Wheat gluten films prepared at high temperature and low pH decrease degradation by rumen microorganisms

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Introduction
Encapsulated amino acids, vitamins, and other nutrients are gaining popularity in the ruminant feed industry. The purpose of encapsulation is to provide protection from premature digestion in the rumen, making it possible to increase bioavailability of the core ingredient in the small intestine. Encapsulated products are more effective at delivering a targeted amount of a limiting nutrient than the traditional methods of heat or chemically treating protein, which result in an excess supply of other nutrients. The main limitation of feeding encapsulated products is cost. These products are expensive because of the cost of the film forming/encapsulating materials used. Wheat gluten is an inexpensive alternative and has natural film-forming capabilities.

Processing factors that influence the extent of degradation in the rumen and subsequent uptake in the post-ruminal digestive tract have not been fully elucidated. The objective of our research was to identify the initial processing conditions under which wheat gluten will provide sufficient protection from microbial degradation in the rumen. Temperature and pH, in particular, have a large effect on the final properties of the film because of their ability to alter the protein structure of the wheat gluten.

Experimental Procedures
We conducted an in vitro study to investigate effects of three pH levels (3.0, 5.0, and 7.5) and three temperature levels (104°F, 131°F, and 167°F) of the film-forming solution on final film stability in the rumen. An in vitro protein degradation assay was used to determine susceptibility of the protein-based film to degradation by ruminal microorganisms. Degradation of the films was measured after 0, 2, 4, 6, and 8 hours of fermentation.

Films were prepared by mixing wheat gluten (18% of the solution) into 100 mL of 95% ethanol and then slowly adding 50 mL of water. Glycerol was added at 1% as a plasticizing agent. The pH of film-forming solutions was appropriately adjusted with glacial acetic acid or 6 M ammonium hydroxide. The solutions were sheared for 5 minutes and then stirred and heated to the appropriate temperature under continuous reflux. Heated solutions were held at the appropriate temperature for 10 minutes and then centrifuged at 959 × g for 5 minutes at room temperature (68°F) to remove any remaining insoluble gluten. The supernatant was poured onto a Teflon-coated tray and allowed to dry at ambient air temperature. When dry, the films were subjected to a protein degradation assay to determine degradability under rumen conditions.

Results and Discussion
There was an interaction between pH and temperature (P<0.01); low pH (pH 3) and high temperature (167°F) films were most resistant to microbial degradation (Figure 1).
There was no interaction between temperature and time (P>0.05), but there was an interaction between pH and time (P<0.01) as well as a quadratic effect (P<0.01) of pH on degradability. Degradability values were smallest at pH 3 and largest at pH 5. The film prepared at pH 5 may be partially soluble in the rumen because the rumen pH is close to the pH used to prepare the film. Film formation at pH 7.5 was hindered by poor protein dispersion because the isoelectric region of wheat gluten is near pH 7.5. Poor film formation at the isoelectric region will compromise integrity of the film, making it more easily degraded by ruminal bacteria.

Film degradability decreased with increasing temperature (P<0.01); films manufactured at 167°F had the lowest degradability. The linear decrease of degradability with increased temperature of the film-forming solution may indicate increased cross-linking through covalent S-S bonds. The mechanism behind this may be that heated film-forming solutions denature wheat gluten proteins, thereby reducing existing S-S bonds and revealing previously unexposed SH groups. Upon drying, covalent S-S bonds formed by air oxidation cross-link protein molecules. This would contribute to the films’ strength and resilience to microbial degradation.

Implications
Low pH and high processing temperatures yield encapsulating films that are substantially resistant to degradation by ruminal microorganisms. These films are inexpensive to produce and may be useful for encapsulating amino acids and vitamins to improve rumen bypass of these nutrients.

**Figure 1.** Protein degradation of films after 8 hours of ruminal fermentation.