-assimilation pathways. Therefore, this study aimed to evaluate the effects of different amounts of ammonia and AA supplementation on enzyme activities in some important assimilation pathways using *in vitro* batch culture technology. Furthermore, the temporal changes of enzyme activities of assimilating ammonia and populations of total bacteria, *Ruminococcus albus*, *R. flavefaciens*, *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvebs*, and *Prevotella ruminicola* were investigated during *in vitro* batch culture processes.

2. Materials and methods

2.1. Animals and diets

All procedures involving the care and use of the animals were approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

Four ruminally fistulated mature Liuyang black wether goats (a local breed in southern China) with initial body weight of 24.3 ± 1.1 kg (mean BW $\pm$ SE) were used as ruminal fluid donors. Animals were housed individually in stalls with feed-boxes and free access to drinking water. The goats received a total mixed ration supplied as two equal portions at 0800 and 1800 h daily, which could provide 1.4 times maintenance requirement of metabolic energy according to Lu et al. (1996). The ingredients of total mixed ration were (per kg): 500.0 g maize stover, 226.0 g corn meal, 60.0 g soybean meal, 1.5 g rapeseed meal, 180.0 g wheat bran, 8.0 g urea, 6.0 g NaCl, and 20 g premix (contained per kg: 169.93 g $MgSO_4 \cdot H_2O$, 2.54 g $FeSO_4 \cdot 7H_2O$, 0.81 g $CuSO_4 \cdot 5H_2O$, 3.03 g $MnSO_4 \cdot H_2O$, 5.03 g $ZnSO_4 \cdot H_2O$, 0.01 g $NaSeO_3$, 0.04 g KI, 0.03 g $CoCl_2 \cdot 6H_2O$, 3.72 g multi-vitamins, and 814.86 g sepiolite), with the following chemical composition (per kg dry matter): 137.8 g CP, 54.2 g undegradable protein, 83.7 g ruminal degradable protein, 259.8 g ADF, 378.7 g NDF, 358.8 g structural carbohydrate, 304.7 g non-structural carbohydrate, and 9.42 MJ metabolic energy.

2.2. Experimental design

The experiment was designed according to a 3×3 factorial arrangement with 3 levels each of ammonia supplied as ammonium chloride (NH_4Cl), and AA supplied as a mixture (992 g casein acid hydrolysate, 1.4 g L-cysteine and 8.68 g L-tryptophan) which was based on the composition of casein. Extra cysteine and tryptophan were added to compensate the loss during acid hydrolysis. Thus, there were 9 treatments in total. The concentrations of ammonia were 1, 4, and 15 mM while AA mixture was added at 0, 1, and 15.5 g/L of culture media. Because 50 mg/L (approximately 3.6 mM) NH_3–N concentration has been reported as the concentration beyond which ammonia concentration had no effect on microbial protein production (Satter and Slyter, 1974), in this experiment 1 mM NH_4Cl concentration was used to create an ammonia-limited environment, 4 mM NH_4Cl concentration was moderate for ruminal microbial growth, and 15 mM NH_4Cl provided enriched NH_3–N environment. The principle of AA supplementation was as follows: 1 g/L addition of total AA mixture was similar to total AA concentrations determined in ruminal fluid, while 15.5 g/L addition of total AA mixture was equivalent to the 10 g peptides (in the form of pancreatic casein hydrolysate) per litre employed routinely in many growth medias *in vitro* (Atasoglu et al., 1998). To name the treatments, we used N and A instead of ammonia and amino acid, respectively. So, the 9 treatments were named as N1A0, N1A1, N1A15.5, N4A0, N4A1, N4A15.5, N15A0, N15A1, and N15A15.5.

2.3. In vitro batch culture trial

On each experimental day 500 mL of rumen fluid was withdrawn from each goat at 0700 h prior to the morning feeding via the rumen fistula. The rumen fluids were