

In Vitro Dry Matter Disappearance of Three Different Forage Sources

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ABSTRACT

Fresh 7 day growth Bermudagrass-clipping lawn pellets (*Cynodon dactylon* L.), alfalfa hay, sun-cured, mid-bloom pellets (*Medicago sativa* L.), and Coastal hay, sun-cured, 43-56 days growth bermudagrass hay pellets (*Cynodon dactylon* L.) were used to study the effects of forage source on dry matter disappearance in an in-vitro ruminal culture system. Rumen fluid was collected from a cannulated steer receiving 100% bermudagrass hay diet and transported to the Texas Tech University Ruminant Nutrition Laboratory. Tubes were prepared in triplicate with substrate and McDougall's Buffer. Incubation times of cultures with forage substrate were 12, 24, and 48 hours. As expected, there was a linear increase ($P < .0005$) in dry matter disappearance for both the Coastal hay pellets and the alfalfa pellets over the 12h, 24h, and 48h time periods. Furthermore, there was a significant ($P < .05$) quadratic increase in dry matter disappearance over the 12h, 24h, and 48h, time period for the Coastal hay pellets. However, while the bermudagrass-clipping lawn pellets had adequate dry matter disappearance, there was no difference ($P > .05$) between the 12h, 24h, and 48h time period.

Differences amongst treatment diets at hour 12 and hour 24 were different ($P < .05$) between the alfalfa pellets, Coastal hay pellets, and the bermudagrass-clipping lawn pellets. However, at hour 48, dry matter disappearance was higher for the alfalfa pellets, but was not significantly different for the Coastal hay diet or the bermudagrass-clipping lawn pellets. Therefore, this study indicates that alfalfa pellets are most digestible in an in vitro culture system followed by the bermudagrass-clipping lawn pellets, and the Coastal hay pellets.

Keywords: Ruminant Nutrition

INTRODUCTION

Many studies have discovered differences in digestibility between C_3 and C_4 plants. It has been well documented that C_3 plants, in most situations, are more digestible than C_4 plants. In general, C_4 plants contain a greater proportion of vascular bundles than C_3 plants whereas, C_3 plants have a larger proportion of leaf mesophyll tissues that have

large intracellular spaces that allows for digestion to take place more rapidly in the rumen (Hanna et al., 1973). C₄ plants also tend to produce more dry matter that is less digestible than C₃ plants.

Stage of maturity is also an important factor that plays a role in plant digestibility. As the plant matures, the amount of cellulose, hemi-cellulose, and lignin begin to increase. This causes the plant to become less digestible to the animal.

Common bermudagrass from domestic lawns and Coastal bermudagrass hay are C₄ plants. Alfalfa is a legume that is a C₃ plant. Research has indicated that alfalfa should be more digestible than the grasses. However, stage of maturity may play a big role in the digestibility of these forages. The objective of the study was to determine the digestibility of bermudagrass-clipping pellets, alfalfa pellets, and Coastal bermudagrass hay pellets.

MATERIALS AND METHODS

Bermudagrass-clipping lawn pellets, alfalfa pellets, and Coastal bermudagrass hay pellets were analyzed in an *in vitro* fermentation culture to determine dry matter disappearance. Chemical composition of bermudagrass-clipping lawn pellets, alfalfa pellets, and Coastal bermudagrass hay pellets is listed in Table 1. Dry matter disappearance was determined by a modified two-stage Tilley and Terry procedure (Galylean, 1997). Twenty liters of ruminal fluid were collected from a cannulated steer receiving a 100% forage diet, strained through four layers of cheesecloth to remove feed particles, and stored in a plastic container for transporting. Upon arrival, rumen fluid was buffered with McDougall's artificial saliva. Before ruminal fluid collection, approximately 0.5 g of each sample was placed in a 50-mL plastic centrifuge tube for digestion (triplicate tubes per sample). One part strained ruminal fluid was mixed with four parts McDougall's artificial saliva and 30 mL of mixed solution were added to each *in vitro* tube. Oxygen was displaced with the flow of CO₂ into the top of each tube and capped to maintain anaerobic conditions. Triplicate blank tubes for each incubation time were filled with 30 mL of one part strained ruminal fluid with four parts McDougall's artificial saliva and were incubated in 39° C water bath as for sample tubes. Samples were incubated for 12, 24, and 48 hours.

After incubation, samples were removed from water bath and frozen at -4° C to stop fermentation. Tubes were subsequently thawed at 22° C and centrifuged for 15 minutes at 2,000 x g. After centrifugation, ruminal fluid was suctioned off by vacuum, leaving undigested substrate and microbial residue in tube. Thirty milliliters of acidified pepsin solution were then added to each tube and incubated in 39° C water bath for 48 hours. The pepsin solution was prepared by adding 6.6 g of pepsin, and 100 mL of a 1 N HCl solution to a 1-L flask and filling to volume with deionized water. Following incubation with acidified pepsin, tube contents were filtered onto a pre-weighed Whatman 541 filter paper (Whatman International Ltd., Maidstone, England) using a Buchner funnel and vacuum pump. Filter papers containing the residue from each tube were dried in a 60° C drying oven for approximately 24 hours, after which filter papers were weighed to calculate IVDMD. The mean residue weight from the three blank tubes per incubation time was subtracted from each sample residue weight. The IVDMD values that were calculated for each incubation time were determined by dividing final

substrate weight – blank from each incubation period by initial substrate weight and multiplied by 100.

Table 1. Chemical Composition of Alfalfa Pellets (*Medicago sativa* L.), Bermudagrass-Clipping Pellets (*Cynodon dactylon* L.), and Coastal Bermudagrass Hay Pellets (*Cynodon dactylon* L.)^a

	Alfalfa	Coastal Hay	Bermudagrass
DM, %	92.81	92.1	89.25
Ash, % ^a	12.3	6.08	15.35
CP, % ^a	18.81	10.65	24.23
Ca, % ^a	1.19	0.43	1.12
P, % ^a	0.22	0.15	0.3
NDF, % ^a	45.52	72.31	58.51

^a DM Basis

Statistical Analysis: Time expressed as 12h, 24h, and 48h was analyzed as a completely randomized design in the GLM procedure of SAS (1999). Tube was considered the experimental unit with three replications per treatment group.

Treatment expressed as alfalfa pellets, Coastal hay pellets, and bermudagrass-clipping lawn pellets were also analyzed as a completely randomized design in the GLM procedure of SAS (1999). Tube was again considered the experimental unit with three replications per treatment group.

RESULTS

The effects of time at 12h, 24h, and 48h, was different ($P < .05$) for the alfalfa pellets and the Coastal hay pellets. The alfalfa pellets had a DM disappearance over the 12h, 24h, and 48h time period that was linear ($P < .0005$) but was not quadratic. The Coastal hay pellets were linear ($P < .0001$) as well as quadratic ($P < .05$). The bermudagrass-clipping lawn pellets were not different ($P > .05$) for dry matter disappearance over the 12h, 24h, and 48h time period. The bermudagrass-clipping lawn pellets were also not linear or quadratic ($P > .05$) for DM disappearance over the 12h, 24h, and 48h time frame. The effects of time on treatment for in vitro dry matter disappearance are listed in Table 2.

At hour 12, the alfalfa pellets had the highest amount of DM disappearance. The alfalfa pellets had a much higher ($P < .001$) DM disappearance than the coastal hay pellets and a slightly higher difference ($P < .05$) than the bermudagrass-clipping lawn pellets.

At hour 24, the alfalfa pellets became more different ($P < .0001$) than the Coastal hay pellets and the bermudagrass-clipping lawn pellets ($P < .0005$). The Coastal hay pellets and bermudagrass-clipping lawn pellets were also different ($P < .02$) for DM disappearance.

At hour 48, the alfalfa pellets were again different from the Coastal hay pellets ($P < .0001$) and the bermudagrass-clipping lawn pellets ($P < .0002$). The Coastal hay pellets and bermudagrass-clipping lawn pellets were not different ($P > .05$) at hour 48 for DM disappearance. The effects of treatment on time for in vitro DM disappearance are listed in Table 3.

Table 2. Effects of Time on Treatment for In Vitro Dry Matter Disappearance, %

Item	Time			SEM	Contrast	
	12h	24h	48h		Linear	Quadratic
Alfalfa Pellets	40.71 ^a	52.05 ^b	59.22 ^c	1.66	P<.0005	NS
Coastal Hay Pellets	23.94 ^a	26.51 ^b	34.50 ^c	0.711	P<.0001	P<.05
Bermudagrass-Clipping Pellets	34.28 ^a	34.27 ^a	39.24 ^a	2.3	NS	NS

^a Means within the same row with different superscripts differ ($P < .05$)

Table 3. Effects of Treatment on Time for In Vitro Dry Matter Disappearance, %

Item	Treatment			SEM
	Alfalfa Pellets	Coastal Hay Pellets	Bermudagrass-Clipping Pellets	
12h	40.71 ^a	23.94 ^b	34.28 ^c	1.84
24h	52.05 ^a	26.51 ^b	34.27 ^c	1.54
48h	59.22 ^a	34.50 ^b	39.24 ^b	1.66

^{a,b,c} Means within the same row with different superscripts differ ($P < .05$)

DISCUSSION

The ultimate indication of the quality of forages is obtained by feeding them and measuring the animal responses of interest, such as milk production or weight of gain. Such feeding trials account for all important factors, including unknown or unexpected factors (Collins and Fritz, 2003). However, feeding and grazing trials can become expensive. Therefore, laboratory chemical analysis can provide useful and relatively inexpensive information about potential animal responses.

In these analyses, alfalfa showed a linear increase in digestibility over time and was also more digestible than the Coastal hay pellets or the bermudagrass-clipping lawn pellets. Collins and Fritz (2003) stated that forage species can differ markedly in forage quality. In general, legumes are higher in quality than grasses. Therefore, results indicating higher digestibility of alfalfa *in vitro* agrees with these findings.

Coastal hay pellets also increased ($P < .05$) in DM disappearance over time showing a linear and quadratic increase in digestibility. Collins and Fritz (2003), state that grasses are generally higher in NDF than cool-season legumes. This may partially explain the lower digestibility of the Coastal hay pellets when compared to the alfalfa pellets (Table 1). Another factor to consider when explaining digestibility between the Coastal hay pellets and the alfalfa pellets is the difference between C3 and C4 morphology. While legumes are C3 plants, grasses such as coastal bermudagrass hay are C4 plants. C4 plants tend to have a greater percentage of area occupied by less digestible vascular bundles, epidermis, and sclerenchyma tissues than C3 plants (Akin and Burdick, 1975). Therefore, results of digestibility between the alfalfa pellets and Coastal hay pellets are not uncommon.

Results for bermudagrass-clipping lawn pellets were much different from alfalfa and Coastal hay pellets. While bermudagrass-clipping lawn pellets were more digestible than Coastal hay pellets and less digestible than alfalfa pellets, they were not more digestible over time. This suggests that the majority of digestion took place in the first twelve hours. One reason for this may be due to the maturity of the plant. Because the bermudagrass-clippings were harvested at such a premature state (7 day growth), digestibility of the forage was extremely high. Collins and Fritz (2003) stated that forage quality nearly always declines as forages age or undergo reproductive development. In fact, maturity at harvest is usually considered to be the primary factor affecting forage quality.

Other factors effecting digestibility of the bermudagrass-clipping lawn pellets may be the amount of NDF (Table 1). The bermudagrass-clipping pellets tend to have a higher amount of NDF than the alfalfa pellets but a lower percent of NDF than the Coastal bermudagrass hay. This would be expected because alfalfa is a C3 legume and the grasses are C4 plants. Legumes tend to have faster cell wall digestion and particle size reduction and therefore the rumen empties more quickly and allows more forage to be consumed. Ash and silica content could also further reduce digestibility (Johnson, et. al., 1998)

IMPLICATIONS

Alfalfa is a C3 legume that is more digestible than common bermudagrass from domestic lawns or Coastal bermudagrass hay which are both C4 grasses. The difference

in plant morphology, NDF, and plant maturity are major factors in the digestibility of these forage sources. While bermudagrass-clippings from domestic lawns were not as digestible as alfalfa, crude protein levels were higher. Therefore, more protein can be provided to the animal from the bermudagrass-clipping lawn pellets than from the alfalfa pellets.

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