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Bacterial Protein Correction on *In situ* Digested Samples with Purine or NDF Methods

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Abstract

In situ ruminal digestion procedure is known procedure for estimating ruminal digestion. In situ procedure requires correction for microbial contaminations, and the purine method has been used to correct microbial attachments. The neutral detergent fiber (NDF) method has been proposed as an alternative to the purine method. Objectives of this experiment were to compare purine and NDF method for microbial correction. Alfalfa hay, bermudagrass hay, tall fescue hay, corn and soybean meal samples were selected. Chemical analyses of dry matter (DM), crude protein (CP), NDF, Acid detergent fiber (ADF), NDF-protein (NDIP), ADF-protein (ADIP), lignin, crude oil and ash were determined on selected samples. In the experiment, samples were inserted into the rumen of a cannulated cow for 6, 12, 24, 72, and 96 h to determine in situ digestion. The average microbial purine to nitrogen ratio was determined to be 11.43. In situ digested tall fescue microbial protein corrected CP values with purine and NDF methods were similar within all hours, except 96 h. Other In situ digested sample purine procedure had some interference during sample reading and standard errors were not acceptable level. There were not enough data to compare both methods for other samples.

Key Words: In situ ruminal digestion, bacterial protein, purine, neutral detergent fiber.

INTRODUCTION

The *in situ* rumen procedure is a technique that generates data used to predict ruminal digestion [12, 16]. It has been reported that microbial attachment to the feed substrate can cause a lower estimate of protein digestion. Microbial attachment needs to be corrected for the *in situ* method. If rumen microbial protein is not correctly estimated and corrected for the digested samples, microbial protein appears as an undigested protein in the feed. Estimation of protein digestion can change significantly.

Purines are adenine, guanine, hypoxanthine and xanthine [5]. Hypoxanthine and xanthine are important intermediates in synthesis and degradation of adenine and guanine. Adenine and guanine are major compounds for ribonucleic acids. Ribonucleic acid is present in bacteria, protozoa and feed. Marshak and Vogel [9] reported a method that determines microbial purines and pyrimidines in biological materials. The method combines hydrolysis of nucleotide, precipitation of free purines and pyrimidines, and measured spectrophotometrically.

Microbial protein was estimated by the ratio of purines to nitrogen of isolated values, which is accepted as a constant for given conditions [3]. They suggested that purine determination gives more accurate results for estimating microbial population. Over the years, purine procedure was improved by Zinn and Owens [17], Obispo and Dehority [11] and Makkar and Dehority [7]. The method can estimate net microbial protein synthesis in the rumen. The ratio of purines to nitrogen of isolated bacteria can be estimated and can give total protein value of the bacterial protein. However, purine procedure requires several steps that increase variation. The purine method has been used to correct microbial attachment. The purine procedure requires both skilled technicians and laboratory equipment such as the spectrometer.

The neutral detergent fiber method is a common procedure that is used in most labs, but it is a new method for correcting microbial attachment [8] and needs to be evaluated for *in situ* digested samples. The primary objectives of these experiments were to evaluate neutral detergent fiber method with purine procedure to estimate microbial attachment for *in situ* digested samples.

MATERIALS AND METHODS

Sample preparation and chemical analyses: Air-dried samples of alfalfa hay, bermudagrass hay, tall fescue hay, corn and soybean meal were used in this experiment. A total of 200 g sample was collected and ground to pass through a 2 mm screen and a subsample weighing 50 g ground to pass through 1 mm in a Wiley mill.

Sample DM was determined by drying samples at 100º C for 12 h [2]. Ash content was determined in a temperature-controlled furnace at 600º C for overnight [2]. Sample ether extract was determined with AOAC [2] standard procedure. Samples were analyzed for NDF, ADF and ADL (sulfuric acid method) using the ANKOM200 Fiber Analyzer [1]. Feedstuffs were analyzed for total N, neutral detergent fiber insoluble protein (NDIP), and acid detergent fiber insoluble protein (ADIP) using a nitrogen analyzer [4].

Animal: A Jersey cow fitted with a ruminal cannula was used. The cow was managed according to Knoxville Experiment Station dairy farm standard procedures and fed individually. The cow was used under a protocol approved by The University of Tennessee Animal Care and Use Committee. Alfalfa cubes (80%) and concentrate feed (20%) were offered twice daily (0800 and 1600). Animal had free access to mineral salt block containing 0.4% Zn, 0.2% Fe, 0.2% Mn, 0.03% Cu, 0.007% I, 0.005% Coin 94% minimum NaCl. The animal was fed a maintenance energy level diet during the study from February 8 to March 1, 2002. The animal was adapted to the diet for ten days. Diet chemical analyses were done and are reported in Table 1. Samples were inserted into the rumen at 11 to 14 days. The animal remained on the diet for 3 days after the removal of the samples and then a second run was conducted.

Table 1 Chemical analyses of diet feed ingredients[a] in the experiment

Chemical analyses	80% Alfalfa Cubes	20% Corn base supplements	Total diet
DM, % AF	91.79	88.50	-
CP, % DM	13.68	8.61	12.66
NDF, % DM	57.00	10.11	47.62
ADF, % DM	43.26	2.45	35.09
ASH, % DM	10.00	0.98	8.19

[a] Based on DM as fed basis.

In situ method: The *in situ* method was used to evaluate the disappearance of DM using #R1020 Dacron bags (10 cm x 20 cm, 50-µm to 70-µm pore size). Duplicate ground samples (5 g: 2 mm screen) were placed into identified Dacron bags. Dacron bags placed in to mesh bags. Mesh bags (used to prevent loss of *in situ* bags) contained a maximum of 12 Dacron bags . Mesh bags were soaked in 39 °C water for 15 min in a thermos container after that it was placed in the rumen under the ruminal particulate mat. Samples were inserted sequentially (96, 72, 24, 12, 6, and 0 h prior to removal) and removed at the same time. Upon removal, samples were pre washed with cold water and then the *in situ* bags were rinsed in a washing machine until bags were clear as described by Vanzant et al. [16]. Bags were dried at 100 °C in a forced draft oven for 12 h. Dry weights were recorded for all samples. Zero hour samples were not inserted into the rumen. They were soaked in 39 °C water for 20 min. After the soaking, the zero hour samples received the same treatment as the ruminal samples.

Ruminal fluid collection: Two kg of ruminal contents was collected four hour post feeding at the 11th and 17th days. The ruminal fluid was stored in a thermos container until brought to the laboratories. Two kg ruminal fluid was weighed and mixed with ice-cold saline solution (9 g of NaCl/L). It was blended in a high-speed blender and strained through two layers of cheesecloth into 250 mL bottles. The rumen fluid was then centrifuged at 500 x g for 20 min (to separate protozoa and feed particles). Supernatant fluid was collected into 250 mL bottles and centrifuged at 30,000 x g for 20 min. The supernatant fluid was discarded and water was added. Bacterial pellets were pooled and re-suspended. These steps were repeated three times. Separated bacteria were lyophilized, ground and stored in a glass jar until further analyses.

The purine procedure was used to correct microbial protein attachments to feed substrate as modified from Ushida et al. [15], Zinn and Owens [17], Obispo and Dehority [11], and Makkar and Dehority [7]. Additional analyses included CP, NDF, and NDIP analyses.

Statistical Methods: Data were analyzed using Mixed Model Procedures in SAS [13]. Analyses of variance were done using a complete randomized design. PDMIX800 and MMAOV macros were used for this SAS procedure (Saxton, personal communication [14]).

RESULTS AND DISCUSSION

Chemical analyses of selected feedstuffs and data are shown in Table 2. Nutrient Requirements of Beef Cattle [10] reported nutrition values were compared to our findings. There are some differences between our findings and the reported values due to plant differences (maturity, year, fertilization etc.), drying and storage.

Soybean meal had the highest CP value (53.86%) among the selected feedstuffs. Bermudagrass and corn had the lowest CP values among the feedstuffs, (8.14 and 8.61%, respectively). Alfalfa had the highest CP value (19.8%) among the forages. NDIP and ADIP values were to be higher than the literature. Madsen and Hvelplund [6] reported that larger variations were seen on nitrogen analyses than on other analyses between laboratories.

NDF values were similar to those reported in the literature. Forages had NDF values ranging from 40% to 76%. Corn and soybean meal had lower NDF levels, 10.11% and 13.76%, respectively. Lignin values were close to reported values, except corn and soybean meal had higher lignin levels than reported values, 7.04 and 6.71%, respectively. Alfalfa had the highest lignin value 14.93%. Fat content was similar to the reported values for corn (4.18%) and soybean meal (1.68%). Other feedstuff fat values were slightly less than the reported values. Ash content was lower for bermudagrass (5.52%), tall fescue (5.72%), and corn (0.98%). Others ash values were similar to the reported values.

Selected samples dry matter disappearance percentages are shown in Figure 1. Alfalfa dry matter disappearances were 34% at zero hour for Dacron bags. The zero hour value represents a sample that was soaked in warm water and washed following the standard washing procedure used for all samples. It was mostly soluble fractions that were lost in this process. Alfalfa had higher CP (19%) than other forages and the zero hour loss was higher than in other forages. Most of the DM disappearance was at 24 h.

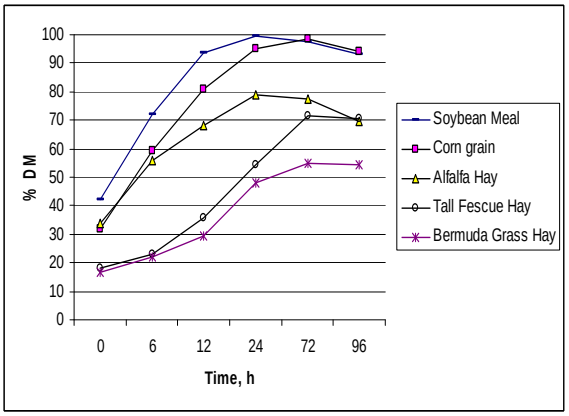

Figure 1 *In situ* dry matter disappearances for selected feed stuff.

Table 2 Chemical analysis of selected feedstuffs[a]

Common name	DM, % AF	CP, % DM	NDF, % DM	Lignin, % NDF	NDIP, % CP	ADF, % DM	ADIP, % CP	Fat, % DM	Ash, % DM
Alfalfa hay	90.13	19.8	43.22	14.93	15.78	30.77	6.64	1.29	9.26
Bermudagrass hay	92.05	8.14	76.74	9.67	50.61	39.56	11.71	0.65	5.52
Tall fescue hay	90.49	11.56	73.91	7.85	50.40	41.19	7.12	1.82	5.72
Corn	88.50	8.61	10.11	7.04	32.90	2.45	5.27	4.18	0.98
Soybean meal	89.05	53.86	13.76	6.71	6.49	9.48	3.91	1.68	6.54

Bermudagrass had the highest NDF values (76%) among the feedstuffs used. Bermudagrass dry matter disappearances were 16% at zero hour with Dacron bags. Dry matter disappearances were 55% at 96 h for Dacron bags. The digestion trend was similar for bermudagrass and tall fescue; however, 96 h disappearance was lower than tall fescue. Tall fescue dry matter disappearances were 18% at zero hour. Tall fescue DM disappeared slowly and after 72 h, it did not change much due to a higher NDF value (73%). Dry matter disappearances were 70% at 96 h. Corn dry matter disappearances were 31% at zero. Since corn is high in starch, DM rapidly disappeared. Extended dry matter disappearances were 94% at 96 h (Figure 1). Soybean meal dry matter disappearances were 42% at zero hour. At 24 h, soybean meal had the highest DM disappearance rate (99%). Extent of dry matter disappearances were 93% at 96 h.

Table 3 *In situ* digested tall fescue samples microbial protein corrected values

Time	Purine	NDF
	Corrected CP, % (DM)	
6	$7.55 + 0.33^{a,b,c}$	$7.43 + 0.33^{b,c,d}$
12	$8.43 + 0.23^{a}$	$8.14 + 0.23^{a,b}$
24	$8.19 + 0.33^{a,b}$	$7.66 + 0.23^{a,b,c}$
72	$6.48 + 0.23^{d,e}$	$5.88 + 0.23^{e}$
96	$6.86 + 0.23^{c,d}$	$5.97 + 0.23^{e}$

^{a,b,c,d,e} Least square means with unlike letters differ ($P < 0.05$).

Microbial purine (111.83 mg purines/g DM) and nitrogen (9.78 % DM) ratio were determined to be 11.43. *In situ* digested tall fescue microbial protein corrected protein values are presented in Table 3. *In situ* digested tall fescue sample microbial purine concentrations were determined and N contamination corrected based on the microbial purine:protein ratio. *In situ* digested tall fescue samples were also washed with NDF and nitrogen was determined on the same samples before and after NDF wash. *In situ* digested tall fescue CP values were compared after microbial corrections. Data from purine and NDF methods were similar within the same time, except 96 h purine corrected samples. Purine analyses were also run several times for other samples; however, purine procedure results were not consistent for other samples; on the other hand, the standard was consistent for the each run. There was some interference for the reading of the samples because of the many fine particles in solution as reported by Ushida et al. [15]. *In situ* digested samples standard errors were not acceptable for purine procedure. After reruns there were not enough samples left to run NDIP or purine except tall fescue.

In situ digested tall fescue results were similar for microbial correction with purine and NDF. If there were more data available, the two methods could be better compared; however, the neutral detergent fiber could replace purine method as suggested by Mass et al. [8] and Klopfenstein et al. [3].

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