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Stimulatory and Inhibitory Effects of Protein Amino Acids on Growth Rate and Efficiency of Mixed Ruminal Bacteria

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ABSTRACT

Mixed ruminal bacteria were incubated in vitro with glucose, xylose, cellobiose, and various protein amino acids replaced isonitrogenously with 25% (i.e., 25 mg of N/L) of ammonia-N, to determine the growth rate and the amount of sugar consumed in the exponential growth phase. The growth rate and efficiency (grams of bacteria per gram of sugars) increased by 46 and 15%, respectively, when a mixture of 20 amino acids was added. On the other hand, neither growth rate nor efficiency increased when any one of these amino acids was added singly, except for Glu and Gln, each of which produced significant but small improvements. The stimulatory effect of the combined amino acids on bacterial growth declined when each of Leu, Trp, Tyr, Glu, Met, Phe, and Val was removed from the original group of 20. When a mixture of only these seven amino acids was used as a supplement, their stimulatory effects on growth rate and efficiency were only 21 and 25%, respectively, of the effects that the mixture of 20 amino acids showed. The effects increased to 76 and 72% on growth rate and efficiency, respectively, when Gly, Cys, and His were supplied in addition to the seven amino acids. The growth rate and efficiency of the ruminal bacteria were inhibited by an addition of each of Ile, Thr, Cys, Phe, Leu, Lys, or Val to ammonia-N, and the effects of the first five of these amino acids were highly significant. Isoleucine, threonine, and phenylalanine were each inhibitory even at a low concentration (1 mg of N/L), while cysteine and leucine showed inhibitory effects at higher concentrations (more than 10 mg of N/L). A higher growth rate of the ruminal bacteria when supplemented with amino acid mixtures was accompanied with a higher growth efficiency, which was attributable to a relatively smaller proportion of energy expended on maintenance according to the Pirt derivation.

(**Key words:** rumen bacteria, amino acid, inhibition, stimulation)

Abbreviation key: SAA = seven amino acids.

INTRODUCTION

Estimation of microbial synthesis in the rumen is one of the main components in the metabolizable protein system (Fox et al., 1990; AFRC, 1993; National Research Council, 1996). Since the amount of dietary energy available to the microorganisms often restricts the amount of microbial protein synthesized in the rumen, improving the efficiency of microbial growth (i.e., the amount of microbes synthesized per dietary energy consumed) would increase postruminal protein supply. Most ruminal bacteria can grow with nonprotein N, such as ammonia, as their sole N source (Bryant and Robinson, 1962), but supplementation with amino N can improve the growth yield (Maeng et al., 1976; Argyle and Baldwin, 1989), rate (Van Kessel and Russell, 1996), and efficiency (Cotta and Russell, 1982) of ruminal microbes. Several studies showed that certain amino acids or amino acid subgroups stimulated in vitro growth yields of mixed ruminal bacteria, although to a lesser degree than the stimulation provided by whole amino acid mixtures (Maeng et al., 1976; Argyle and Baldwin, 1989). But those reports did not identify the amino acids that were truly essential for improving microbial growth. Several amino acids, on the other hand, are known to inhibit microbial growth (De Felice et al., 1979), and it follows that some mixtures of amino acids might include inhibitory members that diminish the benefits provided by the other members.

The present study was designed to determine 1) which ones among the 20 α-amino acids that are commonly found in proteins would stimulate, and which would inhibit, microbial growth; 2) which amino acid would be indispensable to the improvement of microbial synthesis that amino acid mixtures are expected to show; and 3) which combination of amino acids would be most efficient for microbial growth in the rumen. These determinations were made by analyzing the growth rate and efficiency of mixed ruminal bacteria

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during the exponential growth phase in the presence or absence of single or combinations of specific amino acids.

MATERIALS AND METHODS

Animals and Diet

Two ruminally fistulated nonlactating Holstein cows (580 kg of average BW) were housed in individual stalls, and were each fed a diet (3.3 kg average DM per meal) consisting of timothy hay (67% on DM), steam-flaked corn grain (21%), and soybean meal (12%) twice a day, at 0900 and 1700 h. This diet satisfied the cows' energy requirement for maintenance and balanced N degradation and consumption in the rumen based on the level 2 model of the National Research Council (1996). Water was freely given.

Preparation of Mixed Ruminal Bacteria

Liquid and solid portions of the ruminal content, taken by a suction pump and by hand grasp, respectively, were provided through a fistula just before morning feeding. Equal amounts (wt/wt) of these portions were mixed, ground with a homogenizer (model MN-2, Nihon Seiki Co., Tokyo, Japan) at 250 W for 1 min, and squeezed through four-layered gauze. The squeezed fluid was left undisturbed for 30 min at 39°C to separate the feed particles. The fluid obtained from a middle section of the bottle of the undisturbed sample was slowly centrifuged (at 750 × *g* for 10 min at 10°C) to remove protozoa and then centrifuged again (at 10,000 × *g* for 15 min at 10°C) to harvest the mixed ruminal bacteria, in which no protozoa was microscopically detected. The mixed ruminal bacteria were washed twice with a buffer (pH 6.8) containing 50 m*M* Na_2HPO_4, 10 m*M* KH_2PO_4, 4.2 m*M* $Na_2S{\cdot}9H_2O$, and 4 μM sodium resazurin, and resuspended in the same buffer in a volume equivalent to the original. Anaerobic conditions were maintained throughout the procedure by using an N_2 gas stream.

Incubation of Mixed Ruminal Bacteria

The mixed ruminal bacteria were diluted 20 times, thereby attaining an optical density (at 600 nm) of 0.15, into a medium (pH 6.5) containing 2.4 m*M* K_2HPO_4, 1.8 m*M* KH_2PO_4, 2.1 m*M* NaCl, 0.1 m*M* $MgSO_4{\cdot}7H_2O$, 0.01 m*M* $CaCl_2{\cdot}2H_2O$, 4.2 m*M* $Na_2S{\cdot}9H2O$, 4 μM sodium resazurin, 38 m*M* Na_2CO_3, and the same amounts of vitamins, VFA, and trace minerals as described by Cotta and Russell (1982). The mixed bacteria were anaerobically incubated at 39°C with 4 m*M* glucose, 4 m*M* xylose, and 2 m*M* cellobiose. For the NH_3 only treatment, 3.57 m*M* (i.e., 100 mg of N/L) of ammonium sulfate was added as a sole N source. In experiments 1 and 3, 25% of ammonia in the NH_3 only treatment was isonitrogenously replaced by a single amino acid or by an amino acid mixture in which the same amounts of amino acids were included on isonitrogenous basis. In experiment 2, various amounts of ammonia were isonitrogenously substituted by individual amino acids, each of which had been shown in experiment 1 to inhibit growth. Growth of bacteria was terminated when the optical density (at 600 nm) reached 0.7, which was the middle of the exponential growth phase, by adding formalin (1% in final concentration). Cells were separated by centrifugation (at 15,000 × *g* for 15 min at 4°C), and the supernatant was stored at –20°C until sugar analysis.

Analysis

Animal feeds were analyzed by the methods of AOAC (Cunniff, 1996) and Van Soest and Wine (1967). The chemical composition of the diet was as follows (% on DM basis): OM, 94.3; CP, 13.1; ether extract, 2.0; NDF 49.5. Sugars in the medium were analyzed by capillary electrophoresis (^{3D}CE, Hewlett Packard, Waldbronn, Germany) with a capillary column (50 μm i.d., 80.5 cm length) using 20 m*M* 2,6-pyridinedicarboxylic acid and 0.5 m*M* cetyltrimethylammonium bromide (pH 12.1) as an electrolyte after the sample was filtered through an ultrafiltration unit (Ultrafree-MC, 30,000 NMWL, Millipore Corp., Bedford, MA; Soga and Heiger, 1998). The DM of the cells was identified after drying at 105°C for 16 h. The protein content of the cells was measured by the method of Lowry et al. (1951) after hydrolyzation with 0.2 *M* NaOH at 100°C for 15 min; protein content was 165 μg/ml at an optical density (at 600 nm) of 1.0 and 44% of cell DM, which was kept constant during the exponential growth phase.

Statistics

Incubations with each N source were performed in triplicate for each cow. Data are described as both the actual values and percentages of the NH_3 only treatment for experiments 1 and 2 and percentages of the effect of the 20 amino acids for experiment 3. The statistical model is described below:

$$Y_{ij} = \mu + N_i + C_j + e_{ij},$$

where

Y_{ij} = observation,
μ = overall mean,