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Passage of bundle sheath cells in warm-season grasses from the rumen appears to be a mechanism allowing proteins to escape rumen degradation.

Summary

This two-year study was conducted on monocultures of switchgrass and big bluestem to: (1) determine the concentration of the protein ribulose-1,5-bisphosphate carboxylase (Rubisco), found only in the bundle sheath cells of warm-season grasses, in omasal, masticate and fecal samples of grazing cattle; and (2) estimate rumen-escape Rubisco via bundle sheath cells. A quantifying enzyme-linked immunosorbent assay, along with estimates of rumen and lower tract digestibilities, indicated as much as 11% of Rubisco in big bluestem and 13% in switchgrass escaped rumen degradation and was absorbed in the lower tract. Realizing these amounts of escape Rubisco represent a significant level of soluble protein, bundle sheath cells may provide a mechanism allowing soluble protein to escape ruminal degradation.

Introduction

Significant amounts of dietary protein in warm-season grasses pass from the rumen undegraded and are digested and absorbed in the lower tract. Previous research indicates bundle sheath cells in warm-season grasses may play a role in protein escape from the rumen. In warm-season grasses, Rubisco, the enzyme responsible for CO_2 fixation in C3 photosynthesis, is located exclusively in bundle sheath cells. In previous research, Rubisco was detected in omasal and fecal samples from cattle grazing monocultures of switchgrass or big bluestem. This past research hypothesized that a portion of the ingested Rubisco and associated proteins escape the rumen via bundle sheath cells which are structurally weakened by rumen activity. They proposed the proteins are digested in the lower tract following further degradation of the weakened bundle sheath cells. The hypothesis has not been verified, nor has the amount of Rubisco escaping the rumen or disappearing in the lower tract been estimated.

The purpose of this study was to determine if passage of bundle sheath cells from the rumen represents a mechanism for protein to escape from the rumen. The specific objectives were to (1) determine the concentration of Rubisco in masticate, omasal and fecal samples of ruminally-fistulated beef cattle grazing switchgrass and big bluestem; and (2) estimate rumen-escape Rubisco via bundle sheath cells. Presence of Rubisco in a sample would indicate presence of intact bundle sheath cells, as the highly soluble Rubisco is found only in bundle sheath cells of warm-season grasses.

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Procedure

Monocultures of switchgrass and big bluestem were grazed in the summers of 1995 and 1996 at the University of Nebraska Agricultural Research and Development Center near Mead, Nebraska. Each pasture was grazed by three ruminally fistulated cows (1,350 lb) in 1995 and four ruminally fistulated steers (650 lb) in 1996. Pastures were grazed at vegetative and late elongation/early reproductive stages for each grass species. Cattle strip-grazed each pasture at each stage of development for six days and samples were collected on day 7. Animals were moved daily and herbage allowance on the switchgrass and big bluestem monocultures remained above 330 lbs AUD^{-1} in 1995 and above 440 lbs AUD^{-1} in 1996.

Rumen contents of each animal were evacuated and samples of the omasal contents were obtained by hand through the reticulo-omasal opening on day 7. Before replacing the rumen contents, masticate samples were collected after a 30-minute grazing period to represent the undegraded forage being grazed. Fecal samples also were obtained on day 7 of the grazing period.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) along with a western immunoblotting procedure were used to determine the presence of Rubisco in the masticate, omasal and fecal samples. Enzyme-linked immunosorbent assay analysis was performed to quantify Rubisco concentrations.

Dry Matter Disappearance

Dry matter loss in the rumen and lower tract of the animal was estimated for calculation of Rubisco disappearance at the two points in the digestive tract. Acid insoluble ash (AIA) was used as an internal marker for estimation of digestibility. Percentage dry matter disappearance (DMD) in the rumen and lower tract was calculated using the following equations.

DMD in rumen = 100 - 100 (% AIA masticate sample / % AIA omasal sample)

DMD in lower tract = 100 - 100 (% AIA omasal sample / % AIA fecal sample)

Calculations of Rumen-Escape Rubisco

Rumen-escape Rubisco, or Rubisco disappearing in the lower tract (%RE RuBPCase), was calculated (± S.E.) as a percentage of total Rubisco using the following equation:

$$\% \text{ RE Rubisco} = \frac{(OR \times 1 - RD) - (FR \times 1 - RD \times 1 - LD)}{MR}$$

where OR is omasal Rubisco concentration, RD is rumen digestibility, FR is fecal Rubisco concentration, LD is lower tract digestibility and MR is masticate Rubisco concentration. The equation accounts for dry matter (DM) loss in the rumen and lower tract so the estimate of percentage rumen-escape Rubisco is based on intake Rubisco.

Concentrations of Rubisco escaping the entire digestive tract (%TE Rubisco) was estimated (±S.E.) as a percentage of total Rubisco using the following equation:

$$\%\text{TE Rubisco} = \frac{FR \times (1 - RD) \times (1 - LD)}{MR}$$

where FR is fecal Rubisco concentration, RD is rumen digestibility, LD is lower tract digestibility and MR is masticate Rubisco concentration. Fecal Rubisco concentrations represent the percentage of intake Rubisco found in the feces.

Results and Discussion

Extraction of N-containing substrates from the switchgrass and big bluestem samples was very effective. Over 97% of the N was extracted consistently from the masticate, omasal and fecal samples. Rubisco was detected in undegraded leaf, masticate and omasal extracts of switchgrass and big bluestem by western immunoblotting following SDS-PAGE. Negligible amounts of Rubisco were detected in the fecal extracts of switchgrass and big bluestem. Identification of Rubisco in omasal samples of big bluestem and switchgrass verified previous research results.

Concentrations of Rubisco in masticate, omasal and fecal samples (Table 1) were used in conjunction with estimates of DM loss in the rumen and lower tract to calculate Rubisco disappearance in the rumen and lower tract (Table 2). Disappearance of big bluestem Rubisco in the rumen was between 63.6% and 65.3%, except in the 1996 vegetative stage. Percentage switchgrass Rubisco disappearing in the rumen was 15 to 25% lower than for big bluestem, except for the 1996 vegetative samples. Percentage switchgrass Rubisco disappearance of the 1996 vegetative samples was nearly two-fold higher than the other switchgrass samples. The relatively high amounts of Rubisco disappearing in the rumen for the switchgrass and big bluestem vegetative samples in 1996 cannot be explained.

Disappearance of Rubisco in the lower tract ranged from 10.6% to 19.5% for big bluestem and 13.3% to 39.6%

Table 1. Percentage Rubisco concentrations (±S.E.) in omasal, masticate and fecal samples of beef cattle grazing big bluestem or switchgrass in vegetative or elongation/reproductive stages of growth.

	Vegetative		Elongation/Reproductive	
Species	1995	1996	1995	1996
	%			
Big bluestem				
Omasal	2.9 (.06)	5.0 (.30)	3.7 (.28)	4.3 (.28)
Masticate	3.9 (.26)	11.9 (3.0)	5.2 (.96)	6.6 (.96)
Fecal	3.2 (.16)	3.2 (.24)	4.2 (1.3)	2.6 (.20)
Switchgrass				
Omasal	4.6 (.12)	3.5 (.20)	4.8 (.16)	4.6 (.38)
Masticate	5.5 (.70)	12.3 (2.8)	5.0 (.10)	7.2 (2.2)
Fecal	4.1 (.16)	1.5 (.26)	4.4 (.16)	2.1 (.28)

Table 2. Percentage intake Rubisco (±S.E.) disappearing in the rumen and lower tract, and escaping the entire digestive tract of beef cattle grazing big bluestem or switchgrass in vegetative or elongation/reproductive stages of growth.

	Vegetative		Elongation/Reproductive	
Species	1995	1996	1995	1996
	%			
Disappearing in rumen				
Big bluestem	63.6 (3.4)	80.8 (6.8)	65.3 (13.3)	63.8 (9.0)
Switchgrass	49.6 (9.6)	84.1 (2.3)	39.3 (8.9)	47.9 (9.4)
Disappearing in lower trace				
Big bluestem	14.4 (.42)	10.6 (2.4)	14.7 (5.7)	19.5 (5.3)
Switchgrass	21.7 (8.9)	13.3 (2.4)	27.1 (3.8)	39.6 (.54)
Escaping entire trace				
Big bluestem	22.2 (3.0)	9.1 (3.4)	19.9 (7.6)	14.2 (2.3)
Switchgrass	28.7 (1.0)	4.8 (.9)	33.5 (6.0)	21.8 (3.2)

for switchgrass over stages of growth and years. Disappearance of Rubisco in the lower tract indicates a significant portion of ingested bundle sheath cells escape the rumen to be degraded in the lower tract. Bundle sheath cells entering the lower tract may be structurally weakened due to rumen activity and their contents, including Rubisco, may become available to digestive enzymes in the lower tract. Mean percentages of Rubisco escaping the entire digestive tract were above 10% for both species, except for the 1996 vegetative samples. A portion of bundle sheath cells apparently escaped the entire digestive tract.

Our results indicate a significant part of intake Rubisco escapes rumen degradation via bundle sheath cells and disappears in the lower tract. The Rubisco, which we used as a marker of bundle sheath cell integrity, represents only a portion of the available protein in bundle sheath cells. Because concentration of Rubisco in bundle sheath cells has not been determined for switchgrass and big bluestem, we cannot accurately estimate amount of protein escaping the rumen via these cells. Composition of bundle sheath cells and total soluble protein relative to Rubisco, however, has been determined for such warm-season, agronomic grasses as corn and millets. For example, we used the Rubisco concentrations in millet, along with our values of rumen-escape Rubisco, to determine if the amount of rumen-escape protein via bundle sheath cells was biologically significant. Estimates of rumen-escape protein ranged from 7% to 32% of the total crude protein content for switchgrass and 7% to 14% for big bluestem. Rumen-escape protein estimates were lower for the vegetative stage and higher for the elongation/reproductive stages. Our estimates of escape protein via bundle sheath cells are about 50% less than estimates of total rumen-escape protein for big bluestem and switchgrass reported in the literature. Our values are low compared to other estimates partially because we are not accounting for the non-bundle sheath cell protein that escapes. However, our example calculations indicate that the bundle sheath cell mechanism may account for as much as 50% of the rumen escape protein in big bluestem and switchgrass.

In conclusion, passage of bundle sheath cells from the rumen appears to provide a mechanism which allows protein escape from the rumen. Also, our results indicate Rubisco and associated proteins found in a portion of the escaping bundle sheath cells disappear in the lower tract. The amount of Rubisco and associated proteins escaping the rumen via bundle sheath cells could represent a significant portion of rumen-escape protein in big bluestem and switchgrass. Understanding the mechanisms involved in rumen escape protein should improve the efficiency of livestock feeding systems and assist in the selection of improved forage species.

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