

Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 1 *Cattleman's Day (1993-2014)*

Article 668

1994

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Recommended Citation

Vanzant, E.S.; Cochran, R.C.; Stafford, S.; St Jean, G.; and Olson, K. C. (1994) "Continuous-culture fermentation as a tool for forage evaluation," *Kansas Agricultural Experiment Station Research Reports*: Vol. 0: Iss. 1. <https://doi.org/10.4148/2378-5977.2071>

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Continuous-culture fermentation as a tool for forage evaluation

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CONTINUOUS-CULTURE FERMENTATION AS A TOOL FOR FORAGE EVALUATION

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Summary

Ruminal degradation of organic matter and protein in alfalfa and prairie hay were evaluated in vivo, using cannulated cows, and in vitro, using a continuous-culture fermenter to simulate ruminal fermentation. Estimates of organic matter degradability, microbial N flow per unit feed N input, and efficiency of microbial growth were not different ($P>.10$) between the in vivo and in vitro systems. However, for both forages, estimates of nitrogen degradability were greater with the in vitro system. Despite the differences between in vivo and in vitro techniques for some variables, continuous-culture fermentation will allow us to compare the effects of dietary treatments on forage digestion and will aid in the formulation of supplements to meet specific nutrient requirements for cattle consuming forage-based diets.

(Key Words: Alfalfa, Prairie Hay, Ruminal Digestion, Protein.)

Introduction

The nutritive value of forages to ruminant animals depends on the extent to which forage components are digested and utilized within the rumen. Because measurement of ruminal digestion in the live animal presents various difficulties, other approaches have been developed to simulate ruminal digestion. One such approach is the use of continuous-culture fermentation. With this technique,

ruminal microorganisms are maintained in glass fermentation vessels and are fed similar diets to those fed the live animal. Collection of the materials flowing out of the fermentation vessels allows for calculation of the extent of degradation. This technique deserves particular attention as a means for measuring forage protein degradation. New protein systems for cattle require knowledge about the quantity of dietary protein that is degraded in the rumen and, consequently, the quantity that passes to the small intestine without degradation by ruminal microorganisms. Most of the research in this area has dealt with concentrates, particularly grains and protein supplements. For cows and stocker cattle that receive most of their crude protein (CP) from forages, we have little information relating to the amount of rumen degradable and nondegradable protein in their diets. Thus, we are unable to assess accurately their needs for supplemental degradable and undegradable protein. This study was undertaken to evaluate the ability of continuous-culture fermentation to simulate ruminal fermentation of both high- and low-quality forages.

Experimental Procedures

Degradation of alfalfa (16.8% CP) and prairie hay (5.8% CP) in continuous-culture fermenters was compared with in vivo degradation measurements obtained using cannulated cows. The in vivo measurements were made using six Angus $\times$ Hereford cows that had been fitted surgically with cannulas

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in the rumen and duodenum. Samples were collected from the diet, the rumen, and the duodenum and were analyzed for organic matter; crude protein; and an internal marker, indigestible acid detergent fiber, which allowed us to calculate the amount of undegraded forage constituents flowing through the duodenum each day. We measured the quantity of these constituents derived from ruminal bacteria by determining the concentration of purine nitrogen in the duodenal contents and in bacteria that were isolated from the rumen. By assuming that all purine nitrogen at the duodenum comes from bacteria, we were able to determine the amount of organic matter and protein that came from bacteria. After estimating contributions to duodenal flow coming from animal cells and enzymes, the remainder of the flow through the duodenum was assumed to be undegraded forage constituents. For the *in vitro* technique, we fed each forage into a continuous-culture fermenter, a laboratory apparatus designed to simulate ruminal fermentation. The fermenter contained ruminal microorganisms harvested from a ruminally cannulated steer and maintained temperature, oxygen levels, and acidity within levels that allowed for continuous growth and digestion by the ruminal microorganisms. The amounts of organic matter and protein flowing into and out of the fermenter were measured, and microbial contribution was subtracted from the outflow as described above for the *in vivo* technique.

Results and Discussion

The amounts of organic matter and CP fed are shown in Table 1. Estimated ruminal digestibility of organic matter was greater ($P<.01$) for alfalfa than for prairie hay with both techniques. Although the difference between the forages was numerically greater when measured *in vivo*, differences between techniques were not significant. An interaction between forage type and measurement technique existed ($P=.05$) for estimated ruminal protein degradability, because protein degradability for alfalfa was similar between measurement techniques,

whereas it was significantly greater for the *in vitro* technique with prairie hay. The flows of nitrogenous constituents out of the respective systems were calculated as fractions of the amount of feed N put into each system to account for the fact that much greater total amounts of N were required *in vivo* than *in vitro*. The values for nonammonia N in Table 1 represent a combination of N from both microbial cells and undegraded feed. The total nonammonia N flow out of each system as a fraction of the feed N put into the system was greater ($P<.01$) for prairie hay than for alfalfa and was greater ($P=.01$) *in vivo* than *in vitro*. The amount of bacterial N flowing out of each system as a fraction of the feed N put into the system was also greater ($P<.01$) for prairie hay than for alfalfa but was unaffected ($P>.10$) by the technique used. The large N outflows for prairie hay as compared with alfalfa, when expressed as a percentage of N input, are indicative of the small amount of ruminally degradable N provided by the prairie hay. Nonammonia N outflow can exceed feed N input, as demonstrated by *in vivo* outflow in excess of 100% of input for prairie hay. This is possible because ruminal bacteria utilize ammonia that is recycled back into the rumen to synthesize bacterial protein. Efficiency of bacterial growth is measured by the amount of bacterial N produced per kg of organic matter digested in the system. The efficiency of bacterial growth was unaffected by either forage type or measurement technique.

The similarity between the techniques with respect to estimates of bacterial N exiting the system as a proportion of feed N or per unit organic matter fermented suggests that continuous culture can provide a reasonable estimate of the ability of a forage to sustain bacterial protein production. This would allow us to predict the amount of bacterial N available to the animal consuming a forage diet. Thus, the need for additional ruminally undegradable N could be assessed. Further study is necessary to determine whether more accurate predictions of *in vivo* organic matter and N degradability are possible using our continuous culture fermenters.

In conclusion, specific values for degradability of protein measured with continuous-culture fermentation did not match values obtained from in vivo measurements. However, continuous-culture techniques provided a means to evaluate differences between the organic matter and

protein degradability of forage diets and allowed for prediction of bacterial protein production supported by both high-quality and low-quality forages. Use of these techniques will allow for more accurate formulation of supplements to meet the nutrient requirements of ruminants consuming forage-based diets.

Table 1. Digestibility and Nitrogen Outflow for Alfalfa and Prairie Hay as Affected by Measurement Technique

Item	Alfalfa		Prairie Hay		P-value ^b			
	in vivo ^a	in vitro	in vivo	in vitro	SE	F×T	F	T
Feed input, g/d								
Organic matter	6337	13.5	6080	13.6	-	-	-	-
Crude protein	1149	2.4	366	.8	-	-	-	-
Digestibility ^c , %								
Organic matter	61.6	60.5	45.9	53.9	3.73	.24	<.01	.36
Crude protein	83.0	90.7	54.1	75.5	3.26	.05	<.01	<.01
Nitrogen outflow, % of feed N input								
Non-ammonia N ^d	55.8	40.9	122.1	97.8	7.22	.53	<.01	.01
Bacterial N	38.8	31.5	76.2	73.4	7.42	.77	<.01	.51
Bacterial N flow, g/kg DOM ^e	17.8	16.2	16.5	14.1	2.56	.90	.54	.47

^aMeasurement techniques: in vivo = measurements made using cannulated cows; in vitro = measurements made using continuous culture fermenters.

^bProbability of a greater F-value. F×T = forage type × measurement technique interaction; F = forage type effect; T = measurement technique effect.

^cDigestibility values for in vivo technique represent true ruminal digestibility (corrected for bacterial contribution).

^dNonammonia N corrected for estimated endogenous N flow with in vivo technique and, therefore, represents sum of bacterial N and undegraded feed N for both techniques.

^eDOM = organic matter digested in rumen or fermenter, corrected for microbial organic matter.