

# Ruminal Ammonia Production



## ***Inhibition of Hyperactive Amino Acid-Degrading Ruminal Bacteria and the Potential for Decreasing Wasteful Ammonia Production***

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### Introduction

In ruminant animals, feeds are fermented in the rumen prior to gastric and intestinal digestion. Ruminant animals have the benefit of fiber utilization, but this method of feed digestion produces heat, methane and ammonia. Because these end-products decrease the efficiency of animal production and create environmental pollution, rumen microbiologists and ruminant nutritionists have sought methods of reducing ruminal fermentation losses. Most ruminal bacteria can utilize ammonia as a nitrogen source for growth, but ruminal microorganisms often produce an excess of ruminal ammonia. Excess ruminal ammonia is converted to urinary urea and excreted.

In the early 1960's, Bladen et al. (1961) examined the capacity of various ruminal bacteria to deaminate protein hydrolysate, but most strains produced little ammonia. Based on the activities and numbers in the rumen, they concluded that *Prevotella (Bacteroides) ruminicola* was probably the most important ammonia-producing bacterium. Later work, however, indicated that this species could not account for all the ammonia accumulation in vivo. Hungate (1966) noted that he had "encountered" rumen bacteria "able to digest casein and requiring no carbohydrate," but he never isolated these bacteria.

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**“The obligate amino acid-fermenting bacterium differ greatly in their pH sensitivities.”**

In the late 1980's, enrichments on a high concentration of protein hydrolysate yielded three ruminal bacteria with very high specific activities of ammonia production, and these bacteria could not utilize carbohydrates as an energy source (Russell et al. 1988, Chen and Russell 1989a). These bacteria only account for a small proportion of the ruminal bacteria, but they have very high activities of ammonia production. Because they can be inhibited by the feed additive, monensin, ruminant nutritionists have another avenue of decreasing wasteful ammonia production.

## Taxonomy

One of the obligate amino acid-fermenting bacteria was a peptostreptococcus (*Peptostreptococcus anaerobius*), but the other two could not be readily classified by traditional methods. Ribosomal rRNA sequencing has provided an alternative and more biochemically valid method of grouping and classifying bacteria. 16S rRNA sequence analyses indicated that one of the obligate amino acid-fermenting bacteria was *Clostridium sticklandii*, and the other one was a new species, *Clostridium aminophilum* (Paster et al. 1993). All three of the obligate amino acid-fermenting bacteria were found in the same 16S rRNA cluster, and clostridia were their closest relatives. Clostridia are usually described as “spore-forming, anaerobic rods,” but none of the isolates readily formed spores. *P. anaerobius* is a large coccus, and this contrast illustrates the potential limitations of morphological traits as criteria for taxonomic classification.

## Physiology

Amino acid fermentation is an energetically difficult process that yields little ATP, and the obligate amino acid-fermenting bacteria must ferment more than 20 amino acids in order to get enough ATP to polymerize a single amino acid into protein. They have circumvented this constraint by very rapid rates of metabolism. The carbohydrate fermenting ruminal bacteria have amino acid degradation rates less than 20 nmol/mg

protein/min, but the obligate amino acid fermenting-bacteria have rates that are at least 15-fold faster.

The rumen is a sodium-rich environment that has been compared to an “inland sea,” and all of the obligate amino acid-fermenting ruminal bacteria have extremely fast, sodium-dependent transport systems for amino acids (Chen and Russell 1989b, Chen and Russell 1990, Van Kessel and Russell 1992). *Clostridium aminophilum* has a membrane bound, botin-dependent glutacoyl CoA decarboxylase reaction that pumps sodium out of the cell (Chen and Russell 1990) and *Clostridium sticklandii* uses an ornithine efflux mechanism to expel sodium (Van Kessel and Russell 1992). *P. anaerobius* has a glutamine electrogenic uptake mechanism that generates a membrane potential from the chemical gradient of glutamine across the cell membrane (Beck and Russell 1994).

The rumen is well buffered by the bicarbonate of salivary secretions, but ruminal pH can decrease when the rate of starch fermentation is rapid. The effect of pH on ruminal deamination has not been extensively examined, but Erfle et al. (1982) noted a dramatic (10-fold) decrease in deaminase activity when they decreased the pH of their mixed culture chemostats from 6.7 to 5.5. The obligate amino acid-fermenting bacteria differ greatly in their pH sensitivities. *C. aminophilum* grew in continuous culture at pH 5.2, and is one of the most acid resistant bacteria in the rumen (Chen and Russell 1989b). Its pH resistance is mediated by its ability to decrease its intracellular pH, maintain a small pH gradient across the cell membrane and prevent a toxic accumulation of volatile fatty acid anions (Russell 1991). *P. anaerobius* tolerates pH values as low as 5.6 (Chen and Russell 1988b), but *C. sticklandii* was unable to grow at pH values less than 6.0 (Chen and Russell 1989).

## Ecology

The obligate amino acid-fermenting bacteria have little proteolytic capacity, but the rumen has a variety of proteolytic bacteria that can degrade pro-

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tein to peptides and amino acids. The obligate amino acid-fermenting bacteria have different preferences for individual amino acids, and this amino acid specificity decreases competition (Table 1). *P. anaerobius* converts branched-chain amino acids to branched-chain volatile fatty acids, and these acids are required by the predominant cellulose digesting ruminal bacteria.

The obligate amino acid-fermenting bacteria must compete with carbohydrate-fermenting bacteria for amino acids. Carbohydrate-fermenting bacteria like *P. ruminicola* only deaminate amino acids when the rate of carbohydrate fermentation is slow (Russell 1983), and <sup>15</sup>N studies indicated that approximately two-thirds of the ruminal deamination was carbohydrate-sensitive (Russell et al. 1983). The remainder (one-third), was carbohydrate-insensitive. Because the obligate amino acid-fermenting bacteria cannot utilize carbohydrates as energy sources for growth (Table 1), it appeared that they might be producing as much as one-third of the ruminal ammonia.

## Enumeration

Because most carbohydrate-fermenting ruminal bacteria cannot utilize amino acids as an energy source for growth (Bladen et al. 1961), it was possible to enrich the obligate amino acid-fermenting with amino acid sources. Most probable number estimates indicated that the obligate amino acid-fermenting bacteria were less than 10% of the total ruminal count (Yang and Russell 1993), but other work indicated that not all strains grew readily in the laboratory (Paster et al. 1993). Because the obligate amino acid-fermenting bacteria had unique 16S rRNA sequences, it was possible to design nucleic acid probes that could be used for in vivo enumeration. rRNA hybridization indicated that the obligate amino acid-fermenting bacteria made up approximately 3.5% of the ruminal population (Krause and Russell 1996). *C. aminophilum* was present at greater numbers than *P. anaerobius* and *C. sticklandii*, but these differences were not statistically significant.

## Importance

Given the observation that mixed ruminal bacteria have specific activities of approximately 30 nmol/mg protein/min (Yang and Russell 1993) and the obligate amino acid-fermenting bacteria have specific activities of approximately 300 nmol/mg protein/min (Russell et al. 1988, Chen and Russell 1989a), it was possible to estimate the roles of the obligate amino acid-fermenting and carbohydrate-fermenting ruminal bacteria in ruminal ammonia production. If the obligate amino acid-fermenting bacteria account for 3.5% of the population, the carbohydrate-fermenting bacteria must have a specific activity of 20 nmol/mg protein/min:

$$0.035 (300 \text{ nmol/mg protein/min}) \times 0.965 (20 \text{ nmol/mg/min}) = 1.00 (30 \text{ nmol/mg/min})$$

and the obligate amino acid-fermenting must produce approximately 35% of the ruminal ammonia:

Table 1.  
Some general characteristics of the obligate amino acid-fermenting ruminal bacteria.

	<i>Clostridium anaerobius</i>	<i>Peptostreptococcus aminophilum</i>	<i>Clostridium sticklandii</i>
Gram stain	+	+	+
Growth in presence of O <sub>2</sub>	no	no	no
Ferment carbohydrates	no	poorly	poorly
Proteolytic	no	no	no
Fermentation Products			
Acetate	+	+++	+++
Propionate	-	-	+
Butyrate	-	++	+
Isobutyrate	+	-	+
Isovalerate	+	-	+
Valerate	+	-	-
Isocaproate	++	-	-
Hydrogen	+	-	-
Amino acids most rapidly fermented	leu ser thr gln phe met	glu gln his ser thr	arg ser lys gln

**“... work indicated that monensin might be able to decrease ruminal ammonia production ...”**

350 mg monensin per day, the specific activity of ammonia production decreased 28 to 38%, the steady state concentration of ruminal ammonia decreased 30 to 54%, and the numbers of obligate amino acid-fermenting bacteria decreased nearly 10-fold (Yang and Russell 1993).

Studies using 16S rRNA probes indicated that monensin could completely eliminate *C. sticklandii* and *P. anaerobius* from the rumen, but monensin had little effect on *C. aminophilum* (Krause and Russell 1996). The ability of *C. aminophilum* to persist in the rumen appears to be a slow growth rate-dependent property that is not observed with rapidly growing cultures, and this effect compromises the benefit of monensin in amino acid sparing. Based on a population of 1.4%, a total bacterial protein concentration of 1.1 mg/ml, a yield of 3.3 mg protein/mmol amino acid fermented, a molecular weight of 100 (average amino acid), a fluid dilution rate of 0.07 h<sup>-1</sup>, and a ruminal volume of 70,000 ml, the additional loss of amino acids due to *C. aminophilum* would be:

$$0.014 \times 1.1 \text{ mg bacterial protein/ml} \div 3.3 \text{ mg protein/mmol amino acid} \times 100 \text{ mg/mmol amino acid} \times 70,000 \text{ ml} \times 0.07 \text{ h}^{-1} \times 24 \text{ h} = 54,879 \text{ mg or } 55 \text{ g of amino acids/day}$$

Since the protein intakes of the cows were 630 g/day, it appeared that *C. aminophilum* might be wasting approximately 9% of the feed protein.

$$[0.035 \text{ (300 nmol/mg protein/min)}] \div [1.00 \text{ (30 nmol/mg protein/min)}] = 35\%$$

## Inhibition

The ionophore, monensin, has been used as a feed additive in beef cattle rations since 1976, and it was originally marketed as a methane inhibitor (Russell and Strobel 1989). Early work indicated that monensin might be able to decrease ruminal ammonia production (Dinius et al. 1976), and this action is consistent with the ability of monensin to inhibit the obligate amino acid-fermenting bacteria (Russell et al. 1988, Chen and Russell 1989a). When cattle were fed

## Conclusions

The rumen has a previously unidentified group of obligate amino acid-fermenting bacteria that can be inhibited by the feed additive monensin. This inhibition decreases deamination, spares amino acids and decreases urea nitrogen excretion.

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