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Development of an in vitro procedure to determine ruminal availability of protein (1998)

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DEVELOPMENT OF AN *IN VITRO* PROCEDURE TO DETERMINE RUMINAL AVAILABILITY OF PROTEIN

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Summary

A series of *in vitro* experiments was conducted to determine the ruminal availability of protein from grains. Procedures were based on assumptions that 1) ruminal availability of protein is first-limiting to microbial growth, 2) accumulation of microbial cells accurately predicts ruminal protein availability, 3) cytosine can be used to accurately estimate microbial cell mass, and 4) cytosine is present in microorganisms but not in feeds. Cytosine content of *in vitro* cultures was measured by high performance liquid chromatography. Early experiments determined that adding 0.75 g soluble starch provided enough energy that culture growth depended on available protein. In the final experiment, microbial cytosine was measured for several processed grains and for graded levels of sodium caseinate (as a standard for comparison). Cytosine increased as sodium caseinate levels increased. Heat-processed grains yielded less cytosine than grains processed without heat. Cytosine accumulation during *in vitro* fermentation provides a useful measure of ruminal protein availability.

(Key Words: Cytosine, Protein Degradability, Microbial Growth.)

Introduction

The proportion of dietary protein that is ruminally degradable vs. undegradable is a component of modern ration formulation systems. Protein degradability can be measured in numerous ways; the most common is the *in situ* procedure, in which feeds are placed into nylon bags and suspended in the rumen for digestion. Although somewhat expensive and labor-intensive, it assesses the protein availability for a variety of feedstuffs. However, questions have been raised about the *in situ* procedure for feedstuffs that are low in protein. Our objective was to develop a method for measuring ruminally degradable protein that would be appropriate for all feedstuffs, regardless of protein level.

Experimental Procedures

Samples of various feedstuffs (0.5 g) were measured into 50-ml centrifuge tubes. A 30-ml aliquot of a mixture of rumen fluid/McDougall's buffer was added to each tube. The tubes were flushed with carbon dioxide, sealed with one-way valve rubber stoppers, and allowed to incubate for 12 hours at 39°C. At the end of the incubation period, the microbial fermentation was stopped by adding 0.1 ml formalin and refrigerating the tubes. Solids were harvested by centrifuging for 15 minutes at 30,000 x *g*. The resulting pellet was dried in a 55°C forced-air oven for 48 hours. The dry residue was hydrolyzed in perchloric acid, and high performance liquid chromatography was used to measure cytosine. All feed samples were run in quadruplicate.

Experiment 1. Dry-rolled wheat, grain sorghum, and corn were prepared by cracking whole kernels and then grinding them through a 1-mm mesh screen. Steam-flaked

corn was simulated by autoclaving corn for 45 minutes under dry steam, then grinding through a 1-mm mesh screen. High-moisture corn was produced by reconstituting cracked corn, allowing 4 weeks for fermentation, and then grinding through a 1-mm mesh screen using dry ice. Dry samples were kept in sealed bags at room temperature. Wet samples were kept frozen in sealed bags. Pure soluble starch was used as a control (an energy source without protein). Incubations of 3, 6, 9, 12, and 24 hours were tested to determine appropriate fermentation times for microbial growth.

Experiment 2. Previously ground wheat and grain sorghum samples were combined with 0.25 or 0.5 g soluble starch to determine if the fermentation system was energy deficient. Grain samples also were supplemented with sodium caseinate (0.05 g) to determine the effect of degradable protein on microbial growth. Samples without additives served as controls.

Experiment 3. The ground wheat and grain sorghum used in experiments 1 and 2 were supplemented with identical caseinate levels. In this experiment, soluble starch was added at 0.5, 0.75, or 1.0 g to evaluate effects of energy addition on microbial growth.

Experiment 4. Samples of wheat and corn were autoclaved to simulate flaking. Samples of autoclaved wheat and corn were compared to the same grains dry rolled without heat. Samples of soybean meal and nonenzymatically browned soybean meal (a high escape protein source) also were tested. Sodium caseinate, a protein source that is completely rumen-degradable, was used to develop a standard curve. Starch was added to all incubations at 0.75 g per tube to ensure that energy was not limiting.

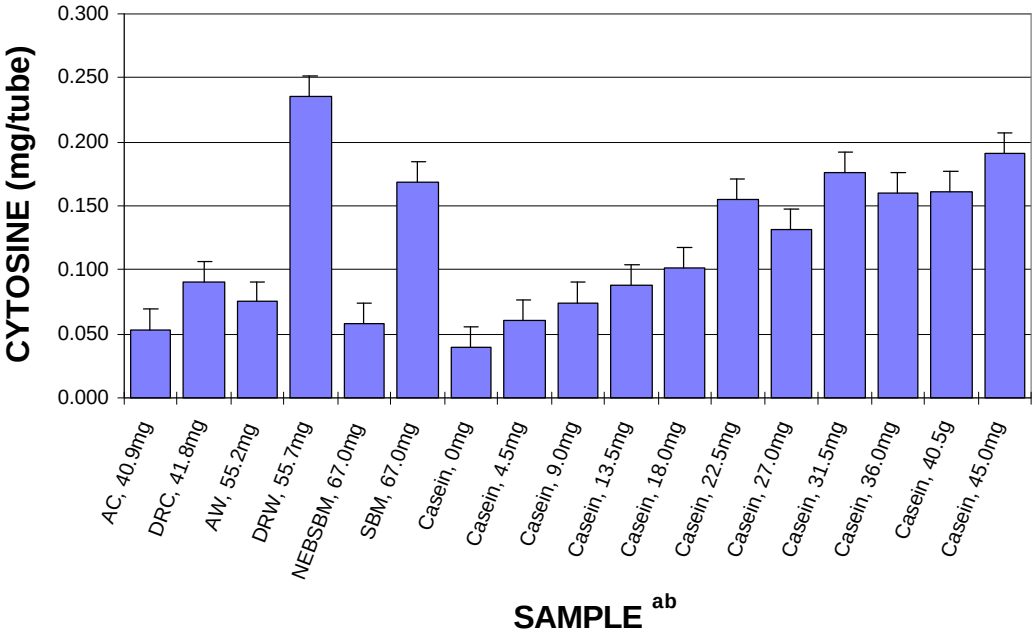
Results and Discussion

Experiment 1. Microbial growth was higher ($P<.05$) for samples containing soluble starch, indicating that energy, not protein, was first-limiting. Microbial growth reached a maximum at 12 hours of fermentation, so this incubation period was used.

Experiment 2. As the level of starch supplementation increased, cytosine content increased in a linear manner ($P<.01$), indicating that microbial growth was stimulated. Cytosine amounts also increased ($P<.01$) when sodium caseinate was added to fermentation samples. No interactive effects occurred when caseinate and starch were added in combination.

Experiment 3. Adding soluble starch at levels above 0.5 grams had no effect on microbial growth ($P>.7$), so energy was no longer limiting above the level. Cytosine increased ($P<.01$) when sodium caseinate was added to grain samples, indicating that microbial growth was responding to added degradable protein.

Experiment 4. Microbial growth increased ($P<.05$) with additions of sodium caseinate up to levels of about 0.03 g of protein (Fig. 1). Thus, the availability of protein appeared to be limiting microbial growth. Cytosine concentrations resulting from fermenting dry-rolled wheat and soybean meal were significantly higher ($P<.01$) than those from autoclaved wheat or the nonenzymatically browned soybean meal, indicating that heat processing in those feeds decreased the availability of protein to rumen microbes. Cytosine contents were numerically, but not significantly, higher for dry-rolled than autoclaved corn. This *in vitro* procedure currently is being applied to a wider range of feedstuffs for further validation.



[a]AC=autoclaved corn; DRC=dry-rolled corn; AW=autoclaved wheat; DRW=dry-rolled wheat; NEBSBM=nonenzymatically browned (high escape) soybean meal; SBM=soybean meal; casein=sodium caseinate.
[b]Numbers following abbreviations display mg of crude protein provided by samples.

Figure 1. Average Cytosine Contents after Fermentation of Processed Feeds and Additions of Graded Levels of Sodium Caseinate.