THE APPLICATION OF FORAGE DENSIFICATION TECHNOLOGIES TO FEEDING SYSTEMS FOR SMALL RUMINANT PRODUCTION IN THE CARIBBEAN

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ABSTRACT

Determining the potential of densified forage as a prospective substitute to commercial feed in small ruminant production systems in the Caribbean is critical. This may lead to the development of more self-sufficient feeding systems which incorporate more locally available ingredients and reduce the dependence on imported commercial feeds. There is currently a lack of information on the use of, or effect of, densified forage on performance in regional small ruminant production. Additionally, information is limited on the nutritive value of prospective forages, to which these technologies can be applied. Therefore, the aims of the thesis were to 1) determine the nutritive value of a range of tropical forages in the Caribbean that are used in regional small ruminant production systems; 2) determine the effect of densified diets comprising different levels of forage on intake in growing lambs; and 3) determine the effect of densified diets comprising forage on growth performance and digestibility in lambs. The results of the thesis showed that there is a range of forages of varying nutritive value that can be used to develop more sustainable feed systems for small ruminants in the Caribbean. Further, the results of the thesis showed that when *Trichanthera gigantea* (an abundantly available forage) was densified and fed to growing lambs, it resulted in similar intakes, digestibility and growth performance in growing lambs to that of commercial concentrates. The findings of the research can be used as a platform for future studies on the application of densification technologies to feeding systems for small ruminants in the Caribbean.

DEDICATION

I dedicate this thesis to God my creator, my source, my strength, my courage, my wisdom and guide. Without His grace, enduring this very intense doctoral programme will not have been possible. I also dedicate this work to my parents, Harrington Jack and Miriam Jack, who have not held back their love, prayers, words of encouragement and overall support. To my dearest sister, Ria Jack, for her love, patience and good sense of humour which often lightened up the very intense period of my data collection. Ria sacrificed many things, including her weekends, to ensure that I had the on-farm support required for the duration of the data collection activities. To my other siblings Ted, Andre, Harrington-Junior, Legena and Rhonda, who have all been very supportive of me throughout the process. To the late Dr Norman Gibson who was always like a second father and a mentor to me. His patience, kindness and trustworthiness were inspiring. He was the epitome of a true leader/mentor. To my dearest friends from my church community including Michelle Chloke, Allison St. Brice, Takiyah Gordon and Nandi Mitchell for their encouragement and words of wisdom offered throughout the process.

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CHAPTER 1 General Introduction

The global demand for animal protein has increased and, by 2050, there will be an additional two billion people, raising this demand by 60 to 70% (FAO-UN, 2014). This observed trend is similar for the Caribbean Community (CARICOM) which may be a direct result of rising affluence and urbanisation, as well as a rapidly increasing population projected to rise from 18 million to 22 million by 2050 (Delgado et al., 2001; FAO-UN, 2014; Valdes et al., 2017). Further, small ruminants, including sheep and goats, are a significant component of the agricultural landscape in the Caribbean region and there is a high demand for animal protein from small ruminants (CFC and FIGMDP, 2010; FAO-UN, 2014; Hosein et al., 2013; Lallo, 2009; Lallo et al., 2016b). Nevertheless, local meat production from the sector only meets 20 to 25% of the regional demand and some of the major constraints to production may require addressing, particularly the high dependence on costly imported concentrate feeds (Lallo et al., 2016b; Singh et al., 2006b; Valdes et al., 2017). The increasing complexity and uncertainty in global grain markets, competing demands for grain supply (biofuel), higher frequencies of extreme weather events and growing foreign exchange volatility within the Caribbean Community (CARICOM), all increase the risks of depending on imported feed ingredients (Gaughan et al., 2009; Lallo, 2015; Prakash and l'agriculture, 2011). One approach to addressing this challenge is increasing the utilisation of locally available feeds, including forages, which may allow for the development of more sustainable feeding systems for small ruminants in the Caribbean (Avril et al., 2011; Hughes et al., 2013; Miller et al., 2003).

However, forages have various challenges which must be treated with, to improve their utility in regional, small ruminant, production systems. For example, unpredictable yields is a critical challenge of forages given the increasingly pronounced dry periods that are now a feature of Caribbean agriculture (Hughes et al., 2012; Lallo et al.,

2016b). Further, the labour intensive nature of harvesting forages (John et al., 2010; Palmer et al., 1998) and the difficulties with storage and transportation are other challenges of the resource (Tumuluru et al., 2011; Tumuluru et al., 2010a). Moreover, inconsistent quality which is dependent on climate, geographic location, soil conditions, harvesting time and cultural practices are other challenges of forages (Assefa and Ledin, 2001; Ball et al., 2001; Newman et al., 2006). These may all deter the adoption of forage as an alternative to imported concentrate feed despite its high cost. In fact, approximately 62% of regional farmers use concentrates that account for up to 65% of the total feed rations (Lallo, 2009). This suggests that the Caribbean small ruminant production systems cannot be built simply on the introduction of highly nutritive tropical forages. Therefore, there is a need for the adoption of a strategy that treats with these challenges.

Forage conservation technologies, including pellets and cubes, have been used to address some of the previously outlined challenges of forage systems (ASABE, 2016; Tumuluru et al., 2011). This technology has been used to preserve abundant high-quality forages yielded during the wet periods for improved access and use during the drier resource-scarce months (ASABE, 1997; Dougnon et al., 2012; Hau, 2014). Further, the technology improves the storage and transportation of bulky materials through conversion into more dense forms (Orden et al., 2014; Tumuluru et al., 2011; Wanapat et al., 2013). Additionally, densified feeds are nutrient-dense and are associated with improved animal performance compared to feed offered in fresh low-bulk density forms (Coleman and Lawrence, 2003; El-Deek and Brikaa, 2009b; Hau, 2014). This technology has been explored across both temperate and tropical farm systems, however, it has been seldom examined or applied to small ruminant production systems in the Caribbean. Therefore, the main objective of this study is to

determine the effect of forage densification (pelleting) on feeding systems for small ruminants in the Caribbean and the related aims are to:

1. Review the literature on small ruminant production systems in the Caribbean and on the application of forage densification technologies to feed systems for ruminant production;
2. Identify the range of forage species available and used in small ruminant production systems in the Caribbean and to describe these based on their nutritive value;
3. Determine the nutritive value and effect of a densified diet comprised of different levels of forage on intake in growing lambs;
4. Determine the effect of a densified diet comprised of forage on growth performance and digestibility in lambs; and
5. To discuss the findings of the previous chapters and to conclude with recommendations on the way forward.

CHAPTER 2 Literature Review

2.1 Overview

The Governments of the Caribbean Community (CARICOM) regard small ruminants as significant to Caribbean agriculture (CFC and FIGMDP, 2010; Lallo, 2015; Singh et al., 2006a). The commodity is an important source of animal protein and calorie intake for the region and many derive income from it (Asiedu, 2001; FAO-UN, 2014). Further, small ruminants provide a model of livestock production which complements the geophysical and socioeconomic construct of the Caribbean islands (Hosein et al., 2013; Mohammed, 2013). For instance, small ruminants have a lower requirement for land space compared to large ruminants, making them more suitable for the limited land resources of the Small Island Developing States (SIDS) of CARICOM (Maharaj and Singh-Ackbarali, 2014). Additionally, the ability to thrive on marginal forages and the overall low investment per head when compared to large ruminants, makes the species suitable for small-scale farming systems which are common to the region (Lallo et al., 2016b). Small ruminants provide a more economically viable livestock production system due to the lower dependence on costly grain imports compared to the swine and poultry livestock classes (Terrill, 1986). They are a key part of regional cultural events and celebrations, as well as cuisine (Lallo et al., 2016b). With respect to religion, there are no religious taboos attached to small ruminant products which restrict their consumption (FAO-UN, 2014; Hosein et al., 2013; Singh et al., 2006a). Despite the great potential of the commodity, the region remains a large importer of small ruminant meat and meat products with a mere 25% of consumption being met by local production (Lallo, 2015). One of the current efforts of CARICOM involves reducing the food import bill of US $5 billion, and, in 2011, the import of meat and meat products accounted for 10% of this bill (CARCIOM, 2020). Improving regional production requires treating with the major challenges of the sector, a critical one being

the high dependence on costly imported concentrate feeds (FAO-UN, 2014; Hosein et al., 2013; Singh et al., 2006a). Currently, feed inputs account for over 60% of the cost of production for regional small ruminant systems (Singh et al., 2006a). The overall high cost of production has resulted in reduced price competitiveness when compared to that of imports (see Table 2.1) (Singh et al., 2006a).

Country	Average cost of regionally produced Mutton/Chevron (Wholesale (USD/kg))	Average cost of imported Mutton (Wholesale (USD/kg)) [a]	Average cost of imported Chevron (Wholesale (USD/kg)) [a]
Trinidad and Tobago	11.17	7.91	5.61
Jamaica	8.88	5.62	3.32
Barbados	8.8	5.59	-
St Lucia	7.68	4.42	2.12
Guyana	6.61	3.35	-
Belize	5.87	2.61	-
St. Vincent and the Grenadines	5.17	1.91	-

Table 2.2 Comparison of costs between regionally produced and imported mutton/chevron

Source: Singh et al. (2006a)

[a] Cost includes 15% CET (Common External Tariffs) and Importers' margin

Forages have been identified as a potential alternative to costly feed imports. (Hernández and Sánchez, 2014; Patersen et al., 1992; Valdes et al., 2017). Although there are many challenges related to the production, harvesting and feeding of forages, their utility in small ruminant systems may be improved through the application of forage densification technologies. However, there is a lack of information on the use or effect of forage densification on regional feed systems for small ruminants. Therefore, the objectives of the literature review are to:

1) Define and describe small ruminant production systems within the Caribbean; and
2) Assess the potential of forage densification for use in sheep and goat production systems by:
    i. Outlining the various ways to regulate the nutritive value of forage inputs prior to processing
    ii. Describing forage densification technologies and the impact of these on ruminant production systems

2.2 Characteristics of small ruminant production systems within the Caribbean

2.2.1 Scale of agriculture operations

The land resources of the islands of the English-speaking CARICOM is less than that of the larger producers of small ruminants including Australia and New Zealand (See Tables 2.2 and 2.3). The comparably lower and more vulnerable land resources prompt the need for more rigorous management systems which encourage efficient utilisation of these limited resources (Lallo et al., 2016b).

Table 2.2 Land distribution for the Caribbean region and developed Oceania

Country	Total Land Area (ha)	Total Agriculture Land (ha)
Antigua and Barbuda	44,000	9,000
Bahamas	1,001,000	14,000
Barbados	43100	14,000
Belize	2,296,600	160,000
Cayman Islands	26,400	2,700
Dominica	75400	25,000
Grenada	34,400	8,000
Guyana	19,685,000	1,680,000
Jamaica	1,083,000	444,000
Saint Kitts and Nevis	26,100	6,000
Saint Lucia	61,600	10,600
Saint Vincent and the Grenadines	38,900	10,000
Trinidad and Tobago	512,800	54,000
Australia	76,823,000	365,913
New Zealand	26,331,000	11,116, 000

Source: (FAOSTAT, 2018; Trading Economics, 2018)

Table 2.3 Farm size distribution across the Caribbean

Size (acres)	%
Landless	2.2
1	33.3
> 1 to 5	26.9
>5 to 15	17.1
>15	20.6

Source: (Lallo et al., 2009; Lallo et al., 2016b)

2.3 Production systems

There are three main types of production systems for small ruminants within the Caribbean. These are intensive, semi-intensive and extensive production systems (Hernández and Sánchez, 2014; Lallo et al., 2009; Lallo et al., 2016b).

In extensive production systems, animals are left to graze and browse all day with minimal to no supplemental feeding (Hernández and Sánchez, 2014). These systems are associated with minimal management and are often low cost as a result (Hernández and Sánchez, 2014). However, the generally poor quality of pasture and overgrazing results in lower production rates (Singh et al., 2006a). Additionally, there is a higher risk of loss through praedial larceny and dog predation (Hernández and Sánchez, 2014). In addition to the increasing number of farmers who are either landless or have limited land resources, these challenges mean that such a system is becoming less popular with a minority of 13% farms practicing extensive sheep and goat production (Lallo, 2009).

Intensive systems are those where animals are housed throughout the day and forages are fed primarily through cut and carry. One of the challenges with cut and carry is that forages offered are typically selected on a volume rather than nutritional basis which may impact negatively on animal performance (Hernández and Sánchez, 2014; Singh et al., 2006a). Based on a region-wide survey conducted on small ruminant production systems, approximately 34% of farmers practice intensive production (Lallo, 2009). Additionally, there is the semi-intensive production systems where animals are left to graze pastures primarily during the day and are housed at night where the risks of praedial larceny and dog predation increase (Hernández and Sánchez, 2014). Lallo (2009) estimated that a total of 61% regional farmers operate under such systems. Overall, approximately 97% of farms within the Caribbean implement housing structures for animals (Lallo, 2009).

Of all the systems present, the intensive or variations of it (semi-intensive systems), are becoming more popular across the region (Hernández and Sánchez, 2014). These systems are costly as they require more investment for the construction and maintenance of housing, as well as the purchase of costly concentrate feeds which support higher production to offset investment costs (Hernández and Sánchez, 2014; Thomas, 1997).

2.4 Breeds of sheep and goats found within the region

Increasing meat production has been the primary objective of small ruminant farming systems in the Caribbean (Asiedu, 2001; Mohammed, 2013). As a result, most sheep

and goats in the region are crossbred primarily as a part of the regional strategy to improve meat production (Hernández and Sánchez, 2014; Mohammed, 2013).

The main type of sheep found in the Caribbean is the tropical hair sheep. Some of the major breeds include the Barbados Blackbelly, Virgin Island White, West African, Katahdin and the Blackhead Persian (CARDI, 2006b; Thomas, 1997). The most popular sheep breed used across CARICOM is the Barbados Blackbelly which was developed in the region (Hernández and Sánchez, 2014). This is the preferred breed because of the high prolificacy, high resistance to internal parasites and the superior meat quality (Hernández and Sánchez, 2014; Thomas, 1997). However, the Barbados Blackbelly is a smaller breed (live weight (adult ram): 60 to 90 kg; live weight (adult ewe): 40 to 60 kg) with low growth rates (90 g/d) (DAGRIS-IS, 2005; Thomas, 1997). Consequently, the Barbados Blackbelly is often cross-bred with the Dorper which has high growth rates (average daily gain: 180 g/d ; average weaning weight: 36 kg (three to four months)) and the Katahdin which is a larger breed (Adult ram: 82 to 144 kg; Adult ewe: 54 to 73 kg) (DAGRIS-IS, 2005; Thomas, 1997). This results in the production of animals with characteristically higher carcass yield (Hernández and Sánchez, 2014). Unlike the West African and the Virgin Island White, the more prolific Barbados Blackbelly requires high quality feed and careful management making them more suited for intensive systems where the nutrition and health of these animals are carefully managed.

Some of the main goat breeds found in the region include the Native Creole Goat, Boer, Saanen, Anglo Nubian, British Alpine and Toggenburg (CARDI, 2006a). The Native Creole goat and the Boer are meat breeds and low maintenance animals (Lallo, Unpublished). In addition to the meat breeds, there are dairy breeds which are high maintenance animals which are typically housed and demand more nutrient-dense

diets to meet their higher nutrient requirements (Lallo, Unpublished). The British Saanen, British Toggenburg, Anglo Nubian and British Alpine are some of the main dairy breeds (CARDI, 2006a). The Anglo Nubian and British Alpine are dual purpose animals which are reared for milk and meat (CARDI, 2006a). The Saanen, Toggenburg and Alpine are high-milk producers and the combination of greater energy efficiency, higher milk production and good temperance make these some of the more preferred breeds in the region (Lallo, Unpublished).

2.5 Nutrition and Feeding

Within the Caribbean and the wider tropics, feed is the principal factor limiting performance (Devendra, 1986; Patersen et al., 1992; Singh et al., 2006a). Therefore, inefficiencies in feed management often limit the full expression of the genetic potential of animals.

2.5.1 Forage resources in the Caribbean

Forages are a significant source of food for sheep and goat production systems in the Caribbean (CFC and FIGMDP, 2010; FAO-UN, 2014; Valdes et al., 2017). The main forages include grasses, legumes and non-leguminous multipurpose trees which are commonly fed as either fresh forage, hay or silage (Devendra and Gohl, 1970; Hernández and Sánchez, 2014; Patersen et al., 1992).

Grasses

The main grass species found in the Caribbean include species of the *Panicum, Bothriochloa, Dicanthium, Brachiaria, Digitaria, Cynodon, Panicum and Pennisetum* genera (CARDI, 2008; CIFSRF, 2013; Patersen et al., 1992). Generally, grasses in the

region like most tropical grasses, have higher yields and vigour than that of temperate grasses (Givens et al., 2000; Proverbs et al., 1992). This is related to their efficient C4 photosynthetic pathway which allows more efficient use of sunlight, water and nutrients (Lara and Andreo, 2011). However, tropical grasses, in comparison to temperate grasses, are higher in crude fibre which accounts for 300 to 350 g/kg DM (Devendra and Gohl, 1970; Oyenuga, 1957; Proverbs et al., 1992). The crude protein (CP) and metabolisable energy (ME) content of tropical grasses are within the range of 20 to 200 g/kg DM and 5 to 11 MJ/kg DM, respectively, which is lower than that of their temperate counterparts (7 to 13 MJ/kg DM and 60 to 250 g/kg DM for CP and ME, respectively) (Wilkins, 2000). Under harsh conditions, the CP of native grasses may be reduced to 30 g/kg DM or less CP and, for improved species, to 50 g/kgDM CP which, in both instances, are lower than that of the minimum CP requirement of rumen micro-organisms (70 /kg DM CP). Overall, the dry matter digestibility of these grasses is 10 to 13% lower than that of temperate species (Devendra and Gohl, 1970; Proverbs et al., 1992). Few studies have investigated the mineral status of forages in the islands within the region and the concentration of calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), zinc (Zn) and manganese (Mn) were often reported as abundant in forages; iron (Fe) was frequently reported as being at high or at toxic levels; and sodium (Na) and copper (Cu), reported as being low or deficient in forages (Bernard et al., 2019; Devendra, 1977; Mohammed et al., 2017; Youssef and Brathwaite, 1987). Mineral licks and salt blocks are commonly used as mineral supplements across regional farm systems (Hernández and Sánchez, 2014). With respect to vitamins, Vitamin A is of particular concern to the region in instances where animals are fed primarily on weathered hay, by-product-based diets, and roughages for prolonged periods, as these feeds are low in vitamin A. Overall, poor quality pasture

fed alone, without supplements, will not support the body weight of even dry non-productive animals (Paterson et al., 1992). However, with appropriate management, fresh, green and actively growing grass will support high levels of animal growth and moderate levels of milk production (Proverbs et al., 1992).

Legumes

There is a range of leguminous forages available including the tree/shrub type legumes as *Leucaena leucocephala* and *Gliricidia sepium* and trailing vine-type legumes like *Stylosanthes guianensis*, *Pueraria phaseoloides* (Kudzu), *Macroptilium atropurpureum* (siratro) and *Neonotonia wightii* (glycine). Legumes tend to have a higher protein and mineral content as well as an overall higher digestibility than grasses (Dianingtyas et al., 2017; Hau, 2014; Osuji and Odenyo, 1997). With their deeper root systems, legumes tend to be more robust than grasses, less susceptible to seasonal changes and more capable of supporting year around animal production (Patersen et al., 1992). They are, therefore, an important supplement to poor quality pastures during the drier months of the year, and are recommended to occupy 25 to 40% of grass-legume stands for maintaining animal production (Osuji and Odenyo, 1997; Patersen et al., 1992). However, despite the higher nutrient value, digestibility and hardiness of legumes, the total dry matter yield is lower than that of grasses making grasses the mainstay of animal production for much of the year (Patersen et al., 1992).

Multipurpose non-leguminous species

Multipurpose trees may include both leguminous and non-leguminous species. Some of the common non-leguminous species may include *Morus spp., Trichanthera gigantea and Moringa oleifera* among others (Valdes et al., 2017). The foliage of most multipurpose tree species contain CP levels two to three times (120 to 420 g/kg

DM CP) that of tropical grasses and, in some instances, higher than commercial concentrate feed which typically contains between 140 to 180 g/kg DM CP (Hernández and Sánchez, 2014; Patersen et al., 1992). Further, the *in-vitro* digestibility of dry matter is high and may be comparable or superior to that of concentrates.

Forages in the Caribbean are primarily rain fed or rely heavily on rainfall for growth (Lallo, 2015). The yield and quality of forages is seasonal being higher during the wetter periods of the year and lower during the drier periods (Hughes et al., 2012; Lallo et al., 2009; Lallo et al., 2016b). The lower quality and yield during the drier months have often resulted in reduced livestock performance and the need for supplements to maintain production levels (Avril et al., 2012). With 'Climate Change' and the projected reduction in rainfall and extended dry periods, this challenge may be further exacerbated (Lallo et al., 2016b).

2.5.2 Alternative feedstuffs and supplements

There are many alternative feeds and/or supplements that are used to replace or supplement fresh forages (Devendra and Gohl, 1970; Hernández and Sánchez, 2014). These include conserved forages, roots and tubers; crop and agro-industrial residues; miscellaneous and concentrates.

Conserved forages

Forages are conserved primarily as hay or silage (Patersen et al., 1992). Hay made from native grasses with a shorter growing period is difficult to produce as cutting takes place in the wet period where limited sunlight makes it hard to produce high quality hay. Consequently, hay made from these grasses contains about 50 g/kg DM CP or less. For the improved pasture species with a longer growth period, cutting takes place in the dry period which is more suitable for haymaking. Therefore, the quality

of this hay is higher as the CP content may be in excess of 70 g/kg DM. Hay may include legumes which increases its feed value. The coarse nature of grasses, as well as the low soluble sugar content makes it difficult to ensile. As a result, these coarse grasses are chopped and compressed mechanically to remove air and are often treated with a dilution of molasses to provide enough soluble sugar for ensiling. A good silage may contain up to 120 g/kg DM CP.

Roots, tubers and their foliage

Some of the main roots and tubers include cassava (*Manihot dulcis; Manihot esculenta*); dasheen (*Colocasia esculenta*); and sweet potato (*Ipomoea batatas*). The tubers of these plants are highly palatable and are a good source of digestible energy for ruminants (Aregheore et al., 2002). The foliage provides high amounts of energy, degradable (sweet potato) and undegradable (cassava, taro) protein. Cassava foliage has a good amino acid profile and is a rich source of calcium and trace minerals (Heuze and Tran, 2002). The fresh leaves of cassava (Onwuka and Akinsoyinu, 1989; Wanapat, 2009) and hay made from the leaves of sweet potato (Ishida et al., 2000) have a feeding value that is comparable to that of alfalfa (Inthapanya et al., 2011).

Crop residues, agricultural by products and agro-industrial residues

Crop residues and agricultural by-products are often high in fibre and low in protein. These can be supplemented with molasses-urea blocks which are a rich source of degradable protein, vitamins and minerals and are typically used to supplement poor quality roughage (Sansoucy et al., 1992). With respect to agro-industrial residues, these are often combined with grain and crop residues to formulate diets that can meet the requirements of animals. The use of these may only be profitable if they are provided at a subsidised price, or for free (Hernández and Sánchez, 2014).

Grains and concentrates

These are the primary and most effective forms of supplements to forage-based feeding and are used heavily in intensive or intensive-type systems across the region (Hernández and Sánchez, 2014; Thomas, 1997). Imported concentrates are often used to supplement poor quality pasture during the drier periods of the year and are associated with higher animal performance when compared to other supplements used (Avril et al., 2012; Balraj et al., 2018). The cost of grains is high and has been rising for the past decade (Lallo, 2015). This rise in price continues as a result of the competing use of grain for biofuel (Lallo, 2015). In spite of the high cost, farmers continue to use these grains over other supplements or alternative feeds (Lallo, 2009). Ideally, it is recommended that grain constitutes 30 to 40% of the diet, however, inclusion may be up to 65% of diets (Asiedu, 2001; Lallo, 2015).

The main ingredients used in concentrate feeds include maize, sorghum, soybean meal, and wheat middling imported mainly from USA, Brazil, Argentina and Belize (Lallo, 2015). There is no known estimate of the percentage of feed cost that is accounted for by concentrate feed in regional small ruminant production systems, however, studies done by Duffus and Jennings (2005) revealed that between 2005 and 2010, concentrate feed accounted for 40% of the cost of milk production in Jamaica.

The increased intensity of dryer periods projected for the region, and the consequent rise in dependence on costly grains by farmers, are challenges which must be addressed. There is a requirement for an unconventional approach which is sustainable and presents an alternative that incorporates locally available materials, providing feeds of comparable nutritive value and supporting similar performance in small ruminants as that of commercial concentrate.

2.6 The application of forage densification technologies to feed systems for ruminant production

More recently, as with the wider tropics, forage conservation technologies including forage densification (pelleting and cubing) have been applied to improve the sustainability of forage systems across both temperate and tropical regions (ASABE, 1997; Dougnon et al., 2012; Hau, 2014; Wanapat et al., 2013). However, prior to processing, the nutritive value of forage inputs must be quantified, which can be done through several methods of forage evaluation.

2.6.1 The evaluation of tropical forages

The nutritive value of forages, compared to other feed resources available, varies considerably as a result of climatic and management conditions (Assefa and Ledin, 2001; Ball et al., 2001; Wilkins, 2000). Therefore, regulating the nutritive value of forage inputs prior to processing is critical (Madsen, Hvelplund, and Weisbjerg 1997). This ensures that the nutritive value of inputs and end-products are consistent (Coleman and Lawrence, 2003; Madsen et al., 1997). Further, forage evaluation can be used to inform guidelines on how forages can be best managed to optimise their use (Madsen, Hvelplund, and Weisbjerg 1997). However, with the lack of access to well-equipped laboratory facilities as well as the characteristically low analytical capacity of laboratories within the Caribbean, the analysis of regional forage resources is not routinely done, nor is it extensively reported on in the literature (Dardenne and Salgado, 2015). Therefore, one of the objectives of the thesis is to provide information

on the nutritive value of a range forage species used in small ruminant production systems in the region.

There are various methods of forage evaluation which can be used to quantify the nutritive value of forages. These include physical or visual appraisal, *in-vivo* and laboratory methods which are briefly discussed in the following sections (Beever and Mould, 2000; Getachew et al., 1998a; Givens et al., 2000; Leng, 1996; Wheeler and Mochrie, 1981).

Visual appraisal

Visual appraisal, according to Schroeder (2009) is critical and the first step towards assessing the value of forages and normally precedes the more complex forage evaluation techniques. It involves drawing conclusions from the value of forages based on the colour of leaves; presence or absence of odours; texture of leaves and stems; the stage of maturity; attack from weeds, insects and diseases; and palatability.

In-vivo feed evaluation or digestibility studies

The *in-vivo* method is the most precise approach for estimating the nutritional value of feedstuffs (Minson, 1981; Minson, 2012; Rymer, 2000; Schneider and Flatt, 1975). It involves the estimation of value through the direct feeding of animals to observe the level of production achieved on any given feed (Rymer, 2000; Schneider and Flatt, 1975). However, because of the laborious, time-consuming and costly nature of the *in-vivo* method, it is an impractical approach for routine and large-scale feed evaluation efforts (Carro et al., 1994; Coelho et al., 1988; Jones and Barnes, 1996; Tilley and Terry, 1963).

Laboratory methods

Prediction of in-vivo digestibility utilising statistical association between gross chemistry and in-vivo digestibility

The gross chemistry can be obtained from wet chemistry methods like proximate analysis and the Van Soest method (Belanche et al., 2013; Getachew et al., 1998a). These involve a series of steps designed to quantify nutrient fractions of feeds (Belanche et al., 2013; Hart and Fisher, 1971; Van Soest, 1967). The advantages of these methods are the absolute values derived for the various fractions of feeds. Conversely, other methods rank feeds based on estimated digestibility that is dependent on animal and diet factors (Mould, 2003). Of the two methods, the Van Soest method is more reliable and robust because it entails a more practical partitioning of nutrient fractions in feeds (Marten, 1981; Van Soest, 1967).

The reference library created through *in-vivo* studies has a range of forages with the concentration of their respective chemical fractions obtained from wet chemistry methods along with their respective *in-vivo* digestibility, both of which are used to establish statistical associations that can help predict the *in-vivo* digestibility of new feeds being analysed (Kitessa et al., 1999). These statistical associations are in the form of a simple correlation (regression equation) which is population-dependant and should not be used on samples outside the population used to derive the equation (Weiss et al., 1992). They are in the form of a cause-effect relationship which is population-independent and can be applied across various feeds (Weiss, 1993). Some of the main chemical fractions considered to have a major impact on digestibility include the cell wall fractions NDF, and ADF and lignin which are often used in regression equations to predict digestibility (Kitessa et al., 1999). Theoretically, the error of prediction decreases when the most indigestible fraction, lignin, is used. In

practice, however, this may not always be the case, and the approach is challenging when predicting the digestibility of feeds like grain that are low in lignin (Kitessa et al., 1999). Others have used a summative approach for calculating the total digestible nutrients (TDN) (Conrad et al., 1984; Weiss et al., 1992) which Aerts et al. (1977) saw as more accurate than utilising the single fibre fractions, when predicting digestibility.

The advantage of equations based on chemical indices is that they are simple, rapid and relatively cheap (Kitessa et al., 1999; Weiss et al., 1992). However, one disadvantage of the method is that it does not account for variation as a result of extrapolation across feed type, differences in chemical fractions used as well as differences in methods used to determine chemical fractions.

Prediction of *in-vivo* digestibility utilising fermentative methods

In-vitro Method (Tilley and Terry 1963)

The technique by Tilley and Terry (1963) is an important tool used for evaluating or analysing and predicting the digestibility of ruminant feedstuffs. This is a two-stage method which involves the incubation of feed samples in strained rumen liquor (rumen liquor digestion – stage 1) representing digestion in the rumen followed by further digestion in acidified pepsin for 48 hours (pepsin digestion – stage 2) which represents digestion in the lower digestive tract. Once *in-vitro* digestibility for a sample is obtained, this is corrected using a regression equation relating *in-vitro* and *in-vivo* digestibility values obtained from a large sample set. Such a method has been recognised as reliable for predicting the *in-vivo* digestibility of a range of ruminant feeds, however, there may be some limitations in predicting *in-vivo* dry matter digestibility of feeds with a low nutritive value (Khazaal et al., 1993). The shorter

time of incubation under *in-vitro* conditions may limit the digestion of high fibre feeds. Animals may gradually proliferate more fibrolytic ruminal microbes in *in-vivo* studies, to digest the higher fibre feeds which is an opportunity that may be limited under *in-vitro* conditions. Some of the major sources of variation that can affect the precision of the method include sample preparation, microbial activity of inoculum and the donor animal (Tilley and Terry, 1963). One advantage of the method is that it is less susceptible to some of the disadvantages of predicting *in-vivo* dry matter digestibility from chemical indices. Factors such as heat treatment, alkali treatment of straws; associative effects and species of animals (once donor animal and test animal are the same species) can be accounted for with this method (Kitessa et al., 1999). However, one major disadvantage of the technique is that it is an endpoint method giving one result after both 48-hour incubation periods which are limited and inadequate for revealing information on the kinetics of fermentation unless extended and laborious time course studies are conducted (Getachew et al., 1998a; Mould, 2003; Tilley and Terry, 1963). Without information on the kinetics of digestion, feed cannot be differentiated based on the rate at which nutrients are made available. This is critical as feed degradability determines rumen retention time and, therefore, feed intake (Mould, 2003). Other disadvantages of the technique include the need for costly donor animals and the associated animal welfare issues, susceptibility of rumen liquor to antibiotics in compound feeds, and the time-consuming nature of the method which requires at least two days per batch of sample. The length of time has, however, been reduced in modifications to the technique (Weiss et al., 1992).

Enzyme methods (in-vitro assay)

The enzymatic methods utilise commercially produced enzymes instead of microorganisms for predicting the dry matter digestibility of forages and is an

alternative approach to the *in-vitro* Tilley and Terry (1963) method. There are many variations of the method, one of which is described by Roughan and Holland (1977) and involves the digestion of feed substrate in acid pepsin enzymes followed by cellulase enzymes. Once the cellulase digestibility is obtained, the prediction of its dry matter digestibility is done through the application of a regression equation established from correlating the cellulase digestibility of a range of reference forages to their *in-vivo* digestibility (Roughan and Holland, 1977). An advantage of the technique is that it provides an alternative to the rumen liquor method of Tilley and Terry (1963) which depends on costly fistulated animals maintained solely for rumen liquor (Jones and Barnes, 1996; Macheboeuf et al., 1998). It is more rapid than the Tilley and Terry (1963) method (24 hours less) and enzyme preparations are more uniform providing greater repeatability than biological inoculum. However, these enzymatic methods like Tilley and Terry (1963), utilise endpoint digestibility procedures and, therefore, share similar disadvantages of endpoint measures of degradability. One other disadvantage of the technique may be that the results of the system are not as extensively validated by *in-vivo* values as with the Tilley and Terry (1963) technique. Further, enzymes, unlike microorganisms, are insensitive to factors such as associative effects of toxins which can affect microbial degradation (Kitessa et al., 1999). Additionally, digestibility determined by these methods may not factor in possible interactions between microbial species in the rumen and the modification of this by the diet of the host animal (Getachew et al., 1998a). With respect to more precision in the estimation of digestibility for diets with a low nutritive value, using a combination of fibrolytic enzymes for diets of low nutritive value or a starch-digesting enzyme for high starch diets, will improve estimations (Kitessa et al., 1999).

In-vitro gas method

The *in-vitro* gas method measures both the extent and rate of degradability (primarily carbohydrates) through a measure of the volume of gas produced over time when substrate is incubated in rumen fluid (Herrero et al., 1996; Menke et al., 1979). Generally, all *in-vitro* gas methods share similar procedures requiring milled substrate (feed), an anaerobic medium and an inoculum with microbes from the rumen. The substrate (feed) is weighed and placed into a medium which is warmed to ~39 ^{O}C and rumen fluid is added as inoculum. When feeds are incubated in rumen fluid *in-vitro*, the carbohydrates in the rumen are fermented into short-chain fatty acids or volatile fatty acids (VFAs) including acetate, butyrate, propionate and gases (primarily carbon dioxide (CO_2) and methane) (Beuvink and Spoelstra, 1992; Getachew et al., 1998a). The resulting gas production is used as a measure of the degradability of substrate which can be recorded as an endpoint measurement, or at time intervals where a cumulative gas profile is plotted (Herrero et al., 1996; Menke et al., 1979; Rymer et al., 2005). The cumulative gas produced *in-vitro* can be fitted to mathematical models (France et al., 2000). These models are used to estimate *in-vitro* gas production kinetics, or the rate and extent a substrate or feed has been fermented and/or degraded (France et al., 2005; Üçkardeş and Efe, 2014).The fermentation of carbohydrates results in the highest volume of gas, while protein and fats produce negligible gas volumes (Getachew et al., 1998b; Menke, 1988; Wolin, 1960). The gases produced are formed directly through fermentation or indirectly through buffering. The direct production occurs through the fermentation of substrate to acetate and butyrate (Getachew et al., 1998a). Substrate that is converted to propionate produces no gas and, as a result, *in-vitro* analysis requires a bicarbonate buffer that reacts with propionate to produce CO_2. Propionate can be measured indirectly from the CO_2 produced (Rymer et al., 2005). Additionally, regression equations have been used to

correlate gas produced through *in-vitro* methods with *in-vivo* digestibility. The technique was built on the assumption that gases produced during the fermentation of feed substrate incubated in rumen fluid *in-vitro* are related to *in-vivo* digestibility and, thus, the energy value of feeds for ruminants (Menke et al., 1979; Rymer et al., 2005).

Some of the advantages of the techniques are that, unlike enzyme and *in situ* methods, they are not subject to errors from washing, filtration and weighing of residue (Kitessa et al., 1999). However, these may be offset by variances related to measurement and modelling of gas production. The method is potentially susceptible to underestimating the *in-vivo* digestibility of feeds that may have slow gas production but are high *in-vivo* digestibility (Getachew et al., 1998a; Kitessa et al., 1999).

In situ, in sacco or nylon bag method

In this method, feed samples and replicates are placed in porous bags (polyester, nylon or dacron) which are then placed in the rumen of a cannulated animal (Huntingdon and Givens, 1995). After incubation in the rumen for a predetermined time, the bags are taken out, washed, dried and DM loss recorded. This method is used to measure the rate and extent of degradation of feed constituents in the rumen (primarily protein and carbohydrates). This gives an indication of the rate and extent to which nutrients from feed are made available to the microorganisms (rumen-degradable protein and rumen-degradable carbohydrates) and the animal (microbial protein (amino acids), non-degradable protein from feed and fermentable metabolisable energy (FME) from volatile fatty acids (VFAs)) (Mehrez and Ørskov, 1977; Orskov et al., 1980). The method has been used to measure the rate of degradation at different sections of the gastro-intestinal tract and the total tract digestibility which is determined from the relationship between *in-vivo* digestibility and dry matter disappearance (Kitessa et al., 1999). Dry matter disappearance is either measured directly from a 48-hour incubation

(Gasa et al., 1989) and/or from a 48-hour incubation followed by digestion in acid pepsin (Aerts et al., 1977). The data obtained may be fitted to models which describe the rate of degradability of substrate. For example, $p = a + b (1- e^{-ct})$, where *p* is DM disappeared at time t, (a+b) represents the total potentially degradable DM (a and b representing the rapidly and slowly degradable fraction, respectively), and c represents the rate of DM degradation (Orskov and McDonald, 1979). Disadvantages of the technique include a lack of standardisation of procedures; the small number of samples processed at any given time; the requirement for fistulated animals; the laborious nature; and the requirement for a large number of samples. These all make routine laboratory screenings of large numbers of sample difficult. Further, the method may be open to substantial error related to microbial contamination of samples which may increase weight and lead to an underestimation of dry matter loss (Dewhurst et al., 1995; Getachew et al., 1998a). Moreover, the technique may lead to an overestimation of fermentation for feeds that lose substrate before fermentation takes place, for example, feeds with a high soluble content (sugar and starch) and a high unfermentable fibre content (Dewhurst et al., 1995).

Prediction of *in-vivo* digestibility utilising infrared technologies

Infrared spectroscopy is a measure of molecular vibrations that occur when molecules interact with infrared radiation (IR) or energy (Belanche et al., 2013; Rodríguez, 2000). When a molecule is exposed to thermal energy coming from a hot source or source of infrared radiation, it absorbs heat at a frequency that stimulates vibrations unique to it (Belanche et al., 2013; Rodríguez, 2000). The absorption of IR by molecules is represented graphically as spectra which can be used to generate chemo-structural

information about substrate. As a result, the technique has been applied to nutrition for determining the chemical composition of feeds.

NIRS, which is one of the more commonly used methods of spectroscopy, provides a rapid and non-destructive physical technique for evaluating feeds (Stuth et al., 2003). It measures the chemical components of feeds including CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and *in-vitro* dry matter digestibility with standard errors of 0.95, 3.1, 2.5, 2.1 and 3.5, respectively, as reported in studies by Brown et al. (1987) and Norris et al. (1976). It has been used for the prediction of intake of forages from gross composition (Minson, 1982; Park et al., 1997; Steen et al., 1998); for the prediction of fermentable energy obtained through *in-vitro* gas production methods (Herrero et al., 1996); and for the prediction of the nitrogen degradable fractions derived *in situ* (Waters and Givens, 1992). The method relies on chemometrics which utilises mathematical models to relate spectral data to the chemical composition of feeds. Some of the main advantages include the speed and overall convenience of the technique and the standardised network of equipment giving more uniform analyses from laboratories for digestibility and the different components and properties of feeds (Kitessa et al., 1999).

2.6.2 Forage conservation using forage densification technologies

Increasing the scope of forage conservation techniques in the Caribbean may improve forage-based feeding for small ruminant systems regionally. Hay and silage making are the two most popular forms of forage conservation used in regional, small ruminant, production systems (Devendra and Gohl, 1970; Hernández and Sánchez, 2014; Patersen et al., 1992). However, very limited work has been done on conservation techniques which involve the densification of forages into compressed or

densified packages including pellets which are an agglomeration of forage materials which have been ground or cubes which are an agglomeration of forage chops (ASABE, 2016; Samson et al., 2005; Sokhansanj and Turhollow, 2004).

2.6.3 Processing densified forages

The densification process involves the compression of low-bulk density materials into more compact or dense forms through the application of pressure in the presence of moisture and temperature treatments (Thomas et al., 1997; Tumuluru et al., 2010b). Raw materials are first harvested, dried (mechanically or sun cured), ground if products are being pelleted; conditioned (treated with moisture in the form of steam (heat and moisture) or liquid to increase the compressibility of raw materials) and compressed through a perforated metal structure known as a die from which dried densified forage packages emerge. Figure 2.1 is a flow diagram of the major steps of the densification process.

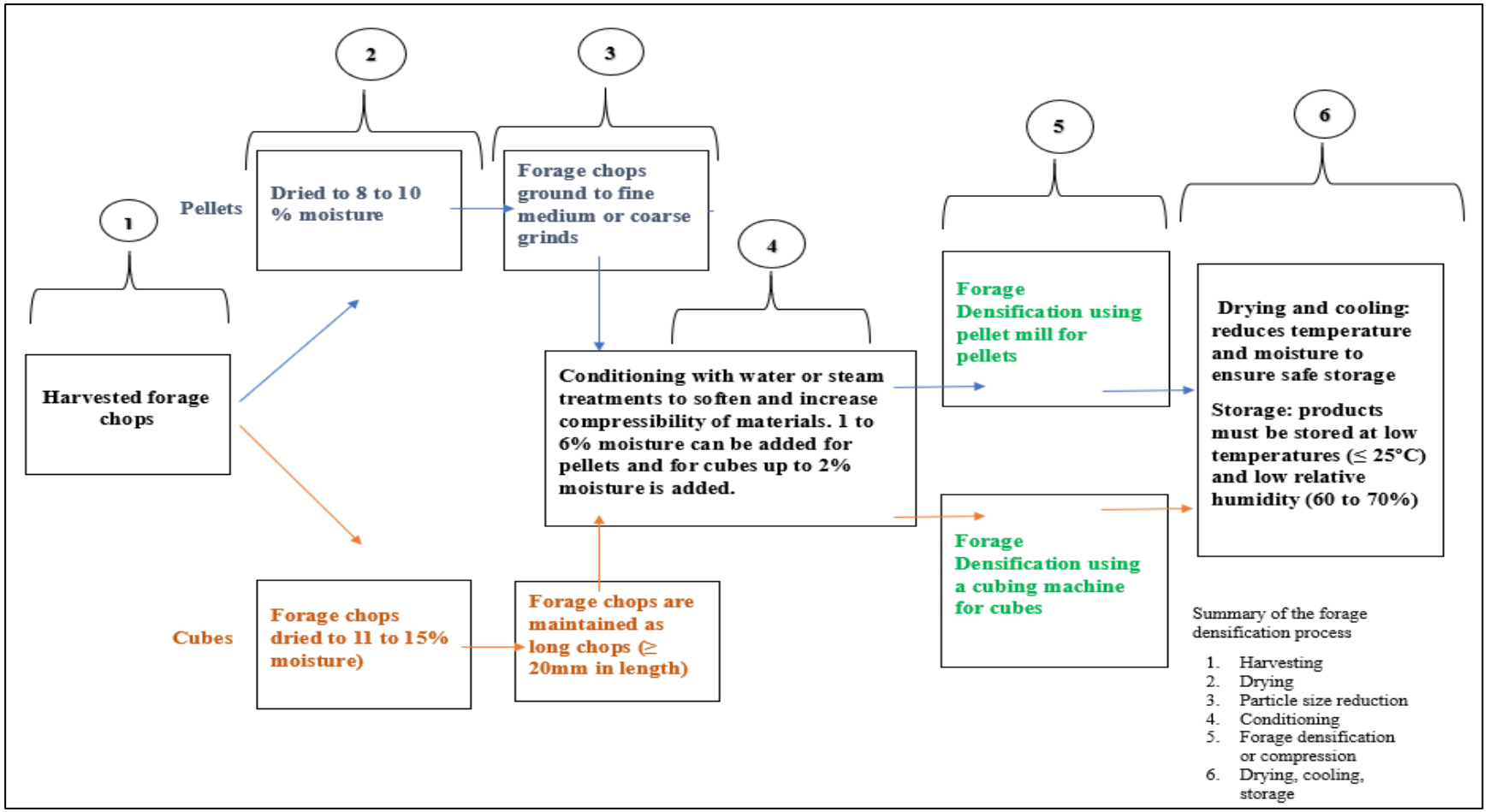

Figure 2.1 Major steps in the densification of forages (Source: Kaliyan and Morey (2009); Khoshtaghaza et al. (1999); Turner (1995))

2.7 Advantages of forage densification technologies

Densified forages may provide another means of conserving forage resources (Dianingtyas et al., 2017; Hau, 2014) in the Caribbean. This type of conservation technology offers a more convenient system which has important advantages over systems that rely heavily on the low bulk density forms of preserved forage (hay and silage) (Bilanski et al., 1985; Coleman and Lawrence, 2003). Some of these advantages include increased bulk density and, therefore, greater ease of handling, storage, transportation, distribution and feeding (Dianingtyas et al., 2017; Dobie, 1961; Long et al., 1955). If regional farm systems had access to these forage packages, it may reduce the demand for, and inconvenience of time-consuming harvesting of cut-and-carry forage which may be both labour intensive and costly (John et al., 2010; Palmer et al., 1998). In fact, farmers may be more willing to pay a premium price for the convenience of these products, which may be lower than the price for pelleted grain feeds (Orden et al., 2014; Srivastava et al., 1981). Cubes, which are comprised of longer fibrous forage chops (forage chops ≥ 20 mm in length (Dobie, 1975; Sokhansanj and Turhollow, 2004; Wallace et al., 1961), tend to be comparable in terms of physical quality and digestibility to cut forages. Forage cubes may, therefore, provide an alternative to cut-and-carry forages which are compressed and more easily handled; more consistent in quality; and potentially more cost effective (Coleman and Lawrence, 2003).

More critically, densified forage packages improve animal performance (Dobie, 1975; Dougnon et al., 2012; Huyen et al., 2012; Magill et al., 1958). For instance, the overall increase in feed intake has been emphasised in various studies (Coleman and Lawrence, 2003; Zhong et al., 2018). This increased feed intake may be related firstly

to the higher acceptability and palatability of densified forages to ruminants in comparison to bulky loose forage forms (Dobie, 1975; Wallace et al., 1961). The higher nutrient density and rapid passage rate through the gastro-intestinal tract result in the higher feed intake associated with densified forages (Blaxter and Graham, 1956; Minson, 1963). Feed intake in ruminants, particularly with younger animals, is often affected by both the unit dimensions and particle grind or chop size of forage packages (Mark, 1963; Tetlow and Wilkins, 1972; Wilkins et al., 1972). With the overall smaller physical dimensions (diameter: 4.8 to 19.1 mm; length: 12.7 to 25.4 mm (California Pellet Mill Co (CPM), 2012; Tumuluru et al., 2011) of pellets, feed intake tends to be higher than that of cubes with larger dimensions (Cross section: 25 to 32 mm; Length: 40 to 100mm (Khoshtaghaza et al., 1999; Wilkins et al., 1972). Further, even with the higher unit density (961 to 1121 kg/m3) and, therefore, greater hardness of pellets, their smaller size makes pellets more preferred than wafers with a lower unit density (481 to 641 kg/m3). Additionally, high acceptance of pellets may be due to the faster rate of satiety achieved with the characteristically higher intake of pellets (Minson, 1963).

However, finely ground material is often associated with compromised rumen health and reduced performance in ruminants (Mertens, 1997; Mertens, 2002). Some of these challenges related to finer particles may be addressed through increasing the particle size range or inclusion of coarser grinds (Mirzaei-Aghsaghali and Maheri-Sis, 2011). The inclusion of coarse grinds must never exceed the maximum levels required to support optimum rumen conditions, as above this level intake and access to nutrients may be limited (SRNS, 2016). With respect to wafers, reducing size may improve palatability. In studies done by Tetlow and Wilkins (1978), the smaller-sized wafers

were more prehensile and, therefore, more easily and readily eaten by young ruminants including lambs and calves compared to larger wafers.

Densified rations result in a greater live weight gain for a given weight of feed compared to loose feeds (Minson, 1963; Wanapat et al., 2013). Both the increased feed intake and live weight gain associated with densified forages were strongly associated with the quality of the parent material, with lower quality forages having a greater increase in production when densified compared to its loose forms (Beardsley, 1964; Minson, 1963). Further, feed intake, daily gain and feed efficiency can be increased by 25%, 100% and 35%, respectively, when densified forage of marginal quality is offered (Beardsley, 1964). Moreover, carcass yield, quality and milk production increased when densified forages were used (Al-Saiady et al., 2010). This is often more pronounced with pellets than with cubes (Minson, 1963; Wallace et al., 1961).

Forage densification technologies impact on the digestibility and energy efficiency of forages. Some studies found increases in digestibility (Huyen et al., 2012; Long et al., 1955), whereas, others reported reduced digestibility, particularly of fibre fractions (Al-Saiady et al., 2010; Reynolds and Lindahl, 1960; Uden, 1988). Densified forages may increase the energy utilisation of forages (Wainman et al., 1972). For instance, metabolisable energy retained for production was 28% greater for pellets than for long dried grass and net availability for production was increased from 40% for the unpelleted ration to 52% for the pelleted ration. Other benefits of densified forages include reduced ingredient segregation and less feed wastage (El-Deek and Brikaa, 2009a; Fasina and Sokhansanj, 1996; Hau, 2014; Jones et al., 1958).

2.7.1 Defining and assessing the quality of densified forage packages

The quality of densified forage products can be expressed in terms of strength or durability which increases with stronger inter-particle bonding within densified materials (Kaliyan and Morey, 2009). The strength (compressive resistance or hardness) of densified feeds is measured using the diametrical compression test. This test simulates compressive stress due to the weight of top pellets or cubes on the lower pellets or cubes during storage. This test simulates the crushing of pellets or cubes during mastication in animals. The load or force at which the specimen breaks is recorded as the compressive strength. The durability is the ability to withstand shearing and abrasive action during transportation. It can be measured using the abrasive resistance test which simulates the shearing and abrasive action during transportation (Kaliyan and Morey, 2009; Thomas and Van der Poel, 1996). This test can be done using the Tumbling can, the Holmen tester and the Ligno tester (ASABE, 1997; Franke and Rey, 2006; Winowiski, 1988). Tumbling is the more commonly used of the three methods and it gives the Pellet Durability Index (PDI) or the percentage durability (Kaliyan and Morey, 2009). This involves three steps including tumbling pellets in a tumbling can, followed by sieving these pellets and the fines produced as a result of tumbling, and measuring the weight of intact pellets or pellet pieces retained after sieving (ASABE, 1997; Kaliyan and Morey, 2009). The PDI is the ratio of weight after tumbling to the weight before tumbling, expressed as a percentage (ASABE, 1997).

2.7.2 Factors affecting quality

There are various factors affecting the quality of densified products (Behnke, 1994, 2001; Briggs et al., 1999; Thomas et al., 1997). According to Behnke (1994), Turner (1995)Turner (1995) and Thomas et al. (1997), these factors contribute to pellet quality

in the following proportions: 1. Diet formulation (40%); 2. Particle size (20%); 3. Steam conditioning (20%); 4. Die specifications (15%) and cooling/drying (5%). Based on these proportions, the cumulative effect of factors, such as diet formulation as well as particle size and steam conditioning, have a significant impact on quality and are, therefore, emphasised in the following sections (Kaliyan and Morey, 2009).

Diet formulation

Physical composition of feed affects the quality of densified products. For instance, the types of ingredients, the parts of the plant including leaves, stems and the percentage inclusion of these all impact on quality (Adapa et al., 2005a; Adapa et al., 2005b; Loar II and Corzo, 2011; Loar et al., 2010; Reece, 1966; Rehkugler and Buchele, 1967; Theerarattananoon et al., 2011; Zarate et al., 2004). The chemical composition including starch, protein, fibre, fats, lignin and lignin extractives are other factors that affect the quality of densified products (Kaliyan and Morey, 2009; Muramatsu et al., 2015; Tumuluru et al., 2010a). Starch, protein, lignin and lignin extractives are all natural binders (Bradfield and Levi, 1984; Briggs et al., 1999; Wood, 1987) and all impact positively on the durability of densified products. Starch gelatinises when exposed to heat, moisture (Cai and Wei, 2013; Stevens, 1987) and shear friction when expelled through dies (Kaliyan and Morey, 2009). Further, the combination of heat, moisture and shear friction during densification processes denatures and, subsequently, plasticise proteins which increase its binding capacity (Briggs et al., 1999; Hill and Pulkinen, 1988; Tabil et al., 1997; Thomas et al., 1998; Winowiski, 1988; Wood, 1987). Some sources of protein including wheat, rye, barley, sorghum and soybean meal have a higher binding effect (Stevens, 1987; Winowiski, 1988) than that of corn or other sources (Cavalcanti, 2000). During the conditioning process, lignin softens, developing natural binding properties (Kaliyan and Morey,

2009). However, according to Bradfield and Levi (1984), above a threshold level (34%), both lignin and extractives decrease the durability of densified products.

Fibre (Angulo et al., 1995; Hill and Pulkinen, 1988; Mohsenin, 1965) and fat (Cavalcanti, 2000; Richardson and Day, 1976) can have negative impacts on pellet quality. Fat functions as a lubricant and it reduces shear friction which is important for compression (Kaliyan and Morey, 2009). Additionally, fat has hydrophobic properties that inhibit the binding properties of water soluble feed components including starch, protein and fibre (Thomas et al., 1998). However, natural fats released from the cell wall during conditioning were observed to sometimes have a positive impact on binding (Thomas et al., 1998). Vest (1993) recommended an inclusion level of no more than 1.5% fats in densified products. Fibres can be both water-soluble and water-insoluble. Water-soluble fibres add to the viscosity of ingredients impacting positively on the quality of densified feeds (Kaliyan and Morey, 2009). Conversely, the high resilience of insoluble fibres reduces inter-particle binding, increases fragmentation and, therefore, reduces pellet quality (Rumpf, 1962; Thomas et al., 1998). High water content (10 to 23%) and chemical agents including NaOH, CaO and urea may help to reduce resilience of insoluble fibres. Additionally, minerals impact on the quality of densified products (Behnke, 2006; Turner, 1995) as the degree of abrasiveness of different minerals may have varying effects on the compressibility of feeds (Kaliyan and Morey, 2009).

Moisture content

Moisture content of materials can either be in the form of bound moisture, processing moisture or final moisture (Kaliyan and Morey, 2009). The bound moisture is referred to as the inherent moisture of dehydrated raw materials (Turner, 1995). After being harvested, materials are wilted or dehydrated through mechanical drying (Coleman

and Lawrence, 2003; Hill and Pulkinen, 1988) or through sun-curing (Adapa et al., 2005a; Adapa et al., 2005b) before conditioning and compression (Kaliyan and Morey, 2009). Dehydration is critical as higher bound moisture results in more resistant plant protoplasm, reduced compressibility of materials and, therefore, compromised product quality (Turner, 1995). Raw materials may be dried to 8 to 10% moisture content for the pelleting process (Kaliyan and Morey, 2009) or up to 15% (Castrillo et al., 2013; Mark, 1963) for materials being cubed. During conditioning, moisture may be added at a rate of 1 to 2% for forage chops (cubing process) or 1 to 6% for ground materials (pelleting process) (Maier and Bakker-Arkema, 1992; Pfost, 1964). The processing moisture includes the moisture added during conditioning in addition to the bound moisture. The maximum processing moisture for pelleting is 15% (Castrillo et al., 2013) and up to 23% (Mark, 1963) for the cubing process. During the pelleting process, the processing moisture is often in the form of steam (a combination of moisture and heat) (Thomas et al., 1997). There are optimum steam treatments for the densification of different types of feeds which is important for activating both inherent and added binders that are required for producing permanent bonds in densified feeds (Payne, 1978). Steam conditioning in the pelleting process is critical as the integrity of pellets depend heavily on the release of natural binders which results in the formation of inter-particle bonds in densified forage products. These bonds are initially molten, but are later solidified during the drying and cooling process to form strong compact pellets (Kaliyan and Morey, 2009). For cubes, conditioning through the addition of water may suffice as the major bonds formed are primarily as a result of the interlacing and interlocking of long fibres and less through the activation of inherent binders (Mark, 1963; Pickard et al., 1961). The final moisture is the moisture content of densified forage packages after the drying and cooling process. The final moisture for

safe storage may range between 8 and 10% (Kaliyan and Morey, 2009) for pellets to between 10 and 12% for cubes (Khoshtaghaza et al., 1999; Sokhansanj and Turhollow, 2004).

Binders

Binders are any liquid or solid that forms a bridge, film, matrix, or that reacts chemically to form inter-particle bonds (Kaliyan and Morey, 2009). During processing, steam conditioning is essential for activating binders (Franke and Rey, 2006). There are more than 50 different types of organic and inorganic binders employed during the densification process (Pietsch, 2002) and inclusion in the range of 0.5 to 5% have been used to improve and standardise pellet quality (Tabil, 1996). The commonly used ones in the feed industry include lignosulphonate, bentonite, modified cellulose, molasses, starches and proteins (Payne, 1978; Tabil, 1996; Thomas et al., 1997). Different binders have different levels of effectiveness in improving the quality of densified feeds (Behnke, 1994).

Particle size

The particle size impacts on the quality of densified feeds. For pelleting, particle size is commonly classified as either fine or coarse grind (Minson, 1982). In the pelleting process, the finer grinds result in higher quality pellets as finer grinds increase the surface area over which steam conditioning occurs, more readily stimulating the natural binding capacity of ingredients (Tabil, 1996). Finer grinds, however, have a higher energy requirement and may be more costly to obtain (Kaliyan and Morey, 2009). With coarser grinds, the large particles are not as effectively conditioned as finer particles as there is more fissuring and air spaces which reduce compressibility and compromise quality (Tumuluru et al., 2010b). The difficulty of compressing coarser grinds may be improved through using either dies with a larger length to diameter ratio, synthetic binders, or equipment that increase the compressibility of feeds. These include double pelleting where feeds are pelleted in a two-phase pelleting system or the use of expanders which function to enhance the densification, shear and

mixing of feed mash (Kaliyan and Morey, 2009; Thomas et al., 1997). Both double pelleting and expanders, however, increase the cost of production (Kaliyan and Morey, 2009). One of the important advantages of cubing over pelleting is the capacity of systems to effectively compress raw materials with a larger particle size range (forage chops ≥ 20 mm in length (Dobie, 1975; Sokhansanj and Turhollow, 2004; Wallace et al., 1961). Cubing is, therefore, the more desirable of the two processes, as the use of long chops eliminates the power-consuming grinding process of pelleting. This makes cubing a more energy-efficient and cost-effective system than the pelleting process (Heimann, 2016; Pickard et al., 1961).

2.8 Conclusion

The Governments of the CARICOM regard the small ruminant sector as significant to Caribbean agriculture. Establishing more sustainable production systems requires addressing some of the major constraints to production, a critical one being the high dependence on costly imported commercial feed ingredients regionally. Forages have been identified as a potential cost-effective feed resource and substitute to costly concentrate feeds. Further, the application of forage densification technologies to forages may improve the utilisation of forages and lead to the establishment of more self-sufficient feed systems in the region that are less dependent on costly commercial concentrate. Therefore, as previously outlined, the objectives of this research were to:

i. Review the literature on small ruminant production systems in the Caribbean and on the application of forage densification technologies to feed systems for ruminant production;

ii. Identify the range of forage species available and are used, or have the potential to be used in small ruminant production systems in the Caribbean and to describe these based on their nutritive value;

iii. Determine the nutritive value and effect of a densified diet comprised of different levels of forage on intake in growing lambs;

iv. Determine the effect of a densified diet comprised of forage on growth performance and digestibility in lambs; and

v. To discuss the findings of the previous chapters and to conclude with recommendations on the way forward.

CHAPTER 3 Determining the chemical composition and *in-vitro* digestibility of forage species used in small ruminant production systems in the English-Speaking Caribbean

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3.1 Abstract

The nutritional evaluation of tropical forages in the Caribbean is limited and the aim of this study is to provide information on the nutritive value of 12 forages used in regional small ruminant production systems, utilising three different methods of forage evaluation. Samples of seven grasses (*Brachiaria arrecta, Brachiaria hybrid cv. Mulato II (Brachiaria ruziziensis x B. brizantha x B. decumbens), Cynodon dactylon, Cynodon, nlemfuensis, Digitaria eriantha, Megathyrsus maximus and Pennisetum purpureum*); two leguminous multipurpose tree species (LMPTs) (*Gliricidia sepium* and *Leucaena leucocephala*) and three non-leguminous multipurpose tree species (NLMPTs) (*Moringa* oleifera, *Morus alba* and *Trichanthera gigantea*) were analysed using proximate analysis, *in-vitro* assays and near infrared spectroscopy (NIRS). *Cynodon nlemfuensis* had the highest ($p < .0001$) crude protein (CP) of the grasses and *Leucaena leucocephala* and *Moringa oleifera* the highest ($p < .0001$) CP of the multipurpose tree species (MPTs). *Trichanthera gigantea* had the highest ($p < .0001$) ash content of the MPTs. The NLMPTs, *Morus alba* and *Moringa oleifera* had the highest ($p < .0001$) starch, highest ($p < .0001$) *in-vitro* digestible organic matter in dry matter (IVDOMD) and metabolisable energy (ME) and the lowest ($p < .0001$) neutral detergent fibre (NDF) of the MPTs. There was a strong positive relationship between the IVDOMD and both the CP and starch fractions and a strong negative relationship between the IVDOMD and both the NDF and acid detergent fibre (ADF) fractions. The NIRS predicted values, generated from a calibration model built using temperate forages, had a strong relationship with the proximate CP and NDF and the IVDOMD and ME of the tropical forage species. The correlation of determination (R^2) between proximate CP, NDF, ADF, IVDOMD, ME

and their respective NIRS values were 0.91, 0.86, 0.59, 0.7 and 0.8, respectively. Overall, the forage species were above the minimum CP, IVDOMD and ME required to be classified as good quality forages.

Keywords: Caribbean, forage evaluation, small ruminants, Trinidad and Tobago

3.2 Introduction

There is a growing demand for livestock products within the Caribbean Community (CARICOM). This is thought to be a direct result of rising affluence and urbanisation, as well as a rapidly increasing population which is projected to rise from 18 million to 22 million by 2050. Further, there is a high regional demand for animal protein from small ruminants and local meat production from the sector only meets 20 to 25% of the regional demand (Avril et al., 2011; Lallo et al., 2016b). Increasing production may require addressing some of the major constraints, particularly the high dependence on costly imported concentrate feed in the region (Singh et al., 2006b). One approach to addressing this challenge is increasing the utilisation of locally available feeds including forages which may allow for the development of more sustainable feeding systems (Avril et al., 2011).

There is a wide range of tropical forages that are used in small ruminant production systems in the Caribbean (Hernández and Sánchez, 2014). These include tropical grasses which are often presented as cut-and-carry in the predominantly intensive production systems found in the region (Lallo, 2015). With their efficient C_4 photosynthetic pathway, tropical forages undergo faster maturation than their temperate counterparts, becoming more fibrous and less digestible over a short period of time (Leng, 1990). This reduction in the nutritive value of grasses results in the need for supplementation with commercial feeds, especially during the dry season when the quality of grasses declines severely (Lallo, 2015). There is a wide range of fodder trees or multipurpose tree species (MPTs) which are known for their higher concentrations of protein, vitamins and minerals when compared to the grasses (Wilson, 1969). The high and more consistent nutritive value of these MPTs make

them good supplemental forages which may improve the overall feeding value of the more fibrous tropical grasses (Topps, 1992; Wilson, 1969).

The nutritive value of forages can vary significantly, depending on environmental factors including weather, cultural practices and land topography (Caro-Costas et al., 1976; Hughes et al., 2012; Radcliffe, 1982). This necessitates the ongoing evaluation of these resources to inform their management and optimised use in farming systems. There are several ways to evaluate the quality of forages including *in-vivo* studies and laboratory methods. *In-vivo* studies are often labour intensive, costly and time-consuming and, consequently, laboratory methods which are faster and more cost-effective are commonly used as alternatives to *in-vivo* methods (Carro et al., 1994). However, the application of these laboratory methods to tropical feed is limited because of a lack of access to well-equipped laboratory facilities as well as their characteristically poor analytical capacity (Dardenne and Salgado, 2015). This may explain why the analysis of regional forage resources, utilising these laboratory methods, is not routinely done, nor is it extensively reported on in the literature. Understanding both the nutritive value of forages obtained from various methods of forage evaluation and how they compare, and/or relate, may give increased insight into the nutritive value of these resources. This information can be used to better inform how forages are handled, fed and supplemented for optimised use as feeds for small ruminants (Madsen et al., 1997). Therefore, the following study aimed to provide current information on the nutritive value of a range of forage species (seven grasses, two leguminous multipurpose trees and three non-leguminous multipurpose trees) used in small ruminant production systems in the Caribbean utilising both Proximate analysis, Near Infrared Spectroscopy (NIRS) and *in-vitro* assays. Further, the study

aimed to determine how well the NIRS predicted values compared or related to the respective proximate and *in-vitro* values.

3.3 Materials and Methods

3.3.1 Site description

Samples for all species were collected from one of two sites (Site 1 and Site 2). Site 1 is the Forage Bank at the University of Trinidad and Tobago - Valsayn Campus, Trinidad and Tobago (10.63°N, -61.41°W) and Site 2 is the forage bank at New Wales Manchester, Central Jamaica (17.93°N, -77.52°W). The total rainfall for January 2018 at Site 1 when samples were harvested was 60.8 mm; the minimum and maximum temperatures at the time of harvest were 22.25 and 30.14^{0}C, respectively (Trinidad and Tobago Meteorological Services (TTMS), 2018); and the predominant soil type was the Piarco soil series comprised of terrace sand and gravel clay (characterised as having imperfect drainage; waterlogged in the wet season; and desiccated in the dry season (Brown, 1965)). The total rainfall for January 2018 at Site 2, when samples were harvested, was 77.4 mm; the minimum and maximum temperatures at the time of harvest were 24.4 and 30.5^{0}C, respectively (Meteorological Service Jamaica, 2018); and the predominant soil type was the St. Ann's clay loam which is deep, well-drained dark red to dark reddish brown, moderately fine-textured with a high content of organic carbon to a great depth. They have a good structure and are highly permeable (MoA Jamaica, 1987).

3.3.2 Selection of forage species

Forage species were selected based on informal consultations with regional stakeholders across the Caribbean Community (CARICOM), including farmers who utilise these in their production systems and livestock scientists who have highlighted their potential use in small ruminant production systems in the region. A total of 12

species were selected including seven grass species; two leguminous multipurpose tree species (LMPTs) and three non-leguminous multipurpose tree species (NLMPTs).

3.3.3 Harvesting and preparation of forage samples

On 16 January 2018, samples (n=3) for ten forage species were harvested from Site 1. These included the leaves and stems of five grasses: *Brachiaria arrecta* (*B. arrecta*), *Brachiaria* hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*), *Digitaria eriantha* (*D. eriantha*), *Megathyrsus maximus* (*M. maximus*) *and Pennisetum purpureum* (*P. purpureum);* young and mature leaves and stems of two leguminous multipurpose tree species (LMPTs): *Gliricidia sepium* (*G. sepium*) and *Leucaena leucocephala* (*L. leucocephala*); and young and mature leaves and stems of three non-leguminous multipurpose tree species (NLMPTs): *Moringa oleifera (M. oleifera), Morus alba* (*M. alba*) and *Trichanthera gigantea* (*T. gigantea*)). On 23 January 2018, samples (n=3) of leaves and stem for two grass species (*Cynodon dactylon* (*C. dactylon*) *and Cynodon nlemfuensis* (*C. nlemfuensis*) were harvested from Site 2. At both sites, plots for grass species were cut prior to the harvesting date so that all species had a regrowth of 35 days (Aumont et al., 1995). Samples (n=3) for each grass species were harvested (manually chopped with a machete) at five to seven cm above ground level with each of the three replicates comprising cuts from several individual plants in one of three different locations within plots. Samples (n=3) for each tree species were harvested (manually chopped with a machete) from all parts of the tree canopy with each of the three replicates comprising cuts from several individual trees within the plots (Rosales, 1996). Immediately after harvesting, all samples collected were dried at 60^0C for 48 hours in a forced-air oven. The dried samples were ground before being packaged (wrapped in triple-plastic layers and

boxed) and exported (Export Permit no. 2017065045) to the Food and Nutrition Laboratory, Massey University, New Zealand for analysis. Upon arrival, the samples were ground further with a Thomas hammer mill (screen size:1 mm) and analysed using proximate analysis, *in-vitro* assays and near infrared spectroscopy (NIRS).

3.3.4 Proximate analysis

Samples were analysed for dry matter (DM) by drying at 105°C in a convection oven (AOAC 930.15). The total nitrogen (N) content was determined by combustion (AOAC 968.06) using a Leco CNS 200 Analyser (Leco Corporation, St Joseph, MI, USA), and the crude protein (CP) was computed by multiplying the N values obtained by a factor of 6.25. Starch was determined using an α-amylase Megazyme kit (AOAC 996.11). The neutral detergent fibre (NDF) (with heat stable amylase) and acid detergent fibre (ADF) fractions were determined by the method of Van Soest et al. (1991) and the Tecator Fibretec System (AOAC 973.18). The ash content was determined by total combustion at 550°C (AOAC 942.05) and the organic matter was calculated as the difference between the dry matter content and the ash content. Fat was determined by using the Soxtec method (AOAC 2003.06) and the gross energy using a bomb calorimeter.

3.3.5 *In-vitro* digestibility

The *in-vitro* dry matter digestibility (DMD) and *in-vitro* organic matter digestibility (OMD) were measured using the pepsin-cellulase method of Roughan and Holland (1977). The digestible organic matter content in dry matter (IVDOMD) was calculated from the organic matter (percentage) in the diet multiplied by the OMD. The *in-vitro*

metabolisable energy (ME) of the forages (MJ ME/kg DM) was calculated as DOMD × 0.163 (AFRC, 1993).

3.3.6 Near Infrared Spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) was used to estimate the chemical components of forage samples including CP, NDF, ADF, lipid, OM, ash, digestible organic matter in dry matter (NIRS DOMD) and the metabolisable energy (NIRS ME). The tropical forage samples were scanned using a Bruker MPA NIR spectrophotometer (Ettlingen, Germany). The resulting NIR spectra were then analysed using Optic user software (OPUS) version 5.0. (Ettlingen, Germany). The calibrations for each component were developed using NIRS after scanning finely ground temperate pasture samples in the range of 400 to 2500 nm.

3.3.7 Statistical analysis

Statistical analysis was done in the R environment for statistical computing and visualisation (Team 2013). Data on the nutritive value of forages was fitted to a linear model. An analysis of variance (ANOVA) was then applied to determine the level of significance of treatments in the model utilising both carr (Fox and Weisberg 2011) and Agricolae (de Mendiburu and de Mendiburu 2019) R packages. Means and superscripts were generated using both emmeans (Lenth et al. 2019) and multcomp (Hothorn et al. 2016) R packages which help to separate significantly different means using the Tukey's multiple comparison test. Differences were considered statistically significant if $P \leq 0.05$. Pearson's correlation between the digestibility data and the proximate chemical components, as well as the Pearson's correlation between

proximate chemical components, IVDOMD, *in-vitro* ME and their respective NIRS values were generated using the Corrr package (version 0.2.1 (Jackson 2016)). A simple linear regression was carried out to investigate the relationship between the chemical composition values generated by the NIRS method and those obtained by proximate analysis. Graphs were generated using the ggplot2 (Wickham, 2016) and ggpmisc (Aphalo, 2016) R packages were used to generate graphs.

3.4 Results

3.4.1 Proximate analysis and Near Infrared Spectroscopy (NIRS)

Crude protein

The chemical composition of forage species determined by proximate analysis is presented in Table 3.1. The CP content for all species ranged between 67.6 to 263.6 g/kg DM with grasses measuring between 67.6 to 191.2 g/kg DM and MPTs between 171.1 to 263.6 g/kg DM. The average CP content for the grasses was 113.4 g/kg DM and that of the MPTs was 213.0 g/kg DM. The grass species *C. nlemfuensis* had the highest CP content of 191 g/kg DM. *Brachiaria ruziziensis* had the lowest (p <.0001) CP content of 67.6 g/kg DM and there was no significant difference between *D. eriantha* (87.1 g/kg DM) and *M. maximus* (90.3 g/kg DM). The MPT *L. leucocephala* had the highest CP content (263.6 g/kg DM) and it was not significantly different from *M. oleifera* (232.5 g/kg DM). *Trichanthera gigantea* was the MPT with the lowest (p <.0001) CP content (171.1 g/kg DM).

The chemical composition of forage species determined by NIRS is presented in Table 3.2. *Cynodon nlemfuensis* had the highest (p <.0001) CP concentration (172.6 g/kg DM) of the grasses. *Brachiaria ruziziensis* (51.8 g/kg DM) had the lowest (p <.0001) CP concentration which was not significantly different from those of *D. eriantha* (60.1 g/kg DM) and *M. maximus* (93.6 g/kg DM). *Leucaena leucocephala* had the highest (p <.0001) CP content (272.1 g/kg DM) of all the MPTs. There was no significant difference in the CP concentration observed for the other MPTs which ranged between 190 to 218 g/kg DM.

Carbohydrates

The starch, NDF and ADF fractions for all forages ranged between 1.06 to 28.40, 380 to 756 and 250 to 497 g/kg DM, respectively. Starch ranged between 1.06 to 12.58 g/kg DM for the grasses and between 4.59 to 28.36 g/kg DM for the MPTs. The concentration of starch observed for the NLMPTs was significantly higher (p <.0001) than the concentrations obtained for the other species (23.68 to 28.36 g/kg DM). The NDF content of the grasses and MPTs ranged between 699 to 756 g/kg DM and 380 to 506 g/kg DM, respectively and that of ADF ranged between 383 to 497 for grasses and 250 to 363 g/kg DM for the MPTs. The average concentration of NDF for grasses was 722 g/kg DM and that of the MPTs was 455 g/kg DM. Grasses averaged 434 g/kg DM and the MPTs averaged 316 g/kg DM in ADF concentration. The NDF fraction of the MPTs was variable with LMPTs and *T. gigantea* having significantly higher concentrations (p <.0001) than those of *M. oleifera* and *M. alba*. The lignin content for all the MPTs except *M. oleifera* was higher (p <.0001) than the concentrations observed for the grasses. The concentration of NDF and ADF fractions for grasses using the NIRS method was between 696 to 783 g NDF/kg DM and 372 to 425 g ADF/kg DM, respectively and were higher (p <.0001) than the values obtained for the MPTs which ranged between 308 to 415 and 162 to 237 g/kg DM, respectively.

Organic matter, ash and gross energy

The OM, ash and GE for the species ranged between 684 to 847 g/kg DM, 92.8 to 222.5 g/kg DM and 16.0 to 20.1 MJ/kg DM, respectively. The ash content of *T. gigantea* (225.5 g/kg DM) was higher (p <.0001) and the GE significantly lower (p <.0001) than the concentrations obtained for the other MPTs. The OM and ash concentrations for all species, using the NIRS method, ranged between 809 to 894 g/kg DM, and 66.0 to 116.8 g/kg DM, respectively. The ash content of *L. leucocephala*, *M.*

alba and *T. gigantea* (112.0, 113.8 and 116.8 g/kg DM, respectively) were the highest (p <.0001) of all the MPTs.

Table 3.1 Gross chemical composition (g/k DM) (including the crude protein (CP), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, fat, organic matter (OM), ash) and gross energy (GE, MJ/kg DM) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) (n=3 per forage species)

	CP	Starch	NDF	ADF	Lignin	Fat	OM	Ash	GE
Grasses									
Brachiaria arrecta	109.5^{bc}	3.71^{a}	705^{cd}	449^{def}	77.3^{ab}	17.9^{ab}	820^{b}	122.0^{bcd}	17.4^{def}
Brachiaria hybrid*	67.6^{a}	4.85^{a}	715^{cd}	414^{cdef}	40.5^{a}	18.8^{abcd}	843^{b}	94.1^{a}	17.9^{f}
Cynodon dactylon	142.8^{cd}	12.58^{ab}	748^{cd}	388^{bcdef}	67.0^{a}	12.3^{a}	685^{a}	126.3^{cd}	17.2^{bcd}
Cynodon nlemfuensis	191.2^{ef}	1.06^{a}	699^{c}	383^{bcde}	59.6^{a}	18.3^{abc}	804^{b}	108.4^{ab}	17.3^{cde}
Digitaria eriantha	87.1^{ab}	3.92^{a}	727^{cd}	497^{f}	78.5^{ab}	21.8^{bcd}	838^{b}	95.5^{a}	17.9^{f}
Megathyrsus maximus	90.3^{ab}	1.96^{a}	756^{d}	472^{ef}	57.2^{a}	18.6^{abcd}	791^{b}	137.1^{de}	16.9^{bc}
Pennisetum pupureum	105.5^{b}	1.52^{a}	704^{cd}	436^{cdef}	45.3^{a}	26.1^{cde}	757^{ab}	146.4^{e}	16.7^{b}
LMPTs									
Gliricidia sepium	192.6^{ef}	12.92^{ab}	501^{b}	335^{abc}	188.1^{c}	31.3^{e}	807^{b}	112.0^{bc}	19.1^{g}
Leucaena leucocephala	263.6^{h}	4.59^{a}	505^{b}	347^{abcd}	185.4^{c}	26.3^{de}	847^{b}	92.8^{a}	20.1^{h}
NLMPTs									
Moringa oleifera	232.5^{gh}	28.36^{c}	386^{a}	284^{ab}	99.9^{ab}	46.3^{f}	836^{b}	93.5^{a}	19.8^{h}
Morus alba	205.3^{fg}	25.36^{c}	379^{a}	250^{a}	139.5^{bc}	22.1^{bcd}	755^{ab}	146.8^{e}	17.8^{ef}
Trichanthera gigantea	171.1^{de}	23.68^{bc}	502^{b}	363^{bcde}	196.5^{c}	22.4^{bcd}	684^{a}	225.5^{f}	16.0^{a}
SEM	6.61	2.37	10.4	21.5	12.8	1.54	18.4	3.09	0.097
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

All means carrying the same superscripts within columns are not significantly different (P > 0.05)
*Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

Table 3.2 NIRS predicted values for the chemical composition (g/kg DM) (including the crude protein, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, fat, organic matter (OM), ash) and *in-vitro* metabolisable energy (IV-ME, MJ/kg DM) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs)

	CP	NDF	ADF	Fat	OM	Ash	DOMD	IV-ME
GRASSES								
Brachiaria arrecta	104.4^{bc}	715^{cd}	403^{ef}	17.80^{cd}	889^{ef}	76.8^{abc}	0.507^{a}	6.99^{ab}
*Brachiaria h*ybrid[*]	51.8^{a}	717^{cd}	382^{e}	27.29^{d}	881^{def}	70.1^{ab}	0.517^{ab}	7.08^{ab}
Cynodon dactylon	111.3^{c}	699^{cd}	394^{e}	5.41^{a}	894^{f}	66.0^{a}	0.595^{b}	7.30^{abc}
Cynodon nlemfuensis	172.6^{d}	696^{c}	388^{e}	6.85^{ab}	881^{def}	85.3^{cd}	0.601^{b}	8.25^{cd}
Digitaria Eriantha	60.1^{ab}	709^{cd}	373^{e}	17.94^{cd}	883^{def}	72.7^{ab}	0.570^{ab}	7.69^{bcd}
Megathyrsus maximus	93.6^{abc}	783^{e}	425^{f}	16.52^{bc}	881^{def}	68.9^{ab}	0.496^{a}	6.27^{a}
Pennisetum pupureum	120.8^{c}	739^{d}	372^{e}	20.14^{cd}	864^{cdef}	80.7^{bcd}	0.565^{ab}	7.91^{bcd}
LMPTs								
Gliricidia sepium	215.6^{d}	308^{a}	200^{bc}	38.84^{e}	853^{cd}	88.9^{d}	0.728^{c}	8.66^{de}
Leucaena leucocephala	272.1^{e}	318^{a}	162^{a}	49.14^{f}	846^{bc}	112.0^{e}	0.770^{cd}	9.77^{ef}
NLMPTs								
Moringa oleifera	218.1^{d}	415^{b}	212^{cd}	51.15^{f}	860^{cde}	92.0^{d}	0.759^{cd}	9.90^{f}
Morus alba	190.2^{d}	311^{a}	181^{ab}	58.97^{f}	815^{ab}	113.8^{e}	0.839^{de}	12.21^{g}
Trichanthera gigantea	203.7^{d}	374^{b}	237^{d}	38.55^{e}	809^{a}	116.8^{e}	0.913^{e}	10.25^{f}
SEM	8.96	8.1	6.1	1.95	6.35	2.33	0.017	0.227
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

All carrying the same superscripts within columns are not significantly different (P > 0.05)

[*]Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

3.4.2 *In-vitro* digestibility and ME content of forages

Table 3.3 summarises the digestibility and IV-ME of the different forage species. The IVDOMD for the grasses were between 0.496 to 0.530 and for the MPTs, between 0.585 to 0.655. Metabolisable energy ranged between 8.08 to 8.65 MJ/kg DM for grasses and 9.53 to 10.68 MJ/kg DM for the MPTs. With respect to the MPTs, the digestibility of the NLMPTs was significantly higher (p <.0001) than those of the LMPTs. *Morus alba* had the highest IVDOMD which was not significantly different from that of *M. oleifera*. *Trichanthera gigantea* had the lowest IVDOMD (0.585) and ME (9.53 MJ kg DM) of all the MPTs.

The NIRS DOMD ranged between 0.496 to 0.601 for the grasses, 0.728 to 0.770 for the LMPTs and 0.759 to 0.913 for the NLMPTs. The MPTs, *T. gigantea* and *M.* alba had the highest NIRS DOMD values (0.913 and 0.839, respectively). The NIRS ME values ranged between 6.27 to 8.25 MJ /kg/DM for grasses; 8.66 to 9.77 MJ /kg/DM for LMPTs; and 9.90 to 12.21 MJ /kg DM for NLMPTs. *Morus alba* had the highest (p<.0001) NIRS ME concentration (12.21 MJ ME/kg/DM).

Table 3.3 Digestibility (*in-vitro* dry matter digestibility (IVDMD, g/g DM); *in-vitro* organic matter digestibility (IVOMD, digestibility coefficient); *in-vitro* digestible organic matter in dry matter (IVDOMD, g/kg DM) and metabolisable energy (ME, MJ/kg DM) of grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) that are used in small ruminant production systems in the Caribbean (n=3 per forage species)

	IVDMD	IVOMD	IVDOMD	ME *
GRASSES				
Brachiaria arrecta	0.581^{b}	0.574^{ab}	0.515^{ab}	8.40^{ab}
Brachiaria hybrid**	0.582^{b}	0.582^{b}	0.526^{b}	8.58^{b}
Cynodon dactylon	0.575^{ab}	0.571^{ab}	0.511^{ab}	8.32^{ab}
Cynodon nlemfuensis	0.592^{b}	0.591^{b}	0.530^{b}	8.65^{ab}
Digitaria eriantha	0.572^{ab}	0.572^{ab}	0.517^{b}	8.42^{b}
Megathyrsus maximus	0.560^{a}	0.553^{a}	0.496^{a}	8.08^{ab}
Pennisetum pupureum	0.586^{b}	0.580^{b}	0.517^{b}	8.42^{b}
LMPTs				
Gliricidia Sepium	0.662^{c}	0.671^{c}	0.599^{cd}	9.76^{cd}
Leucaena leucocephala	0.668^{c}	0.683^{c}	0.614^{d}	10.01^{d}
NLMPTs				
Moringa oleifera	0.705^{d}	0.726^{d}	0.651^{e}	10.62^{e}
Morus. Alba	0.729^{e}	0.749^{d}	0.655^{e}	10.68^{e}
Trichanthera gigantea	0.696^{d}	0.687^{c}	0.585^{c}	9.53^{c}
SEM	0.004	0.005	0.004	0.066
p-value	<.0001	<.0001	<.0001	<.0001

All means carrying the same superscripts within columns are not significantly different (P > 0.05)
* ME = *in-vitro* digestible organic matter (IVDOMD) x 0.163
**Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *Brachiaria brizantha* x *Brachiaria decumbens*)

3.4.3 Correlation coefficients between proximate components and *in-vitro* digestibility

Correlation analysis between chemical components and digestibility parameters showed that CP and starch had a strong positive relationship (0.82 and 0.74, respectively, $P < 0.05$) with the IVDOMD. However, the NDF and ADF fractions had a strong negative correlation with the IVDOMD (-0.98 and -0.86, respectively, $P < 0.05$) (Table 3.4).

Table 3.4 Correlation coefficients between proximate components and *in-vitro* digestibility of forages used in small ruminant production systems in the Caribbean

	Chemical composition and the respective correlation coefficients*						
Variable	Ash	CP	Fat	Starch	NDF	ADF	Lignin
IVDMD	0.27	0.78	0.58	0.80	-0.98	-0.84	0.72
IVOMD	0.17	0.80	0.61	0.78	-0.99	-0.85	0.69
IVDOMD	0.02	0.82	0.66	0.74	-0.98	-0.86	0.65

* Correlation coefficients ≥ 0.310 or ≤ -0.310 are significant at P ≤ 0.05

Terms used: CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; IVDMD: *in-vitro* dry matter digestibility; IVOMD: *in-vitro* organic matter digestibility; IVDOMD: *in-vitro* digestible organic matter in dry matter

3.4.4 Correlation Coefficients between Gross chemical composition and *In-vitro* metabolisable energy values predicted by Near Infrared Spectroscopy (NIRS)

The NIRS predictions for crude protein (CP) and NDF were strongly correlated with the respective values generated by gross chemical analysis having coefficients of 0.94 and 0.94 ($P < 0.05$) respectively (Table 3.5). The correlation between the NIRS-ME and the *in-vitro* assay ME was 0.89 ($P < 0.05$).

Table 3.5 Correlation coefficients between Gross chemical composition and *in-vitro* metabolisable energy values predicted by Near Infrared Spectroscopy (NIRS) for 12 forage species used in small ruminant systems in the Caribbean

Variable	Correlation Coefficients*
Dry matter	0.50*
Ash	0.86*
Crude protein	0.94*
Fat	0.11
Neutral Detergent Fibre	0.94*
Acid Detergent Fibre	0.79*
In-vitro metabolisable energy	0.89*

* Correlation coefficients ≥ 0.310 is significant at $P \leq 0.05$

3.4.5 Linear Relationship between NIRS and the chemical composition

The linear relationship between NIRS and the chemical composition of forages is presented in Figures 3.1 and 3.2. The correlation of determination (R^2) between the proximate CP, NDF, ADF as well as the IVDOMD and IV-ME and their respective NIRS values were 0.91 ($P < 0.05$), 0.86 ($P < 0.05$), 0.59 ($P \leq 0.05$), 0.7 ($P < 0.05$) and 0.8 ($P < 0.05$), respectively.

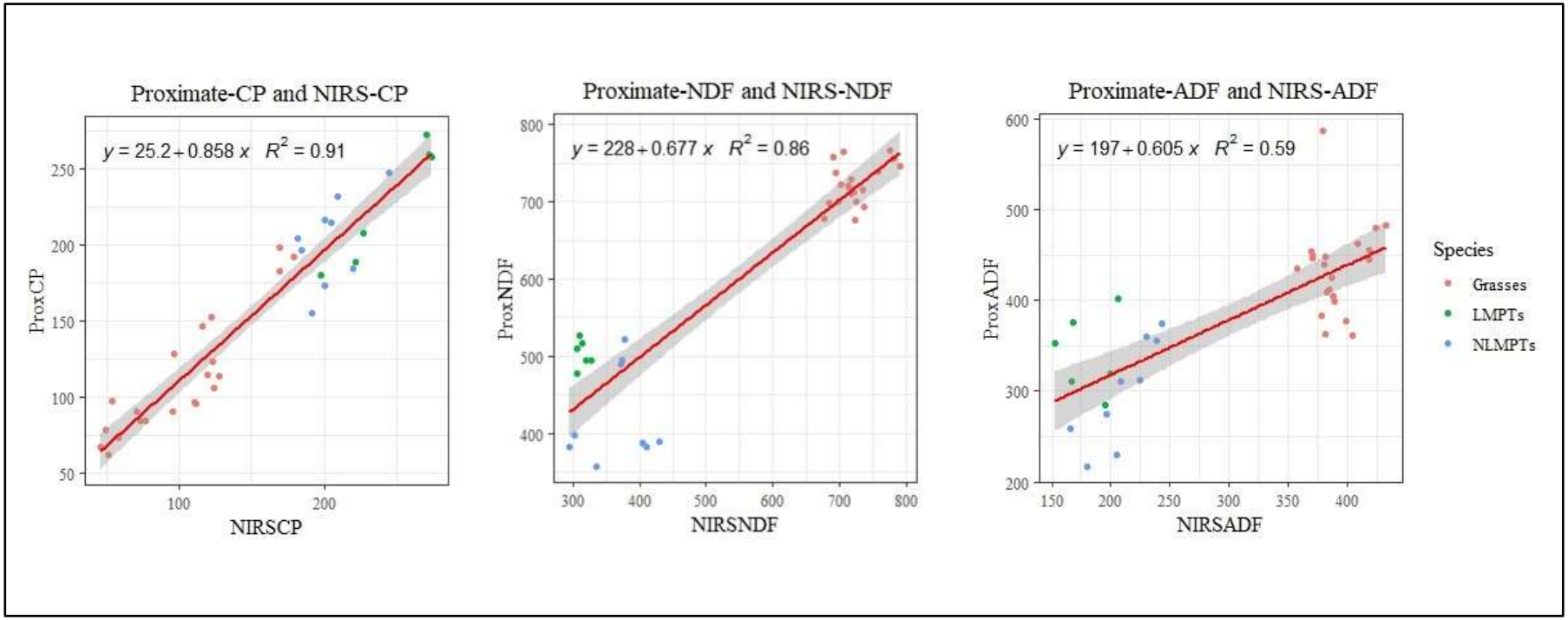

Figure 3.1 The relationship between gross chemicals CP, NDF and NDF with their respective near infrared spectroscopy (NIRS) values

*Terms used: proxCP: proximate crude protein; NIRSCP: crude protein determined by near infrared spectroscopy; proxNDF: proximate neutral detergent fibre; NIRSNDF: neutral detergent fibre determined by near infrared spectroscopy; proxADF; proximate acid detergent fibre; NIRSADF: acid detergent fibre determined by near infrared spectroscopy; LMPTs: leguminous multipurpose tree species; NLMPTs: non-leguminous multipurpose tree species

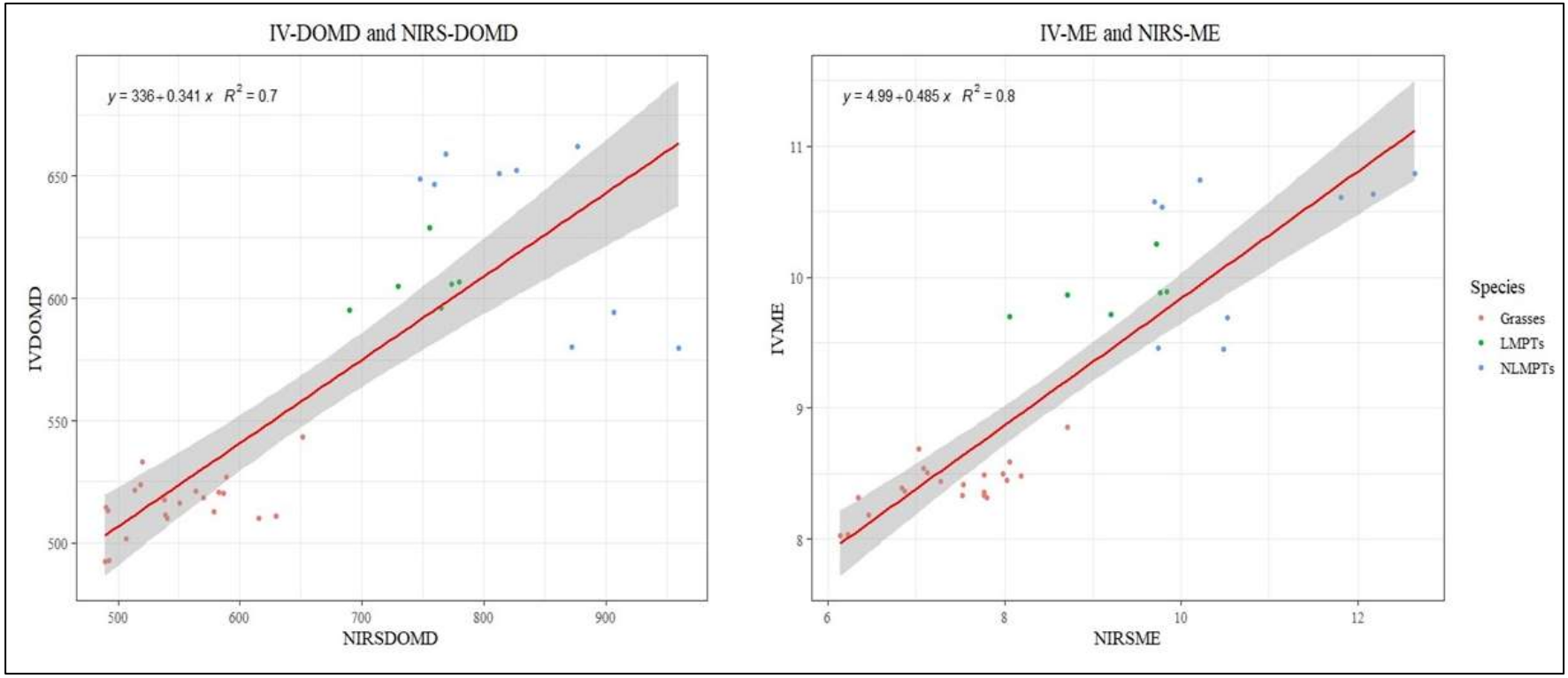

Figure 3.2 The relationship between the *in-vitro* digestible organic matter in dry matter (DOMD) and metabolisable energy (ME) with their respective near infrared spectroscopy (NIRS)-predicted values

3.5 Discussion

In the current study, the CP concentration observed for the MPTs was high with an average proximate CP and NIRS CP concentration of 213.0 and 219.9 g/kg DM, respectively. MPTs are typically known for their high CP content when compared to tropical grasses which makes them suitable high-protein forage supplements, particularly during the drier parts of the year when both the quality and yield of tropical grasses decline severely (Wilson, 1969).

There were differences in the concentration of CP among the various grass species. For example, *C. nlemfuensis* was ranked as having the highest proximate and NIRS CP concentrations for the grass species despite the lower values reported for the species in the Caribbean (98 to 140 g/kg DM) (Aumont et al., 1995; Miller et al., 2004). The differences in the concentration of CP between the studies may be implicated in differences in plant factors (samples comprising leaves or stem; maturity; cultivars) and environmental factors including weather and cultural practices (Hughes et al., 2012; Sarwar, 1999). Conversely, values up to 242 g CP/kg DM have been reported for the species in other tropical regions (Caro-Costas et al., 1976). The *Brachiaria* hybrid (cv.) Mulato II had the lowest proximate CP and NIRS CP (67 and 51.8 g/kg DM, respectively) of all the grass species which, by both methods, were comparable to those of *D. eriantha* (87.1 and 60.1 g/kg DM, respectively) and *M. maximus* (90.3 and 93.6 g/kg DM, respectively). The lower values obtained for *M. maximus* and *D. eriantha* despite the early regrowth (35 days), were consistent with the literature for the species at a similar stage of regrowth (5.3 to 12.0 g CP/kg DM for *D. eriantha* (Chaiwang et al., 2011; Fanchone et al., 2012)) and between 86 to 140 g/kg DM for *M. maximus* (Lima et al., 2013; Melesse et al., 2017) which may indicate the low

quality of these grass species. However, the *Brachiaria* hybrid is one of the improved tropical cultivars and CP concentrations between 110 to 160 g/kg DM, and sometimes up to 210 g CP/kg DM, have been reported for this forage (Argel et al., 2007; Guiot, 2005). The differences in values observed between the studies for the *Brachiaria* hybrid may be implicated in environmental factors, including weather, cultural practice and soil type, as these impact on the nutritive value of forages (Hughes et al., 2012; Sarwar and Saeed, 1999).

With respect to the MPTs, *L. leucocephala* had the highest concentration of proximate CP which was comparable to that of *M. oleifera*. A similar pattern was observed for the NIRS method where *L. leucocephala* had the highest NIRS CP concentration among the MPTs. Both *L. leucocephala* and *M. oleifera* are rich in CP which may contain up to 260 g/kg DM for *L. leucocephala* and over 300 g CP/kg DM for *M. oleifera* (Gill et al., 2007; Heuze et al., 2019). Values obtained were comparable to values reported for *L. leucocephala* (302 to 318 g/kg DM) and *M. oleifera* (160 to 205 g/kg DM) in the Caribbean (Edwards et al., 2012; López et al 2017). The proximate CP-value for *T. gigantea* was the lowest recorded for the MPTs, however, the NIRS value was high (203.7 g CP/kg DM) and comparable to the NIRS CP-value s of other protein-rich species like *G. sepium* (215.6 g CP/kg DM), *M. oleifera* (218.1 CP/kg DM) and *M. alba* (190.2 g CP/kg DM). The CP-value s obtained by both methods for these MPTs were within the range reported in the literature for these MPTs (Fadiyimu et al., 2016; Martín et al., 2007; Rodríguez et al., 2017; Rosales, 1997a).

Carbohydrates are critical precursors to volatile fatty acids (VFAs) which are the main sources of energy for ruminants (Faverdin, 1999; Rosales, 1997b). Tropical grasses, in comparison to other forages, are often higher in the fibrous carbohydrate fractions (Devendra and Gohl, 1970; Oyenuga, 1957). For instance, the proximate NDF

observed for grasses ranged between 699 to 756 g/kgDM. Similar results were observed for the NIRS method where the concentration of both NIRS NDF ranged between 696 to 783 g/kg DM. The proximate NDF values for the MPTs ranged between 379 to 505 g/kg DM and 308 to 415 g/kg DM for NIRS. Unlike the MPTs, the NDF values obtained for grasses were above the critical levels for these fractions (650 g/kg DM) which are associated with restricted intake in ruminants (Van Soest et al., 1991). For the MPTs, the proximate NDF concentrations for the LMPTs (501 to 505 g/kg DM) and *T. gigantea* (502 g/kg DM) were higher than those typically reported for these species. For instance, in studies done by Rosales (1997b), *G. sepium, L. leucocephala* and *T. gigantea* had the lowest concentration of the cell wall fractions ranging between 294 to 308 g/kg DM for NDF and between 217 to 248 g/kg DM for ADF when compared to that of other MPTs which ranged between 318 to 613 g/kg DM for NDF and 264 to 620 for ADF. The lower values obtained by Rosales (1997b) were more comparable to the NIRS NDF and NIRS ADF values reported (308 to 374 g/kgDM and 162 to 237 g/kg DM, respectively). The observed differences between the proximate values of the current study and those of Rosales (1997b) may be as a result of differences in samples used which were comprised of both stems and leaves in the current study and leaves only for the study done by Rosales (1997b).

The generally low CP and high NDF concentration in grasses may limit both dry matter and energy intake and subsequently reduce performance (Arthington and Brown, 2005; Islam et al., 2003). However, supplementation of tropical grasses with the MPTs may increase the CP concentration; reduce the concentration of the cell wall fractions and, therefore, improve the overall intake of diets comprising mainly fibrous tropical grasses of low nutritive value (Wilson, 1969).

The IVDOMD and ME obtained in this study were within the range reported in the literature for tropical grasses and MPTs (Durmic et al., 2017). The IVDOMD is the digestibility parameter most closely related to the ME of feeds (CSIRO, 2007). The average IVDOMD and ME obtained for the MPTs was high, which may be as a result of the characteristically high concentrations of CP and the lower concentrations NDF observed for the MPTs (Arthington and Brown, 2005). The positive impact of CP on the IVDOMD may be demonstrated by the strong positive relationship between CP and the IVDOMD ($r = 0.82$, $P < 0.05$) and the negative effect of cell wall fractions on the IVDOMD may be demonstrated by the strong negative relationship between the NDF ($r = -0.98$, $P < 0.05$) and ADF ($r = -0.86$, $P < 0.05$) with the IVDOMD. Conversely, the low concentrations of CP and high concentrations of NDF in grasses may have resulted in the overall lower digestibility of these species. This negative relationship between both NDF and ADF with the IVDOMD was reported by other authors (Kamalak et al., 2005).

Lignin had a positive relationship with the IVDOMD (0.65, $P < 0.05$), unlike that of the NDF and ADF fractions. Throughout the literature, there have been conflicting reports on the accuracy of using the concentration of lignin to predict the digestibility of feeds. For instance, in some studies, lignin was not as strongly correlated with digestibility as the ADF fraction (Moss and Givens, 1990) whereas, for other authors, lignin was the cell wall fraction more strongly correlated with digestibility (Jung and Allen, 1995). The contradictions in reports may be because the spatial distribution of lignin in the cell wall matrix impacts more readily on the digestibility of feeds rather than its concentration. Therefore, feeds with a higher lignin concentration may not always be the least digestible (Reeves, 1987).

The IVDOMD and ME of grasses were comparable across species, but varied for the MPTs. However, for the MPTs, *M. oleifera* and *M. alba* had the highest IVDOMD (0.651 and 0.655, respectively) and ME (10.62 and 10.68 MJ/kg DM, respectively) which may be related to the overall higher nutritive value of these species in comparison to the other MPTs. *Trichanthera gigantea* and *G.* sepium had the lowest IVDOMD and ME which were comparable to the values reported in the literature for these species (Durmic et al., 2017). The low IVDOMD obtained for *T. gigantea* (0.585) may be better explained by the lower proximate CP (171.1 g/kg DM), and higher proximate NDF (502 g/kg DM). *Trichanthera gigantea* also had the highest ash content of all the MPTs (225.5 g/kg DM). Greater proximate ash content has also been associated with lower digestibility and energy in forage and may also explain the lower IVDOMD obtained for the species (225.5 g/kg DM) (Faverdin, 1999; Lazzarini et al., 2009; Negesse et al., 2009). The lower proximate CP (192.6 g/kg DM) and higher concentrations of proximate NDF (501 g/kg DM) for *G. sepium* compared to *M. alba* and *M. oelifera* may be more aligned to the lower IVDOMD (0.599) than the higher DOMD of the NIRS method (0.728). Although the NIRS DOMD and NIRS ME values obtained for the grasses were comparable to their respective *in-vitro* values, those for the MPTs were higher and the ranking of the species differed between the methods. For instance, based on the *in-vitro* method, both *M. oleifera* and *M. alba* ranked the highest, and *T. gigantea* the lowest, in terms of their IVDOMD and ME, however, for the NIRS method, the DOMD and ME of *T. gigantea* was comparable to that of *M. alba* and higher than that of *M. oleifera*. Overall, species were above the minimum IVDOMD (0.500) and 7.5 MJ/kg DM required for tropical forage species to be classified as good quality forage (Bediye et al., 2007).

There was a strong relationship between the proximate CP ($r = 0.94$, $P < 0.05$), proximate NDF ($r=0.94$, $P \leq 0.05$), IVDOMD ($r = 0.84$, $P < 0.05$) and ME ($r=0.89$, $P < 0.05$) and their respective NIRS values. The high correlations suggest that even with a NIRS calibration model built primarily on temperate pasture, reasonable predictions can be obtained for tropical forages (Figures 1 and 2). The high coefficient of correlation between the proximate CP and NDF with their respective NIRS values was expected as the NIRS prediction models for these are typically precise because of the extensive use of NIRS to measure these components (Dardenne and Salgado, 2015). Further, the results of the study suggest that the NIRS model was effective at predicting the IVDOMD and the *in-vitro* ME of tropical forages. There was a high R^2 between proximate CP, proximate NDF, IVDOMD, *in-vitro* ME and their respective NIRS values. In the case of NDF and ADF, the high R^2 was due to the vast differences in the values obtained for both grasses and MPTs. When the forage type was included in the model as a covariate, the relationship between proximate and NIRS for NDF and ADF was not significant.

3.6 Conclusion

Overall, forage species varied in terms of their nutritive values. The multipurpose tree species (MPTs) compared to grasses were generally of higher nutritive value in terms of their crude protein (CP), neutral detergent fiber (NDF), digestibility and metabolisable energy (ME). This may indicate their potential to improve the quality of diets comprised of grasses with inherently lower CP, higher NDF and generally lower digestibility and ME. Moreover, the MPTs, particularly *Moringa oleifera* and *Morus alba*, having the highest reported digestibility and ME of all the species, may be used as partial alternatives or substitutes to the costly commercial concentrate feed. Further, studies may be required to determine the spatial and temporal effect of management (cutting intervals, fertilizer application, irrigation) and environmental factors (soil, precipitation, temperature) on the nutritive value of forages established under tropical conditions in the Caribbean. The high correlation between NIRS and proximate values may indicate the potential of NIRS to provide routine, rapid and cost-effective evaluation of a range of forages in the Caribbean which may indirectly lead to optimised livestock management and productivity. Overall, although the species varied in terms of their nutritive value, they were generally above the minimum CP, IVDOMD and ME required to be classified as intermediate to good quality forages.

CHAPTER 4 The mineral content of some tropical forages used in small ruminant production systems in the Caribbean

Data presented in this Chapter have been published in:

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4.1 Abstract

Understanding the concentrations of minerals in forages is critical as it informs which and how species can be used to improve the mineral content of diets. Regulating the mineral composition of forages through ongoing forage evaluation is important as deficient minerals can be identified and supplemented. Therefore, the aim of this study was to provide information on the mineral profiles for 12 forages used in small ruminant production systems in the Caribbean. The forages included seven grasses (*Brachiaria arrecta, Brachiaria* hybrid cv. Mulato II *(Brachiaria ruziziensis x B. brizantha x B. decumbens), Cynodon dactylon, Cynodon, nlemfuensis, Digitaria eriantha, Megathyrsus maximus and Pennisetum purpureum*); two leguminous multipurpose tree species (LMPTs) (*Gliricidia sepium* and *Leucaena leucocephala*) and three non-leguminous multipurpose tree species (NLMPTs) (*Moringa* oleifera, *Morus alba* and *Trichanthera gigantea*). *Trichanthera gigantea* had the highest (p <.0001) concentration of calcium (Ca) for the MPTs. *Brachiaria arrecta* and *Digitaria eriantha* had the highest (*p* <.0001) sodium concentration of all the grasses. *Pennisetum purpureum, Brachiaria arrecta* and *Cynodon nlemfuensis* had the highest (*p* <.0001) concentration of potassium (39.1, 34.0 and 32.6 g/kg DM, respectively). Copper concentrations were highest in grasses including *Brachiaria arrecta*, *Cynodon dactylon* and *Cynodon nlemfuensis* and in MPTs including *Leucaena leucocephala* and *Trichanthera gigantea*. *Cynodon dactylon* had the highest (*p* <.0001) concentration of iron (3340 g/kg DM). The concentration of molybdenum in *the Cynodon* species were higher (*p* <.0001 than that of the other grasses. The results of the study suggest that forages varied in mineral concentrations and inclusion levels must be carefully manged

to ensure that the mineral concentrations of diets offered are within the range required for small ruminants.

Keywords: Caribbean, forage, minerals, small ruminants

4.2 Introduction

One of the major sources of minerals for small ruminants is forage (McDowell and Arthington, 2005). Minerals are inorganic nutrients that are required for growth and development, and under-nutrition, as a result of mineral imbalances, has long been held responsible for low production in ruminants of the tropics (McDowell and Arthington, 2005). Few studies have been done primarily on the mineral status of forages in several of the islands within the region. Generally, the concentration of minerals including calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), zinc (Zn) and manganese (Mn) were often reported as abundant in forages; iron (Fe) was frequently reported as being at high or at toxic levels; and sodium (Na) and copper (Cu) reported as being low or deficient in forages (Bernard et al., 2019; Devendra, 1977; Mohammed et al., 2017; Youssef and Brathwaite, 1987). Some of the major challenges experienced in the region as a result of mineral imbalances include nutritional disorders that result in wasting diseases, high mortality, low fertility and non-infectious abortions (Devendra, 1977). These may lead to severe economic losses to farms and the careful management of mineral intakes is required to ensure that the requirements of animals are met and that losses are minimised (Hernández and Sánchez, 2014).

There are within and between variations in the mineral concentration of grasses and multipurpose tree species (MPTs) (Dongall and Bogdan, 1958; Topps, 1992). Even though there are limited reports on the mineral status of MPTs established under tropical conditions in the Caribbean, they are known to be good sources of macro-minerals in comparison to grasses (Goodchild and McMeniman, 1994; Rosales, 1997b; Topps, 1992). This may be related to the relatively deeper root systems of

MPTs, which allows for the exploitation of mineral reserves across the soil profile (Rosales, 1996). Several studies reported variations in the mineral concentrations between different grasses and different MPTs despite being subject to similar growth conditions (Dongall and Bogdan, 1958; Rosales, 1997b). Further, some accumulator species may be more inclined to absorb extremely high levels of specific micro-minerals (metals), although at high concentrations that may be detrimental to the health of animals (McDowell and Arthington, 2005). As a result, understanding the concentration of minerals in forages is critical as it informs which and how species can be used to improve the mineral content of the diet. Assessing the mineral composition through ongoing forage evaluation is important as deficient minerals can be identified and supplemented (Youssef, 2000). Therefore, the aim of the following study is to provide current information on the concentration of minerals (macro-minerals: Ca, P, Mg, Na, K; and micro-minerals: Fe, Cobalt, Mn, Molybdenum, Zn and Cu) for both grasses and multipurpose tree species (MPTs) used in small ruminant production systems in the Caribbean.

4.3 Materials and Methods

4.3.1 Site description

The site description was described in Chapter 3 of this thesis. Samples for all species were collected from one of two sites (Site 1 and Site 2). Site 1 was the Forage Bank at the University of Trinidad and Tobago - Valsayn Campus, Trinidad and Tobago and Site 2 was the forage bank at New Wales Manchester, Central Jamaica.

4.3.2 Selection of forage species and harvesting and preparation of samples

The selection of forages and the harvesting and preparation of forage samples are described in Chapter 3.

4.3.3 Proximate analysis

Samples were analysed for dry matter (DM), nitrogen (N), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, organic matter, fat and the gross energy (GE). The analyses are described in Chapter 3.

4.3.4 Mineral profile of forages

Dried ground samples were digested with concentrated nitric acid and hydrochloric acid at 105 °C for 1 hour; made to volume with Type 1 water and filtered. Elements in the digest including calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K); and micro-minerals including iron (Fe), cobalt (Co), manganese (Mn), molybdenum (Mo), zinc (Zn) and copper (Cu) were measured by inductively coupled plasma optical emissions spectrometry (ICP-OES) with internal standard (ISTD) correction against matrix-matched standards using a Perkin Elmer NexION 300D system in accordance with in-house procedures based on APHA 3030F and 3125. Elements including Mo and Co were measured by inductively coupled plasma mass spectrometry (ICP-MS) with internal standard (ISTD) correction against matrix-matched standards using a Thermo Scientific iCAP 6500 system in accordance with in-house procedures based on APHA 3030F and 3120.

4.3.5 Statistical analysis

A statistical analysis was done in the R environment for statistical computing and visualisation (Team 2013). An ANOVA was used to obtain the *p*-value for the model differences. Where significant differences between the treatment groups were detected, means were separated using the Tukey's test. Differences were considered statistically significant if $p < 0.05$.

4.4 Results

4.4.1 Macro-mineral profile of forages

The macro-mineral concentrations of the twelve forage species are presented in Table 4.1. There was no significant difference in the concentrations of Ca observed for the grasses. The concentration of Ca for the MPTs was wide-ranging. *Trichanthera gigantea* had the highest (p <.0001) Ca concentration (55.68 g/kg DM) among the MPTs measuring up to twice the average Ca value for the other MPTs (29.82 g/kg DM). *Morus alba* had the highest (p <.0001) P concentration (8.28 g/kg DM) of all the MPTs. The P concentration of the LMPTs (2.15 g/kg DM) was lower (p <.0001) than those of the other MPTs except for that of *T. gigantea* (3.19 g/kg DM). The concentration of Mg reported for *T. gigantea* (9.08 g/kg DM) and *G. sepium* (5.81 g/kg DM) were highest (p <.0001) of all the MPTs. All forages were generally low in Na except for *B. arrecta* (0.9626 g/kg DM) and *D. eriantha* (4.4281 g/kg DM). The concentration of K observed for *P. purpureum* (39.1 g/kg DM) was significantly higher (p <.0001) than those reported for all other grass species except *B. arrecta* (32.6 g/kg DM) and *C. nlemfuensis* (34.0 g/kg DM). The Ca:P ratio of grasses ranged between 1:1 to 2:1 and between 3:1 to 17:1 for the MPTs.

Table 4.1 Macro-mineral content (g/kg DM) (calcium, phosphorus, magnesium, sodium, potassium) and calcium to phosphorus ratio of grasses and leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean (n=3 per forage species)

	Calcium	Phosphorus	Magnesium	Sodium	Potassium	Ca:P ratio
Grasses						
Brachiaria arrecta	5.06^{a}	3.65^{bcd}	2.06^{a}	0.9626^{b}	32.6^{efg}	1:1
*Brachiaria h*ybrid *	4.91^{a}	4.94^{cde}	3.84^{c}	0.0871^{a}	19.2^{ab}	1:1
Cynodon dactylon	6.80^{a}	4.67^{cde}	2.05^{a}	0.3134^{a}	28.4^{def}	1:1
Cynodon nlemfuensis	6.29^{a}	5.70^{e}	3.03^{abc}	0.2072^{a}	34.0^{fg}	1:1
Digitaria eriantha	4.50^{a}	4.54^{bcde}	1.95^{a}	4.4281^{c}	14.7^{a}	1:1
Megathyrsus maximus	5.58^{a}	3.55^{abc}	3.95^{c}	0.1670^{a}	26.2^{cde}	2:1
Pennisetum pupureum	5.90^{a}	5.54^{e}	2.47^{ab}	0.0760^{a}	39.1^{g}	1:1
LMPTs						
Gliricidia sepium	30.49^{d}	2.15^{a}	5.81^{d}	0.1959^{a}	16.0^{a}	14:1
Leucaena leucocephala	20.65^{c}	2.15^{a}	2.95^{abc}	0.0660^{a}	19.8^{abc}	10:1
NLMPTs						
Moringa oleifera	16.04^{b}	5.02^{de}	3.01^{abc}	0.2158^{a}	25.1^{bcd}	3:1
Morus alba	26.24^{d}	8.28^{f}	3.58^{bc}	0.1432^{a}	28.5^{def}	3:1
Trichanthera gigantea	55.68^{e}	3.19^{ab}	9.08^{e}	0.1377^{a}	24.6^{bcd}	17:1
SEM	0.837	0.279	0.227	0.122	1.31	-
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	-
Requirement**	2.0 - 8.2	1.6 - 3.8	1.6 - 1.8	0.9 - 1.8	5.0 - 8.0	1:1 - 2:1
Maximum tolerable levels**	20	6	5	90	30	-

All means carrying the same superscripts within columns are not significantly different (P > 0.05)

*Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

Terms used: Ca:P ratio*: Calcium to Phosphorus ratio; McDowell and Arthington (2005); LMPTs: Leguminous multipurpose tree species; NLMPTs: Non-leguminous multipurpose tree species

**Mineral requirement for small ruminants (g/kg DM) NRC (1985); Maximum tolerable levels of macro-minerals for small ruminants (g/kg DM) Kearl (1982)

4.4.2 Micro-minerals

Table 4.2 presents the micro-mineral content of the twelve forages. *Cynodon dactylon* had the highest (p <.0001) Fe concentration (3340 g/kg DM) which was over 10 times the average value for the other grass species (237 mg/kg DM). *Cynodon dactylon* had the highest (p <.0001) Co (1.0477 mg/kg DM), Mn (173.7 mg/kg DM) and Zn (89.7 mg/kg DM) concentrations of all the grass species. Among the grasses, both *C. dactylon* and *C. nlemfuensis* had the highest (p <.0001) concentration of Mo (8.615 and 7.722 mg/kg DM, respectively). All species were low in Cu except for *C. dactylon* (11.50 mg/kg DM), *C. nlemfuensis* (20.24 mg/kg DM), *B. arrecta* (11.95 mg/kg DM), *L. leucocephala* (8.16 mg/kg DM) and *T. gigantea* (16.60 mg/kg DM).

Table 4.2 Micro-mineral content (iron, cobalt, manganese, molybdenum, zinc, copper (mg/kgDM)) and the copper to molybdenum ratio of grasses and multipurpose tree species used in small ruminant production systems in the Caribbean (n=3 per forage species)

	Iron	Cobalt	Manganese	Molybdenum	Zinc	Copper	Cu: Mo ratio*
Grasses							
Brachiaria arrecta	174^{a}	0.0672^{ab}	27.0^{ab}	1.288^{a}	55.6^{f}	11.50^{c}	8.15:1
Brachiaria hybrid **	160^{a}	0.0533^{ab}	20.9^{ab}	2.422^{a}	29.9^{bcd}	3.34^{a}	1.49:1
Cynodon dactylon	3340^{b}	1.0477^{e}	173.7^{e}	8.615^{b}	89.7^{g}	20.24^{e}	1.82:1
Cynodon nlemfuensis	435^{a}	0.2416^{cd}	49.8^{cd}	7.722^{b}	49.4^{ef}	11.95^{c}	1.32:1
Digitaria eriantha	235^{a}	0.0715^{ab}	24.7^{ab}	3.002^{a}	37.9^{cde}	6.46^{b}	2.64:1
Megathyrsus maximus	351^{a}	0.0825^{ab}	29.5^{ab}	1.659^{a}	42.0^{def}	5.90^{b}	4.26:1
Pennisetum purpureum	288^{a}	0.1328^{abc}	40.2^{bcd}	2.712^{a}	36.5^{cde}	7.59^{b}	2.71:1
LMPTs							
Gliricidia sepium	154^{a}	0.1923^{bcd}	14.2^{a}	0.106^{a}	15.5^{a}	3.34^{a}	38.70:1
Leucaena leucocephala	112^{a}	0.1597^{abcd}	34.4^{bc}	0.873^{a}	20.6^{ab}	8.16^{b}	6.33:1
NLMPT							
Moringa oleifera	123^{a}	0.0215^{a}	27.2^{ab}	1.237^{a}	24.7^{abc}	7.75^{b}	4.58:1
Morus alba	173^{a}	0.0406^{a}	40.0^{bcd}	0.507^{a}	34.4^{bcd}	6.91^{b}	13.71:1
Trichanthera gigantea	400^{a}	0.2749^{d}	58.6^{d}	0.675^{a}	37.0^{cde}	16.60^{d}	19.26:1
SEM	154	0.028	3.87	0.59	2.79	0.479	-
p-*value*	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	-
Requirement***	30 - 50	0.05 - 0.1	20 - 40	0.01	20 - 33	8 - 25	2:1 - 4:1
Maximum tolerable levels***	500	10	1000	5	300	25	-

All means carrying the same superscripts within columns are not significantly different (P > 0.05)

*Co:Mo ratio or cupper to molybdenum ratio

***Brachiaria* hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*), Cu:Mo ratio*: copper to molybdenum ratio

***Micro-mineral requirement (mg/kg DM) for small ruminants (Miltimore and Mason, 1971; NRC, 1985); Maximum tolerable levels of micro-minerals (mg/kg DM) for small ruminants (Kearl, 1982)

4.5 Discussion

The aim of this study was to examine the mineral composition of a range of forage species used in small ruminant production systems in the Caribbean. This information is important as there are limited reports on the mineral composition of forages (particularly MPTs), found in the Caribbean.

Overall, the concentrations of all macro-minerals in forages, except for Na, were above the minimum concentrations required by small ruminants (NRC, 1985). Both Devendra (1977) and Bernard et al. (2019) report adequate concentrations of Ca, Mg and P in tropical forages found in Trinidad and Tobago and Jamaica. Even though the values obtained by Devendra (1977) were higher than those observed in the current study, those of Bernard et al. (2019) were more comparable. Overall, both the observed and reported values were within the ranges required by small ruminants for Ca (2.0 to 8.2), Mg (1.6 to 1.8) and P (1.6 to 3.8 g/kg DM) (NRC, 1985). One of the key roles of these minerals is their contribution to bone and skeletal development (Kearl, 1982). Further, Mg plays a critical role in neuromuscular function and carbohydrate metabolism (Kearl, 1982). Further, Leng (1990) emphasise the importance of these minerals to the health of rumen microbes, the efficiency of degradation and intake of feeds. Therefore, supplying adequate concentrations of these minerals is fundamental to maintaining high performance in animals.

Multipurpose tree species are often known to be good sources of macro-minerals having concentrations that are typically within the range required by livestock (Smith, 1992). All MPTs were high in Ca, P, Mg and K. *Trichanthera gigantea* had the highest Ca concentration with a value twice the average Ca concentration for the other

multipurpose tree species (MPTs). The high Ca concentration may be explained by the presence of calcium-rich cystoliths on the surface of epidermal cell walls of the leaves and upper stems of *T. gigantea* (Benton and Benton, 1963; Rosales, 1997b).

Sodium is considered to be the mineral most limiting to livestock production worldwide (Whitehead, 2000). This may have explained the low values obtained for ten out of the twelve species examined in this study (< 0.9 to 1.8 g/kg DM required for small ruminants). For instance, low forage Na was reported by Bernard et al. (2019) for Jamaica (0.41 to 0.58 g/kg DM). The requirement for Na is high in the tropics as a result of the elevated temperatures which lead to losses through perspiration (McDowell and Arthington, 2005). The requirement is further increased in rapidly growing and high-producing animals (McDowell and Arthington, 2005). This gap between forage Na and the animal requirement for the mineral indicates that supplementation of Na is required in the diet of ruminants in tropical regions such as the Caribbean. Although most forages had low Na concentrations, that of *B. ruzizensis* and *D. eriantha* were above the minimum concentration required by small ruminants. Further, the Na content of *D. eriantha* was almost five times that of *B. arrecta* which was expected as *D. eriantha* has relatively high concentrations of Na in its tissues (Heuzé V. et al., 2015). Both species may be used to improve the intake of Na in the diets of small ruminants.

Potassium is typically high in forage which may be linked to the overall complex transport systems of plants which allows access to the mineral in conditions of low and abundant soil K (Arroyo-Aguilu and Coward-Lord, 1974; Morgan and Connolly, 2013). The typically high concentration of plant K reported in the literature was consistent with the results obtained in the current study. For instance, K concentration

in *P. purpureum, C nlemfuensis* and *B. arrecta* were above the maximum tolerable levels. These values, although high, were lower than the range reported by Arroyo-Aguilu and Coward-Lord (1974) (30 to 73 g/kg DM) for tropical grasses. The differences in the K concentrations obtained between the studies may be as a result of the differences in species evaluated, age of material, cultural practices and soil type which are some of the major factors that impact on the mineral composition of forages (Dongall and Bogdan, 1958; Topps, 1992). Further, the current K values obtained for the MPTs were high, yet within the range reported by Guerrero-Cervantes et al. (2012) for MPTs (3 to 56 g/kg DM). In some instances, high K may lead to reduced absorption of Mg and Ca which may predispose animals to hypoglycaemia and hypocalcaemia, especially in high-producing animals, however, the generally high concentration of K in forage may not lead to toxicosis as excess K is often rapidly excreted in animals (McDowell and Arthington, 2005). Potassium (K) plays a role in nerve and muscle health as well as carbohydrate metabolism (Kearl, 1982; McDowell and Arthington, 2005) and, therefore, providing adequate amounts may be critical for optimising performance in animals.

Calcium and P are closely related and a dietary excess or deficiency in one can impact on the utilisation of the other (McDowell and Arthington, 2005). The Ca:P ratio is important and affects various aspects of performance, more critically, growth and bone formation. Ruminants may thrive on a wide range of Ca:P ratios, however, the most optimum ratio ranges between 1:1 to 2:1 (McDowell and Arthington, 2005). In this study, the Ca:P ratio of the MPTs was high, but that of the grasses was between 1:1 and 2:1, which was well within the range required by small ruminants.

The concentration of all micro-minerals except Cu, were above the minimum concentration required by small ruminants (NRC, 1985). Micro-minerals including Fe, Co, Mn and Zn were comparable to those reported by McDowell et al. (1977) for tropical forages: 75% of forages had Fe concentrations between 31 to 500 mg/kg Fe; 49% forages had Co concentrations between 0.06 to 0.20 mg/kg DM; 53% forages had Mn concentrations between 21 to100 mg/kg DM and 44% forages had Zinc concentrations between 31 to 75 mg/kg DM. In the current study, the concentration of Fe in *C. dactylon* was high and above the maximum tolerable levels for small ruminants (500 mg/kg DM) (NRC, 1985). This may be related to the iron-rich bauxitic soils (St. Ann's clay loam) at Site 2 which may have resulted in the high absorption and, therefore, high concentration of Fe observed for *C. dactylon*. The characteristically high pH and Fe concentration of these soils may have resulted in the high absorption of Mo observed for both *C. dactylon* and *C. nlemfuensis* (Greenberg and Wilding, 2007; Schulte, 1992). The ability of these *Cynodon* species to bioaccumulate metals has been reported and may explain the above-average metal concentration (Fe and Mo) observed for these species (Franco et al., 2013).

The Fe concentrations obtained for other species in this study were similar to those reported in the literature (up to 619 g/kg) (Guerrero-Cervantes et al., 2012; Mtui et al., 2006). The Cu concentrations of several species were below the minimum concentrations (8 to 25 mg/kg DM) required by small ruminants. This was expected as Cu is typically low in tropical forages (McDowell and Arthington, 2005; Mohammed et al., 2016). However, the *Cynodon* species, *B. arrecta*, *L. leucocephala* and *T. gigantea* had concentrations of Cu that were within the range required for small ruminants and may be used to improve the Cu concentration in diets.

4.6 Conclusion

Overall, the Ca, P, Mg, Co, Mn, and Zn were within the range required for small ruminants. Although most species were low in Na, adequate concentrations of Na in *B. arrecta* and *D. eriantha* may indicate the potential of these species to improve the Na concentration in diets. Similarly, *C. dactylon*, *C. nlemfuensis*, *B. arrecta*, *L. leucocephala* and *T. gigantea* had adequate concentrations of Cu, unlike most other forage species, and may be used to improve Cu concentrations in diets for lambs. The concentration of K in *P. purpureum*, *B. arrecta*, and *C. nlemfuensis*, the concentration of Fe in *C.* dactylon and Mo in both *Cynodon* species were above the maximum tolerable levels for small ruminants which may require limiting their inclusion in diets. The results of the study suggest that forages varied in mineral concentrations and the toxic and marginal concentrations of specific minerals in various forage species elicits the careful management of inclusion in diets, to ensure that the mineral concentrations are within the range required for small ruminants.

CHAPTER 5 Fermentation kinetics and *in-vitro* digestibility of tropical forages used in sheep and goat production systems in the Caribbean

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5.1 Abstract

The *in-vitro* fermentation kinetics and digestibility of six tropical grasses and five multipurpose tree species (MPTs) found in the Caribbean were determined in the current study. Forage samples were dried, ground and incubated at 39^{o}C in rumen-buffer inoculum for 48 hours. The *in-vitro* gas production was measured continuously using an automated pressure transducer system and fitted to a dual and single pool model to determine the fermentation kinetics of these forages. The average rate of the fast pool (C_1) for grasses was over 20% higher than that of the MPTs. The volume of the fast pool by the dual pool model (V_1) for grasses was 40% lower and slow pool (V_2) 30% greater than that of the MPTs. There was a strong negative relationship between the fast pool of the dual pool model (V_1) and the NDF (r= - 0.781) and ADF (r = - 0.655) concentrations and a positive relationship between the V_1 and the digestible organic matter in dry matter (DOMD) (r =0.537). Based on the single pool model, the average rate of fermentation (c) of the slowly fermentable pool (b) for the grasses was 0.043%/hr and that of the MPTs 0.086%/hr. The average b for the grasses was 15% higher than that of the MPTs. *Moringa oleifera* produced the greatest amount of total gas at 48 hours according to the dual pool model (Vt_{scho}) (132.1 ml/g DM) and single pool model (Vt_{orsk}) (131.5 ml/g DM) at 48 hours which was followed by that of *Gliricidia sepium* (116.1 and 113.5 ml/g/DM, respectively) and *Morus alba* (116.9 and 113.7 g/kg DM, respectively). At 48 hours, the Vt_{scho} and Vt_{orsk} for *Trichanthera gigantea* and *Leucaena leucocephala* produced the least amount of gas (*Trichanthera gigantea*: 70.8 and 65.7 ml/g DM and *Leucaena leucocephala*: 89.1 and 86.2 ml/g DM for the dual and single pool models, respectively). The microbial biomass yield of *Trichanthera gigantea* was approximately 60% above the average yield for the other species. *Moringa oleifera* had the greatest total VFA concentration, whereas

Trichanthera gigantea had the lowest. All MPTs except *Trichanthera gigantea* and *Leucaena leucocephala*, were more fermentable than the grasses and based on chemical composition, *in-vitro* digestibility, fermentation parameters and end products, *Moringa oleifera* and *Morus alba* demonstrated overall high performance, whereas *Trichanthera gigantea* performed poorly.

5.2 Introduction

The nutritive value of feeds for ruminants is determined by both the concentration of its chemical components as well as the rate and extent to which the feed is digested (Getachew et al., 2004). The *in-vitro* gas method is used to determine the rate and extent to which feed is digested and has been based on measured relationships between *in-vivo* digestibility of feeds; *in-vitro* gas production; and the chemical composition of feeds (Menke, 1988).

The *in-vitro* gas method involves the incubation of feedstuffs with buffered rumen fluid *in-vitro* primarily for measuring the digestion of soluble and insoluble carbohydrates (Menke et al., 1979). When incubated, the carbohydrates in feeds are fermented to produce volatile fatty acids (VFAs) including acetate, propionate and butyrate (Menke et al., 1979). In addition to VFAs, other gases (primarily including CO_2 and CH_4) are produced and the microbial biomass (MBM) is increased through the microbial fermentation of substrate (Getachew et al., 1998b). The close association between the cumulative gas production and the fermentation of carbohydrates to VFAs is well known and gases produced are used to reflect the production of these short chain fatty acids (Getachew et al., 2002). Measuring the production and/or concentration of VFAs is critical as these represent a major source of energy for the ruminant, providing up to 80% of their energy requirement (Annison, 1970). Both protein and fats produce gases, but in small and negligible amounts, respectively (Getachew et al., 2004; Wolin, 1960).

The cumulative gas produced *in-vitro* can be fitted to mathematical models (France et al., 2000). These models are used to estimate *in-vitro* gas production kinetics or the rate and extent a substrate or feed has been fermented and/or degraded which can be

used to estimate the potential animal performance when that feed is fed (France et al., 2005; Üçkardeş and Efe, 2014). Both the dual pool logistic model by Schofield et al. (1994) and single pool model by Orskov and McDonald (1979) are commonly used to estimate the kinetics of ruminal fermentation (Peripolli et al., 2014). However, information on the fermentation kinetics, *in-vitro* digestibility and how these relate to the nutritive value of forages in the Caribbean, is lacking. Therefore, the study aimed to determine and report on the fermentation kinetics of a range of tropical forages including six grasses, two leguminous multipurpose tree species (LMPTs) and three non-leguminous multipurpose species (NLMPTs). This was done using the using both the dual pool logistic model by Schofield et al. (1994) and the single pool model by Orskov and McDonald (1979). Additionally, the study aims to determine the *in-vitro* digestibility and fermentation end products including the VFAs and MBM and to determine how the fermentation kinetics of forages relate to their nutritive value.

5.3 Materials and Methods

5.3.1 Site description

Site description was described in Chapter 3 of this thesis. Samples for all species were collected from one of two sites (Site 1 and Site 2). Site 1 was the Forage Bank at the University of Trinidad and Tobago - Valsayn Campus, Trinidad and Tobago and Site 2 was the forage bank at New Wales Manchester, Central Jamaica.

5.3.2 Selection of forage species and harvesting and preparation of samples

The selection of forages and the harvesting and preparation of forage samples are described in Chapter 3.

5.3.3 Proximate analysis

Samples were analysed for dry matter (DM), nitrogen (N), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), ash, organic matter, fat and the gross energy (GE). The analyses have been described in Chapter 3.

5.3.4 Fermentation kinetics parameters and end products

Forage samples (n = 2) were analysed using the Alltech IFM^{TM} system. About 1.4 L of rumen fluid was collected approximately two hours post-morning feed from a lactating dairy cow fed a typical diet consisting of pasture, grass silage and maize silage, 0.5 kg of molasses and 1.5 kg of a pelleted compound feed as part of the regular management of cows through a robotic system (Lely Astronaut). Once collected, the rumen fluid was strained using two layers of cheese cloth and mixed with 250 ml of a

reducing agent and 5.6 L of McDougall (1948) buffer solution resulting in a rumen fluid to buffer ratio of 20:80. For each forage species, approximately 0.5 g of dried sample, ground to a size of 2 mm was weighed into 250 ml bottles in duplicates and incubated at 39^{o}C in 100 ml of rumen-buffer inoculum for 48 hours (Mould et al., 2005). During the incubation period, gas production was measured continuously using an automated pressure transducer system by Pell and Schofield (1993). The cumulative gas production (ml/g DM) after 48 hours was fitted to a dual pool logistic model by Schofield et al. (1994) to estimate the rate of gas production of the fast pool (fast rate, FR %/hour, %/hr); the rate of gas production of the slow pool (slow rate, SR, %/hr); and the respective gas production volumes, including the fast pool (FP ml/g DM) and slow pool (SP ml/g DM) for each forage species. The total gas production after 48 hours (hrs) (Vt_{scho}) was calculated as FP+SP (ml/g DM). The cumulative gas production over 48 hours was fitted to the single pool model by Orskov and McDonald (1979) where the gas production from the immediately soluble fraction (*a* ml/g DM), gas production from the soluble fraction (*b* ml/g DM), the gas production rate constant (*c* %/hr) and the total gas production (Vt_{orsk}) at 48 hours were determined. The apparent dry matter digestibility (aDMD, %) or the percent of incubated feed DM left after the 48h incubation (undigested residue that contains microbial biomass (MBM, mg/g DM), was determined by the Tilley and Terry (1963) method. The true dry matter digestibility (tDMD, %) was measured after the solubilisation of the MBM in the undigested residue and was estimated using the batch culture *in-vitro* digestibility method (Mould et al., 2005; Tilley and Terry, 1963) after treating the residue with a neutral detergent solution (Goering and Van Soest, 1970). The MBM synthesis was estimated as the difference between the aDMD and

the tDMD (Goering and Van Soest, 1970). The DOMD was generated from the tDMD utilising the following formula:

$$DOMD = \frac{[OM\ weight - (NDR\ weight - Ash\ weight)]}{DM\ weight}$$

Where NDR weight = Neutral detergent residue weight (the residues after 48 hr fermentation were treated with NDR solution to remove MBM);

Ash weight = Ash of NDR residue;

OM weight = OM, % substrate x substrate weight;

DM weight = DM, % substrate x substrate weight

The metabolisable energy (ME, MJ/kg DM) was calculated as the DOMD x 0.163 (AFRC, 1993). After 48 hours of incubation of the forage samples, individual and total VFA concentrations (mmol/L) were determined by gas chromatography according to Erwin et al. (1961) using an Agilent GC 7890B (FID detector).

5.3.5 Statistical analysis

Statistical analysis was conducted in the R environment for statistical computing and visualisation (Team, 2013). Data on the nutritive value of forages, *in-vitro* digestibility, fermentation kinetics and fermentation end products were fitted to a linear model. An ANOVA was used to obtain the *p*-value for the model differences. Where significant differences between the treatment groups were detected, means were separated using least significant difference (LSD, $P \leq 0.05$). Pearson's correlation between the digestibility data and the proximate chemical components, as well as the Pearson's correlation between proximate chemical components and fermentation

parameters were generated using the Corrr package (version 0.2.1(Jackson, 2016)). Correlations were considered significant if $P \leq 0.05$.

Cumulative gas production data for both samples were fitted to the dual pool and single pool models using R environment for statistical computing and visualisation (Team 2013) to determine the fermentation kinetics:

Dual pool logistic model by Schofield et al. (1994):

$$Vt_{scho} = \left[\frac{V1}{1 + exp^{(2+4 \times C1\ x\ (L-T))}}\right] + \left[\frac{V2}{1 + exp^{(2+4\ x\ C2\ x\ (L-T))}}\right]$$

where Vt_{scho} = the measured gas volume at time *t*; V_1 and C_1, = the asymptotic cumulative gas volume and fractional degradation rate for pool 1; and V_2 and C_2 = the respective parameters for pool 2. *T* is the time (hours) and *L* is the lag time (hours) for both pools. One value for each parameter V_1, C_1, V_2, and C_2 was obtained for forage samples (n=2 for each species) and averaged to obtain predicted cumulative gas volumes using the dual pool logistics model (Schofield et al., 1994). The cumulative gas volumes were illustrated by graphs using ggplot2 (Wickham, 2016).

The single pool model by Orskov and McDonald (1979):

$$Vt_{orsk} = a + b\,(1 - e^{-c(t)})$$

where Vt_{orsk} = the measured gas volume at time *t*, *a* = gas production from the immediately soluble fraction, *b* = gas production from the soluble fraction, *a*+*b* = the potential gas production and *c* = gas production rate constant. The above fermentation parameters including *a, b* and *c* were predicted by fitting original gas volumes to the

single pool model of Orskov and McDonald (1979). The averages of the parameters *a*, *b* and *c* were used to predict cumulative gas fitted to the single pool model (Orskov and McDonald, 1979) and were illustrated by graphs using ggplot2 (Wickham, 2016)

5.4 Results

5.4.1 Chemical composition

The chemical composition of the forage species were described in Chapter 3 and are presented in Table 5.1.

Table 5.1 Gross chemical composition (g/kg DM) (including the crude protein (CP), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, fat, organic matter (OM), ash and gross energy (GE, MJ/kg DM)) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean (n=3 per forage species)*

	CP	Starch	NDF	ADF	Lignin	Fat	OM	Ash	GE
GRASSES									
Brachiaria arrecta	109.5^{bc}	3.71^{a}	705^{cd}	449^{def}	77.3^{ab}	17.9^{ab}	820^{b}	122.0^{bcd}	17.4^{def}
Brachiaria hybrid**	67.6^{a}	4.85^{a}	715^{cd}	414^{cdef}	40.5^{a}	18.8^{abcd}	843^{b}	94.1^{a}	17.9^{f}
Cynodon dactylon	142.8^{cd}	12.58^{ab}	748^{cd}	388^{bcdef}	67.0^{a}	12.3^{a}	685^{a}	126.3^{cd}	17.2^{bcd}
Cynodon nlemfuensis	191.2^{ef}	1.06^{a}	699^{c}	383^{bcde}	59.6^{a}	18.3^{abc}	804^{b}	108.4^{ab}	17.3^{cde}
Digitaria eriantha	87.1^{ab}	3.92^{a}	727^{cd}	497^{f}	78.5^{ab}	21.8^{bcd}	838^{b}	95.5^{a}	17.9^{f}
Megathyrsus maximus	90.3^{ab}	1.96^{a}	756^{d}	472^{ef}	57.2^{a}	18.6^{abcd}	791^{b}	137.1^{de}	16.9^{bc}
LMPTs									
Gliricidia sepium	192.6^{ef}	12.92^{ab}	501^{b}	335^{abc}	188.1^{c}	31.3^{e}	807^{b}	112.0^{bc}	19.1^{g}
Leucaena leucocephala	263.6^{h}	4.59^{a}	505^{b}	347^{abcd}	185.4^{c}	26.3^{de}	847^{b}	92.8^{a}	20.1^{h}
NLMPTs									
Moringa oleifera	232.5^{gh}	28.36^{c}	386^{a}	284^{ab}	99.9^{ab}	46.3^{f}	836^{b}	93.5^{a}	19.8^{h}
Morus alba	205.3^{fg}	25.36^{c}	379^{a}	250^{a}	139.5^{bc}	22.1^{bcd}	755^{ab}	146.8^{e}	17.8^{ef}
Trichanthera gigantea	171.1^{de}	23.68^{bc}	502^{b}	363^{bcde}	196.5^{c}	22.4^{bcd}	684^{a}	225.5^{f}	16.0^{a}
SEM	6.61	2.37	10.4	21.5	12.8	1.54	18.4	3.09	0.097
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*Table obtained from Chapter 3 of this thesis

Means carrying the same superscripts within columns are not significantly different (P > 0.05)

** *Brachiaria* hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*))

5.4.2 *In-vitro* digestibility

In the current study the aDMD, tDMD, DOMD and ME were measured for all species (Table 5.2). The aDMD ranged between 40.4 to 55.1% for grasses and 34.7 to 61.5% for the MPTs. The tDMD ranged between 55.2 to 64.9% and 59 to 77.4% for grasses and MPTs, respectively. The DOMD for grasses was between 51.2 to 65.1% and for MPTs was between 55.3 to 73.9%. The ME was estimated and ranged between 8.18 to 10.42 MJ/kg DM and 8.85 to 11.83 MJ/kg DM for grasses and MPTs, respectively.

Table 5.2 The apparent dry matter digestibility (aDMD, %), true dry matter digestibility (tDMD, %), digestible organic matter in dry matter (DOMD, %) and metabolisable energy (ME MJ/kg DM) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean

Grasses	aDMD	tDMD	DOMD	ME
Brachiaria arrecta	46.8	58.6	54.8	8.8
Brachiaria hybrid *	45.5	57.4	56.6	9.1
Cynodon dactylon	47.8	60.6	65.1	10.4
Cynodon nlemfuensis	55.1	64.9	62.8	10.1
Digitaria eriantha	47.1	58.9	58.3	9.3
Megathyrsus maximus	40.4	55.2	51.2	8.2
LMPTs				
Gliricidia sepium	46.2	65.5	66.1	10.6
Leucaena leucocephala	37.5	59.0	59.4	9.5
NLMPTs				
Moringa oleifera	61.5	73.7	73.9	11.8
Morus alba	56.9	77.4	73.9	11.8
Trichanthera gigantea	34.7	64.2	55.3	8.9
LSD	5.96	5.27	11.11	1.77
p-value	<.0001	<.0001	0.011	0.011

* Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

5.4.3 Fermentation parameters and gas production

The fermentation parameters after fitting the dual pool logistic model of Schofield et al. (1994) and the single pool model of Orskov and McDonald (1979) to the cumulated gas volumes are presented in Table 5.3. In the dual pool model, the *L* ranged between 1.24 to 1.93 hrs for the grasses and 0.50 to 1.19 hrs for the MPTs. There was no lag time calculated for the single pool model. The fast pool rates (C_1) observed for the dual pool logistic model ranged between 0.22 to 0.37%/hr for grasses and 0.20 to 0.27%/hr for the MPTs, and the slow pool rates (C_2) were between 0.03 to 0.04%/hr for grasses and 0.04 to 0.06%/hr for the MPTs. The rate of gas production (*c*) for the single pool Orskov model ranged between 0.02 to 0.06%/hr for grasses and 0.04 to 0.13%/hr for the MPTs. The total gas production at 48 hours for grasses determined by the dual pool logistic model (Vt_{scho}) and the single pool model (Vt_{orsk}) was 90.3 to 108.1 and 89.8 to 111.5 ml/g DM, respectively, and for the MPTs were 70.8 to 132.1 ml/g DM and 65.7 to 131.5 ml/g DM, respectively.

Table 5.3 The fermentation parameters of the dual pool logistic model by Schofield et al. (1994) and the single pool model by Orskov and McDonald (1979) for grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean

	Dual pool logistic model						Single pool model			
	L	V_1	V_2	C_1	C_2	Vt_{scho}	*a*	*b*	*c*	Vt_{orsk}
Grasses										
Brachiaria arrecta	1.93	22	74.3	0.268	0.038	96.6	4.39	103.4	0.047	95.8
Brachiaria hybrid*	1.18	28	75.1	0.220	0.036	102.7	11.66	105.6	0.043	101.8
Cynodon dactylon	1.41	16	91.9	0.235	0.037	108.1	6.78	127.4	0.036	111.5
Cynodon nlemfuensis	1.60	16	90.2	0.261	0.031	105.9	8.7	154.5	0.023	107.7
Digitaria eriantha	1.78	21	81.3	0.369	0.043	102.5	6.54	108.4	0.053	102.8
Megathyrsus maximus	1.24	21	69.2	0.290	0.044	90.3	7.48	88.4	0.058	89.8
LMPTs										
Gliricidia sepium	0.50	40	76.6	0.269	0.058	116.1	5.06	108.7	0.121	113.5
Leucaena leucocephala	1.18	28	61.2	0.257	0.047	89.1	5.33	82.6	0.081	86.2
NLMPTs										
Moringa oleifera	0.51	51	81.3	0.219	0.060	132.1	2.23	129.6	0.126	131.5
Morus alba	1.14	43	73.7	0.2	0.049	116.9	1.44	114.2	0.09	113.7
Trichanthera gigantea	1.19	26	44.8	0.26	0.038	70.8	14.07	59.1	0.043	65.7
LSD	0.79	15.6	22.64	0.18	0.01	18.89	6.42	37.53	0.03	21.50
p-value	0.037	0.006	0.033	0.797	0.008	0.002	0.031	0.011	<.0001	0.003

* Brachiaria hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)

Terms used: *L*: lag time (hrs); V_1: Fast pool (ml/g DM), V_2: Slow pool (ml/g DM), C_1: Fast rate (%/hr), C_2: Slow rate (%/hr); Vt_{scho}: total gas production by Schofield 1994; *a*: gas production from the immediately soluble fraction (ml/g DM); *b*: gas production from the insoluble or slowly degradable fraction (ml/g DM); *c*: rate of gas production from the slowly degradable fraction (%/hr); Vt_{orsk}: total gas production by Orskov and Mcdonald (1979) (ml/g DM)

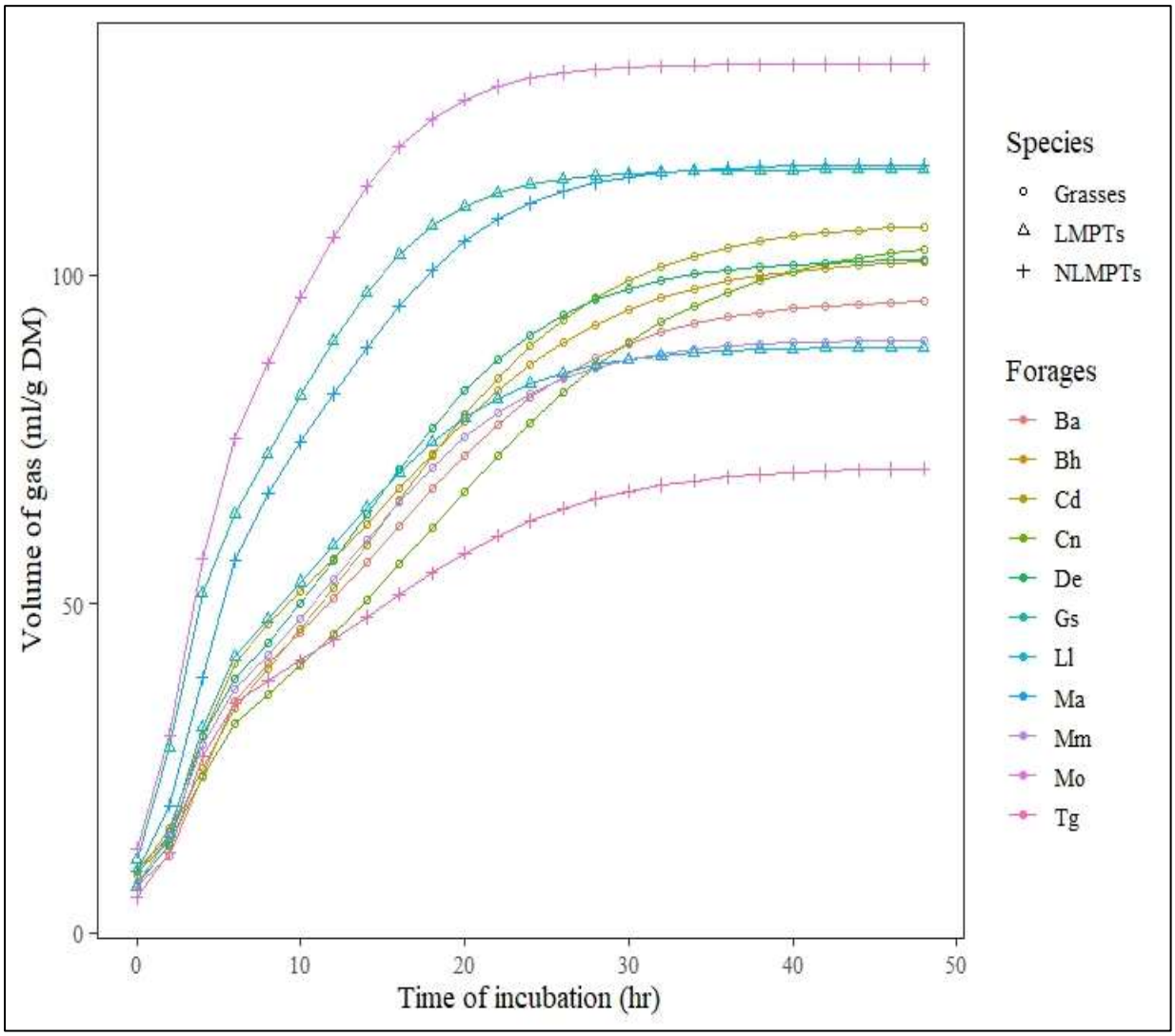

Figure 5.1 The mean cumulative gas volume for all forage species using the dual pool logistic model by Schofield et al. (1994)

Terms used: Ba: *Brachiaria arrecta*; Bh: *Brachiaria hybrid* (*Cv. Mulato II* (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)); Cd: *Cynodon dactylon*; Cn: *Cynodon nlemfuensis*; De: *Digitaria eriantha*; Gs: *Gliricidia sepium*; Ll: *Leucaena leucocephala*; Ma: *Morus alba*; Mm: *Megathyrsus maximus*; Mo: *Moringa oleifera*; and Tg: *Trichanthera gigantea*

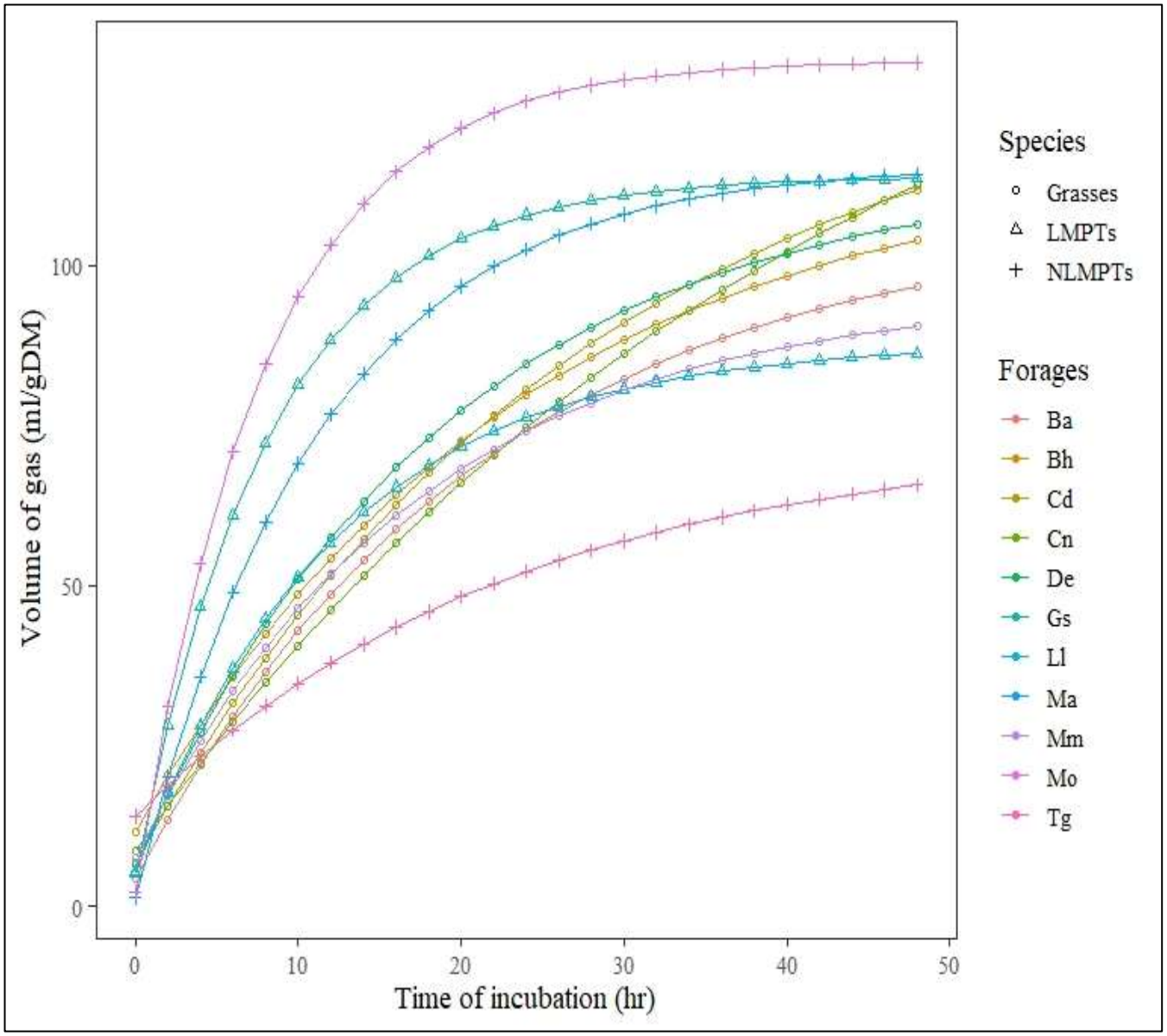

Figure 5.2 The mean cumulative gas volume for all forage species using the single pool model by Orskov and McDonald 1979

Terms used: Ba: *Brachiaria arrecta*; Bh: *Brachiaria hybrid* (*Cv. Mulato II* (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*)); Cd: *Cynodon dactylon*; Cn: *Cynodon nlemfuensis*; De: *Digitaria eriantha*; Gs: *Gliricidia sepium*; Ll: *Leucaena leucocephala*; Ma: *Morus alba*; Mm: *Megathyrsus maximus*; Mo: *Moringa oleifera*; and Tg: *Trichanthera gigantea*

5.4.4 Relationship between chemical composition and fermentation parameters

The relationship between the chemical components of forages and fermentation parameters can be observed in Table 5.4. There was a strong positive relationship between the concentration of fat and the size of the slow pool determined by the dual pool model (V_2) (r = 0.725, P < 0.05) and the gas pool of the slowly degradable fraction for the single pool model (b) (r = 0.811, P < 0.05). The relationship between the NDF concentration and the V_2 and b gas pools were negative (r = -0.651 and -0.75,1 respectively, P < 0.05). There was a significant positive relationship between the Vt_{scho} at 48 hours and fat concentration, GE and the DOMD (r = 0.428, 0.473 and 0.773, respectively, P < 0.05). The relationship between the ash and the Vt_{scho} and the Vt_{orsk} at 48 hours were negative (r =- 0.547 and -0.578, respectively, P < 0.05). There was a significant and negative relationship between the Vt_{scho} at 48 hours and the ADF concentration (r = -0.428, P < 0.05).

Table 5.4 The correlation between chemical components (g/k DM) (including crude protein (CP), fat, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, ash), gross energy (GE, MJ/kg DM), digestible organic matter in dry matter (DOMD, %) and the fermentation parameters for the dual and single pool models

	Dual pool Logistic Model (Schofield et al. 1994)					Single pool Model (Orskov and McDonald 1979)			
	V_1	V_2	C_1	C_2	Vt_{sch}	a	b	c	Vt_{orsk}
CP	0.47*	-0.163	-0.265	0.414	0.195	-0.284	0.046	0.518*	0.135
Starch	0.592*	-0.236	-0.194	0.477*	0.219	-0.151	-0.14	0.500*	0.144
NDF	-0.781*	0.301	0.265	-0.651*	-0.298	0.359	0.122	-0.751*	-0.197
ADF	-0.655*	0.047	0.476*	-0.363	-0.428*	0.335	-0.176	-0.488*	-0.352
Lignin	0.359	-0.539*	0.009	0.476*	-0.21	0.002	-0.481*	0.521*	-0.290
Fat	0.746*	-0.074	-0.136	0.725*	0.469*	-0.341	0.058	0.811*	0.400
Ash	-0.055	-0.585*	-0.05	-0.188	-0.547*	0.431*	-0.517*	-0.216	-0.578*
GE	0.526*	0.111	-0.116	0.615*	0.473*	-0.491*	0.134	0.705*	0.428*
DOMD	0.537*	0.448*	-0.14	0.524*	0.773*	-0.553*	0.476*	0.556*	0.742*

* $P \leq 0.05$ (n=22); Terms used: V_1: fast pool (ml/g DM), V_2: slow pool (ml/g DM), C_1: fast rate (%/hr), C_2: slow rate (%/hr); Vt_{scho}: total gas production at 48 hours by Schofield et al. (1994); *a*:gas production from the immediately soluble fraction (ml/g DM); *b*: gas production from the insoluble or slowly degradable fraction (ml/g DM); Vt_{orsk}: total gas production at 48 hours by Orskov and Mcdonald (1979) (ml/g DM); *c*: rate of gas production from the slowly degradable fraction (%/hr); LMPTs: leguminous multipurpose tree species; NLMPTs: non-leguminous multipurpose tree species

5.4.5 Fermentation end products

The total VFAs ranged between 13.1 to 17.09 mmol/L for grasses and 8.92 to 20.81 mmol/L for the MPTs (Table 5.5). The MBM values obtained for grasses ranged between 112 to 170 mg/g DM and MPTs between 140 to 340 mg/g DM. The MBM yield of *T. gigantea* was 129 mg/g DM higher than the average MBM yield (211 mg/g) of the other MPTs.

Table 5.5 The volatile fatty acid concentrations (% molar proportions); total volatile fatty acid (TVFA, mmol/L); the acetate to propionate ratio (A:P); and microbial biomass (MBM) yield (mg/g DM) after 48-hour incubation of grasses, leguminous multipurpose tree species (LMPTs) and non-leguminous multipurpose tree species (NLMPTs) used in small ruminant production systems in the Caribbean

Grasses	Ac	Pr	Isobut	But	Isoval	Val	TVFA	A:P	MBM
Brachiaria arrecta	58.7	30.6	0.361	8.70	0.563	1.050	15.05	1.92	136
Brachiaria hybrid*	53.2	36.7	0.292	8.33	0.324	1.185	15.00	1.45	137
Cynodon dactylon	60.7	31.8	0.368	5.96	0.522	0.668	13.82	1.93	147
Cynodon nlemfuensis	63.7	31.1	0.383	3.65	0.466	0.707	17.09	2.05	112
Digitaria eriantha	58.4	31.9	0.258	8.28	0.295	0.835	15.68	1.83	135
Megathyrsus maximus	60.4	31.0	0.390	6.63	0.558	1.061	13.16	1.95	170
LMPTs									
Gliricidia sepium	66.1	26.2	0.304	5.77	0.560	1.069	16.01	2.53	222
Leucaena leucocephala	64.9	27.0	0.388	6.07	0.682	0.897	13.09	2.41	247
NLMPTs									
Morus alba	63.0	25.0	0.386	9.48	0.737	1.369	18.32	2.52	235
Moringa oleifera	60.1	26.2	0.469	11.14	0.846	1.242	20.81	2.30	140
Trichanthera gigantea	73.2	24.9	0.126	1.31	0.208	0.253	8.92	2.94	340
LSD	3.94	3.02	0.11	1.29	0.19	0.24	2.15	0.34	21.56
p-value	<.0001	<.0001	0.003	<.0001	0.0004	<.0001	<.0001	<.0001	<.0001

$p \leq 0.05$ (n=22);*_Brachiaria_ hybrid cv. Mulato II (*Brachiaria ruziziensis* x *B. brizantha* x *B. decumbens*))
Terms used: Ac: acetate; Pr: propionate; Isobut: isobutyrate; But: butyrate; Isoval: isovalerate and Val: valerate

5.5 Discussion

Fermentation parameters were derived using the dual pool logistics model of Schofield et al. (1994) and the single pool model of Orskov and McDonald (1979). The different parameters from each of the models gave critical insight into the fermentation kinetics of a range of tropical forages. Tropical grasses are typically high in fibrous fractions which decreases fermentability of forages and in the current study the NDF and ADF content were generally high (NDF: > 650 g/kg DM and ADF: > 400 g/kg DM) (Gemeda and Hassen, 2014; Nussio et al., 1998; Van Soest et al., 1991). From the dual pool model, the size of the slow and fast pools was determined, and the more fibrous grasses had a 40% smaller fast pool (V_1) and a 30% larger slow pool (V_2) than the MPTs. A correlation analysis between the fermentation parameters and chemical composition of the forages in this study suggests that the more digestible species (eg. MPTs) with lower ADF and NDF fractions were strongly associated with a larger V_1 pool, than the generally less digestible grasses with higher fibre fractions. The size of the different pools must be considered in combination with rates of gas production. Surprisingly, the average C_1 for the grasses was over 20% higher than that of the MPTs. There is no clear explanation for the higher C_1 observed for the grasses given their less fermentable nature. However, if the average size and rates of the individual gas pools are considered, grasses were, overall, slower to ferment and produce less gas compared to the MPTs.

Further, fitting the single pool model to the accumulated gas production data provided additional information about the fermentation of the forages studied. An overall estimate of the rate of fermentation (c) of the slowly fermentable pool (b) was obtained and the MPTs fermented (0.086%/hr) twice as fast as the grasses (0.043%/hr). The

lower rate of degradation estimated by the model for grasses may further indicate the lower fermentability of grasses compared to the MPTs. Additionally, the average *b* pool for the fibrous grasses was approximately 16% higher than that of the MPTs which was expected and consistent with the literature (Bezabih et al., 2014). Moreover, the different pattern of the exponential increase in the gas volumes for grasses and MPTs is noted in Figures 1 and 2 which illustrates the rate of fermentation of the different forages. The gas production of the MPTs increased exponentially but plateaued, or was near plateauing, before 48 hours, whereas, the grasses did not appear to plateau until 48 hours, or after. The low degradability of grasses over time may indicate that nutrients are not as readily accessed in tropical grasses. This may explain the generally lower animal performance on these forages and the importance of supplementing with MPTs of higher degradability, to ensure that there is an adequate supply of both bypass and rumen degradable nutrients (Soliva et al., 2008).

The Vt_{scho} and Vt_{orsk} at 48 hours of incubation for the grasses were within the range reported in the literature for tropical species (65.6 to 174.2 ml/g DM) (Gemeda and Hassen, 2014; Teguia et al., 1999). The Vt_{scho} and Vt_{orsk} observed for the MPTs at 48 hours (Table 5.3 as well as Figures 5.1 and 5.2) were wide-ranging. This may be linked to differences in the chemical composition of the individual species and the presence or absence of anti-nutritional factors in the MPTs (Apori et al., 1998; Kafilzadeh and Heidary, 2013). The Vt_{scho} and Vt_{orsk} at 48 hours for *M. oleifera* was at the higher end of the range for gas production which was followed by *G. sepium* and *M. alba*. The comparably greater fermentability of these species is not surprising given their higher nutritive value in comparison to other tropical MPTs (Hernández and Sánchez, 2014; Valdes et al., 2017). At 48 hours, the Vt_{scho} and Vt_{orsk} for *T. gigantea* and *L. leucocephala* were at the lower end of the range for the MPTs. However,

Nguyen and Le (2003) reported a total gas production of 113 ml/g DM after 48 hours of incubation for *T. gigantea* which was almost 60% higher than the value observed in the current study of only 70.8 and 73.2 ml/g DM for the dual and single pool models, respectively. The comparably higher volumes reported by Nguyen and Le (2003) may be primarily as a result of the more digestible leafy samples used compared to the combination of leaf and stems used in the current study. Adiwimarta et al. (2017) reported a total gas production of 234 ml/g DM for *L. leucocephala* after 48 hours of incubation which was almost three times the value observed in the current study (89.1 ml/g DM and 87.9 ml/g DM in the dual and single pool model, respectively). Although the observed values were low, the higher values reported by other studies may indicate the potential of these species as feeds for ruminants.

Further, the lower values observed for the *G. sepium* and *L. leucocephala* may be explained by the high MBM yield. Digestible substrate is either partitioned towards the synthesis of MBM or fermentation gases and there is often an inverse relationship between gas production (or VFA production) and the synthesis or yield of MBM (Blümmel and Bullerdieck, 1997). Microbial biomass represents an important source of amino acids (70 to 80% of supply (AFRC, 1992)) and bypass protein required to support production in ruminants (Nolan, 1981). Therefore, the higher MBM yield of *T. gigantea* may indicate that the species is a good source of bypass protein. Having the right balance of both protein and energy supports high microbial efficiency (Clark et al., 1992). Therefore, forages must be selected on a combination of gas production potential as well as the potential to yield microbial biomass (Hoover and Stokes, 1991; Makar, 2004).

Volatile fatty acids constitute the major source of energy for the ruminant providing 70 to 80% of its energy requirements (Annison, 1970; Bergman et al., 1965; Warner,

1964). In the current study, the total VFA production ranged between 13.1 to 17.09 mmol/L for grasses and 8.92 to 20.81 mmol/L for MPTs. Based on the ranking of feedstuffs by Negesse et al. (2009), all forages in the current study, except *M. oelifera*, had low VFA production which, according to the author, ranged between 11.5 to 19 mmol/L. Further, the values observed for grasses and MPTs were lower than those reported by Singh et al. (2014) for tropical grasses (37.3 to 39.8 mmol) and MPTs (38.6 to 43.1 mmol/L). The overall, lower VFA concentration observed for the forages may indicate the low energy concentration and the requirement to supplement with digestible feeds that may improve the energy of diets comprising these forages (Gemeda and Hassen, 2014).

The molar proportions of VFAs produced is influenced by the substrate fermented which, in turn, influences the amount of gas produced (Beuvink and Spoelstra, 1992). High concentrations of fermentable substrate yield higher concentrations of propionate resulting in lower A:P ratios compared to less rapidly fermented substrate that yield higher concentrations of acetate and butyrate; and lower propionate leading to higher A:P ratios (Janssen, 2010). However, the A:P ratios observed for the more fibrous grasses was at a lower range compared to that of the MPTs in the current study. There is no clear explanation for this as the higher nutritive value and fermentability of the MPTs was expected to result in lower A:P ratios than the tropical grasses of low nutritive value and fermentability. The unexpected A:P ratios observed is similar to other studies where there was no clear relationship between the chemical composition of forages and the molar proportions measured using *in-vitro* incubations (Niderkorn et al., 2011; Rivero et al., 2020). The time at which values were measured may have affected the observed values of the current study and those reported by other authors. For example, Niderkorn et al. (2011) and Rivero et al. (2020) observed that the A:P

ratios was lower at earlier (3.5 hrs) compared to later sampling times (24 hours). Therefore, it is possible that the A:P ratio would have been different if molar proportions were measured at earlier sampling times where there is a greater breakdown of the more fermentable fractions that lead to the production of propionate. Overall, the absolute values obtained for the fermentation parameters measured may have been different if samples were analysed using donor animals from the tropics (Bueno et al., 2015). Tropical sheep may be better adapted to the higher concentrations of fibre in tropical grasses and the likely presence of anti-nutritional factors in the MPTs than donor animals from temperate regions (Khazaal et al., 1993; Makkar, 2003). Several studies have demonstrated significant differences in fermentability of forages based on the species of donor animals used (Bueno et al., 2015). Forage fermentability may also vary intra-species, depending on the previous dietary history of donor animals (Khazaal et al., 1993; Leng, 1989). Although using tropical animals may result in differences in the absolute values obtained, the ranking of the forage species observed in the current study may be similar.

5.6 Conclusion

Overall, the MPTs were more fermentable than the grasses based on the fermentation parameters derived using the dual and single pool models. The correlation analysis between fermentation parameters and chemical composition of the forages observed indicated that the more digestible species (eg. MPTs) with lower ADF and NDF fractions were more rapidly fermented than the species with high fibrous fractions and low digestibility. Overall, the Vt_{scho} and Vt_{orsk} for the grasses at 48 hours were within the range reported in the literature for tropical species. At 48 hours of incubation the

Vt_{scho} and Vt_{orsk} observed for the MPTs were wide-ranging and, on average, higher than those of the grasses. *Moringa oelifera* was at the higher end of the range for total gas production at 48 hours followed by *G. sepium* and *M. alba*. *Trichanthera gigantea* and *L. leucocephala* were at the lower end of the range for total gas production at 48 hours. Although the observed values for *T. gigantea* and *L. leucocephala* were low in the current study, the higher values reported by other studies may indicate the gas production potential of these species. The microbial biomass (MBM) yield of *T. gigantea* was the highest among all species. Based on the overall chemical composition, *in-vitro* digestibility, fermentation parameters and end products, *M. oleifera and M. alba* demonstrated high performance, whereas *T. gigantea* performed poorly. Overall, the absolute values of the species may have been higher if the donor animal used in the analysis were tropical sheep as these may be better adapted to the higher fiber content of tropical grasses and the presence of anti-nutritional factors, particularly in the MPTS, however, the ranking of the forage species may have been similar to that observed in the current study.

CHAPTER 6 The effect of increasing concentrations of dry fallen *Trichanthera gigantea* leaves on the nutritive value and short-term intake of pelleted diets offered to growing lambs reared under tropical conditions in the Caribbean

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JACK, H.A., CRANSTON, L.M., BURKE, J.L., KNIGHTS, M., and **MOREL, P.C.H.** The effect of increasing concentrations of dry fallen *Trichanthera gigantea* leaves on the nutritive value of and intake of pellets offered to growing lambs reared under tropical conditions in the Caribbean.

6.1 Foreword

In the previous chapters on the nutritive value of forages, although *Trichanthera gigantea* (*T. gigantea*) performed poorly compared to the other multipurpose tree species (MPTs), the species was selected for subsequent chapters aimed to determine the effect of densification technology on small ruminant production systems in the Caribbean. Its selection was merited to its overall, acceptable concentrations of crude protein, fiber and minerals as well as its fermentability in terms of its microbial biomass yield. Further, the species had been recently targeted by the Government of Trinidad and Tobago as one of the more favourable forage species in terms of its ease of establishment and higher dry matter yield compared to other MPTs of higher nutritive value as *M. oleifera* and *M. alba*. Additionally, the ease of access to materials in organised plantations in Trinidad contributed to its selection for the following intake study (Chapter 6) and subsequent studies on the effect of densified forage on digestibility and growth performance in lambs (Chapter 7).

6.2 Abstract

Currently, there is limited information on the effect of increasing the concentration of dry, fallen *Trichanthera gigantea* (*T. gigantea*) leaves on the nutritive value and intake of pelleted diets offered to growing lambs reared under tropical conditions in the Caribbean. Twelve crossbred Barbados Blackbelly rams aged five months were randomly assigned to a treatment diet of 4kg (as fed basis) of chopped *Pennisetum purpureum* (9:00hrs) and one of five pelleted diets (500 g (as fed basis)) comprised of either 100% intact commercial pellets or a mixture of ground commercial pellets and fallen *T. gigantea* leaf grinds mixed in the following ratios (*T. gigantea* leaves: ground Commercial pellets): 20%: 80 % (T20); 40%:60 % (T40); 60%: 40 (T60); 80% :20%

(T80) and 100%: 0% (T100). The total intake of the different treatment pellets was measured after 15 minutes (TPI15) and at the end of each day (TPI), and the average daily nutrient intakes of the different treatment diets were calculated. Treatment, day and treatment by day interactions had an effect on the TPI15 ($p = 0.0001$) and treatment had an effect on both the TPI ($p < .0001$) and nutrient intakes. Overall, the more ingestive response to and the adequate intake of nutrients for the pellet treatments with lower inclusion concentrations of *T. gigantea*, suggest that dry fallen *T. gigantea* should replace no more than 40% of commercial concentrate in pelleted small ruminant diets.

Key words: Barbados Blackbelly, Caribbean, pellet, sheep, *Trichanthera gigantea*, West African

6.3 Introduction

The nutritive value of fresh intact *Trichantera gigantea* leaves is attributed to its high protein content which ranges between 150 to 220 g/kg DM (Rosales, 1997a; Rosales and Rios, 1999). The presence of hydrolysable tannins in *T. gigantea* may increase rumen undegradable or bypass protein which is a direct benefit to ruminants when consumed (Edwards et al., 2012b; Rosales, 1997a). Compared to other MPTs at the same stage of maturity, freshly harvested *T. gigantea* is typically higher in non-structural and storage carbohydrates and lower in structural carbohydrate which results in its high rumen degradability (Rosales and Rios, 1999). *Trichanthera gigantea* has cystoliths on the surface of its leaves and stems which results in a high ash content and a large percentage of calcium which is typically over 20% DM (Benton and Benton, 1963; Rosales, 1997b). The higher ash content may be used to improve the mineral concentrations in the diets of livestock in the tropics where mineral deficiencies in tropical pasture is prevalent (McDowell and Arthington, 2005). Despite the potentially lower nutritive value to fresh *T. gigantea* leaves, owing to senescence, leaf fall may be a potential dry season feed for animals (Charlton et al., 2003). During periods of prolonged drought, there is often an abundance of biomass available as leaf fall (Wright and Cornejo, 1990). This may be significant, particularly in the Caribbean where longer and more severe dry periods are projected (Lallo et al., 2016b). While several studies have focused on the use of fresh intact *T. gigantea* leaves, few have evaluated the use of fallen leaves as a prospective feed ingredient for lambs. Further, there is currently no information on the nutritive value and the effect of increasing the concentration of dry fallen *T. gigantea* on the quality of pelleted diets offered to growing lambs reared under tropical conditions in the Caribbean. Therefore, the

objective of this study was to determine the effect of increasing the concentration of dry fallen *T. gigantea* on the nutritive value and intake of pelleted diets offered to growing lambs.

6.4 Materials and Methods

The study was conducted at the Eastern Caribbean Institute for Agriculture and Forestry (ECIAF) – University of the Trinidad and Tobago (Latitude 10.56°N, Longitude -61.32°W) with approval from the Massey University Animal Ethics Committee (MUAEC 18/91). The effect of replacing commercial pellets with dry fallen *T. gigantea* leaf grinds included 0% (T0), 20% (T20), 40% (T40), 60% (T60), 80% (T80) and 100% (T100) in pelleted diets on intake in lambs was examined over two periods; period 1 (10 - 15 May 2019) and period 2 (22 - 28 May 2019). Owing to limitations with the facilities (spacing), all six treatments could not have been compared at the same time and, therefore, the intakes of treatments T0, T20 and T40 were measured during period 1 and the intakes of treatments T60, T80 and T100 were measured during period 2. The intakes of all treatment groups were compared in this study.

6.4.1 Harvesting and pelleting material

Dry fallen *T. gigantea* leaves were collected prior to each study period from the plantation at the "Up the Hill Farms" located in Moruga, Trinidad (Latitude 10.11°N; Longitude -61.29°W). The total rainfall for May was 45.2 mm; the minimum and maximum daily temperatures were 24.3°C and 32.8°C, respectively (AccuWeather, 2019); and the dominant soil types at the site were a combination of the La Retraite and Basseterre soil series (Khan, 2020).

Fallen leaves and a commercial pellet were the primary ingredients used to produce the diets examined in this study. The control diet (T0 or 100% commercial pellets) comprised intact commercial pellets made of 80% wheat middlings and 20 % corn (DM basis), and a vitamin and mineral mix. The other diet treatments included ground commercial pellets and dry fallen *T. gigantea* mixed in the following ratios (*T. gigantea* leaves: ground commercial pellets): 20%: 80 % (T20); 40%:60 % (T40); 60%: 40 (T60); 80% :20% (T80) and 100%: 0% (T100). Firstly, the commercial pellet and dry fallen *T. gigantea* were ground separately to pass through a 0.635 cm screen (the screen was initially 2.54 cm and modified to a 0.635 cm screen) of a Craftsman shredder-hammer mill (Model 247.776380). The ground materials were weighed (as fed basis) according to the ratios for the different pellet treatments. For example, the T20 pellet treatment included 20% *T. gigantea* (dry fallen leaves) and 80% commercial pellet ingredients and was therefore mixed at a ratio of 80% ground commercial pellets to 20% ground *T. gigantea* leaves. After weighing, according to the respective ratios for the different treatment groups, the ground materials were mixed manually for 10 to 15 minutes and pelleted using a Changchai-ZS1115 Pellet Mill (22 Horse-Power Diesel Engine) with a die length and diameter of 2.54 cm and 1.27 cm, respectively. One week prior to periods 1 and 2, one batch of the required amount of treatment pellets was produced and fed throughout the respective periods.

In addition to the pellets, mature (> six to eight weeks regrowth) *Pennisetum purpureum* was manually harvested with a machete each day at a height of 1.5 m from the Eastern Caribbean Institute for Agriculture and Forestry Campus – University of Trinidad and Tobago (ECIAF-UTT) according to Gemeda and Hassen (2014). *Pennisetum purpureum* was used as the basal feed for both periods 1 and 2. For the period of harvest (May 2019), the total rainfall at the location was 50.5 mm; the

average daily minimum and maximum temperatures for the site was 24.3ºC and 32.8ºC, respectively (Trinidad and Tobago Meteorological Services (TTMS), 2019); and the predominant soil type was the Piarco soil series comprised of terrace sand and gravel clay (characterised as having imperfect drainage; waterlogged in the wet season; and desiccated in the dry season (Brown, 1965)). Once harvested, the *P. purpureum* (including leaves and stem) was manually chopped to lengths of about 5 to 10 cm according to Schnaider et al. (2014), for daily feeding.

6.4.2 Lambs and diets

The same 12 crossbred (Barbados Blackbelly and West African) intact rams, aged five months, were used in both periods (periods 1 and 2) to measure the intake of the treatment diets. At the start of the study, the mean live weight of the lambs in Period 1 was 22 kg (± 2.17) and in Period 2 was 27 kg (± 2.38). Before the commencement of the experiments the lambs were subject to a 19-day adaptation period where they were examined; treated for internal parasites; fed a diet of 4kg (as fed basis) of chopped *P. purpureum* (including leaves and stem) and a commercial pellet (approximately 500 g (as fed basis)); and allowed to familiarise with their enclosures before period 1. For period 1, four lambs were randomly assigned to one of three diets (T0, T20 and T40) based on liveweight and measurements were recorded for seven days. Lambs were then subjected to a five day adaptation period using the same diet fed prior to period 1. Following this was period 2 of the study where the lambs (n = 4) were assigned to diet treatments, T60, T80 and T100 and measurements were recorded for seven days. During the experiment, all lambs were confined to well-ventilated individual pens (1.22 m x 1.22 m) and had unrestricted access to water and a mineral block (Alphablock) which contained 55.000 IU vitamin A; 27.500 IU vitamin D3; 300 IU

vitamin E; 30.000 mg calcium; 5.000 mg magnesium; 1.800 mg iron; 2.500 mg manganese; 50 mg cobalt; 1.500 mg zinc; 10 mg selenium; and 35 mg iodine.

6.4.3 Experimental procedure and design

Animals were fed twice daily at 9:00 hrs (forage) and 15:00 hrs (pellets). For the feed at 15:00 hours, pellets were presented to each lamb for 15 minutes. The time of offer and removal of the pellets for each lamb was lagged for one minute to ensure that each animal was presented with their treatment pellets for exactly 15 minutes. The total pellet intake at 15 minutes of exposure to feed (TPI15) and the total daily pellet intake (TPI) were measured. Total dry matter intake (TDMI) was calculated as the sum of the TPI and the total forage intake (TFI).

Sampling and analytical procedures

The TFI and TPI offered and refused for each animal were recorded daily. Feed samples (forage and pellets) were taken at the end of each week for DM determination and chemical analysis. The TFI and TPI per lamb were done through deducting the quantity of the feed refused from the quantity of feed offered for each day. The TDMI intake was a sum of the TFI and TPI per lamb. The total nutrient content of the diet was calculated by firstly determining the concentration of each nutrient (on a DM basis) in both the forage and pellets fed. The concentrations of each nutrient in the forage were then multiplied by the daily TFI and concentrations for each nutrient in the pellets were multiplied by the daily TPI and both were summed to determine the total daily nutrient intake for each lamb in the different treatment diets.

6.4.4 Chemical analysis

Samples were dried at 60ºC for 72 hours and ground to pass through a 2mm sieve using a Thomas Scientific mill. These were then packaged (package included Export permit no. 139517 for Research) and exported to Cumberland Valley Analytical Services (CVAS), US, for analysis. Dry matter for *P. purpureum* (modified method) was determined by drying samples at 105°C for 3 hours (National Forage Testing Association, 2002). Dry matter for pellets and *T. gigantea* was determined by drying samples at 35°C for 2 hours (AOAC method 930.15). ADF was determined using a Whatman 934-AH glass micro-fibre filters with 1.5um particle retention in place of a fritted glass crucible (modification to AOAC method 973.18). NDF was obtained using a Whatman 934-AH glass micro-fibre filters with 1.5um particle retention which was used in place of a fritted glass crucible (a modification to Van Soest et al. (1991)). Ash was determined using 0.35g sample which was ashed for four hours at 535°C (a modification to AOAC method 942.05). Elements including Calcium (Ca), Phosphorus (P), Magnesium (Mg), Potassium (K), Sodium (Na), Iron (Fe), Manganese (Mn), Zinc (Zn) and Copper (Cu)) were determined (modification to AOAC method 985.01) . Sample (0.35 g) was ashed for one hour at 535°C; digested in open crucibles for 20 minutes in 15% nitric acid on a hotplate; diluted to 50ml and analysed using inductively coupled plasma spectroscopy (ICP). Nitrogen (N) was determined by AOAC method 990.03 and crude protein (CP) was determined by multiplying the concentration of N in samples by a factor of 6.25. Soluble protein (SP) was determined by using the Borate-Phosphate procedure (Krishnamoorthy et al., 1982).

6.4.5 Statistical analysis

Statistical analysis was conducted using R environment for statistical computing and visualisation (Team, 2013). Intake measurements obtained from each lamb at different

times were treated as repeated measures and a linear mixed effect model was applied to the data. The model consisted of treatment, day and day x treatment as fixed effects and animal as the random effect. An ANOVA was used to obtain the *p*-value for the model differences. Where significant differences between the treatment groups was detected, means were separated using the Tukey's test. Differences were considered statistically significant if $P \leq 0.05$.

6.5 Results

6.5.1 Chemical composition and nutrient intakes

The chemical composition of the dry fallen *T. gigantea* used for making *T. gigantea* pellets; *P. purpureum* and all pelleted feeds offered to lambs in the current study is presented in Table 6.1 and the average feed and nutrient intakes for the different treatment groups is presented in Table 6.2. The TFI and the TDMI were comparable across all treatment groups ranging between 0.738 to 0.795 kg DM/head/day for TFI and 1.13 to 1.21 kg DM/head/day for the TDMI. Treatment had an effect on the TPI (p <.0001). The TPI of the T0 group was comparable to the T20, T40 and T60 groups, however it was higher (p <.0001) than that of the T100 group. There was no day or day by treatment effect on the TPI.

Treatment had a significant effect on nutrient intakes. The CP intake for the T0 group was higher (p <.0001) than those of the other groups except that of the T20 group (P > 0.05). T0 had the highest (p = 0.0006) SP intake of 0.055 ± 0.001 kg SP/head/day which was not significantly different (P > 0.05) from that of the T20, T40 and T60 groups, however, was significantly different (p = .0006) from those of the T80 and T100 groups. The ADF intake for the T0 group was comparable to that of the T20 and T40 group (P > 0.05) and was approximately 74 g/kg DM lower (p <.0001) than the average ADF intake for the T60, T80 and T100 groups. The intake of ash for the T60, T80 and T100 groups was on average 0.035 kg ash/head/day higher (p <.0001) than the T0 group and 0.027 kg ash/head/day higher (p <.0001) than the T20 and T40 groups. All mineral intakes were high except for Na. All groups had toxic levels of

K and intakes of Mg and Fe were at toxic levels for the T60, T80 and T100 groups.

There was no day or day by treatment interactions for the nutrient intakes.

Table 6.1 The chemical composition (g/kgDM) (including the dry matter (DM), crude protein (CP), soluble protein (SP), acid detergent fibre (ADF), neutral detergent fibre (NDF), ash, organic matter (OM), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu)) and calcium to phosphorus ratio (Ca:P ratio) for ingredients including *Pennisetum purpureum*, dry fallen *Trichanthera gigantea* leaves (TGL) and treatment pellets (T0, T20, T40, T60, T80, T100) used in experimental diets

Ingredients	*P. purpureum*	TGL*	T0**	T20 **	T40 **	T60 **	T80**	T100 **
DM	270	878	870	876	871	854	843	833
CP	150	81	181	145	143	103	109	98
SP	53	19	33	25	28	21	20	13
ADF	425	308	109	142	152	295	287	340
NDF	660	430	351	302	316	365	432	459
Ash	132	194	64	85	88	151	159	159
OM	138	684	806	791	783	703	684	674
Ca	4.20	49.00	11.40	19.60	22.00	41.00	38.00	40.20
P	3.50	3.00	10.10	7.60	7.20	4.90	3.70	2.70
Mg	2.30	16.90	4.70	6.90	7.70	14.00	14.80	17.00
K	39.70	4.00	10.80	9.10	8.90	6.40	6.00	5.20
Na	0.60	0.30	0.90	0.90	0.80	0.60	0.50	0.50
Fe	0.144	1.067	0.307	0.530	0.559	1.041	1.583	1.705
Mn	0.164	0.271	0.129	0.144	0.151	0.198	0.307	0.364
Zn	0.056	0.036	0.096	0.083	0.079	0.058	0.061	0.048
Cu	0.010	0.012	0.013	0.013	0.015	0.013	0.020	0.018
Ca:P ratio	1.2	16.0	1.1	2.6	3.1	8.4	10.3	14.9

* *Trichanthera gigantea* leaves used in the study were fallen leaves collected from the floor/bed of the *Trichanthera gigantea* plantation

** Commercial pellets were offered intact for the control group (T0). The other pellet treatments were comprised of ground commercial pellets mixed with dry fallen *Trichanthera gigantea* (*T. gigantea*) leaf grinds included at 20, 40, 60 and 80% inclusion for the T20, T40, T60 and T80 diet treatments, respectively. For the T100 pellet, dry fallen *T. gigantea* leaf grinds were included at 100%.

Table 6.2 The average daily feed and nutrient intakes for the different treatment groups (n=4 lambs per treatment)

Average daily intake kg DM/hd/d	T0*	T20 *	T40 *	T60*	T80 *	T100*	SEM	*p*-value
Total forage intake	0.770	0.738	0.762	0.764	0.767	0.795	0.023	0.678
Total pellet intake	0.435^{a}	0.438^{a}	0.435^{a}	0.423^{a}	0.417^{a}	0.330^{b}	0.013	<.0001
Total dry matter intake	1.21	1.18	1.20	1.19	1.18	1.13	0.028	0.4049
Average daily nutrient intake (g/kg DM/hd/d)								
Crude protein	0.194^{a}	0.179^{ab}	0.172^{bc}	0.162^{cd}	0.161^{cd}	0.148^{d}	0.003	<.0001
Soluble protein	0.055^{a}	0.052^{ab}	0.051^{ab}	0.051^{ab}	0.049^{bc}	0.045^{c}	0.001	0.0006
Acid detergent fibre	0.374^{a}	0.389^{a}	0.378^{a}	0.459^{b}	0.447^{b}	0.439^{b}	0.010	<.0001
Neutral detergent fibre	0.660	0.640	0.621	0.673	0.689	0.659	0.015	0.0707
Ash	0.129^{a}	0.139^{a}	0.135^{a}	0.168^{b}	0.168^{b}	0.154^{b}	0.003	<.0001
Minerals								
Calcium	8.19^{a}	11.82^{b}	12.66^{b}	20.66^{c}	19.07^{c}	16.50^{d}	0.524	<.0001
Phosphorus	7.08^{a}	6.02^{b}	5.70^{b}	4.83^{c}	4.24^{d}	3.58^{e}	0.082	<.0001
Magnesium	3.81^{a}	4.79^{ab}	5.04^{b}	7.73^{c}	7.94^{c}	7.38^{c}	0.222	<.0001
Potassium	35.2	34.5	33.0	33.9	33.1	32.3	0.903	0.2469
Sodium	0.853^{a}	0.856^{a}	0.788^{b}	0.726^{bc}	0.671^{cd}	0.627^{d}	0.014	<.0001
Iron	0.244^{a}	0.343^{ab}	0.349^{b}	0.554^{c}	0.771^{d}	0.674^{d}	0.022	<.0001
Manganese	0.182^{a}	0.189^{ab}	0.186^{a}	0.213^{b}	0.254^{c}	0.246^{c}	0.006	<.0001
Zinc	0.0848^{a}	0.0794^{ab}	0.0754^{b}	0.0686^{c}	0.0686^{c}	0.0589^{d}	0.001	<.0001
Copper	0.0133^{a}	0.0134^{a}	0.0139^{a}	0.0134^{a}	0.0160^{b}	0.0136^{a}	0.0003	<.0001
Ca:P ratio	1.16:1^{a}	1.96:1^{b}	2.22:1^{b}	4.28:1^{c}	4.63:1^{c}	4.58:1^{c}	0.154	<.0001

Means carrying the same superscripts within rows are not significantly different (P > 0.05)

* Commercial pellets were offered intact for the control group (T0). The other pellet treatments were comprised of ground commercial pellets mixed with dry fallen *Trichanthera gigantea* (*T. gigantea*) leaf grinds included at 20, 40, 60 and 80% inclusion for the T20, T40, T60 and T80 diet treatments, respectively. For the T100 pellet, dry fallen *T. gigantea* leaf grinds were included at 100%.

6.5.2 Total pellet intake after 15 minutes (TPI15) and total pellet intake (TPI)

Treatment had an effect (p = .0001) on the TPI15 for all treatment groups (Table 6.3). Throughout the 7 days of the study period, there was no difference between the TPI15 of the T0, T20 and T40 groups ($p > 0.05$). The TPI15 for T0 was higher (p = .0001) than that of the T60, T80 and T100 groups for the first 4 days of the study. The intake of T0 was significantly higher (p = .0001) than the intake of the T100 group for all 7 days of the study.

There was an effect of day (p = .0001) on the TPI15 (Table 6.3). For the T0 and T20 groups, there was no significant difference between the TPI15 for day 1 and those recorded for days 2 to 7 ($P > 0.05$). There was no difference in the TPI15 for day 1 and those for days 2 and 3 ($P > 0.05$) for the T40 group. The TPI15 for the T40 group on day 1 was significantly lower than those observed for days 4, 5 and 6. There was no difference between the intakes on day 1 and those of days 2, 3 and 4 ($P > 0.05$) for the T60 group. The TPI15 for day 1 was significantly lower than those observed for days 5, 6 and 7. There was no difference in the TPI15 for day 1 and those of days 2 to 6 ($P > 0.05$) for the T80 group. The TPI15 for the T80 group on day 1 was significantly lower than that observed for day 7. There was no difference in the TPI15 of day 1 to those of days 2, 3, 4, 5, 6 and 7 ($P > 0.05$) for the T100 group. Table 6.4 shows that the TPI for all groups was over 70% of pellets offered.

Table 6.3 The total pellet intake after 15 minutes (TPI15) for the diet treatment groups (kg DM) (n=4 lambs per treatment)

	Treatment							*p*-value		
Day	T0*	T20*	T40 *	T60*	T80 *	T100 *	SEM	Treatment	Day	Treatment x Day
1	0.430^{ax}	0.355^{abx}	0.318^{abx}	0.168^{cdx}	0.275^{bcx}	0.118^{dx}	0.032	0.0001	0.0001	0.0001
2	0.434^{ax}	0.391^{abx}	0.329^{abxy}	0.176^{cxy}	0.260^{bcx}	0.125^{cx}				
3	0.418^{ax}	0.433^{ax}	0.364^{abxy}	0.228^{bcxyz}	0.270^{bcx}	0.134^{cx}				
4	0.424^{ax}	0.417^{ax}	0.419^{ay}	0.242^{bcxyz}	0.265^{bx}	0.119^{cx}				
5	0.426^{abx}	0.438^{ax}	0.422^{aby}	0.272^{cyz}	0.292^{bcx}	0.161^{cx}				
6	0.431^{ax}	0.427^{ax}	0.425^{ay}	0.277^{bz}	0.299^{abx}	0.109^{cx}				
7	0.411^{abx}	0.423^{ax}	0.400^{abxy}	0.270^{byz}	0.402^{aby}	0.121^{cx}				

Means carrying the same superscripts (a,b,c,d) within rows are not significantly different (P > 0.05); Means carrying the same superscripts (x,y,z) within columns are not significantly different (P > 0.05)

* Commercial pellets were offered intact for the control group (T0). The other pellet treatments were comprised of ground commercial pellets mixed with dry fallen *Trichanthera gigantea* (*T. gigantea*) leaf grinds included at 20, 40, 60 and 80% inclusion for the T20, T40, T60 and T80 diet treatments, respectively. For the T100 pellet, dry fallen *T. gigantea* leaf grinds were included at 100%.

Table 6.4 The total pellet intake (TPI) for the diet treatment groups (kg DM) (n=4 lambs per treatment)

	Treatment							*p*-value		
Day	T0*	T20*	T40*	T60*	T80*	T100*	SEM	Treatment	Day	Treatment x Day
1	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.402^{ax}	0.418^{ax}	0.319^{bx}	0.0179	0.0001	0.6092	0.8518
2	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.426^{ax}	0.421^{ax}	0.316^{bx}				
3	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.427^{abx}	0.421^{abx}	0.354^{bxy}				
4	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.427^{ax}	0.419^{ax}	0.322^{bxy}				
5	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.427^{ax}	0.411^{ax}	0.376^{ay}				
6	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.427^{ax}	0.412^{ax}	0.316^{bx}				
7	0.435^{ax}	0.438^{ax}	0.435^{ax}	0.427^{ax}	0.414^{ax}	0.309^{bx}				

Means carrying the same superscripts (a,b,c,d) within rows are not significantly different (P > 0.05); Means carrying the same superscripts (x,y,z) within columns are not significantly different (P > 0.05)

*Commercial pellets were offered intact for the control group (T0). The other pellet treatments were comprised of ground commercial pellets mixed with dry fallen *Trichanthera gigantea* (*T. gigantea*) leaf grinds included at 20, 40, 60 and 80% inclusion for the T20, T40, T60 and T80 diet treatments, respectively. For 100% *T. gigantea* inclusion (T 100), *T. gigantea* was the sole ingredient used.

6.6 Discussion

The daily CP intakes of the T0 , T20 and T40 groups were generally within the CP range required for moderately growing lambs between 20 to 30 kg liveweight (140 to 167 g CP/kg DM) (NRC, 1985). The daily CP intakes of the T60, T80 and T100 groups were lower but close to the recommended daily CP intake required for lambs and, therefore, growth rate may not be restricted on these diets. The SP is recommended to be between 30 to 35% of CP in order to optimise rumen function (Hoover and Miller, 1996). Based on the CP intake, the daily intake of SP was within the required amounts for growing lambs (0.05 to 0.07 kg DM/h/d) in all treatment groups except the T80 and T100 groups. The intake of ADF and NDF for all treatment groups were below the concentration of 440 g/kg DM for ADF and 660 g/kg DM for NDF and were less likely to restrict intake in ruminants (Van Soest et al., 1991). All groups were above the maximum tolerable levels for K (>30 g/kg DM) (NRC, 1985). Under optimum conditions, however, K toxicity is not a practical problem as excess K is readily excreted (McDowell and Arthington, 2005). For the T0, T20 and T40 groups, intakes of the other minerals did not exceed the maximum tolerable levels and, therefore, concentrations will not be toxic to animals (NRC, 1985). However, this was different for the T60, T80 and T100 groups where the intake of Mg and Fe exceeded the maximum recommended levels for these minerals. Although Mg toxicity is not very common, toxic levels may upset the metabolism of Ca and P (McDowell and Arthington, 2005). Iron toxicity may be associated with reduced intakes; lower daily gains; and toxic levels may interfere with the metabolism of Cu and P (McDowell and Arthington, 2005). Based on the nutrient intakes, particularly minerals, dry fallen *T. gigantea* should replace no more than 40% of commercial pellets.

The inclusion of MPTs above 50% in ruminant diets are often associated with reduced intake as a result of anti-nutritional factors inherent to these species (Min et al., 2003; Reed, 1995). While there is no known report of anti-nutritional factors that limit the intake of *T. gigantea* (Rosales, 1997b; Wanapat, 2009), the results of the current study corroborate the findings of Min et al. (2003) and Reed (1995) where the intake of pelleted diets with low concentration of *T. gigantea* (< 50%) were higher than the intake of pellets with higher concentrations of *T. gigantea* (> 50%).

Apart from the presence of anti-nutritional factors, there are other important components of the diet that impact the short-term intake of feeds. Some of the more critical ones include protein, the cell wall fractions and, in some instances, ash (Faverdin, 1999; Lazzarini et al., 2009; Negesse et al., 2009). An adequate supply of protein from diets is associated with increased efficiency of microbial fermentation; improved digestion; increased throughflow from the rumen and, therefore, increased intake. Higher concentrations of cell wall fractions including ADF and NDF are associated with accelerated rumen fill, reduced throughflow and, therefore, reduced intake of feed. Further, high ash is often associated with lower digestibility and may impede intake (Negesse et al., 2009). The combination of the high CP and SP intake as well as the lower intake of ash and ADF may have contributed to the overall higher TPI15 of the T0, T20 and T40 groups in comparison to the T60, T80 and T100 groups. Additionally, while the TPI of T20, T40, T60 and T80 groups were comparable to that of the T0 group, that of T100 remained significantly lower and may be due to the comparably lower CP and SP intake as well as the overall higher intake of ADF and ash when compared to the T0 group.

More critically, the observed differences in the TPI15 between the T0, T20 and T40 pellets from the T60, T80 and T100 pellets may be as a result of the newness factor

described by Kertz et al. (1982). With *T. gigantea* being a relatively new ingredient to the lambs, pellets with higher levels of inclusion of *T. gigantea* may have resulted in lower intakes particularly under restricted exposure (15 minutes) to the pellets. This may have explained why the TPI15 for the T20 and the T40 groups were more comparable to that of the commercial pellet (T0) than that of the T60, T80 and T100 groups with higher amounts of *T. gigantea*. On average, 98% of pellets offered were consumed by 15 minutes for the T20 and T40 groups compared to an average of 55% for the T60, T80 and T100 groups. The overall lower TPI15 of the T60, T80 and T100 groups when compared to the commercial pellet (T0), may be an indication of an aversion to these pellets. According to Kertz et al. (1982), the intake of new feeds, when offered as a single choice, is often associated with lower intakes for the first few days of exposure to the feed. Mejía and Vargas (1993) studied the preference of sheep for various local feeds and concluded that reduced intake was primarily associated with the degree to which animals were accustomed to consuming a given feed. However, despite the lower TPI15 of the T60, T80 and T100 groups, intake may be improved when animals are given more time to adapt to new feeds (Mejía and Vargas, 1993). This was observed in the current study where the TPI15 for T40, T60 and T80 groups were increased after days 3, 4 and 6, respectively. Although the TPI15 of T60, T80 and T100 groups were lower than that of the T0 group, the TPI for all treatment groups was, on average, over 70% of pellets offered.

Another factor that may have impacted on the lower intake of pellets with high concentrations of *T. gigantea* (≥ 60% inclusion), in comparison to the T0 group, may be the moderate to low palatability commonly reported for *T. gigantea* (Mejía and Vargas, 1993). However, although the TPI15 for pellets with ≥ 60% inclusion of *T. gigantea* were comparably lower than the T0 group, ultimately the TPI was high for

these pellets. This may be a result of the pelleting process which is often associated with higher levels of palatability and the presentation of more favourable forms of the feed (Dobie, 1975; Wallace et al., 1961). For instance, the hirsute nature of *T. gigantea* is a major cause of lower palatability of this MPT (Kertz et al., 1982; Mejía and Vargas, 1993), however, the process of pelleting can be used to address this through the drying and grinding leaves. The mixing and compression of ground leaves with more favourable ingredients may further improve palatability, reduce selection, and, therefore, increase the intake of the forage (Wanapat et al., 2013). Moreover, the smaller unit size of pellets makes it more prehensile and easier to ingest compared to the bulkier form of unprocessed forage. Additionally, the smaller, denser form of the feed is associated with more rapid flow of feed through the gastro-intestinal tract, resulting in its characteristically higher intake when compared to the bulkier unprocessed forage (Blaxter and Graham, 1956; Minson, 1963). There are no current studies on the impact of pelleting on the intake *T. gigantea* leaves in small ruminants, however, according to Beardsley (1964), pelleting can increase intake of forage feeds by up to 25%. Therefore, pelleting may provide an opportunity for improving the intake of and, thus, performance on *T. gigantea*.

6.7 Conclusion

Throughout the experimental period, although the total daily intakes of all pellet treatments were above 70% of the total pellets offered, within the first 15 minutes of exposure, the total intake of pellets containing up to 40% *T. gigantea*, were comparable to that of the commercial pellets. This demonstrates a more ingestive response to pellets with ≤ 40% *T. gigantea* which was further validated by the shorter adjustment period of 1 to 2 days for pellets with ≤ 40% *T. gigantea*, compared to the longer

adjustment period of over 4 days, required for pellets with ≥ 60% *T. gigantea*. Further, nutrient intakes for groups offered pellets with ≤ 40% *T. gigantea* were more comparable to that of the group offered the commercial pellets and were more within the range required for small ruminants compared to groups offered pellets with higher concentrations of *T. gigantea* (≥ 60%). The results suggest that dried fallen *T. gigantea* leaves can replace up to 40% commercial pellets in densified diets without compromising dry matter intakes in growing lambs.

CHAPTER 7 Part 1. Determining the *in-vivo* digestibility of *Pennisetum purpureum* in tropical hairsheep of the Caribbean, using the faecal collection method

7.1 Foreword

In the previous Chapter, results suggest that *T. gigantea* can replace up to 40% commercial concentrate without compromising intakes in growing lambs. As a result, the following chapter aims to determine the effect of replacing 40% of commercial concentrate with *T. gigantea* leaves in pellets, on digestibility and growth performance in lambs. The chapter is comprised of two parts. Part 1 aimed to determine the *in-vivo* digestibility of *P. purpureum* in tropical hairsheep sheep of the Caribbean using the faecal collection method. The results of the study was used to compare with and validate data obtained on the digestibility of diets comprising *P. purpureum* fed in combination with pelleted diets, in Part 2 of this chapter.

7.2 Abstract

The aim of the following study was to determine the *in-vivo* digestibility of *Pennisetum purpureum* (*P. purpureum*) in tropical hairsheep using the faecal collection method. The results of the study were used to compare and validate data obtained in the subsequent section on the digestibility of diets comprising *P. purpureum* fed in combination with pelleted diets. A total of six Barbados Blackbelly type ram lambs, aged five months with an average weight of 26 kg ± 4.52, were used in this study. Lambs were subject to a 21-day adaptation period where they were left to familiarise themselves with their enclosures, diet, harnesses and faecal collection bags prior to the subsequent five-day faecal collection period. Animals were fed a sole diet of 3.1 kg (as fed basis) of *P. purpureum* twice daily at 9:00 and 15:00 hrs. The *in-vivo* digestibility coefficients for dry matter (DM), organic matter (OM) and protein were

0.789, 0.843 and 0.842, respectively. The content of digestible dry matter (DDMi), organic matter (DOMi) and protein (DPi) per kg DM for *P. purpureum* were 0.789, 0.680 and 0.131 g/kg DM, respectively. Overall, the digestibility of *P. purpureum* was high and the results of this study suggest that *P. purpureum* is a potential high-quality forage for hairsheep in the Caribbean.

Key words: *In-vivo* digestibility, *Pennisetum purpureum*, Caribbean, small ruminants

7.3 Introduction

Pennisetum purpureum (*P. purpureum* commonly known as Elephant grass) is a tropical grass species that is used in ruminant production systems in the tropics (Francis, 2004; Heuze et al., 2016). This rhizomatous, tufted perennial is native to central and eastern Africa and was introduced to the Caribbean in the 1950s (Heuze et al., 2016; Kariuki, 1998). *Pennisetum purpureum* has several advantages compared to other tropical grasses including its comparably higher-yielding capacity, sometimes attaining yields of over three times the average of those for other tropical grasses (Boonman, 1993). However, this advantage of higher dry matter (DM) yield, is dependent on the corresponding DM intake as both factors form the basis for determining how well yield supports production in livestock (Kariuki, 1998). Another advantage of *P. purpureum* is its versatility as it can be established and utilised successfully under a wide range of conditions (dry or wet conditions) and systems (smallholder or large-scale agricultural systems) (Mannetje, 1992). Generally, the species is known to be resilient and robust and has been used successfully in the region, even under the harsher growing conditions of the Eastern Caribbean islands (Francis, 2004; Paterson et al., 1992). These advantages have contributed to its overall popular use in the Caribbean and wider tropics.

Variations in the nutritive value of *P. purpureum* may result in differences regarding intake and performance in animals (Islam et al., 2003). The nutritive value may vary, depending on rainfall, soil and fertilizer regimes (Hughes et al., 2012; Sarwar, 1999). Additionally, nutritive value may be affected by plant factors including cultivars and stage of maturity. For example, Islam et al. (2003) observed vast intra-species' differences in the botanical fractions and nutritive value of a range of cultivars of *P.*

purpureum which the author concluded may have important implications for intake and productivity in animals. Several studies have demonstrated the negative effect of increasing maturity on the nutritive value of maturing forage, primarily as a result of decreasing protein and energy concentrations and increasing concentrations of structural carbohydrate (Arthington and Brown, 2005; Sarwar, 1999). As a result, more mature forage is often associated with restricted intake and reduced digestibility (Leng, 1990). However, it has been suggested that this reduction in nutritive value with increasing maturity may not be as pronounced with *P. purpureum* as it is for other tropical grasses. For instance, Orodho (2006) concluded that *P. purpureum* retained a given digestibility for a longer period than other tropical grasses, while Nogueira Filho et al. (2000) reported a higher digestibility for *P. purpureum* compared to those of other tropical grasses of similar maturity and subjected to similar growth conditions. Other studies have demonstrated improved quality of diets comprised of *P. purpureum* through supplementation with more nutrient-dense feeds that improve overall rumen function and growth performance in livestock (Clark et al., 1992; Mpairwe et al., 2003). These factors that affect the nutritive value of forage are, therefore, critical, and must be considered in efforts to optimise the quality of grass in livestock production systems.

Although there have been several efforts to investigate the potential of *P. purpureum* for supporting livestock production in the broader tropics, the primary focus of work in the Caribbean has been on establishing best management practices for optimising the nutritive value of the forage (CARDI, 1990; Paterson et al., 1992; Proverbs and Quintyne, 1986). Consequently, very limited work has been carried out using animal trials to determine the quality of *P. purpureum*. One of the important aspects of the quality of forage is the digestibility of the forage, or the extent to which nutrients are

available for metabolism to support production. Therefore, the aim of the following study was to determine the *in-vivo* digestibility of *P. purpureum* in tropical hairsheep sheep of the Caribbean using the faecal collection method. The results of the study were used to compare and validate data obtained on the digestibility of diets comprising *P. purpureum* fed in combination with pelleted diets, in the second section of this chapter.

7.4 Materials and Methods

The experiment took place in April 2019 at the Eastern Caribbean Institute for Agriculture and Forestry (ECIAF Campus) – University of the Trinidad and Tobago (Latitude 10.56°N, Longitude -61.32°W) with approval from the Massey University Animal Ethics Committee (MUAEC 18/92).

7.4.1 Harvesting of materials

Pennisetum purpureum was manually harvested daily with a machete at an approximate height of 1.5 m according to the guidelines of Gemeda and Hassen (2014). For the period of harvest, the total rainfall at the location was 8.3 mm; the average daily minimum and maximum temperatures for the site was 23.2ºC and 32.4ºC, respectively; and the predominant soil type at the site is the Piarco soil series comprising terrace sand and gravel clay with imperfect drainage (Brown, 1965). Once harvested, *P. purpureum* (including leaves and stem) was manually chopped with a machete into lengths of about 5 to10 cm.

7.4.2 Lambs, diets and experimental procedure

Six Barbados Blackbelly type intact ram lambs, aged five months with an average weight of 26 kg ± 4.52, were used in this study to measure the digestibility of *Pennisetum purpureum*. All lambs were confined to well-ventilated individual pens (1.22 m x 1.22 m) with slatted wooden floors, which were each equipped with one feeder and a waterer. Lambs were subject to a 21-day adaptation period where they were examined, treated for internal parasites and left to familiarise with their enclosures and diet of chopped *P. purpureum* and commercial pellets. During the third week of the adaptation period, lambs were fitted with faecal collection bags with

plastic bag liners and harnesses in preparation for the subsequent five day faecal collection period (Jonker and Cosgrove, 2017). Animals were fed a sole diet of 3.1 kg (as fed basis) of chopped *P. purpureum* (leaves and stem) at 9:00 hrs and 15:00 hrs each day. The lambs had unrestricted access to water and a mineral block (Alphablock) which contained 55.000 IU vitamin A; 27.500 IU vitamin D3; 300 IU vitamin E; 30.000 mg calcium; 5.000 mg magnesium; 1.800 mg iron; 2.500 mg manganese; 50 mg cobalt; 1.500 mg zinc; 10 mg selenium; and 35 mg iodine at all times.

7.4.3 Sample collection and processing

Samples of forage offered, forage refused and faeces (collected over the seven day experimental period) were collected. The weight of the forage offered and refused was recorded daily for each lamb. Samples of the forage offered were collected weekly and pooled for DM determination and chemical analysis. The total faecal output for each lamb was collected daily at 6:30 hours, weighed and recorded. This total faecal output included faeces collected in bags and those that escaped the bag (on the slatted floor). Once the total faecal output for each lamb was weighed, the intact faeces contained in faecal bags were collected, pooled over the five day collection period and stored at -20^{o}C. This resulted in the storage of a total of 6 pooled faecal samples (1 sample per lamb). At the end of the faecal collection period, each pooled sample was thawed, mixed for approximately 10 minutes and a 10% sub-sample of the pooled faecal output for each lamb was used for chemical analysis. The remaining faeces for each lamb was repackaged and stored at -20^{o}C. All forage and faecal samples collected were dried at 60^{o}C for 72 hours in a forced air oven and ground to pass through a 2mm sieve using a Thomas Scientific mill. These were then packaged (package included Export permit

no. 139517) and exported to Cumberland Valley Analytical Services (CAVS), US, for chemical analysis.

7.4.4 Intakes and digestibility of Pennisetum purpureum

The individual feed intake on (as fed basis) was calculated from the weight of the feed offered - weight of feed refused. The feed intake (as is basis) was then multiplied by the DM% of feed offered to estimate the dry matter intake (DMI).

The digestibility coefficients for dry matter (DMD), organic matter (OMD), energy and protein were calculated using the following formula:

$$\frac{\text{Total nutrient in feed (kg)} - \text{nutrient in faecal output) (kg)}}{\text{digestible nutrients in feed (kg)}}$$

The total digestible nutrient content per kg *P. purpureum* was calculated as follows:

$$\text{Nutrient value of feed x digestibility coefficient of nutrient}$$

7.4.5 Chemical analysis

Both feed and faecal samples were analysed at the Cumberland Valley Analytical Services (CAVS), US. Samples were dried at 60^{o}C for 72 hours and ground to pass through a 2mm sieve using a Thomas Scientific mill. These were then packaged (package included Export permit no. 139517 for Research) and exported to Cumberland Valley Analytical Services (CVAS), US, for analysis. Dry matter for *P. purpureum* (modified method) was determined by drying samples at 105°C for three hours (National Forage Testing Association, 2002). Dry matter for pellets and *T. gigantea* was determined by drying samples at 35°C for two hours (AOAC method 930.15). ADF was determined using a Whatman 934-AH glass micro-fibre filters with 1.5um particle retention in place of a fritted glass crucible (modification to AOAC

method 973.18). NDF was obtained using a Whatman 934-AH glass micro-fibre filters with 1.5um particle retention which was used in place of a fritted glass crucible (a modification to Van Soest et al. (1991)). Ash was determined using 0.35g sample which was ashed for four hours at 535°C (a modification to AOAC method 942.05). Elements including Calcium (Ca), Phosphorus (P), Magnesium (Mg), Potassium (K) and Sodium (Na) were determined (modification to AOAC method 985.01) . Sample (0.35 g) was ashed for one hour at 535°C; digested in open crucibles for 20 minutes in 15% nitric acid on a hotplate; diluted to 50ml and analysed using inductively coupled plasma spectroscopy (ICP). Nitrogen (N) was determined by AOAC method 990.03 and crude protein (CP) was determined by multiplying the concentration of N in samples by a factor of 6.25. Soluble protein (SP) was determined by using the Borate-Phosphate procedure (Krishnamoorthy et al., 1982). Non-fibrous carbohydrates (NFC) were computed as follows:

$$\mathrm{NFC} = 100\% - [\mathrm{CP}\% + (\mathrm{NDF}\% - \mathrm{NDFICP}\%) + \mathrm{EE}\% + \mathrm{Ash}\%]$$

where NDFICP % is NDF insoluble CP; and EE% is the ether extract% or fat%.

7.4.6 Statistical analysis

The mean DMI, digestibility coefficients, digestible nutrient intakes and digestible nutrient content of *P. purpureum* were generated and presented with the standard deviation in the results section.

7.5 Results

7.5.1 Chemical composition of *Pennisetum purpureum*

The chemical composition of *P. purpureum* is presented in Table 7.1.

Table 7.1 The chemical composition and mineral profile of *Pennisetum purpureum*

Chemical composition	g/kg DM
Dry matter	189.8
Organic matter	806.6
Ash	97.4
Crude protein	156.0
Soluble protein	49.0
Rumen degradable protein	102.0
Neutral detergent fibre (aNDF)*	648.0
Acid detergent fibre	377.0
Non-fibrous carbohydrate	85.6
Macro - minerals g/kg DM	
Calcium	4.9
Phosphorus	3.3
Magnesium	1.8
Potassium	36.5
Sodium	0.2
Micro - minerals mg/kg DM	
Iron	107
Manganese	98
Zinc	59
Copper	10

* Terms used: aNDF: ash free NDF

7.5.2 The *in-vivo* digestibility of *Pennisetum purpureum*

The *in-vivo* digestibility coefficients and the digestible nutrient content of *P. purpureum* are presented in Table 7.2.

Table 7.2 Showing the *in-vivo* dry matter, organic matter and crude protein digestibility coefficients and digestible nutrient content of *Pennisetum purpureum* in Barbados Blackbelly sheep (n = 6)

In-vivo digestibility coefficient[a]	Mean ± sd
Dry matter digestibility	0.789±0.050
Organic matter digestibility	0.843±0.074
Digestible crude protein	0.842±0.035
Digestible nutrient content (g/kg DM *P.* purpureum)[b]	Mean± sd
Digestible dry matter	789±46.11
Digestible organic matter	680±54.35
Digestible crude protein	131±4.96

7.6 Discussion

The aim of this current study was to determine the *in-vivo* digestibility of *P. purpureum*. The results of this chapter are used to compare with digestibility values observed for diets comprised of *P. purpureum* and nutrient dense pellets in the second section of this chapter.

Pennisetum purpureum is typically classified as a low to medium quality forage because of its generally low CP concentration averaging 100 g/kg DM (Njoka-Njiru et al. 2006; Francis 2004); high structural carbohydrates (typically over 650 g/kg DM in NDF and over 400 g/kg DM in ADF) and commonly moderate DMD and OMD (Rusdy, 2016). However, for the following study, the overall quality of *P. purpureum* was higher than those typically reported for the species. For instance, the digestibility coefficients for DM and OM observed for *P. purpureum* in the current study (0.789 and 0.843, respectively) were significantly higher than those reported by Butterworth (1963) and Sarwar (1999) (0.489 to 0.615 for DMD and 0.512 to 0.647 for OMD) for the species. The difference between the studies may have been as a result of the comparably lower CP (71 to 127 g /kg DM) and higher structural carbohydrate concentrations reported by these studies (NDF: 706 to 791 and ADF: 408 to 499 g/kg DM (Sarwar, 1999). The positive impact of higher protein and lower structural carbohydrates on increased efficiency of microbial fermentation and, therefore, improved digestion is well known and may have explained the differences between the studies (Arthington and Brown, 2005; Faverdin, 1999; Lazzarini et al., 2009). However, in other studies, the digestibility coefficients of DM and OM were more comparable to those observed in the current study. For instance, digestibility coefficients between 0.709 to 0.899 were obtained for DM and between 0.703 to 0.722

for OM of *P. purpureum* (Chen et al., 2006; Kozloski et al., 2003; Nogueira Filho et al., 2000). The higher digestibility coefficients reported in this study may indicate the potential of *P. purpureum* to provide nutrients that can be readily accessed to support production in ruminants.

The DCP of a feed is closely related to its CP content (Miliford and Minson, 1965) and the high CP content currently reported may explain the high digestibility coefficient of CP (0.842) observed for *P. purpureum* in this study. The value obtained was comparable to those reported by several authors on the digestibility coefficient of CP for tropical grasses (0.717 to 0.732) (Kariuki, 1998; Shinoda et al., 1999). Additionally, the digestibility of the CP content (131 g/kg DM) aligns with values obtained when the prediction equations derived by both Miliford and Minson (1965) (DCP = 0.899 x CP (in DM) - 3.25) and Hvelplund et al. (1995) (DCP = 0.930 x CP in DM - 3) for the prediction of DCP were used (137 and 142 g/kg DM, respectively). The concentration of DCP in *P. purpureum* was within the range required for weaned lambs between 20 to 30 kg bodyweight and growing between 100 to 200 g/day (95 to 163 g/kg DM) (Kearl, 1982). These protein fractions are critical as they provide a readily available source of protein for rumen microbes and at the required levels which are often associated with improved microbial efficiency and feed digestibility (Clark, Klusmeyer, and Cameron 1992).

The overall higher quality of *P. purpureum* reported in this study may be an effect of the high CP; low structural carbohydrate fractions; high digestible or readily available protein in combination with the presence of readily available energy (NFC, though limited (<200 g/kg DM)) (Melesse et al. 2017; Rêgo et al. 2010). These may have provided the right balance of nutrients to support a high microbial efficiency and the overall high digestibility reported for the forage (Clark et al., 1992).

The quality of diets that are comprised of tropical grasses (eg. *P. purpureum*) may be affected by combined feeding with protein-rich supplements (Mpairwe, Mutetikka, and Tsumbira 2003; Clark, Klusmeyer, and Cameron 1992). The second section of this chapter aims to determine the effect of supplementing *P. purpureum* with pellets comprised solely of grain or a combination of grain and high-protein forage, on the digestibility and growth performance in growing lambs.

7.7 Conclusion

The overall higher quality of *Pennisetum purpureum* reported in this study may be a combined effect of the high crude protein; low structural carbohydrate fractions; high digestible or readily available protein in combination with the presence of digestible energy. These may have all provided the right balance of nutrients to support a high microbial efficiency and the generally high digestibility reported for the forage. Overall, the results of the study suggest that *Pennisetum purpureum* is a potential good quality forage for hairsheep in the Caribbean.

Chapter 7. Part 2. **The effect of the partial replacement of commercial feed with *Trichanthera gigantea* in pellets, on digestibility and growth performance in growing lambs**

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JACK, H.A., CRANSTON, L., BURKE, J.L., KNIGHTS, M., and **MOREL, P.C.H.** The effect of the partial replacement of commercial feed with Trichanthera gigantea in pelleted diets, on digestibility and growth performance in growing lambs

7.8 Abstract

There are limited studies on the effect of the partial replacement of commercial feed with *Trichanthera gigantea* (*T. gigantea*) in pellets, on digestibility and growth performance in small ruminants. Fourteen crossbred (Barbados Blackbelly x West African) rams, aged four to five months, averaging 27 ± 2.67 kg body weight were randomly assigned to one of two dietary treatment groups. Dietary treatments included a control diet comprising a basal feed of *Pennisetum purpureum (P. purpureum)* + a commercial pellet (T0 pellet) or a *T. gigantea* diet comprising a basal feed of *P. purpureum* + pellets comprising 40% *T. gigantea* and 60% commercial pellet (T40 pellet). Animals were subjected to a 21-day adaptation period followed by a 48-day experimental period where the digestibility and growth performance was measured. The digestibility coefficients of dry matter, energy, organic matter, and protein were 0.7274, 0.7299, 0.7423 and 0.7800, respectively, for the T0 diet and were 0.7165, 0.7169, 0.7320 and 0.7615, respectively, for the T40 diet. The dry matter intake, average daily gain (ADG) and feed conversion ratio for the T0 diet were 1.360 kg DM/d, 176 g/d and 6.19, respectively and that of the T40 were 1.371 g/d, 158 g/d and 5.54, respectively. The digestible energy (DE) and protein (DCP) per kg ADG were 103 MJ DE and 886 g DCP/ kg ADG, respectively, for the T0 diet and 106 MJ DE and 944 g DCP/kg ADG, respectively, for the T40 diet. Overall, the results of the study suggest that *T. gigantea* has the potential to replace 40% of commercial concentrate without compromising the digestibility, average daily gain and feed required per gram gain in Barbados Blackbelly sheep.

Key words: Barbados Blackbelly, Caribbean, pellet, *Pennisetum purpureum*, sheep, *Trichanthera gigantea*

7.9 Introduction

Currently, small ruminant producers in the Caribbean depend heavily on imported feed ingredients (Lallo, 2015; Walmsley, 1995). High cost, increasing complexity and uncertainty in global grain markets; competing demands for grain supply (biofuel); higher frequencies of extreme weather events and growing foreign exchange volatility within the Caribbean Community (CARICOM) all increase the risks of being dependant on imported feed ingredients (Gaughan et al., 2009; Lallo, 2015; Prakash and l'agriculture, 2011). These identified challenges elicit the need for more secure regional systems which facilitate the increased use of locally available materials and reduced dependence on imported feeds (Mills, 2011). Multipurpose tree species, (MPTs) may be a suitable substitute for these costly imports as they are a rich source of locally available feed which can enhance ruminant diets through the provision of valuable protein, vitamins and minerals (Datt et al., 2008; Miller et al., 2003; Wilson, 1969). These resources may, therefore, allow for the development of more sustainable feed systems for small ruminant production within the Caribbean (Hernández and Sánchez, 2014; Miller et al., 2003).

However, one of the major challenges of relying on forages as a source of feed for small ruminants in regional production systems are seasonal fluctuations which can impact directly on the quality and quantity of forage available for feed (Hughes et al., 2013; Lallo et al., 2016a). For instance, during the wetter, cooler months of the year, there is a high biomass yield and, during the drier warmer periods, yield and quality decline (Hughes et al., 2013; Lallo et al., 2016a). Further, with the spectre of climate change, these drier periods are projected to intensify (Lallo et al., 2016b). Therefore, there is a requirement to build management systems which buffer the impact of such

challenges. For instance, forage conservation technologies can be used to preserve abundant biomass yielded during the wet periods of the year for use during the drier resource scarce months (ASABE, 1997; Dougnon et al., 2012; Hau, 2014).

Currently, the main forms of forage conservation that are used in regional small ruminant production systems include ensiling, hay-making and leaf meal production (Hernández and Sánchez, 2014; Patersen et al., 1992). However, forage densification is another form of forage conservation which can be used to preserve abundant yields during the wetter periods of the year. The densification process involves the compression of low bulk density materials such as fresh leaves and stems into more compact or dense forms through drying; particle size reduction of material (chopping and/or grinding); followed by the application of pressure in the presence of moisture and temperature treatments (Thomas et al., 1997; Tumuluru et al., 2010b).

There are many advantages to forage densification technologies. The end products have a characteristically high bulk density resulting in greater ease of handling including storage, transportation and distribution; as well as improved animal performance including increased intake, increased daily gain, improved feed conversion ratio; and improved efficiency of nutrient utilisation (Beardsley, 1964; Dianingtyas et al., 2017). Despite the significant benefits, there is no information on its impact on the performance of tropical hairsheep in the Caribbean.

Trichanthera gigantea (*T. gigantea*) is a multipurpose tree species (MPTs) used in small ruminant production systems in the Caribbean (Hernández and Sánchez, 2014). The species is rich in crude protein (CP), fermentable carbohydrates and minerals and is low in anti-nutritional factors (Rosales, 1997a). Despite the comparably lower nutritive value of *T. gigantea* demonstrated in Chapters 3 and 5 of the thesis, compared

to the other MPTs, its abundance, timing of these animal trials and previous studies on its nutritive value warranted further investigation of *T. gigantea* in diets of ruminants in the Caribbean. There are limited studies on the effect of the partial replacement of commercial feed with *T. gigantea* in pellets, on digestibility and growth performance in small ruminants. In previous studies *T. gigantea* has been used as an alternative ingredient for monogastric species or *T. gigantea* is often presented in the bulkier forms of the forage including fresh or dried leaves, rather than in pelleted form (Avril et al., 2012; Balraj et al., 2018). Therefore, the following study aimed to determine the effect of replacing 40% of commercial concentrate with *T. gigantea* leaves in pellets, on digestibility and growth performance in lambs.

7.10 Materials and Methods

The study took place between June - September 2019 at the Eastern Caribbean Institute for Agriculture and Forestry (ECIAF Campus), University of the Trinidad and Tobago (Latitude 10.56°N, Longitude -61.32°W) with approval from the Massey University Animal Ethics Committee (MUAEC 18/92). The aim of the study was to determine the effect of replacing 40% of commercial concentrate with *T. gigantea* leaves in pellets, on digestibility and growth performance in lambs. The treatment diets fed included either a control diet comprising a basal feed of *Pennisetum purpureum (P. purpureum)* + a commercial pellet (T0) or a *T. gigantea* diet comprising a basal feed of *P. purpureum* + pellets made up of 40% *T. gigantea* and 60% commercial pellet (T40). The digestibility (digestible coefficients, digestible nutrient content) and growth performance including intake, liveweight gain, average daily gain (ADG), feed conversion ratio, efficiency of energy and CP utilisation/kg average daily gain ADG for both diets were measured and compared in this study.

7.10.1 Harvesting of materials

Pennisetum pupureum (basal feed) was manually harvested daily with a machete at a height of 1.5 m according to the guidelines of Gemeda and Hassen (2014) from forage banks at the ECIAF-UTT. For the period of harvest, the total rainfall at the location was 50.5 mm; the average daily minimum and maximum temperatures was 24.26ºC and 32.84ºC, respectively (Trinidad and Tobago Meteorological Services (TTMS), 2019); and the predominant soil type was the Piarco soil series made of terrace sand and gravel clay (characterised as having imperfect drainage; waterlogged in the wet season; and desiccated in the dry season (Brown, 1965)). Once harvested, *P.*

purpureum (including leaves and stem) was manually chopped to 15 to 20 cm lengths for feeding. Both young and mature *T. gigantea* leaves and stems for the *T. gigantea* diet (T40) was harvested mechanically in weekly batches from all parts of the tree canopy from a plantation at the University of the West Indies - Field Station (10.64°N, -61.43°W), using a STIHL telescopic tree pruner (model HT 101). During the period, the total rainfall was 526.8 mm and the minimum and maximum temperature for the period was 24.1 and 32.0ºC, respectively (Trinidad and Tobago Meteorological Services (TTMS), 2019). The predominant soil type of the site is the River estate soil series comprised of loams (characterised as free-drained soils (Brown, 1965)).

7.10.2 Pelleting of diets

After harvesting, *T. gigantea* leaves were stored at a sheltered, well-ventilated location where they were wilted for 1 to 3 days before drying mechanically to approximately 15% moisture. For the control diet, intact commercial pellets (T0) comprising 80% wheat middlings (DM basis), 20% (on a DM basis) corn, and a vitamin and mineral mix were used (die dimensions: length:5mm; diameter: 5mm). The *T. gigantea* pellets were made by combining dried ground *T. gigantea* leaves and ground commercial pellets. The dried *T. gigantea* leaves and commercial pellets were ground separately to pass through a 0.635 cm screen (Screen size modification: reduced from 2.54 cm to 0.635 cm) of a Craftsman shredder-hammer mill (model 247.776380). The ground materials were then weighed to give a ratio of 40% dried and ground *T. gigantea* leaves to 60% ground commercial pellets; mixed manually for 10 to 15 minutes; and pelleted using a Changchai-ZS1115 Pellet Mill (22 Horse-Power Diesel Engine) with a die length and diameter of 2.54 cm and 1.27 cm, respectively. The pellets which were fed were made prior to feeding in weekly batches throughout the experimental period.

7.10.3 Lambs, diets and management

The study was comprised of a 21-day adaptation period (D0 to D21) followed by a seven day digestibility study (faecal collection (D22 to D28)) and a 48-day growth study (D22 to D69). A total of 14 crossbred (Barbados Blackbelly x West African) intact rams aged four to five months averaging 27 ± 2.74 kg were used. All 14 lambs were used for the growth study, however, only 12 of the lambs were used for the digestibility study due to the availability of faecal collection bags. Lambs were confined to individual well-ventilated, raised, wooden, slatted floor pens (1.22 m x 1.22 m) which were each equipped with one feeder and a waterer. A mineral block (Alphablock) which contained 55.000 IU vitamin A; 27.500 IU vitamin D3; 300 IU vitamin E; 30.000 mg calcium; 5.000 mg magnesium; 1.800 mg iron; 2.500 mg magnesium; 50 mg cobalt; 1.500 mg Zinc; 10 mg selenium; and 35 mg iodine, was on offer at all times. From D0, all lambs were randomly assigned to one of two treatment diets T0 or T40. The T0 diet was comprised of *P. purpureum* + a commercial pellet (T0 pellet) and the T40 diet was comprised of *P. purpureum* + *T. gigantea* pellets (comprising 40% *T. gigantea* and 60% commercial pellet). During the 21-day adaptation period, lambs were examined, treated for internal parasites and left to familiarise with their enclosures and diets. Between D15 and D21 12 of the lambs were fitted with faecal bags and harnesses to allow them to acclimatise to wearing the apparatus before the seven day faecal collection period commenced (D22 to D28). Between D15 and D44, lambs were offered forage *ad libitum* (10:00 hrs and 17:00 hrs) and 500g of either the T0 or T40 pellets (as fed basis). Between D45 to D69 of the growth study, *P. purpureum* was limited and, therefore, lambs were offered a fixed

amount of 3kg forage (as fed basis) (10:00 hrs and 17:00 hrs) with 700 g of either the T0 or T40 pellets (17:00 hrs).

Forage and pellets offered and refused were recorded daily to determine the feed intake. Samples of the feed offered and refused were taken daily and pooled for DM determination. For the digestibility study, the total faecal output (faeces collected in bags) for each lamb was collected daily at 6:30 hours; weighed and recorded. Once weighed, a 10% sub-sample of the intact (uncontaminated) faeces was collected separately for each lamb; pooled over the seven day collection period and refrigerated at -20^{o}C. The apparent digestibility coefficients for dry matter (DMD), organic matter (OMD), energy and protein for the two treatment diets (forage + T0 and forage + T40) were calculated:

The apparent digestibility coefficients for DM, energy, OM, and CP were calculated using the following formula:

$$\frac{\text{Total nutrients in feed (kg)} - \text{nutrient in faecal output (kg)}}{\text{Total digestible nutrients in feed (kg)}}$$

The total digestible nutrient content per kg of the diet (formula):

$$\text{Nutrient value of feed x digestibility coefficient of nutrient}$$

A multiple regression equation was applied to the data to obtain the apparent digestibility coefficients for the forage, T0 and T40 pellets. These were then used to calculate the total apparent digestible nutrient intake content (DM, energy, OM and CP) of *P. purpureum* (forage), T0 and T40 pellets.

Throughout the experimental period, all animals were weighed weekly before the morning feed. The liveweight gain, average daily gain, feed conversion ratio, energy efficiency and protein efficiency/kg ADG were calculated.

All forage and faecal samples used for analysis were dried at 60ºC for 72 hours in a forced air oven and ground to pass through a 2mm sieve using a Thomas Scientific mill. These were then packaged (package included Export permit no. 139517) and exported to Cumberland Valley Analytical Services (CAVS), US, for chemical analysis.

7.10.4 Chemical analysis

Forage, feed, refusals and faecal samples were analysed for DM by drying at 105°C in a convection oven (AOAC method 930.15). The total nitrogen (N) content was determined by combustion (AOAC method 968.06) using a Leco CNS 200 Analyser (Leco Corporation, St Joseph, MI, USA) and CP was computed by multiplying N by a factor of 6.25. The neutral detergent fibre (NDF) (with heat stable amylase) and acid detergent fibre (ADF) fractions were determined by the method of Van Soest et al. (1991) and Tecator Fibretec System (AOAC method 2002.04), respectively. The ash content was determined by total combustion at 550 °C (AOAC method 942.05) and the organic matter was calculated as the difference between the DM content and the ash content. The gross energy (GE) was determined using a bomb calorimeter.

7.10.5 Statistical analysis

Statistical analysis was done in the R environment for statistical computing and visualisation (Team, 2013). Measurements obtained from each lamb at different sampling times were treated as repeated measures and a linear mixed effect model was applied to the data. The model consisted of treatment, day and day x treatment as fixed effects and animal as the random effect. An ANOVA was used to obtain the standard

error and *p*-value for the model differences. Where significant differences between the treatment groups was detected, means were separated using the Tukey's test. Differences were considered statistically significant if $p \leq 0.05$. In addition, apparent digestibility coefficients for DM, OM, CP and GE were calculated using multiple regression equations (Schneider and Flatt, 1975) for *Pennisetum purpureum* and both pellets (T0 and T40)

$$y = a+bX_1+cX_2 + dX_3$$

where y = digestible DM, OM, CP or GE in the diet (g or MJ/kg DM), a = intercept, b = digestibility coefficient (DM, OM, CP or GE) of the forage or (X_1), c = digestibility coefficient (DM, OM, CP or GE) of the *Trichanthera gigantea* pellet (T40 or (X_2)), d = digestibility coefficient (DM, OM, CP or GE) of the control pellet (T40 or (X_3)) X_1 = amount of nutrients provided by the forage (g or MJ/kg DM), X_2 = amount of nutrients (DM, CP or GE) provided by the *Trichanthera gigantea* pellet (T40) in the ration (g or MJ/kg DM) and X_3 = amount of nutrients (DM, CP or GE) provided by the control pellet (T0) in the ration (g or MJ/kg DM). All graphics were done using the package ggplot 2 (Wickham, 2016).

7.11 Results

7.11.1 Chemical composition

The chemical composition of feeds is presented in Table 7.3. The CP content of *P. purpureum* was 129 g CP/kg DM. The NDF and ADF were 664 and 389 g/kg DM, respectively. The CP content of the T0 pellets was 170 g/kg D and that of the T40 pellets was 173 g CP/kg DM. The concentration of the NDF and ADF fractions were 332 g NDF/kg DM and 98.1 g ADF/kg DM, respectively, for the T0 pellet and 342 g NDF/kg DM and 169.4 g ADF/kg DM, respectively, for the T40 pellet.

Table 7.3 The chemical composition ((g/kg DM)) (dry matter, crude protein, starch, neutral detergent fibre, acid detergent fibre, ash, organic matter) and gross energy (MJ/kg DM) of *Pennisetum purpureum*, T0 pellet (Commercial pellet) and T40 pellet (*Trichanthera gigantea* pellet)

Nutrients	*Pennisetum purpureum*	T0 (Commercial pellet)*	T40 (*Trichanthera gigantea* pellet)*
Dry Matter	220	796	781
Crude protein	129	170	173
Starch	1.12	307.65	189.37
Neutral Detergent Fibre	664	332	342
Acid Detergent Fibre	389.7	98.1	169.4
Ash	110.0	71.6	149.5
Organic matter	110	724	632
Gross energy	17.5	18.1	16.5

*Terms used: T0: Commercial treatment pellet with 0% *T. gigantea* (dried ground leaves); T40: Treatment pellet with 40% *T. gigantea* (dried ground leaves) Chemical composition of *T. gigantea* used in *T. gigantea* pellet (T40): Crude protein:157 g/kg DM; Neutral Detergent Fibre:457 g/kg DM; Acid Detergent Fibre:331 g/kg DM; Ash:270 g/kg DM

7.11.2 Digestibility coefficients and digestible nutrient content

The digestibility coefficients and digestible nutrient content are presented in Table 7.4. The digestibility of DM, DE, OM and CP for the T0 and T40 diets were 0.7274, 0.7299, 0.7423 and 0.7800, respectively, for the T0 diet and 0.7165, 0.7169, 0.7320 and 0.7615, respectively, for the T40 diet. The digestibility of DM, DE, OM and CP for *P. purpureum* obtained in the current study were 0.7899, 0.8025, 0.7980 and 0.8592, respectively. The digestibility coefficients for DM, energy, OM and CP were 0.5996, 0.5866, 0.6332 and 0.6567, respectively, for the T0 pellet and 0.5461, 0.5058, 0.5715 and 0.5935 for the T40 pellet. The DE of the T0 and T40 pellets were 14.03 and 10.63 MJ/kg DM, respectively, and the digestible crude protein (DCP) was 111 and 103 g/kg DM, respectively.

Table 7.4 Predicted digestibility coefficients and digestible nutrient content of Pennisetum purpureum, T0 pellet (Commercial pellet) and T40 (Trichanthera gigantea pellet)

In-vivo digestibility coefficients	*Pennisetum purpureum*	T0 pellet*	T40 pellet*
Dry matter digestibility	0.7899	0.5996	0.5461
Digestible energy	0.8025	0.5866	0.5058
Organic matter digestibility	0.7980	0.6332	0.5715
Protein digestibility	0.8592	0.6567	0.5935
Digestible nutrient content			
Dry matter g/kgDM	790	600	546
Energy MJ/kg DM	14.04	10.63	8.33
Organic matter g/kg DM	710	588	486
Crude protein g/kg DM	110	111	103

*Terms used: T0: Commercial treatment pellet with 0% Trichanthera gigantea (dried ground leaves); T40: Treatment pellet with 40% Trichanthera gigantea (dried ground leaves)

7.11.3 Growth performance and digestible nutrient utilisation for gain

The growth performance and nutrient utilisation data are reported in Table 7.5. The intake of the T0 pellet was higher (p <.0001) than that of the T40 pellets. The LWG, ADG, FCR as well as the efficiency of utilisation of both ME and DCP/kg ADG were not significantly different (P > 0.05) between the treatment groups (T0 and T40).

Table 7.5 Performance parameters including intakes, liveweight changes, digestible nutrient intakes and efficiency of utilisation of digestible energy and digestible crude protein over the 48-day growth period (n=7)

Performance parameters	T0 diet*	T40 diet*	SEM	*p*-value
Intakes				
Forage intake kg DM/h/d	0.8820	0.9050	0.0332	0.625
Pellet intake kg DM/h/d	0.4798a	0.4656b	0.0012	<.0001
Total DMI kg DM/h/d*	1.3600	1.3700	0.0334	0.846
In-vivo digestibility coefficients				
Dry matter digestibility	0.7274	0.7165	0.0067	0.2798
Digestible energy	0.7299	0.7169	0.0073	0.2385
Organic matter digestibility	0.7423	0.7320	0.0066	0.2958
Protein digestibility	0.7800	0.7615	0.0066	0.0770
Digestible nutrient intake				
Dry matter (g/day)	984	969	26.3	0.6975
Energy (MJ/DM day)	17.5	16.6	0.468	0.2029
Organic matter (g/day)	908	869	23.7	0.2672
Crude protein (g/day)	151	148	3.69	0.5705
Growth performance				
Initial Liveweight (kg)	27.6	25.5	0.964	0.1544
Final liveweight (kg)	36.1	33.1	1.24	0.1218
Liveweight gain (kg)	8.46	7.61	0.566	0.3096
Average daily gain (ADG) (g/day)	176	158	11.8	0.3096
FCR* (average daily intake: ADG)	6.19	5.54	0.337	0.1941
DE* and DCP* required per kg ADG				
DE per kg ADG (DE:ADG) MJ/kg ADG	103	106	6.46	0.7265
DCP per kg ADG (DCP:ADG) g/kg ADG	886	944	56.50	0.4792
ME* per kg ADG(ME:ADG) MJ/kg ADG**	84.2	86.9	5.29	0.7265

* Terms used: T0: Commercial treatment pellet with 0% Trichanthera gigantea (dried ground leaves); T40: Treatment pellet with 40% Trichanthera gigantea (dried ground leaves); DMI: dry matter intake; FCR: feed conversion ratio calculated as g feed required per gram gain; ADG: DE: digestible energy; DCP: digestible crude protein; ME: metabolisable energy

** ME: Calculated value (0.82 x DE ((Agricultural Research Council . Technical Committee on the Nutrient Requirements of Farm Livestock, 1965))).

The estimate cost per kg for the diets was calculated and reported in Table 7.6. T0 cost USD 0.42 per kg and T40 USD 0.74 per kg. The estimated cost/kg *P. purpureum* was USD 0.81 and the total cost of the T0 and T40 diets were USD 1.23 and USD 1.55, respectively.

Table 7.6 Estimated cost of T0 and T40 diets offered to lambs over a 90-day growth period from 27 kg at 5 months to a market weight of 38 kg at 8 months

Cost of Pennisetum purpureum						
Cost Description	Input rate	Rate (TTD)	No. of: Units/days/wks/months	Cost per 4.4 Acres (TTD)	Cost per kg of feed (TTD)	Cost per kg (USD)
Harvesting						
1. Labour wages		200TTD/day	90 days	$ 18,000.00	$ 4.44	$ 0.66
Maintenance						
1. Fertilizer inputs	150kg/2.2acres/year	4.52TTD/kg	12 months	$ 1,356.00	$ 0.33	$ 0.05
2. Labour	2 workers	200TTD/day	3 days	$ 1,200.00	$ 0.30	$ 0.04
3. Tractor used for harvesting	1 tractor	500TTD/month	3 months	$ 1,500.00	$ 0.37	$ 0.06
Total				$ 22,056.00	$ 5.45	$ 0.81
Cost of Producing *Trichanthera gigantea*						
Harvesting						
1. Labour wages	2 workers	200TTD/day	6 days	$ 2,400.00	$ 2.22	$ 0.33
Maintenance						
1. Fertilizer inputs	160kg/2.2acres/year	4.52TTD/kg	12 months	$ 1,446.40	$ 1.34	$ 0.20
2. Labour	2 workers	200TTD/day	3 days	$ 1,200.00	$ 1.11	$ 0.17
Grinding Material						
1. Labour	1080kg	200TTD	6 days	$ 1,200.00	$ 1.11	$ 0.17
2. Fuel consumption	200TTD per 156 kg forage	200TTD	1080kg	$ 1,385.00	$ 1.28	$ 0.19
Total				$ 7,631.00	$ 7.07	$ 1.05
Cost of pelleted feed	-	-	-	-	Cost/kg (TTD)	Cost/kg (USD)
Total cost/kg of Commercial pellet (T0) (35 kg Bag at 98.64 TTD)	-	-	-	-	$ 2.82	$ 0.42
Cost of Trichanthera pellet	-	-	-	-	-	-
Cost of Trichanthera leaves at 40% inclusion	-	-	-	-	$ 2.83	$ 0.42
Cost of Commercial ingredients at 60% inclusion	-	-	-	-	$ 1.69	$ 0.25
Cost of pelleting (10% increase in total cost of unpelleted material)					$ 0.45	$ 0.25
Total cost of Trichanthera pellet/kg	-	-	-	-	$ 4.97	$ 0.74

Assumptions (*Pennisetum purpureum* production): Biomass yield/1.1 ha: 10, 000 kg DM/acre; mechanically harvested; recommended fertilizer application 150 kg/ha/year (Heuze et al., 2016);
Assumptions (*Trichanthera gigantea* production): Farm size: 50 animals; Plantation is established and requires maintenance; Field is on site (farm); Biomass yield/1 ha/year = 9200 kg DM (Rosales, 1997a); Optimum fertilizer application: 160 kg/ ha/yr (Ha and Phan, 1995); *T. gigantea* is sun-dried; Pelleting increases cost by 10%; One farm-head can manually harvest 400 kg Fresh forage in one day (92 kg DM); One farm-head can grind 180 kg DM *Trichanthera gigantea* leaves per day; Animal age/weight: 5 months/26.55 kg; Market age/weight: 8 months/38 kg; Total days from 5 months to market: 90; Total intake/animal/d (kg) = 1.5; Forage intake (kg/hd/Day): 0.900 Pellet intake (kg/hd/d) = 0. 600; *T. gigantea* intake (kg/hd)/d: = 0.240; Commercial pellet intake (kg/hd/d) = 0.36; Conversion rate: 1 USD = rounded off to 7 TTD;
Prices for labour and materials were informed by Mohammed (2014) and local suppliers in Trinidad

7.12 Discussion

The digestibility and growth performance observed for both the T0 and T40 groups were comparable. The digestibility of DM, DE, OM and CP for the T0 and T40 diets were high measuring 0.7274, 0.7299, 0.7423 and 0.7800, respectively, for the T0 diet and 0.7165, 0.7169, 0.7320 and 0.7615, respectively, for the T40 diet. The daily DE intake for the T0 and T40 groups were 17.5 and 16.6 MJ/g, respectively, giving an estimated ME intake of 14.35 and 13.61 MJ/kg DM for the T0 and T40 groups, respectively (ME= 0.82% DE (NRC, 1985)). These were above the daily ME requirement of 11.29 to 11.71 MJ required to achieve moderate to rapid growth rates (300 to 325 g/day) in tropical lambs weighing between 20 to 30 kg (NRC, 1985)). Further, Kearl (1982) recommended a daily intake of 9.2 to 12.34 MJ ME for lambs between 20 to 30 kg LW growing at 300g /d. The average DCP for the T0 and T40 diets were 151 and 148 g/kg DM, respectively, which were within the requirement for growing lambs weighing 20 to 30 kg and achieving a daily gain of 300g (146 to 198 g/day). The results suggest that *T. gigantea* can replace 40% commercial pellets without compromising the DE and DCP intakes of growing lambs. Despite the adequate intakes of DE and DCP, the ADG observed in the current study (176 and 158 g/day for the T0 and T40 pellets, respectively) were almost half those predicted by NRC (1985) and Kearl (1982).

However, though the ADG observed was lower than those reported by NRC (1985) and Kearl (1982), the ADG of the group supplemented with the *T. gigantea* pellet (T40) was comparable to the treatment group supplemented with 100% commercial ingredients (T0 pellets). This was different from the reports of other studies in the Caribbean where growing lambs were offered diets comprising *T. gigantea*. For

example, when *T. gigantea* leaves (in bulkier loose form) substituted 40% of commercial feed, growth rates in lambs were a third those supplemented with 100% pelleted commercial feed. It is possible that in the current study, the presentation of the *T. gigantea* leaves in pelleted form resulted in a more efficient partitioning of energy for growth and a more comparable performance to the pelleted commercial supplement. Avril et al. (2012) report up to 78.2 g/d reduction in ADG for *T. gigantea* supplemented lambs compared to concentrate supplemented lambs which may be due to the higher intake of *T. gigantea* (88% DMI) versus the lower intake (16% DMI) in the current study. The results indicate that the inclusion percentage in the diet and form of *T. gigantea* presented may impact on performance in small ruminants.

In Trinidad and Tobago, the target market weight for lambs is 38 kg at 6 to 8 months (Mohammed, 2014). Therefore, with a recommended weaning weight of 13 to 20 kg at 2 to 3 months, animals must achieve growth rates between 100 to 208 g/d to be weaned at 6 to 8 months and 3-month-old weaners must achieve growth rates between 106 to 256 g/d to achieve the desired weight by 6 to 8 months of age. In this study 5-month-old lambs with an average initial LWT of 27.6 kg for T0 and 25.5 kg for the T40 treatment groups, had an ADG of 176 and 158 g/d, respectively. In the current study, 5-month-old animals meeting the target market weight of 38 kg at 6 to 8 months and having an initial LWT of 27.6 kg for the T0 group and 25.5 kg for the T40 group will be growing at a minimum of 116 g/d or 138 g/d for the T0 and T40 groups, respectively. Therefore, the observed ADG for the T0 and T40 groups (176 g/d for the T0 group and 158 g/d for the T40 group) were above the projected ADG for either group. This indicates the diets had nutrient contents that were above the threshold level required for maintenance. It may demonstrate the potential of *T. gigantea* to

replace up to 40% commercial concentrate in pellets without compromising liveweight gain in growing lambs.

The efficiency at which animals utilise nutrients can be influenced by several factors including previous dietary and health history, heat stress and the presence of internal and external parasites (Leng, 1989). Feed conversion ratio is a measure of the amount of feed required to achieve one unit of liveweight gain and represents the efficiency at which feed is converted into production (meat and milk) in animals. The FCR for T0 and T40 groups were 6.19 and 5.54, respectively. This was similar to those reported by Mason (1980) for growing lambs of various breeds of tropical hairsheep. The efficiency for growth obtained for the T40 diet was higher than diets comprising *P. purpureum* supplemented with high quality forage. For instance, *P. purpureum* supplemented with *Sesbania sesban* which had a high CP content of 248 g/kg DM, NDF of 399 g/kg DM and ADF of 299 g/kg DM resulted in a FCR of 35.32 (Taye, 2008). While the interactions between feed and performance is dependent on various factors, including physiological stage of animal, breed, activity, diet, nutrition history of animals, ambient temperatures among others, these results demonstrate the high efficiency potential of the T40 diet in comparison to other diets recommended for lambs grown under tropical conditions.

Another critical index used to measure the performance on diets are the units ME and DCP required for every kilogram ADG (Salah et al., 2014). The ME/kg ADG and DCP /kg ADG for the T0 and T40 groups were 84.2 and 86.9 MJ ME/kg gain and 0.881 and 0.939 kg/gain, respectively. This translated into a daily ME and DCP requirement of 14.82 MJ ME and 156 g DCP/kg ADG, respectively, for the T0 group and 13.73 MJ ME and 147 g DCP/kg ADG, respectively, for the T40 group. The

observed ME (MJ)/kg ADG for both treatment groups were within the range reported in the literature for tropical sheep (14.7 to 19.7 MJ ME or a mean of 17.6 MJ ME per kg ADG) but the observed DCP/kg ADG fell outside the published range for tropical (0.190 to 0.300 g DCP or a mean of 0.200 g DCP per kg liveweight gain) and temperate (0.200 g/kg ADG) sheep breeds (Salah et al., 2014). The higher protein requirements observed in the current study, relative to that of temperate breeds, may be partly explained by the higher temperatures in the tropics which are often associated with an increase in the requirement for absorbed amino acids for growth (Bunting et al., 1992; Salah et al., 2014). Despite the differences from those reported for tropical breeds, DCP requirements observed were comparable to those obtained by Salah (2015) for Barbados Blackbelly lambs (0.749 g/kg ADG).

The estimated cost per kg for the diets was calculated and based on the assumptions outlined in the caption of Table 7.6. With a cost of USD 0.42 per kg T0 pellet, USD 0.74 per kg for the T40 pellet and USD 0.81 per kg *P. purpureum*, the total cost of the T0 and T40 diets were USD 1.23 and USD 1.55, respectively. Therefore, the cost/kg gain on both diets were USD 6.99 for the T0 diet and USD 9.94 for the T40 diet. The estimated cost was comparably higher for the T40 diet as the production of T40 pellets was not based on large-scale commercial operations used to produce the T0 pellets. Perhaps future work will focus on commercial cost comparisons to derive more precise cost differences between the diets.

While the estimated cost per kg of feed and gain were higher on the T40 diet, digestibility and growth performance were all comparable for both groups, indicating the potential of *T. gigantea* as a partial substitute for commercial concentrate feed. This is critical as there is a requirement for the development of more self-sufficient feeding systems that utilise locally available materials.

7.13 Conclusion

The T0 and T40 diets were comparable in terms of the digestibility and growth performance. This suggests that *T. gigantea* has the potential to replace 40% of commercial concentrate without compromising the digestibility, average daily gain and feed conversion ratio in Barbados Blackbelly sheep.

CHAPTER 8 General discussion and conclusion

8.1 Introduction

Forage densification technologies may be used to develop more sustainable feed systems for small ruminant production in the Caribbean. These technologies encourage increased year round access to and more efficient utilisation of forages. Further, they are associated with improved handling (storage, transportation, feeding) and quality (consistent and standard quality, more nutrient-dense, prolonged shelf life) of forages as well as improved performance in ruminants (Beardsley, 1964; Dianingtyas et al., 2017). These advantages may increase the overall use of locally grown forages eliciting less dependence on costly imported concentrate feeds (Beigh et al., 2017). There is a wide range of forages which is used in small ruminant production systems within the Caribbean and, currently, the main forms of processing applied include ensiling, hay-making and leaf meal production (Hernández and Sánchez, 2014; Patersen et al., 1992). However, there is a lack of information on the use or effect of forage densification on regional feed systems for small ruminants. Further, there is limited information on the nutritive value of prospective forages, to which these technologies can be applied. Therefore, the aim of the thesis was to determine the nutritive value of a range of forages used in regional small ruminant production systems and the effect of applying densification technologies to one of the forages selected based on its nutritive value and availability to support animal trials.

In summary, Chapters 3, 4, and 5 give insight into the nutritive value of forages used and available for use in small ruminant production systems in the Caribbean. Forage samples were collected from one site in Jamaica and one site in Trinidad based on their ease of access and were analysed to determine their nutritive value. In Chapter 3, the chemical composition of a range of forage species was obtained utilising proximate

analysis and near infrared spectroscopy (NIRS) and the *in-vitro* digestibility was obtained using *in-vitro* assays. In Chapter 4, the concentration of minerals (macro-minerals: calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K); and micro-minerals: iron (Fe), cobalt (Co), manganese (Mn), molybdenum (Mo), zinc (Zn) and copper (Cu)) of forage species were investigated. This information can be used to understand how forages are fed and supplemented to improve the mineral content of diets for small ruminants (Youssef, 2000). Chapter 5 aimed to examine the fermentation kinetics, fermentation end products and the *in-vitro* digestibility of forages and to determine how the fermentation kinetics of forages relate to their nutritive value. This provided information on the rate and extent to which nutrients were available in forages of different nutritive value. Overall, the availability of forage was used as the basis for selecting one forage to which forage densification technologies was applied in Chapters 6 and 7. The forage selected was *Trichanthera gigantea* (*T. gigantea*) as a result of its nutritive value and the ease of access to large volumes of forage (at one site) for the animal trials conducted in the thesis.

Chapters 6 and 7 examined the effect of densified (pelleted) ingredients comprising forage, on intake, digestibility and growth performance in growing lambs. There is currently no information on the effect of different concentrations of fallen *T. gigantea* leaves on the intake of pellets in growing lambs reared under tropical conditions in the Caribbean and, therefore, Chapter 6 aimed to determine this. Chapters 7 comprised two parts, 7.1 and 7.2. Chapter 7.1 aimed to determine the *in-vivo* digestibility of *P. purpureum* which was used to compare with and validate data on the digestibility of diets comprised of *P. purpureum* and pelleted forage in the second section of this chapter. There is no information on the effect of the partial replacement of commercial

feed with *T. gigantea* leaves in pellets, on digestibility and growth performance in small ruminants in the Caribbean and, therefore, Chapter 7.2 aimed to determine this.

8.2 Summary of main findings and conclusions

8.2.1 Nutritive value of forages

There are a wide range of forages that can be used to establish more sustainable small ruminant production systems in the Caribbean (Chapters 3, 4 and 5). However, one of the major challenges of regional forage systems is the low nutritive value of tropical grasses which undergo faster maturation than their temperate counterparts, becoming more fibrous and less digestible over a short period of time (Leng, 1990). The present study has demonstrated that multipurpose tree species (MPTs) can be used to improve the nutritive value of diets comprising tropical grasses in the Caribbean (Topps, 1992; Wilson, 1969). For example, the MPTs had high concentrations of crude protein (CP) (171.1 to 263.6 g/kg DM) and low concentrations of cell wall fractions, including neutral detergent fibre (NDF) (379 to 505 g/kg DM) and acid detergent fibre (ADF) (284 to 363 g/kg DM) (Chapter 3). Further, the non-leguminous multipurpose tree species (NLMPTs) were of higher nutritive value than the leguminous multipurpose tree species (LMPTs) where both *Morus alba* (*M. alba*) and *Moringa oleifera* (*M. oleifera*) had high CP concentrations and the highest starch and lowest NDF concentrations (Chapter 3).

In the Caribbean, while the concentration of macro-minerals calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg) and micro-minerals Zinc (Zn) and manganese (Mn) were often reported as abundant in forages, both sodium (Na) and copper (Cu) were often reported as being low or deficient in forages, and iron (Fe) and molybdenum (Mo) reported as being toxic (Bernard et al., 2019; Devendra, 1977; Fick

et al., 1978; Mohammed et al., 2017; Youssef and Brathwaite, 1987). This was consistent with the findings of Chapter 4 where most minerals were within the requirement for small ruminants, except Na, Cu, Fe and Mo. Over 70% of grasses and 100% of MPTs were low in Na and 57% of grasses and 60% MPTs were low in Cu. However, both *Brachiaria arrecta* (*B. arrecta*) and *Digitaria eriantha* (*D. eriantha*) were high in Na and may be used to improve Na concentrations in diets. *Cynodon dactylon* (*C. dactylon*), *C. nlemfuensis*, *B. arrecta*, *Leucaena leucocephala* (*L. leucocephala*) and *T. gigantea* were rich in Cu and may be used to improve the Cu concentration in diets. Additionally, there were toxic concentrations of Fe in *C. dactylon* and toxic levels of Mo in both *C. dactylon* and *C. nlemfuensis*. However, these were the only two forages that were sampled in Jamaica and the toxic levels may be related to the iron rich bauxitic soils (St. Ann's clay loam) of the site from which the samples were collected. Further, these soils have a characteristically high pH and Fe concentration which may have resulted in the high absorption of Mo observed for both *C. dactylon* and *C. nlemfuensis* (Greenberg and Wilding, 2007; Schulte, 1992). Therefore, inclusion of these species in diets must be carefully managed, particularly when established on soils with a high bioavailability of metals as the bauxitic soils of Jamaica (Howard and Proctor, 1957). Bauxitic soils are unique and result in an unusual chemical composition of vegetation (Howard and Proctor, 1957). It is possible that metal concentrations may be in a more suitable range if the species were established on a different site with a different soil type. Selenium (Se) is also one of the critical micro-minerals for small ruminants and incidents of deficiency are more typically reported than toxicity (McDowell and Arthington, 2005). Selenium functions as an antioxidant mineral which supports growth and reproduction and secures the integrity of tissues (McDowell and Arthington, 2005). Although this micro-mineral was not

covered in the current work, it needs to be addressed in future work on the mineral profile of tropical forages.

Based on the *in-vitro* assays and Near Infrared Spectroscopy (NIRS) methods used in Chapter 3 and the Tilley and Terry (1963) method used in Chapter 5, the species were generally above the minimum digestible organic matter in dry matter (DOMD) (50%) and metabolisable energy (ME) (7.5 MJ/kg DM) required to be classified as medium to good quality tropical forages (Bediye et al., 2007). Overall, the high nutritive value of the MPTs, particularly the NLMPTs *M. oleifera* and *M. alba,* may explain the generally higher DOMD observed for these species (65.1 and 65.5%, respectively) (Chapter 3).

Moringa oleifera and *T. gigantea* had advantages over the other species in terms of their fermentation and fermentation end products (Chapter 5). For example, *M. oleifera* had the highest total gas production at 48 hours and volatile fatty acid (VFA) production. This is critical as the volatile fatty acids constitute the major source of energy for the ruminant providing 70 to 80% of its energy requirements (Annison, 1970; Bergman et al., 1965; Warner, 1964). Additionally, *T. gigantea* had the highest microbial biomass yield (MBM). Microbial biomass represents an important source of amino acids (70 to 80% of supply (AFRC, 1992)) and bypass protein required to support production in ruminants (Nolan, 1981). Therefore, a higher MBM yield indicates that the forages could be a good source of bypass protein. Digestible substrate is either partitioned towards the synthesis of MBM or fermentation gases and there is often an inverse relationship between gas production (or VFA production) and the synthesis or yield of MBM (Blümmel and Bullerdieck, 1997). The high MBM yield reported for *T. gigantea* may explain why the species was at the lower end of the range

for gas and total VFA production. However, having the right balance of both protein and energy is critical for high microbial efficiency. Therefore, forages cannot be selected solely on gas production potential (only reflects VFA production) but on other attributes as the potential to yield microbial biomass (Hoover and Stokes, 1991; Makar, 2004).

The evaluation of forages indicates that both grasses and MTPs have advantages that must be carefully considered to optimise the use of these resources. However, one of the critical challenges of small ruminant production systems in the Caribbean is the high dependence on imported commercial concentrates (Lallo, 2015; Walmsley, 1995). Decreasing the dependence on nutrient dense commercial feed may require targeting locally grown forages which are comparable in nutritive value. Based on the evaluation of forage resources in chapters 3, 4 and 5, the MPTs may be more suitable substitutes to commercial concentrates because of their high nutritive value. Although *T. gigantea* was generally lower in nutritive value than the other MPTs, it was selected for the subsequent studies on the application of the densification process in the thesis. Additionally, it was selected because of its availability at the time that the studies were being conducted, in combination with the high concentration of CP, the adequate fibre fractions, mineral profile and high MBM yield.

8.2.2 Nutritive value and dry matter intakes

Trichanthera gigantea, unlike other MPTs, is typically found in organised plantations in Trinidad and forage was readily available and sourced for conducting animal trials (Chapter 6 and 7.2). However, severe drought conditions during the intake study led to the scarcity of fresh leaves and an abundance of fallen *T. gigantea leaves*. As a result, the fallen leaves were used in the intake study reported in Chapter 6 which

aimed to assess the effects of different concentrations of *T. gigantea* in pellets on intake in lambs. The results of Chapter 6 showed that lambs required a significantly shorter adaptation period (one to two days) to adjust to pellets comprised of ≤ 40% fallen *T. gigantea* leaves than lambs fed pellets comprised of ≥ 60% fallen *T. gigantea* leaves (four to six days of adaptation). Further, Chapter 6 showed that the intakes of pelleted diets comprising ≤ 40% fallen *T. gigantea* leaves was higher than that of pellets comprising ≥ 60% fallen *T. gigantea* leaves, under a restricted time of exposure to the feeds. Further, CP and soluble protein (SP) intakes for groups offered pellets comprising ≤ 40% fallen *T. gigantea* leaves, were within the requirement for growing lambs (Hoover and Miller, 1996; NRC, 1985), whereas the groups offered higher concentrations of *T. gigantea* (≥ 60%), had adequate CP intakes but SP intakes were below that required for growing lambs (Hoover and Miller, 1996; NRC, 1985). This demonstrates the potential of *T. gigantea* leaves to be consumed, even at the minimum nutritive value observed for the fallen leaves. Further, the nutritive value is likely to be higher if fresh *T. gigantea* leaves were used. For example, the proximate CP reported in this thesis was approximately two times greater and the NIRS CP almost three times greater for fresh leaves (Chapters 3 and 7.2) than that observed for the fallen *T. gigantea* leaves (Chapter 6). This may have explained why the CP observed for the T40 pellets comprised of 40% fresh leaves (Chapter 7.2) was approximately 20% higher than that of the T40 pellets comprised of 40% fallen leaves (Chapter 6). Furthermore, this demonstrates how the nutritive value of forages affects the nutritive value of pelleted products. Factors, including forage species, and management practices (irrigation, cutting intervals, fertilizer application) that affect the nutritive value of forages, may impact on the nutritive value of resulting pellets and subsequent animal performance.

The concentration and intake of NDF and minerals in fresh and fallen *T. gigantea* leaves and *T. gigantea* pellets (T40) comprised of either fresh or fallen leaves were examined (Chapters 3, 6 and 7.2). The NDF concentration of fallen (430 g/kg DM) and fresh *T. gigantea* leaves (457 to 502 g/kg DM) were at concentrations where the NDF may be digestible and the bio-availability of nutrients is not restricted (Chapters 3, 6 and 7.2) (Belyea and Ricketts, 1993). For the T40 pellets where either fresh or fallen leaves were included up to 40%, the NDF was even lower (316 g/k DM for pellets comprised of fresh leaves and 342 g/k DM for pellets comprised of fallen leaves) (Chapter 6 and 7.2). Fallen leaves had toxic levels of Ca, Mg, K and Fe and fresh leaves had toxic levels of Ca and Mg only (Chapters 3 and 6). Fallen leaves were deficient in both Na and Cu while fresh leaves were deficient in Na (Chapters 6 and 3, respectively). However, when fallen leaves were included at up to 40% of pellets Ca, Mg and Fe concentrations were within a suitable range for small ruminants (Chapter 6). Further, intakes of all minerals, except K, when fed with forage, were within the range required by small ruminants (Chapter 6) (Kearl, 1982; NRC, 1985). In some instances, excess K may be antagonistic to Mg absorption and utilisation which may lead to grass tetany (McDowell and Arthington, 2005). However, K toxicity is not usually a practical problem as excess K is readily excreted (McDowell and Arthington, 2005).

Overall, the concentrations and intakes of CP, SP, and NDF of groups fed pellets comprised of ≤ 40% *T. gigantea*, were within the requirements for growing lambs (Chapters 6 and 7.2). Overall, the more ingestive response to *T. gigantea* pellets comprised of 40% *T. gigantea* (Chapter 6) and the overall high nutritive value of pellets comprised of up to 40% *T. gigantea* (Chapters 6 and 7.2) suggests that

commercial concentrate can be replaced by 40% of *T. gigantea* in pellets for growing lambs.

8.2.3 Digestibility and growth performance

The thesis investigated the digestibility of treatment diets offered in Chapters 7.1 and 7.2. Previous studies reported varying digestibility for fresh *P. purpureum* (DMD: 0.489 to 0.615 and OMD: 0.512 to 0.647) (Butterworth, 1963; Sarwar, 1999). However, values observed in Chapters 7.1 and 7.2 were comparable to the higher published values (DMD: 0.709 to 0.899 and OMD: 0.703 to 0.722) (Chen et al., 2006; Kozloski et al., 2003; Nogueira Filho et al., 2000). Despite the later stage of harvest and the lower nutritive value observed for *P. purpureum* in Chapter 7.2 compared to that in Chapter 7.1, when fed with nutrient-dense pellets in Chapter 7.2, the digestibility of the *P. purpureum*-based diets was comparable in both chapters. Several studies have demonstrated improved quality of diets comprising *P. purpureum* when supplemented with more nutrient-dense feeds (Clark et al., 1992; Mpairwe et al., 2003).

The thesis showed that *T. gigantea* can be used as a substitute for commercial concentrate without compromising average daily gain (ADG), feed conversion ratio (FCR), digestible crude protein (DCP)/kg ADG and ME/kg ADG (Chapter 7.2). The digestibility, ADG, FCR, the DCP (g)/kg ADG and ME (MJ)/kg ADG of groups supplemented with 100% commercial ingredients (T0 pellets) was comparable to the *T. gigantea* group (supplemented with pellets comprised of 40% *T. gigantea* and 60% commercial ingredients). Conversely, Balraj et al. (2018) observed when *T. gigantea* leaves (in bulkier loose form) substituted 40% of commercial feed, growth rates in lambs were one-third those supplemented with 100% pelleted commercial feed. It is

possible that in the current study, the presentation of the *T. gigantea* leaves in pelleted form resulted in a more efficient partitioning of energy for growth and a more comparable performance to the pelleted commercial supplement. Avril et al. (2012) reported up to 78.2 g/d reduction in ADG for *T. gigantea* supplemented lambs compared to concentrate supplemented lambs which may be due to the higher intake of *T. gigantea* (88% DMI) versus the lower intake (16% DMI) in the current study. In Chapter 7.2, the comparable performance observed between treatment groups suggests that *T. gigantea* leaves can substitute up to 40% imported commercial concentrate in densified diets without compromising digestibility and growth performance in growing lambs. The comparable performance between commercial and *T. gigantea* pellets is critical given the relatively lower nutritive value of *T. gigantea* to other MPTs, particularly *M. oleifera* and *Morus alba*. It is possible that pellets comprised of MPTs of higher nutritive values may result in animal performance that surpasses that of commercial pellets.

8.3 Methodological considerations and future work

The thesis aimed to determine the nutritive value of a range of forage species that are used in small ruminant production systems in the Caribbean (Chapters 3, 4 and 5). Values obtained fell within the range of those previously reported for tropical grasses and MPTs in the Caribbean and wider tropics (Aumont et al., 1995; Devendra, 1977; Devendra and Gohl, 1970). However, there are both the seasonal and spatial variations (local and regional) in the nutritive value of forages which may affect the quality of densified products. Therefore, future studies on the variations in the nutritive value of forages and the effects on pellet quality should be examined to derive guidelines for optimising and standardising densified forage products. This is critical, particularly

for large-scale commercial operations where processes and the quality of end products are expected to be consistent.

The effect of varying concentrations of fallen *T. gigantea* leaves on short-term intakes in lambs was examined (Chapter 6). However, future work should investigate the long-term effect of densified diets comprised of different concentrations of fallen and freshly harvested leaves, on intakes, digestibility and growth performance in lambs. This may help to obtain critical information on the quality of the diets (Kaitho et al., 1996).

Further, the thesis aimed to determine the effect of pellets comprised of *T. gigantea* on animal performance including intake (Chapter 6), digestibility and growth (Chapter 7.2). Changes in the quality (durability, strength) of pellets as a result of differences in processing methodology may affect animal performance (Cutlip et al., 2008; Moritz et al., 2002). However, this was not examined in the current research and, therefore, future work should aim to address this.

The estimated cost/kg feed and cost/kg ADG of *T. gigantea* pellets (T40) based on small-scale operations was determined and compared to the cost of commercial pellets which were produced using more large-scale commercial operations (Chapter 7.2). However, the economies of scale have important implications for cost and, therefore, future studies should focus on determining more large-scale commercial cost differences between the pelleted diets.

8.4 Practical implications and recommendations

8.4.1 Identifying suitable forage resources

A wide range of forage species can be used to develop more sustainable feed systems for small ruminants in the Caribbean. Reduced dependence on imported commercial concentrate require local feeds of comparable quality. The results of the present research suggest that MPTs are of overall high nutritive value and may provide more suitable substitutes to commercial concentrates when presented in a densified form. Although the effect of nutrient composition on pellet quality was not determined in this research, reports in the literature suggest that feed materials with higher concentrations of natural binders, including protein and starch and lower cell wall fractions, result in higher quality pellet products (Angulo et al., 1995; Bradfield and Levi, 1984; Briggs et al., 1999; Cavalcanti, 2000; Wood, 1987). This does not rule out the application of densification technologies to grasses, as studies have shown where the application of forage densification was more likely to improve the nutritive value and production response of fibrous forages (Beardsley, 1964; Minson, 1963); and improve year round access to grasses which are more severely impacted by harsh weather conditions (Datt et al., 2008; Wilson, 1969). However, tropical grasses are often of lower nutritive value and, therefore, pelleting of MPTs may provide more suitable substitutes to commercial concentrate feed.

8.4.2 Development of more sustainable feeding systems

Forage densification technologies may be used for the establishment of more sustainable feed systems for small ruminants in the Caribbean. The results of the present study indicate that dried fallen leaves can be used to replace up to 40% commercial concentrate without compromising the DMI, CP, SP and NDF requirements of animals (Chapter 6). Fresh *T. gigantea* leaves have the potential to

replace up to 40% commercial concentrate in densified diets without compromising digestibility and growth performance in lambs (Chapter 7.2). This may lead to the development of more self-sufficient systems where there is an increased utilisation of locally available forages and a commensurate decline in the dependence on imported commercial feeds.

8.4.3 Feeding

The presentation of new feeds to animals may result in some aversion to feeds (Kertz et al., 1982). Based on intake studies in Chapter 6, animals required between one to two days for pellets with lower inclusions of *T. gigantea* (≤ 40% DM) and between four to six days for higher inclusions (≥ 60% DM). Therefore, animals may take between one to seven days to adjust to new pelleted feed, particularly when inclusion as a percentage of total DM offered is high. According to Kertz et al. (1982) the intake of new diets is often associated with lower intakes for the first few days of exposure to the feed. However, intake may be improved when animals are given more time to adapt to new feeds (Mejía and Vargas, 1993).

8.4.4 Estimated cost of feed

The estimated cost/kg of *T. gigantea* pellets (T40) was over 70% higher than that of the T0 diet which was produced using more large-scale commercial operations (Chapter 7.2). Consequently, the cost/kg of the T40 diet was almost 30% higher than that of the T0 diet and the cost/kg ADG for the T40 diet was over 40% more than that of the T0 diet. This may have been due to differences in the economies of scale where T0 pellets were produced utilising more large-scale commercial operations compared

to the small-scale operations used to produce the T40 pellet. Therefore, costs may be more comparable using more large-scale commercial cost comparisons.

8.5 Overall summary and conclusions

The overarching objective of the thesis was to determine the effect of applying densification technologies to feeding systems for small ruminants in the Caribbean. The results of the thesis showed that there is a range of forage species with varying advantages to which densification technologies can be applied. The higher protein and the generally higher starch content of the multipurpose tree species (MPTs) may indicate the suitability of these forages for densification systems where ingredients with higher natural binders such as protein and starch are typically associated with higher quality pellets. Further, the lower fiber content of the MPTs is notable and favourable to pellet quality as lower fiber content may result in less resistance, more compression and the lower occurrence of fissures and fractures which compromise the quality of pellets. Therefore, the MPTs may provide inputs that are more favourable to the densification process resulting in the production of pellets of both high nutritive value and quality.

The focal forage species selected for the forage densification studies in this research was *Trichanthera gigantea* (*T. gigantea*). Although the species was at the lower end of the range for most of the measured parameters, its intermediate concentration of crude protein, good mineral profile, high fermentability in terms of the microbial biomass yield and its availability and accessibility in organised plantations in Trinidad, all contributed to its selection as the focal species of this research. Moreover, *T. gigantea* was recently selected by the Government of Trinidad and Tobago as one of the more favourable forage species because of its ease of establishment and relatively

higher dry matter yield compared to species of comparably higher nutritive value as *Moringa oleifera* and *Morus alba*.

The results of the densification studies demonstrated the potential of densified *T. gigantea* to substitute 40% commercial pellets without compromising intake, digestibility and growth performance in lambs. These findings are notable considering the more mature (flowering stage) and lower quality *T. gigantea* leaves used in this research. The results for pellets comprising *T. gigantea* may have been improved if younger leaves of higher nutritive value were used. Further, pellets comprising other forages including *Moringa oleifera* and *Morus alba* that were of higher nutritive value than *T. gigantea* may have resulted in a performance that surpassed that of the commercial pellets.

The overall potential of densified forage was demonstrated in this research. This elicits the need for further investigation into how the densification process can be optimised to secure densified forage products of high nutritive value and quality. There are pre, intra and post-densification processes that must be the principal components of future research to secure optimised yield and quality of forage inputs and densified forage end products. Further, targeting other locally available materials, including crop residue and co-products of agro-processing, may increase the options of locally available materials to which densification technologies can be applied. However, the infrastructure required to conduct the required research is inadequate. Although there are feed mills located across several of the islands which are equipped with suitable machinery, these operate commercially with the principal input being imported concentrate feed. Therefore, the challenge of limited infrastructure and skilled personnel required to maintain and operate densification equipment must be addressed

to secure robust research that drives the development and application of the technology in the region.

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