KINETICS OF AMMONIA PRODUCTION IN VITRO FROM WHEAT FORAGE[1]

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Story in Brief

Two experimental compounds, monensin and processed corn were evaluated in batch cultures of mixed rumen bacteria for effects on rate of ammonia production and disappearance rate constant of soluble carbohydrates. Final concentrations of experimental compounds 1 and 2 and monensin in the cultures were, respectively, 20, 27.5 and 6.25 mg/liter. Compound 1 decreased rate of ammonia production more than compound 2 or monensin, and may have potential for decreasing the wasteful degradation of dietary protein in cattle grazing wheat pasture. Addition of readily fermentable starch as ground steam-flaked corn to the cultures, markedly decreased rate of ammonia production. This is consistent with the concept that grain supplementation of cattle grazing wheat pasture increases performance by increased "capture" of rumen ammonia as microbial protein.

(Key Words: Ammonia Production, Wheat Forage, Rumen Fermentation.)

Introduction

Wheat forage is a high quality forage and commonly contains 25 to 30% crude protein. Rate of ruminal degradation of the nitrogenous components of forages is important with regard to efficiency of microbial protein synthesis and post-ruminal protein supply for grazing ruminants. Studies by Zorrilla-Rios et al. (1985) and Vogel et al. (1987) have shown that the crude protein of wheat forage of two stages of maturity exist kinetically as two distinct pools with different rates of in situ ruminal disappearance. About 50 to 75% of total forage nitrogen disappeared from a "very rapid disappearance" pool at rates of 13 to 28% per hour. Compounds that would slow the rate of ammonia production from wheat forage may improve performance of cattle grazing wheat pasture by decreasing the wasteful degradation of dietary protein in the rumen. The objective of this study was to evaluate two experimental compounds in batch cultures of mixed rumen bacteria for effects on rate of ammonia production and disappearance rate constant of soluble carbohydrates. Effects of addition of readily fermentable starch as ground, unheated or steam-flaked corn were also evaluated.

Materials and Methods

Rumen contents were obtained from a mature, non-lactating Holstein cow 1.5 hours after the morning feeding, and were strained through 4

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layers of cheesecloth. The cow was fed twice daily a diet of 50% timothy hay and 50% pelleted commercial dairy concentrate. The ruminal fluid was allowed to stand in an erlenmeyer flask at room temperature in the lab until layers separated. Liquid from the bottom of the flask was anaerobically pipetted into defined anaerobic medium which served as the inoculum (rumen bacterial suspension) for the batch cultures. The inoculum contained 20% (v/v) rumen fluid, and composition of the defined medium is shown in Table 1.

Forty ml of the rumen bacterial suspension were dispensed anaerobically into 125-ml serum bottles containing substrates and experimental compounds as described below for the individual experiments. The serum bottles were capped with butyl rubber stoppers, gently mixed and placed in a 39 C water bath.

Experiment I

Treatments consisted of a "control" (i.e., no added compound) and experimental compounds 1 and 2[5], and wheat forage samples (400 mg/culture bottle) of 5 harvest dates. Experimental compounds 1 and 2 were dissolved in acetone and ethanol, respectively, and .2 ml of each was added to the batch cultures at time zero using 21 gauge disposable needles and syringes. Final concentrations of compounds 1 and 2 in the cultures were 20 and 27.5 mg/liter, respectively. Dates of harvest of the wheat forage samples, and total N content and in vitro dry matter digestibility are shown in Table 2. The forage samples were hand clipped, immediately frozen in liquid N and remained frozen until lyophilization.

Samples were withdrawn from the cultures, using a 21 gauge, 1 1/2 inch disposable needle and syringe, at 0, 1, 2, 3, 4, 5 and 6 hours after addition of inoculum for ammonia and soluble carbohydrate analyses. Ammonia was determined by the colorimetric method of Chaney

Table 1. Composition of defined medium[a].

Component	mg/Liter water
$K_2HPO_4 * 3H_2O$	292
$K\ H_2PO_4$	240
Na_2SO_4	480
NaCl	480
$MgSO_4 * 7H_2O$	100
$CaCl_2 * 2H_2O$	64
Na_2CO_3	4000
Cysteine	600

[a]Adjusted to pH 6.7 with 10% NaOH and bubbled with oxygen-free CO_2.

[5]Courtesy of Lilly Research Laboratories, Greenfield, IN.

Table 2. Dates of harvest and composition of wheat forage samples.

Harvest #	Date	Total N	Crude Protein	IVDMD, %
		% of DM		
I	12-17-85	2.65	16.6	58.6
II	1-8-86	3.08	19.2	72.2
III	2-21-86	3.87	24.2	75.3
IV	3-12-86	4.35	27.2	76.4
V	4-29-86	1.82	11.4	64.4

and Marbach (1962). Concentrations of soluble carbohydrates were determined by the phenol-sulfuric acid procedure with glucose as the standard.

Rate of ammonia production was assumed constant after an appropriate lag time, and a linear equation $C(t) = a + rt$ where $C(t)$ is ammonia concentration at time t, a is the intercept and r is the rate of ammonia production. The parameters a and r were fit using simple linear regression. Disappearance of soluble carbohydrates was assumed to follow first order kinetics, $C(t) = C_o e^{-kt}$ where $C(t)$ is soluble carbohydrate concentration at time t, C_o is initial concentration and k is the disappearance rate constant. C_o and k were determined using a nonlinear least square minimization procedure.

Experiment II

Effects of addition of fermentable carbohydrate, as finely ground unheated or steam-flaked corn, to cultures were evaluated in experiment II. In addition, the two experimental compounds were compared to monensin. Wheat forage of harvest date III was used as the forage substrate (400 mg) in all treatments, and 0 or 100 mg of the two types of corn were added for treatments 1, 2 and 3. Experimental compounds 1 and 2 were included at the same concentrations as in experiment I (treatments 4 and 5). Monensin[6] was dissolved in ethanol and 0.1 ml was added to treatment 6. Final concentration of monensin in the cultures was 6.25 mg/liter. The sampling schedule and method for determination of the kinetics of ammonia production and loss of soluble carbohydrates were the same as described for experiment I.

Results and Discussion

Experiment I

Rates of ammonia production and disappearance rate constants for soluble carbohydrates are shown in Figure 1. Rates of ammonia production of control cultures were highest for harvest dates III and IV, which contained the greatest amounts of crude protein. While both experimental compounds decreased rate of ammonia production in all

[6] Sigma Chemical Company, St. Louis, MO.

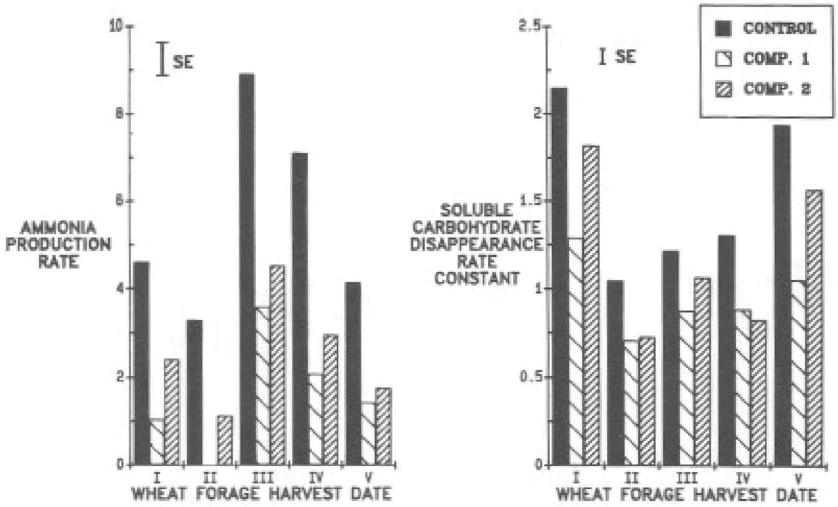

Figure 1. Rates of ammonia production and disappearance rate constants of soluble carbohydrates (Experiment I).

cultures, compound 1 generally had the greatest effect. Both compounds generally decreased the disappearance rate constant for soluble carbohydrates. With the exception of harvest dates II and IV, compound 1 decreased disappearance rate constants for soluble carbohydrates more than compound 2. Reduction in rate of fermentation of soluble carbohydrates of wheat forage would be undesirable because of the very high N:DOM ratio of wheat forage.

Experiment II

Results of experiment II are shown in Figure 2. Addition of ground unheated or steam-flaked corn decreased rate of ammonia production, and steam-flaked corn was much more effective than ground unheated corn. This would be attributed to the faster rate of starch fermentation of steam-flaked corn.

As in experiment I, compound 1 decreased both rate of ammonia production and disappearance rate constant of soluble carbohydrates more than compound 2. Both experimental compounds decreased rate of ammonia production and disappearance rate constant of soluble carbohydrates more than monensin.

Results of these experiments indicate that experimental compound 1 may decrease rate of ammonia production in the rumen of cattle grazing wheat pasture, and therefore may increase protein flow to the small intestine. This should be further evaluated in experiments with cattle grazing wheat pasture.

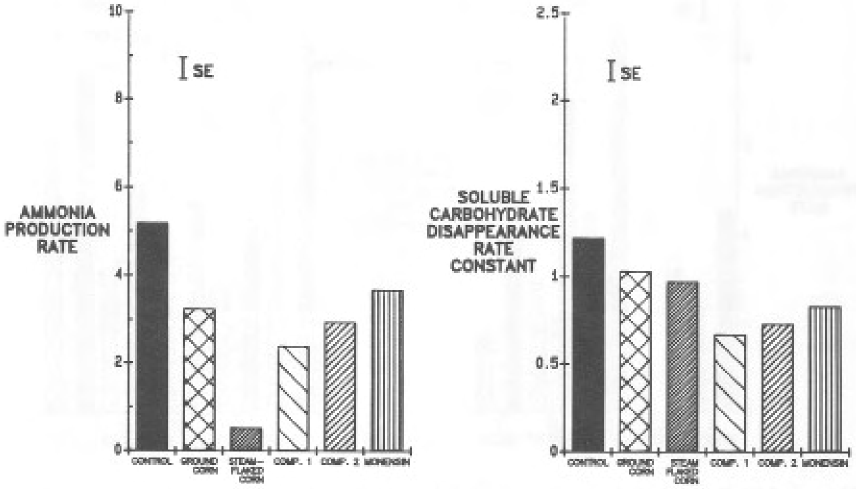

Figure 2. Rates of ammonia production and disappearance rate constants of soluble carbohydrates (Experiment II).

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