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# Effects of Corn Bran and Degradable Protein Source on Microbial Protein Estimated From Spot Urine Samples in Heifers

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## Summary

*A metabolism trial was conducted in finishing heifers to determine if allantoin in spot urine samples could be a predictor of microbial CP (MCP) supply. When corn bran replaced high moisture corn, ruminal pH was higher and microbial efficiency and flow were greater. Estimated microbial efficiency and flow were not different for SBM compared to urea as a source of degradable protein. Daily variation in intake was reflected in MCP estimates. Within day variation for MCP estimates was consistent and small. Estimates of MCP from allantoin in spot urine samples followed NRC estimates. Results demonstrate that allantoin is an effective predictor of MCP flow.*

## Introduction

Purines are one of the building blocks used in the synthesis of DNA and RNA. Feed purines are essentially totally degraded in the rumen leaving only purines of microbial origin for passage to the duodenum. Therefore, purines have commonly been used to estimate microbial crude protein (MCP) flow in studies assessing protein status of beef cattle. However, this requires duodenal fistulation resulting in laborious experiments with few cattle. Urinary allantoin is derived from the breakdown of purines. Recent research (2001 Nebraska Beef Report, pp. 115-116) has shown a strong linear relationship

between allantoin excretion and purine flow to the duodenum. Previous research analyzed urine samples from total daily collections. This technique is noninvasive but is still laborious and limits cattle numbers because it requires total collection. Creatinine may be used as a marker of urine volume because it is excreted at a constant rate relative to body weight. Using creatinine, spot sampling of urine could be used in a larger number of cattle in typical production settings. Our primary objective was to feed diets that would create differences in MCP supply and determine if allantoin to creatinine ratios in spot urine samples could predict these differences.

## Procedure

Six ruminally cannulated cross-bred yearling heifers (1311 ± 103 lb BW) were used in a 3 × 6 Latin rectangle. Each of three periods

consisted of 9 day for adaptation to diet and 5 day for collection. Base diets (Table 1) were a high-moisture corn (HMC) diet and a diet with corn bran (BRAN) replacing 20% (DM basis) HMC. Urea was added to base diets at 0.9% of diet DM. Addition of corn bran provided a highly digestible fiber source that was expected to increase ruminal pH and subsequently increase microbial efficiency and flow. The BRAN diet also was fed with 7.8% soybean meal (SBM) (DM basis) replacing HMC. The levels of urea and SBM were calculated to provide equal amounts of degradable intake protein (DIP). The SBM diet was included to provide microbes a source of true protein and amino acids that was not available when using urea as the supplemental DIP source. The hypothesis was that true protein and amino acids would increase microbial efficiency resulting in increased MCP flow. Heifers were fed for ad libitum

Table 1. Composition of finishing diets (% of DM).

Ingredient	Diet <sup>a</sup>		
	HMC	BRAN	SBM
High-moisture corn	88.3	68.3	60.5
Corn bran	—	20.0	20.0
Soybean meal	—	—	7.8
Cottonseed hulls	6.7	6.7	6.7
Dry supplement <sup>b</sup>	5.0	5.0	5.0
Fine ground milo	1.68	1.67	2.98
Limestone	1.50	1.49	1.36
Urea	0.90	0.90	—
Potassium Chloride	0.43	0.45	0.17
Salt	0.30	0.30	0.30
Tallow	0.10	0.10	0.10
Trace mineral premix	0.05	0.05	0.05
Rumensin premix	0.02	0.02	0.02
Tylan premix	0.01	0.01	0.01
Vitamin premix	0.01	0.01	0.01

<sup>a</sup>HMC=high-moisture corn diet, BRAN=corn bran diet, SBM=soybean meal diet.

<sup>b</sup>All diets supplemented to contain a minimum of 0.6% Ca, 0.24% P, 0.6% K, and 0.1% S (DM basis). All diets contained 32 g/ton monensin and 11 g/ton tylosin (DM basis).

**Table 2. Effect of HMC replacement with corn bran or supplemental degradable protein source on digestion, ruminal pH, and microbial estimates.**

Item	Diet <sup>a</sup>			SEM	Contrasts <sup>b</sup>	
	HMC	BRAN	SBM		1	2
DMI, lb/day	22.2	25.1	23.8	1.5	0.14	0.35
Digestible DMI, lb/day	18.7	20.5	18.7	1.3	0.49	0.22
Ruminal pH	5.44	5.78	5.88	0.18	0.01	0.46
Time pH below 5.6, min	887	576	428	212	0.09	0.49
A:C <sup>c</sup>	1.05	1.30	1.23	0.09	0.02	0.38
Urine volume, L/day <sup>d</sup>	31.6	32.2	25.8	9.4	0.71	0.38
MCP, g/day <sup>e</sup>	740	966	913	88	0.02	0.47
Microbial efficiency <sup>f</sup>	38.8	46.0	48.6	4.0	0.05	0.54

<sup>a</sup>HMC=high-moisture corn diet, BRAN=corn bran diet, SBM=soybean meal diet.

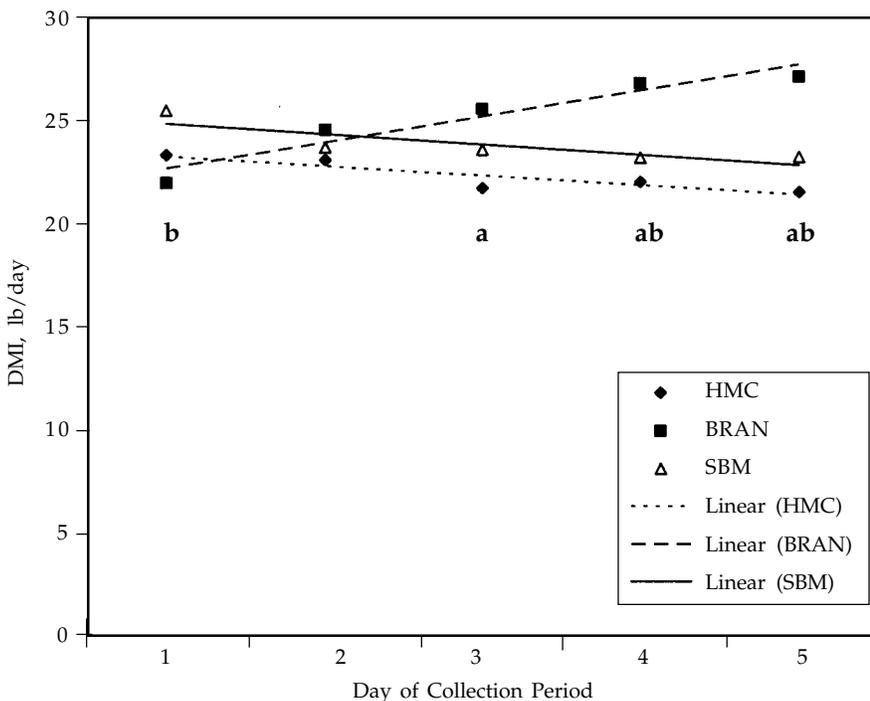
<sup>b</sup>Contrast 1 is comparison of HMC to average of both corn bran containing diets; Contrast 2 is comparison of BRAN and SBM.

<sup>c</sup>Molar ratio of allantoin to creatinine.

<sup>d</sup>Urine volume calculated from creatinine output.

<sup>e</sup>Microbial crude protein.

<sup>f</sup>Microbial efficiency calculated as g MCP/lb digestible DMI.



**Figure 1. Daily variation in dry matter intake. SEM=1.5. Treatment x day ( $P<0.01$ ). Linear effect of day within HMC ( $P=0.19$ ). Linear effect of day within BRAN ( $P<0.01$ ). Linear effect of day within SBM ( $P=0.13$ ). Differences in means on a particular sampling day are denoted by letters beneath points: <sup>a</sup> Contrast comparing HMC to both corn bran containing diets ( $P<0.10$ ) and <sup>b</sup> Contrast comparing BRAN and SBM ( $P<0.10$ ).**

intake once daily at 0900. Chromic oxide was used as an indigestible marker to calculate total-tract digestion and was administered through the ruminal cannula twice daily at 0800 and 1700. Feed intake and

ruminal pH were monitored continuously with feed weight and ruminal pH recorded every minute during the 5 day collection period. Spot urine and fecal grab samples were obtained on each

collection day at 0800, 1100, 1400, and 1700. The molar ratio of allantoin to creatinine was calculated for each urine sample. We assumed daily urinary creatinine output was equal to 16.4 mg/lb BW. To calculate daily allantoin output (moles/day), the allantoin to creatinine ratio was multiplied by assumed daily creatinine output. Estimated MCP flow was calculated from daily allantoin output according to equations outlined previously (2002 Nebraska Beef Report, pp. 66-68). Estimates of microbial efficiency (g/lb DM) were calculated by dividing estimated MCP supply by digestible DMI.

Data were analyzed as repeated measures using the Mixed procedure of SAS. For intake and ruminal pH analyses, collection day represented repeated observations. For urine data analyses, collection day and time of collection were the repeated observations. To determine the effect of corn bran addition, a contrast was used to compare the HMC diet to both of the corn bran containing diets. To determine the effect of supplemental DIP source, a contrast was used to compare the BRAN and SBM diets. Additionally, linear and quadratic effects of time and day were tested with orthogonal contrasts. The REG procedure of SAS was used to compare MCP estimates from urinary allantoin to estimates from Level 1 of the NRC (1996) model.

## Results

Ruminal pH below 5.6 is generally used to define subacute acidosis. Results of the current trial indicate the presence of subacute acidosis. Heifers consuming the HMC diet had an average ruminal pH of 5.44 (Table 2). Additionally, those heifers spent almost 15 hours of each 24-hour collection below a pH of 5.6 (Table 2). Intakes were variable and treatment and collection day interacted ( $P<0.01$ ) with

(Continued on next page)

DMI increasing linearly ( $P<0.01$ ) for the BRAN diet (Figure 1). This may be an indication that the adaptation period was not long enough. The significance of differences in DMI for HMC compared to corn bran containing diets increased toward the end of the collection period (day 3,  $P=0.09$ ; day 4,  $P=0.08$ ; day 5,  $P=0.04$ ).

Estimates of MCP flow and microbial efficiency from allantoin to creatinine ratios in spot urine samples followed the pH and intake responses for dietary addition of corn bran. Treatment effects for estimates of MCP interacted with collection day ( $P=0.02$ ), and MCP was lower on day 2 ( $P=0.02$ ), day 4 ( $P=0.01$ ), and day 5 ( $P<0.01$ ) for HMC versus the average of the corn bran containing diets (Figure 2). On average, corn bran addition increased MCP estimates by 27% ( $P=0.02$ ; Table 2). The interaction for MCP estimates is explained by the interaction found for intake.

When intake variation was taken into account by calculating microbial efficiency, the interaction was no longer present ( $P=0.28$ ), and there was no main effect of collection day. However, microbial efficiency increased ( $P=0.05$ ) by 22% with corn bran addition (Table 2). Because there were no differences in digestible DMI (Table 2), the lower MCP value for HMC is attributable to lower microbial efficiency as a result of lower ruminal pH.

The present study also was designed to evaluate supplementing SBM versus urea based on the hypothesis that providing microbes a source of true protein and amino acids would increase microbial efficiency and flow. There was no effect on DMI, digestible DMI, or ruminal pH when SBM was supplemented relative to urea (Table 2). Evaluation of the treatment by collection day interactions discussed previously for intake (Figure 1) showed that DMI was higher for SBM relative to BRAN on day 1 ( $P=0.05$ ) and lower on day 4 ( $P=0.05$ ), and day 5 ( $P=0.03$ ). Evalu-

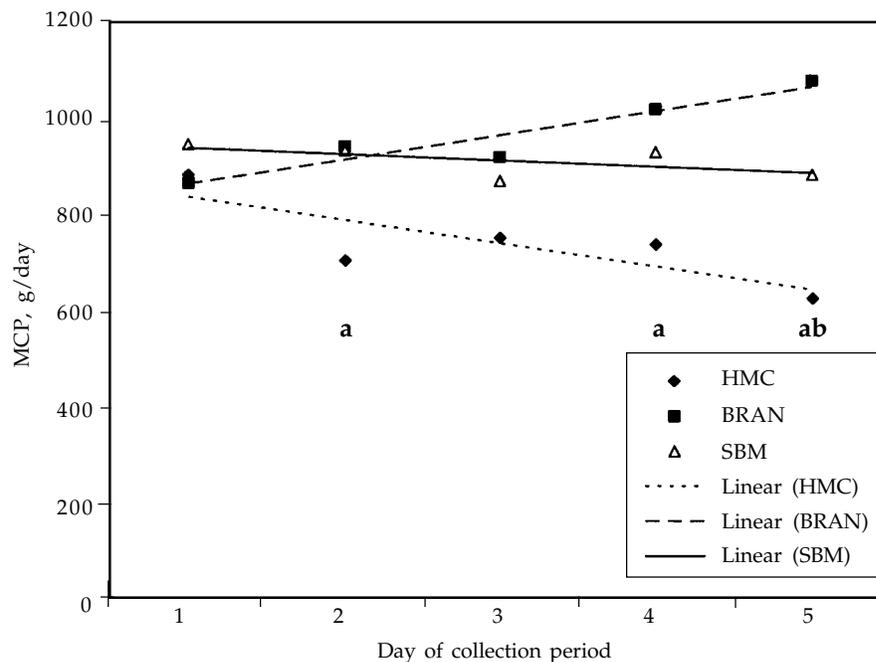


Figure 2. Daily variation in microbial protein estimates. SEM=103. Treatment  $\times$  day ( $P=0.02$ ). Linear effect of day within HMC ( $P=0.01$ ). Linear effect of day within BRAN ( $P<0.01$ ). Linear effect of day within SBM ( $P=0.45$ ). Differences in means on a particular sampling day are denoted by letters beneath points: <sup>a</sup> contrast comparing HMC to both corn bran containing diets ( $P<0.10$ ) and <sup>b</sup> contrast comparing BRAN and SBM ( $P<0.10$ ).

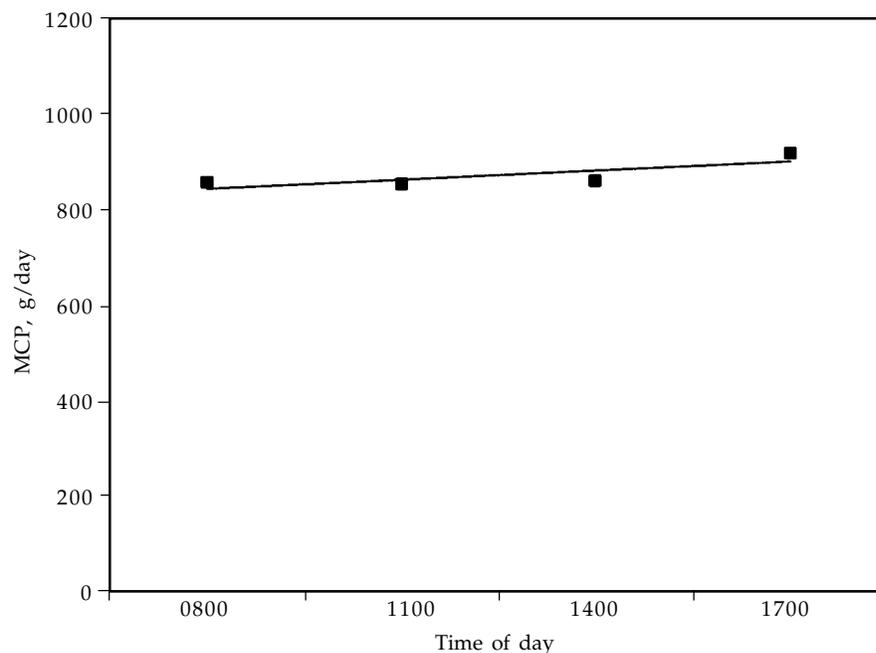


Figure 3. Diurnal variation in microbial protein estimates. SEM=75. Treatment  $\times$  time ( $P=0.19$ ). Main effect of time ( $P=0.10$ ). Linear effect of time ( $P=0.06$ ).

ation of the treatment by collection day interaction for MCP estimates (Figure 2) indicates that MCP was higher ( $P=0.05$ ) only on day 5 of the collection period for BRAN compared to SBM. Microbial efficiency

did not differ between SBM and BRAN (Table 2) treatments. These results are in conflict with some previous reports that indicated MCP flow and efficiency were higher for SBM versus urea supple-

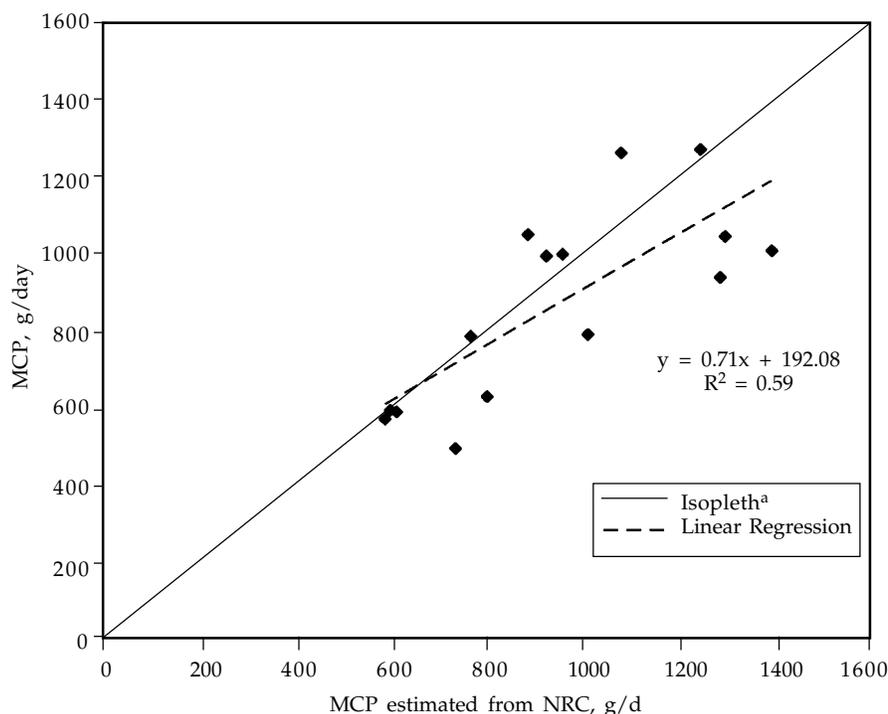


Figure 4. Relationship between allantoin and NRC estimates of microbial protein. <sup>a</sup> Isopleth is the line where estimates are equal and  $r^2$  is equal to 1. Intercept (SE=161;  $P=0.25$ ). Slope (SE=0.16;  $P<0.01$ ).

mented diets. However, those trials were conducted with DRC-based finishing diets, and it seems plausible that the higher DIP value for HMC relative to DRC would provide microbes with more true protein and amino acids decreasing the response to SBM as a supplemental DIP source. Additionally, this trial compared SBM and urea in corn bran containing diets where risk of acidosis had been reduced.

Estimates of urine volume (L/day) did not differ with treatment and were comparable to estimates found in other research trials. The allantoin to creatinine ratio was only different ( $P=0.02$ ) when comparing HMC to the corn bran

containing diets representing the increased MCP flow. Some previous studies have found allantoin to creatinine ratios and estimates of MCP display diurnal variability. This variability is acceptable as long as it is consistent and predictable. Our most important finding in this regard is that there was no time of day by collection day interaction for estimates of MCP ( $P=0.22$ ). We did show some diurnal variability with a MCP estimates increasing linearly ( $P=0.06$ ) from 0800 to 1700 (Figure 3). However, MCP estimates only increased by 63 g or 7% from the first sampling time to the last. In addition, there was no treatment x time of day interaction for MCP

estimates. Using an assumed creatinine output of 16.4 mg/lb BW resulted in MCP estimates that were in agreement with estimates from the NRC (1996) model (Figure 4).

In conclusion, we observed an increase in MCP flow and efficiency estimated from allantoin excretion in spot urine samples when average ruminal pH was increased by adding corn bran to HMC-based finishing diets. Additionally, there were no differences in ruminal pH, intake, or digestibility between diets containing corn bran that were supplemented with urea or SBM resulting in no differences in MCP flow or efficiency. There was little diurnal variation in MCP estimates, and daily variation due to changes in intake was removed when microbial efficiency was calculated. Estimates of MCP followed NRC (1996) estimates, but creatinine outputs may need to be adjusted on an individual animal basis to more accurately and precisely estimate MCP. This trial was conducted in a metabolism setting with a small number of animals. However, the goal of developing a spot sampling technique is to be capable of sampling large numbers of animals in a more typical production setting. Results of a companion finishing trial are also reported in this publication (pp. 32). In that trial, 120 head of heifers were fed the same diets and spot sampled across the feeding period.

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