January 2001

Changes to the Purine Assay Improve Purine Recovery and Assay Precision

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Changes to the Purine Assay Improve Purine Recovery and Assay Precision

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Modifications of the purine assay improved recovery of purines, increased assay precision, and accounted for particle-attached bacteria.

Summary

Three experiments tested modifications of the purine assay designed to improve estimation of microbial crude protein supply. In Experiment 1, changing hydrolysis conditions from 12 normal perchloric acid to 2 normal increased purine recovery and lowered the coefficient of variation. In Experiment 2, phosphate buffer yielded greater recovery of purines than acetate buffer and using the extraction solution as a wash was less variable than silver nitrate in .005 molar hydrochloric acid. In Experiment 3, purine nitrogen:total microbial nitrogen ratios for five forages were determined by incubating NDF in situ and analyzing them before and after neutral detergent extraction.

Introduction

The original purine assay has two problems. First, the current method of isolating rumen microbes, known as differential centrifugation, may not yield a representative proportion of particle-attached microorganisms. Therefore, the measured purine nitrogen:total microbial nitrogen ratio of those microbes may not be accurate. Second, purine recovery and the precision associated with that recovery are poor. Two laboratories (in Germany and at Ohio State University) have proposed separate modifications to the assay that improve recovery of purines. The objectives of this research were to develop a method of estimating the purine nitrogen:total microbial nitrogen ratio of particle-attached microorganisms and to investigate the effect combinations of these modifications have on purine recovery and precision.

Procedure

Three experiments were conducted. Samples for Experiments 1 and 2 were collected from five ruminally and duodenally fistulated steers (ave wt. = 800 lb) that were fed alfalfa hay and smooth bromegrass hay for seven days each. Cattle were fed hay ad libitum for six days and omasal, duodenal and fecal samples were collected on the seventh day. All samples were lyophilized and ground by Wiley mill to pass through a .008" screen. Experiment 3 was an in situ experiment that used both the alfalfa and smooth bromegrass hays from the previous experiments as well three other hays: switchgrass hay, high-quality meadow hay and low-quality meadow hay. The quality of these forages is described in Table 1.

In Experiment 1, the 10 omasal samples were used. The objective was to test the effect of perchloric acid concentration on purine recovery within the original protocol of Zinn and Owens. Treatments were 12 or 2 normal perchloric acid. In Experiment 2, the effects of buffer type and wash solution within the Zinn and Owens procedure were tested in a 2 x 2 factorial arrangement. Samples tested were the duodenal samples that correspond to the same sampling time as the omasal samples in Experiment 1. Buffers tested were .2 molar acetic acid and .2 molar ammonium phosphate. Wash solutions tested were .005 molar silver nitrate and the original precipitation solution of the procedure. The perchloric acid used in Experiment 2 was 2 normal.

In Experiment 3, the objective was to estimate the purine nitrogen:total microbial nitrogen ratio of particle-attached rumen microorganisms. Neutral detergent fiber (NDF) was generated for each forage and dried NDF was incubated in situ for 12 hours. Three bags each were incubated in a steer fed 2% of body weight of smooth bromegrass (8% CP) and another steer fed 2% of body weight of a diet containing 70% of the DM as that smooth bromegrass hay and 30% of the DM as a 50:50 blend of dry-rolled corn and soybean meal. Nitrogen and purine concentrations were measured on the NDF before ruminal incubation and on the in situ residue both before and after neutral detergent extraction.

Results

Results of Experiment 1 are shown in Table 2. Use of 2 normal perchloric acid increased purine recovery over the standard 12 normal acid. Estimates of microbial crude protein concentration increased from 2.23% to 7.43% of the DM. The less concentrated acid also lowered the coefficient of variation (CV), an estimation of the method's error rate, from 14.87% (when 12 normal acid was used) to 3.14%. These results are in agreement with two other research publications. The more concentrated acid generates chemicals in the sample that interfere with the detection of purines, thereby reducing purine concentrations. This effect occurs only when NDF is present in the sample; this

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Table 1. Quality of hays used in experiments.

Forage	CP[a]	UIP	NDF
Alfalfa	22.4	4.0	32.8
Smooth bromegrass	7.8	3.2	63.3
Switchgrass	4.5	2.4	73.2
High-quality meadow hay	8.7	3.5	62.3
Low-quality meadow hay	8.6	2.7	62.9

[a]CP, UIP (in situ NDIN procedure), and NDF on a DM basis.

Table 2. Effect of acid concentration on purine recovery (Experiment 1).

	perchloric acid normality			
Item	2 N	12 N	SEM	*P*-value
MCP[a]	7.43[c]	2.23[d]	.51	.0001
C.V.[b]	3.14[c]	14.87[d]	2.61	.0053

[a]Microbial crude protein (DM basis), as estimated by purines (purine nitrogen:total nitrogen ratio assumed to be .2).
[b]Coefficient of variation for MCP estimates, % of mean value.
[c,d]Means within a row with different superscripts differ (*P*-value shown).

Table 3. Effects of buffer and wash solution type on purine recovery (Experiment 2).

	acetate buffer		phosphate buffer		Effects[a]		
	AGNO3[b]	ORIGINAL	AGNO3	ORIGINAL	buffer	wash	buffer *wash
MCP[c]	1.39	1.91	5.39	6.74	.0001	.003	.16
CV[d]	6.87	12.60	9.85	2.83	.210	.812	.02

[a]P-values for effects listed.
[b]AGNO3 is silver nitrate wash solution; original is the same wash solution as is used for the extraction.
[c]Microbial crude protein (DM basis), as estimated by purines (purine:N ratio assumed to be .2).
[d]Coefficient of variation for MCP estimates, % of mean value.

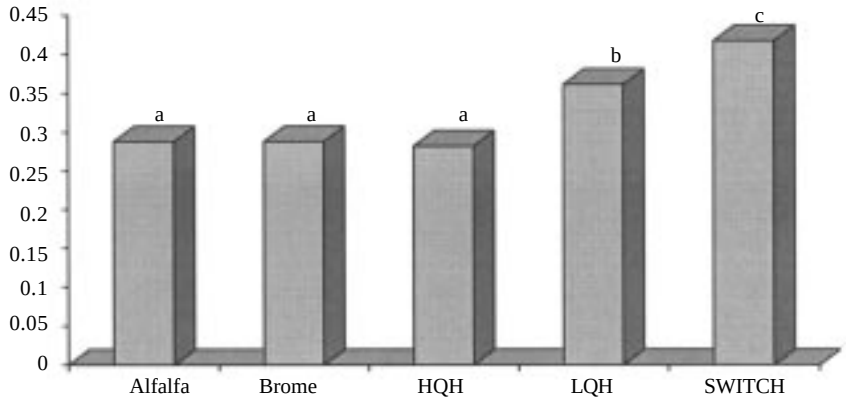

Figure 1. Purine nitrogen:total microbial nitrogen ratio of particle-associated rumen microorganisms on NDF of different forages (Experiment 3). HQH, LQH, SWITCH = high and low-quality meadow hay, and switchgrass, respectively.
[a,b,c]Bars within unlike letters differ ($P \leq .06$).

is the case with omasal and duodenal samples. The reduction of CV when weaker acid is used is also beneficial, as the standard purine assay is known to be variable. Finally, use of weaker acid also makes the assay safer because samples will be less prone to explode.

In Experiment 2, phosphate buffer increased purine recovery when compared to acetate buffer (Table 3). The use of the original precipitation solution as a wash solution also resulted in a significantly greater purine recovery than did the silver nitrate solution. There was no effect of buffer type or wash solution on the CV of the MCP estimate (Table 3). Therefore, the combination of phosphate buffer and the original precipitation solution for washing the sample pellet should be used to achieve the maximum recovery of purines in a sample.

The purine nitrogen:total microbial nitrogen ratios measured in Experiment 3 are shown in Figure 1. There was no effect of fistulated steer diet on the ratios so the data were pooled across diets. Alfalfa, bromegrass and high-quality meadow hay were not different in terms of ratio (~.28). Low-quality meadow hay's ratio (.361) was statistically different than all forages, as was switchgrass (.417). Each of these estimates differs from the previous estimate made at UNL for bromegrass (.137; 1998 Nebraska Beef Report, pp. 90-91) but fall within the range of those estimates made at Ohio State University (ranging from .207 to .587).

Because this ratio converts purine values to MCP values, it has a substantial effect on MCP calculations. Ratio estimates are typically made by the differential centrifugation of rumen contents. This method effectively separates free-floating rumen microbes from forage particles; however, those microbes attached to forage particles tend to remain with the forage. This is problematic because a majority of the rumen microbes are attached to forage particles when forage diets are fed. If the purine nitrogen:total microbial nitrogen ratio of particle-associated microbes differs from that obtained by differential centrifugation, estimates of MCP will be inaccurate. Our data suggest the ratio is variable and can be affected by forage type. This research establishes a modified purine assay that accurately estimates the MCP concentration of a digesta sample. The improved method of estimating the purine nitrogen: total microbial nitrogen ratio that includes particle-associated microbes, a major source of microbial protein, can be used for forage diets.

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