PHOSPHORUS AND OTHER NUTRIENT DISAPPEARANCE FROM PLANTS CONTAINING CONDENSED TANNINS USING *IN SITU* AND MOBILE NYLON BAG TECHNIQUES

A Dissertation

by

SUZIKA PAGÁN RIESTRA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2009

Major Subject: Agronomy

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Approved by:

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ABSTRACT

Phosphorus and Other Nutrient Disappearance from Plants Containing Condensed Tannins Using *In Situ* and Mobile Nylon Bag Techniques. (December 2009)

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Dr. James P. Muir

Plants containing condensed tannins (CT) represent an alternative feed resource for ruminants. However, limited information regarding nutrient disappearance from these plants is available. Two experiments were conducted to evaluate phosphorus (P) and other nutrient disappearance from plants containing CT. In the first experiment, nutrient disappearance from three native Texas species (*Acacia angustissima* var. *hirta, Desmodium paniculatum, Smilax bona-nox*, and *Medicago sativa* as control) were evaluated using the mobile nylon bag technique. For the second experiment, ruminal degradation parameters, ruminal and post-ruminal disappearance of P and other nutrients from a browse containing CT (*Quercus virginiana*) were compared to species without CT (*Cynodon dactylon* cv. Tifton 85, and *Medicago sativa*).

Results from the first experiment indicate that the proportion of nutrient that disappeared during rumen, pepsin/HCl, or intestinal incubation differed among plant species and nutrient evaluated ($P<0.05$) and did not appear to be directly related to relative CT concentrations. Dry matter (DM), inorganic matter (IM), and organic matter (OM) disappearance were greater ($P<0.05$) during rumen incubation than at other stages

for all plants evaluated. Of the plants containing CT, *A. angustissima* demonstrated the greatest overall disappearance of DM, CP, P, and OM. A greater proportion of *A. angustissima* and *D. paniculatum* crude protein (CP) and P disappearance occurred in the intestines compared to *S. bona-nox* and *M. sativa*.

Plants evaluated in the second experiment differed ($P<0.05$) among all degradation parameters (rapidly degradable fraction, slowly degradable fraction, and fractional rate of degradation) for DM, OM, and IM. *Medicago sativa* and *C. dactylon* had high ruminal and post-ruminal nutrient disappearance ($P<0.05$) compared to *Q. virginiana*. The presence of CT appears to reduce total P disappearance and shift disappearance from the rumen to the intestines in some plants containing CT.

To my family:

Ruth Riestra Arroyo

Roberto Pagán Cruz

Roberto Pagán Riestra

Adrián Pagán Riestra

Carmen Cruz Jimenez

Roberto Pagán Quiñones

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NOMENCLATURE

ADFom	acid detergent fiber expressed exclusive of residual ash
ADLom	acid detergent lignin expressed exclusive of residual ash
CP	crude protein
CPD	crude protein disappearance
CT	condensed tannins
DM	dry matter
DMD	dry matter disappearance
ECT	extractable condensed tannins
FBCT	fiber bound condensed tannins
IM	inorganic matter
IMD	inorganic matter disappearance
NDFom	neutral detergent fiber expressed exclusive of residual ash
OM	organic matter
OMD	organic matter disappearance
P	phosphorus
PBCT	protein bound condensed tannins
PEG	polyethylene glycol
PD	phosphorus disappearance
TCT	total condensed tannins

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CHAPTER I

INTRODUCTION

In the past few years, the domestication of native plant species as forage sources has received considerable interest. Because of their adaptation to soil and climatic conditions, these species require few inputs (fertilization and or irrigation) to be productive. Moreover, these species are sought because of their multiple uses, namely cultivated pastures (Springer et al., 2001), wildlife plantings (Gee et al., 1994; Madison and Robel, 2001), and prairie restoration (Jackson, 1999).

Within the wider range of native species, a small but significant group of plants (trees, shrubs, vines, and forbs) contains condensed tannins (CT). In recent years the interest in nutritive value of these has grown due to the multiple benefits of CT for ruminants. Among the benefits associated to the presence of CT in ruminant diets are the reduction of bloat incidence, protection of crude protein (CP) from ruminal degradation, and the reduction of gastrointestinal parasites in small ruminants (Waghorn, 2008).

In Texas, the agronomic characteristics of a few warm season native herbaceous legumes containing CT have been evaluated (Muir et al., 2005, Muir et al., 2008a) while Ott et al. (2004) reported some browse species containing CT consumed by goats. However, limited information exists on the nutritive value of these feed resources.

This dissertation follows the style of the Animal Feed Science and Technology journal.

Evaluation of the nutritive values has been limited to *in vitro* dry matter digestibility (Muir et al., 2008b) and *in situ* CP disappearance (Pawelek et al., 2008) for some species. However, phosphorus (P) disappearance from plant containing CT has not been evaluated.

The objective of this investigation was to evaluate P and other nutrient disappearance in native plants containing varying concentrations of condensed tannins at different bovine gastrointestinal sites. In addition, the ruminal kinetics of P and other nutrient disappearance, and the post-ruminal disappearance of *Quercus virginiana* leaves were compared to common forages used in ruminant diets (*Cynodon dactylon* cv 'Tifton 85' hay, and *Medicago sativa* hay).

CHAPTER II

LITERATURE REVIEW

1. Functions of phosphorus in ruminants

Phosphorus has more known functions than any other mineral in the animal body (NRC, 2001). It is included in multiple molecules involved in most metabolic processes. This element participates in the energy transfer between cells through the molecules adenosine diphosphate and adenosine triphosphate. Additional functions of P includes: structure of cell membrane (phospholipids); cell signaling (i.e. cyclic adenosine monophosphate); and synthesis of deoxyribonucleic acid and ribonucleic acid.

Eighty percent of the P in animals is found in bones and teeth (NRC, 2001). The remaining P has structural and metabolic roles especially maintenance of osmotic pressure and acid-base balance (NRC, 2001). It is component of the saliva (600-800 mg P/L), which is important for neutralization of acidic condition in the rumen providing conditions for microbial growth. Also, represent 80% of endogenous P recycled from the gastrointestinal tract (Care, 1994).

Ruminal microorganisms (bacteria, protozoa, fungi and yeast) require P to maintain their metabolism and growth (Guyton et al. 2003). Durand and Komisarczuk, (1988) concluded that at least 5 g of P/kg organic matter fermented is required for optimal degradation of cell wall and protein synthesis.

2. Absorption of phosphorus in ruminants

Absorption in the rumen, omasum, and abomasum is minimal (Care, 1994). The main site for P absorption is the small intestine, particularly the duodenum (Hays and Swenson, 1993). More recently (Foote, 2009), found that a sodium-dependent secondary active inorganic P transport system is not responsible for P absorption in the proximal portion of the bovine small intestine while it does contribute to the P absorbed in the distal sections of the bovine small intestine. Several factors can influence the absorption of P, including the source of this mineral, calcium: P ratio, and intestinal pH (Hays and Swenson, 1993).

There are two mechanisms involved in P absorption: active transport, influenced by vitamin D (Horst, 1986), and passive transport. The passive transport occurs when a high concentration of P is consumed (Wasserman, 1981), while active transport occurs mainly when diets low or deficient in P are consumed.

3. Mineral availability in forages

Forage ability to supply minerals to ruminants depends on the mineral concentration and the bioavailability of the mineral. O'Dell (1984) defined mineral bioavailability as the proportion of the consumed mineral that is absorbed, transported to its site of action, and converted to a physiologically active form. Measurement of mineral concentration is relatively simple; however, measurement of the bioavailability of forage mineral for a specific function in the animal is difficult (Spears, 1994).

Field (1981) stated that mineral absorption is a function of two processes: the solubilization of minerals in the digestive tract and the fractional absorption of the solubilized fraction. Moreover, the minerals should be first released from the forage in a soluble form (Spears, 1994). Therefore, measurement of mineral solubility may be useful in predicting potential absorption (Field, 1981).

4. Rumen *in situ* disappearance techniques

The *in situ* technique has been used to study the release of minerals from forages and other plant material. Playne et al. (1972) used the technique to evaluate the disappearance of N, P, S and Ca in seed and pods of *Stylosanthes humilis*. They found that after 48 hr in the rumen, solubilization of P was higher compared to the other minerals; Ca was the lowest, while N and S varied in solubilization in relation of the fraction evaluated (seed or pods). The technique has also been used to detect differences between hays of different plants species (*Medicago sativa*, *Stylosanthes humilis*, *Chloris barbata*, and *Heteropogon contortus*) in terms of their mineral solubilization (N, S, P, Ca, Mg, K, and Na; Playne et al., 1978). The legume *Medicago sativa* had the highest proportion of all elements (except Ca and Na) solubilized, while the lowest proportion was observed in *Heteropogon contortus*, a grass. During the first 24 hr a rapid removal of minerals occurred after which the rate of removal tended to parallel the rate of dry matter (DM) digestion. In the evaluated legumes, N continued to be solubilized after 24 hr, as did the P in *M. sativa*. However, in the grasses, N, S, P, Ca, Mg, and Na increased in concentration in the residues after the 24 hr, which was related to their slower rate of

disappearance compared to DM. In another study (Rooke et al., 1983), grass silages showed a rapid release of minerals within 2 hr of rumen incubation. Moreover, the extent of release of this rapid fraction for each mineral was different, and were ranked as P < Zn < Ca < Cu < K< Mg < Na. No differences between minerals were detected in the slow release fraction.

More recent work, evaluating the mineral disappearance in tropical forages, confirms previous observations of differences among plant species. For example, creeping legumes tended to have lower ruminal disappearance of minerals than tree legumes or grasses (Serra et al., 1996).

5. Mobile nylon bag technique

Another technique used to evaluate the disappearance or solubilization of minerals is the mobile nylon bag technique. The advantage of this technique is that it allows the differential determination of nutrient disappearance in the rumen vis-à-vis post-ruminally. The technique was developed primarily in swine studies. However, Kirpatrick et al. (1984) and de Boer et al. (1987) began to use the technique in ruminants. Since that time the technique has been used to evaluate the disappearance of CP and amino acids (Berthiaume et al. 2000; Taghizadeh et al., 2005; Haugen, 2006), as well as starch (Noberg et al., 2007; Dehghan-Banadaky et al., 2008) from different feedstuffs.

To a lesser extent, the mobile nylon bag technique has been used to determine the disappearance or solubilization of minerals from forages or feedstuffs. Only three

studies reported the use of the technique (Emanuele et al., 1991; Riojas et al., 2008; Cherry et al., 2009). Emanuele et al. (1991) found differences among forage species: in general total Ca and P solubilization were greater in legumes vs. grasses species, while total Mg and K solubilization was similar for both groups of forages. In addition, they identified the rumen as the major site of release, except for Ca of dwarf elephantgrass. Based on the maximum extent of release the minerals were ranked as follows: K > Mg > P > Ca.

Cherry et al. (2009) evaluated P disappearance from alfalfa hay, Coastal and Tifton 85 hay, corn silage and ground corn. According to this study, the ruminal + intestinal P disappearance were: ground corn (99.2%); alfalfa hay (94.8%); corn silage (92.2%), Tifton 85 hay (85.4%), and Coastal hay (84.5%). In addition, it was reported that more than 75% of the P in the feedstuffs disappeared in the rumen. Another study using the nylon bag technique (Riojas, 2008) reported that P disappearance in alfalfa and coastal bermudagrass decreased as the maturity (14 to 35 days) of the forage increased. However, regardless of the maturity, P disappearance was greater in the rumen, once again demonstrating that the rumen is the main site for mineral disappearance.

6. Tannins

Tannins are a group of compounds that can be found in a wide range of plant material including grasses, legumes, and other forbs (Waghorn, 2008). These naturally occurring plant polyphenols combine with proteins and other polymers such as cellulose, hemicellulose and pectin, to form stable complexes (Makkar, 2003). Initially the tannins were used to transform animal hides into leather because tannins cross-link to the

collagen chains of hides giving a durable leather resistant to microbial attack (Mangan, 1988).

Based on the chemical composition, tannins can be divided in two groups: hydrolysable and CT. The hydrolysable tannins are esters of phenolic acid and a polyol, usually glucose. Phenolic acid within the molecule can be either gallic acid in gallotannins or other phenolic acid derived from the oxidation of galloyl residues in ellagitannins. Condensed tannins or proanthocyanidins are the second classification, which are polymers of flavan-3-ol units. This group of tannins can be distinguished by the yield of anthocyanidins when heated in acidic media.

Most of the ruminant studies done with CT have been related to their ability to bind proteins, but also to evaluate its anthelmintic value. To a lesser extent, tannin metal complexes have also been evaluated. In their review, Santos-Buelga and Scalbert (2000) stated that the number of functional groups in one molecule of CT determines its essential properties: formation of complexes with proteins, formation of chelates with metal ions and reducing capacity. The structure of CT provides a large number of free phenolic groups that are able to form hydrogen bonds with proteins and carbohydrates, while also complexing with proteins through hydrophobic binding.

7. Biological effects of condensed tannins

7.1 Control of gastrointestinal parasites

Athanasiadou et al. (2000) evaluated the inclusion of different levels of Quebracho (*Schinopsis* spp.) extract (0, 30 and 60 g/kg fresh matter) in sheep diets

during an intestinal infection with *Trichostrongylus colubriformis*. Those sheep that consumed 30 and 60 kg of extract reduced their fecal egg counts compared to the control diet. In another study, the inclusion of Quebracho extract (representing 5% of the diet DM decreased *Haemonchus contortus* egg excretion (Paolini et al., 2003). Moreover, the investigators concluded that reduction in egg excretion was due to a decrease in female fecundity and not a decrease in worm number.

7.2 Protein utilization

The binding ability and strength of tannins to protein complexes depends on the characteristics of both the tannin and the protein, namely molecular weight and structure (Silanikove et al., 2001). Hagerman and Butler (1981) described the characteristics for protein with high affinity for tannins, which include large size, open structure, high proportion of hydrophobic amino acids and high proline content.

7.3 Bloat control

In several trials, CT has been demonstrated to reduce bloat potential in forages. Supplementation (by ruminal cannula) of quebracho CT to steers grazing wheat forage improved animal performance and minimized bloat frequency without deleterious effects to the animals (Min et al., 2006). This is related mainly to a reduction in ruminal microbial activities, biofilm production, and gas production as a result of CT presence.

7.4 Mineral utilization

The influence of CT or other polyphenols on the gut absorption of minerals is limited compared to other nutritional parameters, but may also be significant.

Fe. Condensed tannins have been described as chelators of Fe^{+3} (Santos-Buelga and Scalbert, 2000) which then reduce the bioavailability of Fe for intestinal absorption. This relationship has been evaluated primary in humans, considering the risk of anemia that high consumption of CT can cause. In addition, the CT has been considered as a method to treat iron overload.

Mg, P, and Ca. In trials with New Zeland rabbits, Al-Mamary et al. (2001) fed diets containing low or high tannin sorghum grain (1.4 or 3.5 % catechin equivalent of tannin, respectively) during 4 weeks. The inclusion of tannin sorghum grain did not affect the apparent absorption of Mg. When increased levels of tannins (0, 5, 15, 20 and 25 g/kg DM in diet) and microbial enzyme supplement were fed to broiler chickens an improvement of P ileal digestion was observed while the apparent digestibility of Ca was unaffected (Iji et al., 2004).

Waghorn et al. (1994) fed *Lotus pedunculatus* to sheep and Scharenberg et al. (2007) used polyethylene glycol (PEG) to evaluate the effect of CT from dehydrated or ensiled *Onobrychis viciifolia* (sainfoin) on mineral digestibility. They observed that the PEG treatment in the diet increased the apparent digestibility and retention of Mg, P, and Ca in lambs. Explanation for the change in digestibility and retention was related to the binding of minerals with the CT of the sainfoin.

8. Mineral release from plant material containing condensed tannins

Previous research clearly indicates that CT can affect the utilization of minerals by animals. However, limited information exists on how CT in plant material affects the disappearance of nutrients, particularly P, within the ruminant digestive tract.

Northup et al. (1995) found a strong relationship between release rates of mineral and organic forms of N from *Pinus muricata* litter and litter polyphenols concentrations. As the polyphenol concentrations increased, release rate decreased. This demonstrates that polyphenols have the capability of affecting nutrient release from their own source.

CHAPTER III

PHOSPHORUS AND OTHER NUTRIENT DISAPPEARANCE FROM PLANTS CONTAINING CONDENSED TANNINS USING THE MOBILE NYLON BAG TECHNIQUE

1. Introduction

There is increasing interest in warm season forages other that grasses to improve ruminant nutrition. Perennial forbs and browse, especially legumes can extend the grazing season and contribute more nutrients such as CP to ruminants than grasses (Bowman et al., 1991). These types of plants may also contain CT (Muir et al., 2008b). These naturally occurring plant polyphenols combine with proteins and other polymers such as cellulose, hemicellulose and pectin, to form stable complexes. In their review, Santos-Buelga and Scalbert (2000) stated that the number of functional groups in one molecule of CT dictates its essential properties: formation of complexes with proteins, formation of chelates with metal ions, and other reducing capacities. Most of the studies done with CT have focused on their ability to bind proteins (Hagerman and Butler, 1981; Silanikove et al., 2001) or to evaluate their anthelmintic value (Paolini et al., 2003).

Furthermore, Hess et al. (2006) found that addition of CT to lamb's diets shifted N excretion from the urine to the feces. The authors suggested that shift may contribute to mitigate ammonia N emission from animal excreta, which is an environmental concern in concentrated animal operations. Utilization of CT as an approach for P management has not been evaluated to date. To consider future inclusion of CT in

ruminant diets for nutrient management (especially P), first we must understand how the presence of forage CT affects nutrients degradability in the ruminant gastrointestinal tract.

Our hypothesis was that presence of forage-produced CT affects the extent and site of nutrient, particularly P, disappearance from forage in the bovine gastrointestinal tract. To test this we evaluated the nutrient disappearance of three rangeland plants containing CT using the mobile nylon bag technique in rumen and duodenal fistulated steers compared to *M. sativa* (alfalfa) which have no CT.

2. Methodology

2.1 Plant material

The plant material evaluated consisted of leaves from native Texas *Acacia angustissima* var. *hirta* (prairie acacia), *Desmodium paniculatum* (panicled tick-clover), and *Smilax bona-nox* (greenbriar) compared to *Medicago sativa* (alfalfa), all collected in Stephenville, Texas, USA. Leaves were dried at 55°C for 48 hr, then ground to pass a 2-mm screen in a sheer mill (Wiley Arthur H. Thomas Co., Philadelphia, PA, USA), and stored for later chemical analysis.

2.2 Animals and diet

Two steers with ruminal cannula and two steers fitted with a duodenal intestinal cannula (Streeter et al., 1991) were used for the experiment. Animals were fed a diet consisting of sorghum-Sudan (*Sorghum sudanense* x *S. bicolor*) hay previously

harvested at the Texas AgriLife Research Center at Stephenville, TX USA. Two weeks of adaptation to the diet preceded the rumen and duodenal incubations.

2.3 Incubation

Dried and ground forage samples (2 g) were weighed into dacron bags (ANKOM, Macedon, NY, USA; 50±15 μ porosity, 5 x 10 cm size) to give a surface area:sample ratio of 20 mg:cm^2. Similar to the study by Emanuele et al. (1991), 12 bags per forage were ruminally incubated for 24 hr. Ruminal incubation was replicated five days for a total of 60 bags per species. Bags were inserted randomly into two steers (with ruminal or duodenal fistula) and animals were not used as replications (Haugen et al., 2006; Cherry et al, 2009). At each of these times, once bags were removed from the rumen they were placed in ice water to stop microbial fermentation, and hand washed. For each species, four of the 12 bags within each replication were frozen for later chemical analysis of ruminal nutrient disappearance. The remaining eight bags were placed in a pepsin/HCl solution (Tilley and Terry, 1963) at 37°C and stirred continually for 1 hr. After removal from pepsin/HCl solution, the bags were washed in distilled water and four of the eight bags were frozen for later analysis of ruminal + pepsin/HCl nutrient disappearance. The four remaining bags were used for the intestinal incubation. Four bags of each forage from each of the five replications were used for this last stage. Bags were individually inserted into the duodenal cannula every 30 min and retrieved from the feces using a fecal collection bag, washed until rinse water was clear, and frozen for later analysis of ruminal + pepsin/HCl + intestinal nutrient disappearance.

2.4 Laboratory analysis

Residual material from bags (n=4) from each incubation site for each replication were batched for each forage within each replicate (time N=5) and ground to pass through a 1-mm screen and analyzed for DM (AOAC, 1990; id 934.01), inorganic matter (IM; AOAC, 1990; id 930.05), and organic matter (OM). Phosphorus was determined by a colorimetric method (APHA, 1995). Nitrogen concentrations were determined from samples digested by a Dumas method (AOAC, 1990; id 968.06) using an Elementar Vario Macro C:N analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). Crude protein was calculated using the concentration of N multiplied by a factor of 6.25 (AOAC, 1990). Concentrations of CT fractions were determined as described by Terrill et al. (1992). Condensed tannins from each species were used to develop the standard curve for each species' CT concentrations (Wolfe et al., 2008). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of original samples were measured by a sequential procedure with modifications to standard protocols (Robertson and Van Soest, 1981; Van Soest et al., 1991) by using the ANKOM 200 Fiber Analyzer (ANKOM Technology, Fairport, NY USA; Ankom, 2009). The NDF was assayed with sodium sulfite and with alpha amylase, while ADL was assayed with sulfuric acid. The NDF, ADF and ADL were expressed without residual ash.

2.5 Statistical analysis

Data were statistically analyzed using the PROC MIXED procedure with steers as the random variable in SAS (SAS, 1991). Dependent variables consisted of DM

disappearance (DMD), CP disappearance (CPD), P disappearance (PD), IM disappearance (IMD), and organic matter (OMD). Independent variables considered included plant species, incubation site, replication, and plant species by incubation site interaction. Differences were considered significant at $P < 0.05$. When significant differences were detected, the LSMEANS statement was used to separate multiple means by using the PDIFF option (SAS, 1991).

3. Results

Medicago sativa leaves had greater concentration of CP, P, and IM than the other plant species evaluated (Table 1). Nutrient and CT concentration from plant species containing CT were variable (Table 1).

3.1 Dry matter and organic matter disappearance

A similar pattern in disappearance was observed for DM and OM. *Medicago sativa* had the greatest DMD and OMD in the rumen, while *D. paniculatum* had the lowest (Table 2). For all the species, DMD and OMD occurred primarily in the rumen, and secondly in the intestine. Minimal disappearance occurred during the pepsin/HCl incubation for all the species. Coefficients of total DMD in the three CT-containing

Table 1. Nutrient composition and condensed tannins (CT) fractions from plant species evaluated.

	Plant species			
Item[1]	*M. sativa*	*A. angustissima* var. *hirta*	*S. bona-nox*	*D. paniculatum*
Nutrients (g/kg DM)				
CP	283	193	121	153
P	1.52	1.01	0.97	0.72
IM	93.7	36.9	63.3	60.5
OM	906	963	937	940
NDFom	231	190	373	274
ADFom	154	118	225	184
ADLom	25.7	38.7	58.2	30.4
CT fractions (g/kg DM)				
ECT	nd[2]	37.6	172.7	201.9
PBCT	nd	4.6	26.6	52.7
FBCT	nd	2.9	4.1	6.8
TCT	nd	45.1	203.4	261.4

[1]CP, Crude protein; P, phosphorus; IM, inorganic matter; OM, organic matter; NDFom, neutral detergent fiber corrected for organic matter; ADFom, acid detergent fiber corrected for organic matter; ADLom, acid detergent lignin corrected for organic matter; CT, condensed tannins; ECT, extractable CT; PBCT, protein bound CT; FBCT, fiber bound, CT; TCT, total CT.
[2]nd, non detected.

Table 2. Nutrient disappearance coefficients of plant species evaluated at three incubation sites.

		Incubation site			
Item[1]	Plant species	Rumen	Pepsin/HCl	Intestine	SED[4]
DM	*M. sativa*	0.837A[2]a[3]	0.018b	0.049Bb	0.013
	A. angustissima var. *hirta*	0.435Ba	0.011c	0.188Ab	
	S. bona-nox	0.424Ba	0.011c	0.075Bb	
	D. paniculatum	0.273Ca	0.008c	0.080Bb	
CP	*M. sativa*	0.928Aa	0.015b	0.025Cb	0.014
	A. angustissima var. *hirta*	0.314Ba	0.002b	0.343Aa	
	S. bona-nox	0.266Ca	-0.037c	0.179Bb	
	D. paniculatum	0.148Da	-0.033b	0.176Ba	
P	*M. sativa*	0.895Aa	0.078Bb	-0.024Cc	0.038
	A. angustissima var. *hirta*	0.189Cb	0.091Bc	0.520Ab	
	S. bona-nox	0.501Ba	0.091Bc	0.176Bb	
	D. paniculatum	-0.021Db	0.286Aa	0.221Ba	
OM	*M. sativa*	0.821Aa	0.010b	0.042Bb	0.030
	A. angustissima var. *hirta*	0.409Ba	0.008c	0.146Ab	
	S. bona-nox	0.413Ba	0.008c	0.068Bb	
	D. paniculatum	0.258Ca	0.005c	0.063Bb	
IM	*M. sativa*	0.877Aa	0.032Cb	0.003Ab	0.017
	A. angustissima var. *hirta*	0.497Ca	0.206Ab	-0.127Bc	
	S. bona-nox	0.617Ba	0.141Bb	-0.108Bc	
	D. paniculatum	0.415Da	0.196ABb	-0.264Cc	

[1]DM, dry matter; CP, crude protein; P, phosphorus; OM, organic matter; IM, inorganic matter.
[2]Different uppercase letters within column represent significant differences ($P<0.05$) for each nutrient.
[3]Different lowercase letters within row represent significant differences ($P<0.05$) for each nutrient.
[4]SED: standard error of difference.

plants equaled from 0.36 to 0.67 of that measured in alfalfa (0.904). *Acacia angustissima* var. *hirta* had the greatest coefficient of DMD in the intestine (0.19) compared to 0.049 to 0.08 for the other three species.

3.2 Crude protein disappearance

The rumen was the main site for CPD. However, for *A. angustissima*, and *D. paniculatum,* similar coefficients of CPD were observed during ruminal and intestinal disappearance. In addition, there was an accumulation of CP during incubation in pepsin/HCl for *S. bona-nox* and *D. paniculatum*.

3.3 Phosphorus disappearance

Medicago sativa and *S. bona-nox* plant material released the majority of their P in the rumen. By contrast, for *A. angustissima* the main site for PD was the intestine, while for *D. paniculatum* similar disappearance was observed during the pepsin/HCl, as well in the intestinal incubation. In addition, an accumulation of P occurred in *D. paniculatum* material during ruminal incubation and for *M. sativa* during the intestinal incubation.

3.4 Inorganic matter disappearance

Inorganic matter disappeared mainly in the rumen for all species evaluated, followed by disappearance in pepsin/HCl solution. Greater disappearance

occurred in plant species containing CT while incubated in pepsin/HCl solution compared to *M. sativa*. For all species, an accumulation of IM occurred in the intestine.

4. Discussion

4.1 Effect of plant species and digestion site on nutrient disappearance

The extent of DMD and OMD after 24 hr in the rumen was greater for *M. sativa* compared the other species evaluated in this experiment (Table 2). The lower rumen DMD and OMD in *A. angustissima*, *D. paniculatum*, and *S. bona-nox* could be associated with the presence of CT in these plant species (Acero et al., 2008; Muir et al., 2008b; and Pawelek et al., 2008). In this particular case, *D. paniculatum* had greater concentrations of CT (Table 1) than *A. angustissima*, and *S. bona-nox*. There is no specific mechanism to describe the effect of CT on forage digestion. However, research have demonstrated the ability of CT to bind to proteins (Kariuki and Norton, 2008), ruminal microorganisms (Min et al., 2005) or enzymes produced by microorganisms (Bae et al., 1993); which help to understand the reduction of CT on forage digestion.

For the legumes included in our study, rumen DMD coefficients ranged from 0.273 to 0.837, but disappearance also took place post-ruminally. Similar ranges of DMD were reported by Mupangwa et al. (2003) when evaluating the rumen and post-ruminal degradability of tropical legumes. This indicates that degradation by rumen microorganisms has greater influence on DMD than does post-ruminal (mainly enzymatic) activity (Mupangwa et al., 2003). In addition, most of the post- ruminal disappearance occurred in the intestine rather than during pepsin/HCl incubation.

The proportion of CP that disappeared at each gastro-intestinal location varied with plant species. In the case of *M. sativa*, coefficient of CPD in the rumen was 0.928. This value is in agreement with a Haugen et al. (2006) study, and indicates that protein from *M. sativa* was available for rumen microorganism utilization with less protein from this species escaping rumen degradation than in the CT-containing legume species.

For the plant species containing CT, proportion of CP disappearing at each stage of digestion differed. For example, *D. paniculatum* and *A. angustissima* had similar coefficients for ruminal and intestinal disappearance of CP. These results suggest that some protein from the plants containing CT evaluated in our study can escape ruminal degradation and become available to intestinal digestion. This has been observed previously with *Lotus corniculatus* (Waghorn et al., 1987) and *Onobrychis viciifolia* (Jones and Mangan, 1977). The effect of CT on protein metabolism in ruminants has been related to the ability of these molecules to form tannin-protein complexes (Jones and Mangan, 1977). These tannin-protein complexes inhibit the fermentation of forage protein to ammonia in the rumen, increasing the amount of protein that reaches the small intestine (Barry et al., 1986; Waghorn et al., 1987). Protein that escapes degradation in the rumen can improve protein deposition in meat or milk, which helps reduce N losses to the environment (Aerts et al., 1999). Our research adds to this knowledge by indicating that coefficients of CPD that occurs in the rumen is not strictly related to forage species CT concentrations. Protein and CT complexes have been described as dependent on the characteristics of both molecules, especially their structure, molecular weight and compatibility with binding sites (McAllister et al., 2005; Reed, 1995).

In vitro studies suggest that at low pH (<3.5) CP bound to CT is released (Jones and Mangan, 1977). However, contrary to what was expected for forages containing CT, CPD was minimal during the pepsin/HCl incubation. A recent in vivo study reported that protein was released from the CT complex between the abomasum and terminal ileum (Kariuki and Norton, 2008). In this experiment CPD in the intestine was observed after the pepsin/HCl incubation, which suggests that perhaps the presence of other compounds (pancreatic enzymes or minerals) or its combination with acidic environment are required to release the CP from CT complexes.

Release coefficients of P from *M. sativa* (0.895) and *S. bona-nox* (0.501) indicate that P release occurred mainly in the rumen. Similarly, greater proportions of P from *M. sativa* hay disappearing in the rumen were reported by Emanuele et al. (1991). They concluded that the rumen was the major site for PD for grasses and legumes. Our results indicate that this is not always the case. In the case of *A. angustissima*, the majority of PD occurred during intestinal incubation (0.52). Differences in PD location in the gastro-intestinal tract by *A. angustissima,* and *D. paniculatum* in our study indicate that the presence of CT or the type (molecular weight) of CT molecule present could affect the site and extent of disappearance of this mineral. As in the case of protein-CT complexes, effect of CT in PD can depend on the properties of the CT and the molecules containing P. Moreover, previous studies have confirmed the ability of CT to bind ribulose 1,5-bisphosphate carboxylase (Rubisco; Min et al., 2000; McAllister et al., 2005) which contains P. Therefore, it could be expected that if CT form a complex with

Rubisco or other molecule containing P, reducing its degradation, then at the same time reduce P disappearance.

The possible presence of P-containing CT complexes could explain lower PD in the rumen and during incubation for *D. paniculatum* vis-à-vis the other species in our study. Our findings could be partially confounded by the contamination of the residue with microbial cells that were not totally dislodged during washing of the bags (Ledoux and Martz, 1991; Ibrahim and Zemmelink, 1999) but such large differences in PD location between *M. sativa* and plants containing CT cannot be totally attributed to such a phenomenon.

The majority of IMD occurred in the rumen for all plant species in our study. Emanuele et al. (1991) likewise concluded that the rumen is the major site for Ca, Mg, K, and P disappearance. Incubation of bags with pepsin/HCl contributed to the release of an additional 0.03 to 0.21 units of IM in the species containing CT. Limited IMD in *M. sativa* occurred in the intestine (Table 2). By contrast, in the case of CT-containing species, there were net increases in IM during intestinal incubation. Less IMD was observed for plants containing CT during intestinal incubation than for *M. sativa*. Chemical binding of IM to an indigestible molecule, physical enclosure within fiber matrix, or lack of a concentration gradient between the residue and the digestive tract may affect the release or accretion of elements (Playne et al., 1978). In addition, Emanuele et al. (1991) found a decrease in Ca and Mg release (or an increase in sequestration, as they termed it) after ruminant intestinal incubation, which these authors associated to decreases in pH. In our experiment, CT could have contributed to further

accretion of IMD in the intestines, considering the well-documented binding ability of these molecules. However, depression in disappearance of specific elements has been observed in plants not containing CT, for example in *Heteropogon contortus* (Playne et al., 1978).

4.2 Effect of other plant components on nutrient disappearance

Minerals present in the cell wall are likely to have lower nutritional availability for ruminant nutrition (Whitehead et al., 1985). For example, grasses and legumes contain approximately 1.5-2.0% N as part of the lignin (Van Soest, 1994) which is considered unavailable to the animal. In this study, disappearance of CP and P were greater in *M. sativa* than in *A. angustissima* var. *hirta*, and considerable differences in cell wall component concentrations exist among these species (Table 1). In the case of *S. bona-nox*, the extent of disappearance of CP and P was greater than in *D. paniculatum* despite the greater cell wall concentration of *S. bona-nox*. These relative differences suggest that something besides the relative concentration of fiber fractions affected the nutrient disappearance.

Another compound that sometimes affects the nutrient disappearance is phytic acid. Not only is the P of this molecule not available for non-ruminant utilization, but it can also form complexes with protein, thereby reducing its solubility (Laurena et al., 1994). However, phytic acid is not a concern in forages, since they are normally found in low concentrations (Clark et al., 1986). Moreover, in ruminants, phytic acid does not have the same constraints as in non-ruminant species, because some ruminal bacteria

demonstrate phytase activity (Yanke et al., 1998), rendering associated P available for absorption.

The afore-mentioned components could limit the disappearance of nutrients. However, it is less likely to be the major reason for the large reduction in nutrient disappearance or shift in site of disappearance observed in the present study.

4.3 Implications for P management

Understanding of PD from forages is important to improve the efficiency of P utilization in ruminants. Rumen *in situ* techniques provide some information about what occurs in this compartment; however, our results indicate that for the plant material containing CT, the rumen is not the main site for PD, despite the fact that most DMD, OMD and IMD occurred in the rumen. As a result, PD values derived only from rumen *in situ* studies could result in underestimated values, which can lead to over-feeding this mineral. The mobile nylon bag technique allows not only a determination of the extent of PD, but also the site where it occurs. Such information can be used to select forages based on their ability to release P and where they released it. Furthermore, more precise rations can be formulated using the information from this technique, which can contribute to minimizing the pollution potential associated with overfeeding P. Field (1981) indicated that utilization of minerals is dependent of two processes: (1) release of the mineral from its source, and (2) absorption in the digestive tract. Moreover, that author indicated that absorption of P is mainly a function of the fraction of the mineral that is solubilized in the digestive tract prior to the absorption site. This point to the

importance of evaluating the disappearance of P from forages, not just in the rumen but in the intestinal tract as well.

5. Summary

Knowledge of nutrient release dynamics can guide selection of plants when formulating appropriate rations for ruminants. The study demonstrated that the presence of forage-produced CT in *A. angustissima* var. hirta, *D. paniculatum*, and *S. bona-nox* appeared to directly or indirectly affect the extent and site of nutrient degradation in bovine gastrointestinal tract. As plant CT concentration increased, PD in the rumen decreased. However, further research is needed to evaluate the implications of the shift in location of disappearance of this mineral in ruminants.

Condensed tannins from *A. angustissima* var. hirta, and *D. paniculatum* changed the main site of PD from the rumen to the intestine. This conclusion raises additional questions. For example, will the presence of CT improve the absorption of P by the animal? If it does not improve its utilization by ruminant; will the CT-bound P, and make it less soluble in the feces, reducing P losses as runoff? Research to answer to these questions will contribute to a better understanding the relationship of CT to P, and the potential use of CT in nutrient or waste management.

CHAPTER IV

EVALUATION OF PHOSPHORUS AND OTHER NUTRIENT DISAPPEARANCE FROM PLANT MATERIAL CONTAINING CONDENSED TANNINS USING *IN SITU* AND MOBILE NYLON BAG TECHNIQUES

1. Introduction

Shrubs and browse containing CT represent a feed resource for ruminants especially when quantity or quality of grasses and other forages are limited. Studies evaluating nutrient disappearance of these types of feed resources focus mainly on DM, CP or cell wall disappearance (Kaitho et al., 1993; Ramírez et al, 2000). Nevertheless, considering the roles of P in many metabolic, neurological and cellular functions of the animal (NRC, 2001), it is also important to evaluate the P disappearance from forages and other feeding resources high in CT.

Phosphorus disappearance in ruminants from feeds such as corn, soybean, cereal by-products (Bravo et al., 2000; Fathi Nasri et al., 2006; Mjoun et al., 2008; Cherry et al., 2009), and from forages (Playne et al., 1978; Eys and Reid, 1987; Emanuele and Staples, 1990; Ledoux and Martz, 1991; Flachowsky et al., 1994; Ajayi et al., 2009; Cherry et al., 2009) have been documented. However, few data exist on P disappearance rates from shrubs and browse species. Pagán Riestra et al. (2009) evaluated the P disappearance from herbaceous legume species containing CT and reported not only differences in the extent of P disappearance among species but also along the digestive tract.

Quercus species are considered a CT-containing shrub or tree browse feed resource in tropical and subtropical region for ruminants (Makkar and Singh, 1991a; Makkar and Singh, 1991b; Yousef Elahi and Rouzbehan, 2008; Doce et al., 2009). In more temperate regions such as North America, *Quercus* spp. are also abundant (Vines, 1960). *Quercus virginiana* can be found from southeastern Virginia to Florida, and central Texas (Vines, 1960). However, nutrient disappearance from this specie or its potential as a feed resource has not been evaluated. The objective of this study was to compare the ruminal kinetics and the post-ruminal disappearance of P and other nutrients from *Quercus virginiana* leaves (live oak, a specie containing CT) with two common forages that have no CT, *Cynodon dactylon* cv. Tifton 85 (bermudagrass) hay and *Medicago sativa* (alfalfa).

2. Methodology

2.1 Plant material

The plant material evaluated consisted of *Medicago sativa* and Tifton 85 hay, species that do not contain CT and are commonly used as ruminant feed in North America; these were compared to leaves from *Quercus virginiana* which contain CT (Acero et al., 2008) and is often found in the diet of two browsers, white-tailed deer (Gee et al., 1994) and goats (Packard et al., 2007). Samples from *M. sativa* and 'Tifton 85' hay were collected from multiples bales, while *Q. virginiana* leaves were collected from at least five trees. All plant material was collected in Stephenville, Texas, USA. Samples were dried at 55°C until weight loss cease, then ground to pass a 2-mm screen

in a sheer mill (Wiley Arthur H. Thomas Co., Philadelphia, PA, USA) and stored for later chemical analysis.

2.2 Animals and diet

Two steers with ruminal cannula and two steers fitted with a duodenal intestinal cannula (Streeter et al., 1991) were used for the experiment. Hay of Tifton 85 was available ad libitum throughout the experiment. Nutrient composition of the hay was 86.4 g CP/kg, 678.5 g NDF/kg, 327.2 g ADF/kg DM. Two weeks of adaptation to the diet preceded the rumen and intestinal incubations.

2.3 Incubations

Ground forage samples (2 g) were weighed into dacron bags (ANKOM, Macedon, NY, USA; 50±15 μ porosity, 5 x 10 cm size) to give a surface area:sample ratio of 20 mg/cm^2. The samples were incubated in the rumen at multiple times (0, 2, 4, 6, 12, 24, 48, and 72 hr) with 10 bags of each material per each time period for each steer. Once bags were removed from the rumen, they were placed in ice water to stop microbial fermentation. Bags were machine washed three times for 5 min (Von Keyserlingk et al., 1998). Five bags were frozen for later analysis of ruminal nutrient disappearance and to determine ruminal kinetics. The remaining five bags for the times 0, 12, 24, and 48 hr were placed in Daisy^{II} Incubator (ANKOM Technology, Fairport, NY) jars with pepsin/HCl solution (Tilley and Terry, 1963) to provide constant agitation and temperature (37°C) during 1 hr. After removal from pepsin/HCl solution, the bags

were washed in distilled water and used for intestinal incubation. Bags were individually inserted into the duodenal cannula every 30 min, and retrieved from the feces using a fecal collection bag, bags were machine washed (as previously described) and froze for later chemical analysis. This procedure was repeated twice.

2.4 Laboratory analysis

Samples were batched for each forage within each replicate, time period, or incubation site and ground to pass through a 1-mm screen and analyzed for DM (AOAC, 1990; id 934.01), IM (AOAC, 1990; id 930.05), and OM. Phosphorus was determined by a colorimetric method (APHA, 1995). Nitrogen concentrations were determined from samples digested by a Dumas method (AOAC, 1990; id 968.06) using an Elementar Vario Macro C: N analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). Crude protein was calculated using the concentration of N multiplied by a factor of 6.25 (AOAC, 1990). Concentrations of CT were determined as described by Terrill et al. (1992) and reported as extractable (ECT), fiber-bound (FBCT) and protein-bound (PBCT). Extracted CT from each species was used to develop the standard curve for each species' CT concentrations (Wolfe et al., 2008). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of original samples were performed by the sequential procedure of Van Soest et al. (1991) by using the ANKOM 200 Fiber Analyzer (ANKOM Technology, Fairport, NY USA; Ankom, 2009). The NDF was assayed with sodium sulfite and with alpha amylase, while ADL was assayed with sulfuric acid. The NDF, ADF and ADL were expressed without residual ash.

2.5 Calculations and statistical analysis

2.5.1 Rumen in situ degradability

Ruminal disappearance (g/kg) of each individual nutrient from the nylon bag at any specific time was equal to the amount of the nutrient in the sample minus the amount of the nutrient remaining after incubation for any specific time divided by the amount of the nutrient in the sample. Ruminal nutrient disappearance from each period (n=2) was fitted using a non-linear degradation equation: $D = a + b^{(1-e-ct)}$ given by Ørskov and McDonald (1979). Where D is disappearance after time t, *a* is the slowly degradable fraction, *b* is the rapidly degradable fraction, and *c* is the fractional rate of degradation for *b*. Difference in estimated ruminal parameters between plant species evaluated were analyzed using the PROC MIXED procedure in SAS (SAS, 1991) with steer as a random variable. Differences were considered significant at $P < 0.05$. When significant differences were detected, the LSMEANS statement was used to separate multiple means by using the PDIFF option (SAS, 1991).

2.5.2 Ruminal and post-ruminal disappearance

Ruminal disappearance (g/kg) after 0, 12, 24, and 48 hr of incubation for each individual nutrient was calculated as mentioned above. Post-ruminal disappearance after 0, 12, 24, and 48 hr of ruminal incubation (g/kg) of each individual nutrient was equal to the amount of the nutrient in the residue after rumen incubation minus the amount of the nutrient remaining after post-ruminal incubation divided by the amount of the nutrient in

Table 3. Nutrient composition and condensed tannins (CT) fractions.

Item[1]	Plant species		
	M. sativa	*Q. virginiana*	*C. dactylon*
Nutrient (g/kg DM)			
CP	148.5	87.0	89.9
P	2.7	1.5	1.4
OM	868.2	885.9	888.8
NDF	436.4	409.2	688.1
ADF	345.7	287.5	323.8
ADL	89.7	104.6	34.9
CT fraction (g/kg DM)			
ECT	nd[2]	3.4	nd
PBCT	nd	1.6	nd
FBCT	nd	0.3	nd
TCT	nd	5.3	nd

[1]CT, condensed tannins; CP, crude protein; P, phosphorus; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; ECT, extractable condensed tannins; PBCT, protein bound condensed tannins; FBCT, fiber bound condensed tannins; and TCT, total condensed tannins.
[2]nd, non detected.

the residue after rumen incubation. Data was analyzed using the PROC MIXED procedure in SAS (SAS, 1991).with steer as a random variable. Differences were considered significant at $P < 0.05$. When significant differences were detected, the LSMEANS statement was used to separate multiple means by using the PDIFF option (SAS, 1991). Dependent variables consisted of DM, P, CP, OM, and IM disappearance. Independent variables were plant species, incubation site, ruminal incubation time, and their interaction. Differences were considered significant at $P < 0.05$. When significant differences are detected, the LSMEANS statement was used to separate multiple means by using the PDIFF option (SAS, 1991).

3. Results

3.1 Chemical composition

Medicago sativa hay was characterized by high CP and P concentrations (Table 3). *Quercus virginiana* leaves and *C. dactylon* hay had similar CP, P, and OM concentrations. Both fiber fractions were lower in *Q. virginiana* than in *M. sativa* or *C. dactylon*; however, *Q. virginiana* had the greatest concentration of ADL 104.6 g/kg DM. Only *Q. virginiana* leaves contained CT, which consisted of 3.4, 1.6, and 0.3 g/kg DM of ECT, PBCT, and FBCT, respectively.

Table 4. Ruminal degradation parameters from plant species evaluated.

Item[1]	Plant species	a (g/kg)	b (g/kg)	L (h)	c (/h)
DM	*M. sativa*	367.6b[2]	330.3b	3.2a	0.188a
	Q. virginiana	594.7a	127.4c	1.0b	0.057b
	C. dactylon	274.1c	620.5a	1.1b	0.044b
	SEM	11.3	13.1	0.64	0.02
OM	*M. sativa*	303.2ab	339.9b	2.7	0.157a
	Q. virginiana	451.8a	100.9c	3.5	0.051b
	C. dactylon	214.2b	611.7a	1.1	0.046b
	SEM	37.9	14.9	0.8	0.042
P	*M. sativa*	536.7	161.4a		0.042
	Q. virginiana	437.0	47.5b		0.085
	C. dactylon	590.8	152.3a		0.017
	SEM	38.3	14.4		0.015
CP	*M. sativa*	353.5a	473.7		0.128
	Q. virginiana	136.9b	610.4		0.070
	C. dactylon	132.1b	744.6		0.027
	SEM	38.8	165.0		0.023
IM	*M. sativa*	666.3a	173.7		0.010
	Q. virginiana	221.4b	184.6		0.197
	C. dactylon	553.4a	325.6		0.015
	SEM	38.8	65.4		0.010

[1]*a*, rapidly degradable fraction; L, lag time; *b*, slowly degradable fraction; *c*, fractional rate of disappearance for the slowly degradable fraction; DM, dry matter; OM, organic matter; P, phosphorus; CP, crude protein; IM, inorganic matter.
[2]Different letters within column for each nutrient represent significant differences ($P<0.05$) for each nutrient.

3.2 Ruminal degradation parameters

Plants evaluated differed among all degradation parameters for DM, OM, CP, P, and IM. On a DM basis, the rapidly degradable (*a*) fraction was at least 23% greater for *Q. virginiana* than *M. sativa* and *C. dactylon* (Table 4). The slowly degradable fraction (*b*) was at least 29% greater in *C. dactylon* than *M. sativa* or *Q. virginiana*. Fractional rate of degradation for the slowly degradable fraction (*kd*) was greater for *M. sativa*, but no difference were observed between *Q. virginiana* and *C. dactylon*. *M. sativa* had a greater lag time (3.2 h) than *Q.virginiana* (1.0 h) and *C.dactylon* (1.1 h). For OM, the rapidly degradable (*a*) fraction was at least 15% greater for *Q. virginiana* than *M. sativa* and *C. dactylon*; however, no difference was detected among the last two plant species. For the slowly degradable fraction, and fractional rate of degradation, the OM followed the same pattern as the DM. However, no differences were detected for the time lag. *M. sativa* and *C. dactylon* had similar rapidly degradable fraction for IM. However, no differences were detected for the slowly degradable fraction and the fractional rate of degradation. Only the P slowly degradable fraction was different among the plant species evaluated. While for CP, difference was only detected for the rapidly degradable fraction.

3.3 Ruminal disappearance

Ruminal disappearance was affected by the interaction of length of incubation in the rumen with plant species (Table 5). Within plant species and across time disappearance of DM, OM, IM for all plants, and CP for *M. sativa* and *C. dactylon*

Table 5. Ruminal disappearance at different incubation times.

Item[1]	Plant	Ruminal Incubation Time (hr)			
		0	12	24	48
DM	*M. sativa*	294.1A[2]c[3]	559.0Ab	616.8Aa	625.3Ba
	Q. virginiana	283.2Ad	333.7Bc	364.4Cb	388.8Ca
	C. dactylon	105.4Bd	340.1Bc	487.2Bb	641.9Aa
P	*M. sativa*	739.9Aa	712.5Aa	539.3Ab	605.5Aab
	Q. virginiana	504.6Cb	536.3Ba	499.4Ab	550.7Aa
	C. dactylon	642.5Ba	465.6Cb	446.0Ab	636.3Aa
CP	*M. sativa*	332.9Ac	688.5Ab	842.6Aa	766.4aAb
	Q. virginiana	231.6Aa	393.4Ba	444.5Ba	383.0Ba
	C. dactylon	304.3Ab	419.6Bb	702.1Aa	694.9Aa
OM	*M. sativa*	347.1Bd	616.6Ac	671.8Ab	699.1Aa
	Q. virginiana	388.0Ad	413.9Bc	448.1Cb	471.5Ba
	C. dactylon	174.1Cd	418.6Bc	562.1Bb	711.5Ca
IM	*M. sativa*	715.3Ab	819.4Aa	824.8Aa	802.2Aa
	Q. virginiana	261.7Cb	393.1Ca	354.5Ca	366.6Ba
	C. dactylon	591.5Bc	609.2Bc	638.8Bb	696.0Aa

[1]DM, dry matter; P, phosphorus; CP, crude protein; OM, organic matter; IM, inorganic matter.
[2]Different uppercase letters within column represent significant differences ($P<0.05$) for each nutrient.
[3]Different lowercase letters within row represent significant differences ($P<0.05$) for each nutrient.

increased as length of ruminal incubation increased. However, ruminal PD did not follow this pattern and increased continuously for all plant species while ruminal CPD for *Q. virginiana* did not differ across time.

Within incubation time (0, 12, 24, and 48 hr) *M. sativa* had greater ruminal disappearance, except at 0 hr for OM (*Q. virginiana* had the greatest), and CP in which no differences were detected. No defined pattern of ruminal disappearance for DM, P, CP, OM, and IM was observed for *Q. virginiana* and *C. dactylon*; however, in most cases *C. dactylon* had the second greatest extent of ruminal disappearance.

3.4 Post-ruminal disappearance

As with ruminal disappearance, the post-ruminal disappearance was affected by the interaction of length of incubation in the rumen with plant species (Table 6). Post-ruminal disappearance within plant species decreased for DM, CP, and OM. Phosphorus post-ruminal disappearance within plant species, and IMD for *C. dactylon* increased across time.

4. Discussion

Nutrient concentrations in forages vary according to plant species, soil type, season, and stage of maturity. Moreover, those factors also affect the release of nutrients from forages during its passage through the digestive tract of ruminant. *In situ* techniques provide a method to determine factors that can affect the nutrient disappearance from forages, a precondition for its utilization by animals.

Table 6. Post-ruminal disappearance after different ruminal incubation times.

Item[1]	Plant	Ruminal Incubation Time (hr)			
		0	12	24	48
DM	*M. sativa*	239.6A[2]a[3]	79.4Bb	60.3Bb	74.7Ab
	Q. virginiana	83.8Ca	58.2Cb	57.1Bb	51.0Bb
	C. dactylon	125.6Ba	119.7Aa	87.4Ab	53.2Bc
P	*M. sativa*	159.8Ab	250.6Aab	338.9Aab	437.9Aa
	Q. virginiana	147.1Ab	135.5Bb	188.1Bab	338.3Aa
	C. dactylon	122.3Ac	256.1Ab	356.9Aa	306.2aAb
CP	*M. sativa*	458.3Aa	100.7Bb	71.3Bb	33.9Cb
	Q. virginiana	415.2Aa	392.1Aa	308.4Aa	234.2Aa
	C. dactylon	482.9Aa	385.5Aa	330.7Aab	167.3Bb
OM	*M. sativa*	215.4Aa	63.5Bb	48.5Ab	34.8Ab
	Q. virginiana	77.3Ba	71.8Ba	60.8Aa	38.6Ab
	C. dactylon	107.8Ba	100.2Aa	68.3Ab	35.6Ac
IM	*M. sativa*	100.1Aa	73.3Aa	82.8Aa	102.2Aa
	Q. virginiana	20.8Ca	16.8Ba	33.1Ba	33.3Ba
	C. dactylon	51.0Bb	57.3ABab	73.0Aa	59.3Bab

[1]DM, dry matter; P, phosphorus; CP, crude protein; OM, organic matter; IM, inorganic matter.
[2]Different uppercase letters within column represent significant differences ($P<0.05$) for each nutrient.
[3]Different lowercase letters within row represent significant differences ($P<0.05$) for each nutrient.

4.1 Ruminal degradation parameters

Estimated parameters for ruminal DM and OM disappearance were similar to those reported by Kasuya et al. (2008) and Flachowsky et al. (1994) for *M. sativa*, and different from those reported by Mandebvu et al. (1999) for *C. dactylon*. In all studies, *Medicago sativa* is characterized by similar rapidly and slowly degradable fractions and a high rate of disappearance, while *C. dactylon* has a small rapidly degradable fraction, a greater slowly degradable fractions, and a slow rate of disappearance. These patterns are associated with the characteristics and chemical composition of these two forages. *Medicago sativa* hay has a higher proportion of leaves compared to a grass, which have greater soluble content. The greater slowly degradable fraction in the grass is related to its greater cell wall concentration but low ADL values. In contrast, the lower concentrations of NDF and ADF compared to *C. dactylon*, suggest that *Q. virginiana* is high in soluble material, which explains the greater soluble degradable fraction estimated for this species compared to the other two. However, this species also had the lowest slowly degradable fraction compared to *M. sativa* and *C. dactylon*, for both DM and OM. The high concentration of ADL (104.6 g/kg DM) or presence of CT (5.3 g/kg DM of TCT) can help explain the reduced degradability for *Q. virginiana*.

Lignin is recognized as a limiting factor in digestion of the cell wall in the rumen (Jung and Deetz, 1993). Lignin can form complexes with the fiber matrix, CP, and even minerals, decreasing it solubility from the forage (Jung and Fahey, 1983). On the other hand, it has been demonstrated that CT binds to proteins (Kariuki and Norton, 2008), ruminal microorganisms (Min et al., 2005) or enzymes produced by microorganisms

(Bae et al., 1993), all contributing to the reduction of forage digestion due to the presence of CT.

Ajayi et al. (2009) reported ruminal degradation parameters for P of 180 g/kg for the rapidly degradable fraction, 320 g/kg for the slowly degradable fraction, and 0.042 hr^{-1} for the fractional rate of disappearance in the grass *Panicum maximum*. In addition the authors reported ruminal degradation parameters for P from several herbaceous legumes which included 270-300 g/kg for the rapidly degradable fraction, 510-520 g/kg for the slowly degradable fraction, and 0.068-0.072 hr^{-1} for the fractional rate of disappearance. In the present study the parameters were greater for the slowly degradable fraction, while the slowly degradable fraction and its rate of disappearance were lower; for both the legume (*M. sativa)* and grass (*C. dactylon*). Bromfield and Jones (1972) indicated that of the total P in ground plant material, 60 to 83% is water soluble. This could explain the greater rapidly degradable fraction for P. Moreover, several authors (Emanuele and Staples, 1990; Ibrahim et al., 1990; Serra et al., 1996) have reported P disappearances at 0 hr from 15 to 83%, which can be related to the rapidly degradable fraction, and point the great variability of this parameter for P. The lower slowly degradable fraction of *Q. virginiana* could be associated with the presence of CT. Pagán Riestra et al (2009) reported lower ruminal disappearance of P from plant species containing CT when compared to *M. sativa.* However, as mentioned earlier, high level of ADL could also contributed to the reduced disappearance of this mineral.

The rapidly degradable fraction of CP in the legume and grass of the present study were lower that those reported by Yu et al. (2004) for *M. sativa* and *Phleum*

pratense. Interestingly, the mentioned fraction in *Q. virginiana* was similar to *C. dactylon*; even the first specie contains CT and greater ADL. The fractional rate of degradation for the slowly degradable fraction in *M. sativa* and *C. dactylon* were different to those reported by Yu et al. (2004).

Even when samples were washed mechanically, a method that helps to reduce microbial contamination, samples could be contaminated. This could explain the greater standard error observed for some ruminal parameters for both P and CP.

4.2 Ruminal and post-ruminal disappearance

As observed previously by Pagán Riestra et al. (2009) with *M. sativa* and Cherry et al. (2009) with Coastal and Tifton 85 hay, rumen DMD is greater than post-ruminal disappearance. This indicates that degradation by rumen microorganisms has greater influence on DMD than does post-ruminal (mainly enzymatic) activity (Mupangwa et al., 2003).

The mobile nylon bag provides a method to measure nutrient disappearance at different compartment of the digestive tract. The methodology includes incubation of bags in the rumen for a specific time followed by the post-ruminal incubation. Our results suggest that length of ruminal incubation affect the post-ruminal disappearance for some species. Among plant species, more variability was observed in *C. dactylon* for the nutrients evaluated. While for *M. sativa* and *Q. virginiana,* no differences were detected within species after 12 hr in the rumen and post-ruminal incubation, except for P and OM for *Q. virginiana.* Considering the potential for contamination of the forage

samples with material of microbial origin as ruminal incubation time increased (Flachowsky et al., 1994), it is recommended to incubate samples in the rumen for 12 hr when using the mobile nylon bag technique.

Contrary to what was previously observed with browse containing CT (Pagán Riestra et al., 2009) the presence of these molecules in *Q. virginiana* did not cause a shift in site of CP and P disappearance. The lower concentration of TCT compared to *Acacia angustissima*, *Desmodium paniculatum*, and *Smilax bona-nox* (Pagán Riestra et al., 2009) may explain the lack of shift in site disappearance. Another possibility is that the type of CT molecule present may differ. Condensed tannins complex with other molecules, a relationship described as dependent on the characteristics of both molecules, especially their structure, molecular weight and compatibility with binding sites (Reed, 1995; McAllister et al., 2005).

The intestinal disappearance of DM and nutrients decreased as the ruminal incubation time increased. González et al. (1999) previously reported this pattern for nitrogen, which is related to the enrichment in indigestible nitrogen of feed particles which affects the extent of rumen degradation.

Phosphorus, CP, and IM disappearance rates indicate that *Q. virginiana* breaks down poorly during both rumen and post-ruminally incubations compared to *M. sativa* and *C. dactylon* which had a high disappearance in both incubation sites. Results indicate that these latter two forages provide P, CP, and IM to rumen microorganisms and still provide significant amounts post-ruminally available for animal utilization.

5. Summary

Results indicate that *M. sativa* and *C. dactylon* have high ruminal and post-ruminal nutrient disappearance rates compared to *Q. virginiana* leaves. Even though CP, P, NDF and ADF concentration were similar among *Q. virginiana* and *C. dactylon*, disappearance patterns were different. Such behavior could be attributed either to the presence of CT or to the high concentration of ADL.

CHAPTER V

CONCLUSIONS

Results demonstrated that presence of forage-produced CT in *A. angustissima* var. *hirta*, *D. paniculatum*, and *S. bona-nox* affected the extent and site of nutrient disappearance in bovine gastrointestinal tract. Rumen disappearance of CP and P decreased as the concentration of CT increased; however, the intestinal disappearance of those nutrients increased. Further research is needed to evaluate if a shift in nutrient disappearance from plants containing CT, particularly P would contribute to improve P utilization in ruminants.

In addition, it was demonstrated that *M. sativa* and *C. dactylon* have greater ruminal and post-ruminal nutrient disappearance compared to *Q. virginiana* leaves. The presence of CT or the high concentration of ADL in *Q. virginiana* leaves could be the reason for the difference in the ruminal kinetics parameters and reduction in nutrient disappearance.

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