Lyophilized Rumen Fluid for Use in In Vitro Dry Matter Disappearance Studies with Grain

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Story in Brief

The effect of lyophilization on rumen fluid and its subsequent use in *in vitro* dry matter disappearance (IVDMD) procedures with corn grain was investigated. The rumen fluid, collected via rumen cannula from a mature Holstein steer, was utilized either a) *fresh* as in standard IVDMD procedures, b) *prefrozen rapidly* prior to lyophilization (PR), or c) *prefrozen slowly* (PS) prior to lyophilization. The lyophilized rumen fluid was then stored either frozen (F) or at room temperature (S) preceding its use in the IVDMD evaluation.

In three sequential 24-hr *in vitro* evaluations, no significant differences could be detected in the *in vitro* digestibility of corn between the fresh rumen fluid or any of the lyophilized rumen fluid treatments, PR-F, PR-S, PS-F or PS-S. The variation within each of the treatments, however, was relatively large. This study suggests the potential efficacy of lyophilized rumen fluid for use in IVDMD evaluations of grains and supports the suggestion that continued work with the lyophilized rumen fluid, including potential uses for forages, is warranted.

Introduction

The use of IVDMD procedures for predicting the nutritive value of grains and forages is widespread. One of the main advantages of the *in vitro* technique is the ability to study microbial digestion away from the control and influence of the host animal. This technique yields a rather accurate estimate of the digestibility of different feedstuffs. Such information is useful in comparing feeds, grains or forages. Moreover, many states, either via land grant universities, USDA extension services, or private laboratories, offer forage and feed evaluation services to producers. *In vitro* digestibility is often one of the major criteria evaluated as an indicator of nutritive value.

Previous research clearly demonstrates the variation of the IVDMD procedure between as well as within laboratories. A major source of this variability arises from differences in the rumen fluid itself, due to the diet of the host animal, animal differences or differences in processing or handling of the fluid. Most commonly, each laboratory or testing station will maintain a donor animal and routinely remove

Table 1. Composition of artificial saliva-buffer solution.

Ingredient	Gm/liter of distilled H_2O
$NaHCO_3$	9.80
Na_2HPO_4	3.69
KCl	0.57
NaCl	0.47
$MgSO_47H_2O$	0.12
$CaCl_2$	0.04
Urea	0.91

inoculum as needed. This is a laborious, objectionable practice and tends to be expensive due to maintenance of the donor animal. If the inoculum used in IVDMD procedures could be standardized, then a large amount of the variability of the technique might be eliminated. Similarly, if a product could be developed that would eliminate the need for a donor animal, then perhaps the cost of the evaluation could be reduced and simplified for many laboratories.

Lyophilization, or freeze-drying, has been employed by microbiologists to maintain bacterial cultures with varying degrees of success. In an attempt to standardize the IVDMD procedure, this research was conducted to investigate the effectiveness of a lyophilized rumen fluid product for use in these types of studies.

Experimental Procedure

Five separate samples of approximately 500 ml of whole rumen fluid were removed directly from the rumen of a mature Holstein steer fed 10 lb per day of a 84 percent corn, 16 percent supplement cottonseed hull ration. Water and feed were withheld eight hr prior to sampling to avoid dilution of the microbial population.

Each sample was strained through six layers of cheese-cloth and continuously flooded with CO_2 to promote anaerobic conditions. Two-hundred ml of strained fluid were retained from each of the five samples; one was used fresh. The four remaining samples were then placed in 600 ml lyophilizer flasks, flooded with CO_2 and sealed with parafilm.

Two of these samples were frozen rapidly (PR) by spinning the flasks in dry ice and acetone, and two samples were frozen slowly (PS) by placing the flasks in a conventional freezer at −10 C. Eighty ml of the fresh rumen fluid sample were mixed with 220 ml of artifical saliva or buffer (composition presented in Table 1). Thirty ml of the rumen fluid buffer mixture was then incubated as per standard IVDMD procedure with approximately .40 g of corn grain ground through a 20-mesh screen in a laboratory Wiley mill. Dry matter disappearance was determined by differences after 24 hr of incubation.

When frozen, the remaining four samples were lyophilized to completion on a Virtis 10-100V lyophilizer. After lyophilization, the rumen fluid appeared as a dry, fine powder. One sample of each of the two freezing treatments were then stored either in the freezer (F) or at room temperature (S).

After 24 hours, all lyophilized rumen fluid samples (PR-F, PR-S, PS-F, PS-S) were reconstituted to their original volume with distilled water, which had been prewarmed to 39 C and flooded with CO_2 for 10 minutes. Eighty ml of each reconstituted sample were mixed with 220 ml of artificial saliva and for each sample the same inoculation and incubation procedure as used for the fresh fluid was carried out. A second and third replication of this experiment were conducted on successive weeks.

Table 2. Twenty-four hour IVDMD of ground corn using fresh and lyophilized rumen fluid.

	% DMD		
Treatment	**Week 1**	**Week 2**	**Week 3**
Fresh	65.0[4]	47.4[3]	70.3[1]
PR-F	77.7[1]	38.6[4]	67.7[2]
PR-S	73.9[3]	48.1[2]	58.0[4]
PS-F	63.2[5]	14.3[5]	66.6[3]
PS-S	75.2[2]	55.4[1]	44.4[5]

[1,2,3,4,5]Indicates rank within weeks.

Results and Discussion

Table 2 shows the percent DMD for the fresh and lyophilized rumen fluids. No significant differences (P>.1) due to treatment of the fluid were observed; however, a significant effect due to week was observed. The within treatment variations for all the fluids were large, and may reflect the innate variation within the IVDMD procedure itself. The relative rank of efficiency of DMD for each treatment varied between weeks, suggesting a treatment by week interaction. Because of the vast practical benefit that a storable rumen inoculum product for IVDMD use would afford, these data confirm the efficacy of such a product and suggest that further investigation into the accuracy and repeatability of the lyophilization process is warranted.

Bovine Boots - A New Research Tool

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Story in Brief

To meet the need for precise determinations of forage intake by grazing livestock, an Animal Weight Telemetry System has been developed. Four "boots" containing electronic load cells generate analog signals which are summed, converted to a digital signal and transmitted to a laboratory receiver for interpretation. Minor changes in body weight, coupled with observation of grazing behavior, permit direct and precise measurement of intake continuously or at selected intervals.

Introduction

Two major determinants of forage feeding value are digestibility and intake potential. Evaluation of forages as livestock feed under grazing conditions poses special problems to researchers because of the tendency of grazing animals to "select" plants or parts of plants. Furthermore, livestock eat different amounts of forage when they graze from when they are fed in confinement in pens or stalls. Precise measurement of intake has heretofore been impossible. Expensive and inaccurate estimates of intake have often been assigned to forages for purposes of forage-quality ranking, feed formulation and animal performance prediction. The "bovine boots" will allow development of more reliable feeding programs and promote more efficient and economical livestock nutrition research.

Materials and Methods

A beef steer has been outfitted with boots and accessories needed to determine the pressure exerted on the ground because of its body weight.

The basic components of the "Bovine boots" telemetry system include (1) a laboratory-housed base facility to activate the field equipment, receive data and condition those data, (2) backpack-housed signal conditioning and transmitting equipment, (3) harnesses to support electrical cables on the animal and (4) boots/