Nutritional value of *Chamaecytisus palmensis* (tree Lucerne) browse at different growth stages

by

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DECLARATION

I declare that the dissertation titled **Nutritional value of *Chamaecytisus palmensis* (tree Lucerne) browse at different growth stages** is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

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DEDICATION

This work is above all devoted to THE GIVER of knowledge and wisdom, THE MOST HIGHER GOD, my MAKER for the gift of life;

ALMIGHTY LORD, you have fortified, strengthened and established me;

Endlessly receive great esteem, authority and magnificence.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADFICP	ADF indigestible crude protein
ADL	Acid detergent lignin
ARC	Agriculture Research Council
aNDF	Ash corrected neutral detergent fibre
AOAC	Association of Official Analytical Chemists
Ca	Calcium
CHO	Carbohydrates
CP	Crude protein
CT	Condensed tannins
DDM	Digestible dry matter
DMD	Dry matter digestibility
DMI	Dry Matter Intake
DE	Digestible energy
dTDN	Digestible total digestible nutrients
EB	Early-bloom
ED	Effective degradability
EE	Ether extract
g	grams
h	hour
IADICP	Indigestible acid detergent insoluble crude protein
kg^{-1}	per kilograms
kp	Fractional passage rate
Mcal	Megacalories
Mcal/d	Megacalories expressed the Metabolizable energy intake
ME	Metabolizable energy
MJ	Mega joules
mm	Millimetre
m	Metre
NDF	Neutral detergent fibre
NDF-ADF	Hemicellulose
NDF DM	Neutral detergent fibre

NDFICP	NDF indigestible crude protein
NDFn	NDF corrected for nitrogen
NE	Net energy
NFC	Non-fibre carbohydrate
NSCHO	Non-structural carbohydrates
OM	Organic matter
OMD	Organic matter degradation
P	Phosphorus
PD	Potential digestibility
PB	Post-bloom
S	Sulphur
SCHO	Structural carbohydrates
STDEV	Standard deviation
TDN	Total digestible nutrient
TDN1x	Total digestible nutrient based on intake at maintenance level
TL	Tree Lucerne
RFV	Relative Feed Value
%	Percentage
(ºC)	Degrees celsius

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ABSTRACT

NUTRITIONAL VALUE OF CHAMAECYTISUS PALMENSIS BROWSEABLE COMPONENTS

Chamaecytisus palmensis (tree Lucerne) is a multipurpose tree, which is cultivated mostly for supporting protein nutrition in livestock. Tree Lucerne also serves a critical role in human nutrition and ethno pharmacology. Most tree parts are browseable hence the increase in research focus to optimise utilization of this forage resource. The objectives of this study were to (1) evaluate effects of stage of growth (early-bloom and post-bloom) and postharvest drying method on chemical composition, structural carbohydrate fractions and relative forage value of browseable leafy and woody components of tree Lucerne; (2) Evaluate of drying mature herbage (post-bloom) on rumen degradability of edible components.

Tree Lucerne was harvested in Bela Bela, Limpopo province, South Africa in 2016. Leaves, twigs (< 4 mm) and stems (< 8 mm) were harvested during early-bloom (EB) and post-bloom (PB). Samples of the leaves, twigs and stems were partitioned for whichever air-drying, sun-drying or no drying (fresh material-control) treatment in a complete randomised design yield the following treatments: EBLf (early-bloom fresh leaves); EBLa (early-bloom air-dried leaves); EBLs (early-bloom sun-dried leaves); EBTf (early-bloom fresh twigs); EBTa (early-bloom air-dried twigs); EBTs (early-bloom sun-dried twigs); EBSf (early-bloom fresh stems); EBSa (early-bloom air-dried stems); EBLs (early-bloom sun-dried stems); PBLf (post-bloom fresh leaves); PBLa (post-bloom air-dried leaves); PBLs (post-bloom sun-dried leaves); PBTf (post-bloom fresh twigs); PBTa (post-bloom air-dried twigs); PBTs (post-bloom sun-dried twigs); PBSf (post-bloom fresh stems); PBSa (post-bloom air-dried stems) and PBLs (post-bloom sun-dried stems).

Proximate analysis was done to assess compositional changes caused by drying of EB and PB portions; equations of Traxler *et al.* (1998), Weiss (2004), and Van Soest (1994) and Fox *et al* (2003) were applied to estimate composition of structural and non-structural components. *In sacco* dry matter degradation of PB materials was done to deduce effective degradability. Proximate and degradation data was applied in estimating relative feed value.

Nutritional value of leaves:

Early-bloom leaves: Crude protein content of EB leaves varied within treatment (21-28% DM) with highest level in EBLa and lowest in EBLs. Air-dried and EBLf contents were low in fibre and varied from 21.1-28.7% NDF DM respectively. Non-fibre carbohydrates (NFC) were high (up to 52.6% DM) with highest level in EBLs and lowest in EBLf. Lignin and indigestive CP were both < 2% and 5%, respectively. Leaves had mean of 87.1% TDN, 3.0-3.5 Mcal/kg ME with estimated dry matter intake of 5.7% and relative feed value/ index of 372. The EBLa had the highest forage quality.

Post-bloom leaves: Mean crude protein was 23.2% DM and differences were not observed within treatments. Leaf NDF ranged between 23.2-36.6% DM inversely related to NFC contents (45.7- 49.1%). Lignin content and indigestive CP were < 3%. Leaves varied between 81.5-89.4% TDN, 2.8-3.4 Mcal/kg ME, 213.1-339.3 of RFV with 3.3-5.2% estimated DMI. Although, difference was observed within treatments, PBLa had high RFV and DMD. Mean DMD and NDFD was 81.7 % DM and 66.5% DM respectively with no difference across treatments.

Nutritional value of twigs:

Early-bloom twigs: Although, CP content of twigs varied between 9.8-17.1% DM, no differences were observed. Twigs had 40.7- 48.7% NDF DM. Indigestible CP (ADFICP) was 2% DM. Mean NFC was 26.7% DM and the indigestible fibre CHO was higher in EBTf 42.6% and lower in EBTa (29.8%). The EBTa had the highest RFV (176.7) compared to EBTs (156.3) and EBTf (140.3).

Post-bloom twigs: twigs ranged between 48.3-51.5% NDF DM, 5.7-16.7% CP DM, with greater proportions in PBTf. Mean NFC was 21.7% DM, which related to higher indigestible fibre CHO (29.8; 37.3 and 46.1% DM in PBTa, PBTs and PBTf, respectively). The RFV was low. The PBTa had the highest DMD (63.2%) and NDFD (49.4%) while PBTf was least degradable.

Nutritional value of stems:

Early-bloom stems: Mean CP of stems was 11.5% DM and did not vary. NDF was greater in EBSf (54.9% DM NDF) and least in EBSa (35.7% NDF DM). Lignin content was low in dried material, but about 11% in EBSf associated with higher indigestible CHO C content (46% vs 23.7% DM in EBSa). The EBSa had high NFC content (37.4% DM) and lower in EBSs (7.4% DM). The estimated DMI was < 1.4% BW. *Post-bloom stems*: Drying treatment did not affect crude protein contents, which varied between 8.3-11.6% CP DM. Indigestible CP was 0.3-1.7% DM and NFC were 3.1-18% DM. Drying did not affect DMI and RFV index. At 48h of incubation, DMD was about 56% DM, in the order PBSs< PBSa< PBSf. The NDFD component was between 38-51% NDF.

Findings of this study show that there were less changes in NSCHO components loss, which are essential sources of nutrients.

Keywords: Leaves, twigs and stems, drying, fibre, relative feed value, degradability

CHAPTER 1

INTRODUCTION

Dryland cereal residues constitute a large component of dietary items for ruminants, and irrigated pastures are limited to coastal zones (Creswell & Martin, 1998). Inland cultivation of fodder crops is insignificant relative to animal needs (De Vliegher & Carlier, 2007) and hence the increased loss of stock during the dry season. Forage crops are critical for sustainable livestock production. *Lablab purpureus* (lablab) and *Mucana pruriens* (velvet beans) as well as imported varieties of spineless cactus, forage sorghums and *Chamaecytisus palmensis* (tree Lucerne; TL) could sustain large herds of livestock especially in dry areas (Pande, 1990). Tree Lucerne resembles the herbaceous Lucerne (*Medicago sativa*) in leaf morphology and nutrient quality (Dann & Trimmer, 1986). Both these plants belong to the *Fabaceae* family characterised by trifoliate greenish leaves connecting to the plant via short petioles (Wambugu *et al*, 2006). Tree Lucerne reaches up the height of 6 metres (m) while *M. sativa* grows up to 0.50 m in height. They both have nitrogen-fixing bacteria, but TL is deeply rooted to 10-15 m (Dann & Trimmer, 1986; Gutteridge & Shelton, 1994). At maturity, milky white flowers, and flattening hairy black pods of 12-20 seeds are produced in TL.

The crude protein (CP) and fibre contents of *M. sativa* are 16.45% and 36.84% in stems, 36.87% and 11.92% in leaves, 22% and 25% in petioles, and 26% and 23% in flowers respectively (Popovic *et al.,* 2001). The CP concentration of TL is approximately 20-25% in leaves and 9% in twigs (Oldham *et al.,* 1994; Lindeque & Rethman, 1998). Animals consume the upper part of the stem and bark of *C. palmensis*, although digestible nutrient content is low (Gottesfeld, 1992; Ball *et al*., 2001). Tree Lucerne is palatable, digestible and contains a low concentration of toxic compounds (Kaitho *et al.*, 1998ab).

The browseable trees are perennial and contribute to long-term sustainability of ruminant production systems (Ndlovu & Nherera, 1997*;* Assefa *et al.,* 2012). There is a propagation of TL, with lower concentrations of anti-nutritional factors (Malanot, 2013) although research on feed value in southern Africa is limited. However, with increased global warming in southern Africa; scaling cultivation of climate smart forage is imperative to sustain livestock production (Place & Mitloehner, 2010). Shrubs are more resilient under dryland conditions compared to grasses and herbaceous plants. The existence of plantations in the sub-tropical dry areas and

wetter Mediterranean environment is evidence of the robustness of TL under harsh conditions and high potential for utilization in animal diets (Becholie *et al.,* 2005; Assefa *et al.,* 2008ab; Kitaw *et al.,* 2012; Belachew *et al.,* 2013). Production of TL improved fodder flows for extensive livestock production systems (Edwards et *al.,* 1997b).

MOTIVATION

Tree Lucerne is cultivated worldwide for food, feed and medicinal value. The shrub is adaptable to a broad range of climatic regions (Heuzé *et al.,* 2016). The foliage has high levels of protein and vitamins and is low in secondary organic compounds that are notable for affecting nutrient utilization. Cultivation of TL on communal area fodder banks would therefore improve fodder flows and nutrient intake by ruminant livestock in resource-limited areas. Herbages, including twigs and small stems from TL are edible. No adverse effects were observed in sheep consuming sole diets of TL for two months (Edwards *et al.,*1997a).

Calliandra, Leucaena, Sesbania sesban, Moringa, Faidhebia and other shrubs are cultivated in tropical and sub-tropical areas of Africa to support protein requirements of dairy cattle and other livestock (Norton & Ahn, 1997; Kaitho *et al.,* 1998a; Dubeux *et al.,* 2015). Cultivated shrubs produce fodder within the first year, fodder is conserved in either wet and dry forms to provide nutrient needs of livestock during the dry season. Therefore, evaluation of post-harvest management practises on forage quality will provide information on the nutritional contribution of browseable components of TL to livestock production. The early-bloom period of forage is elongated from vegetative stage to late bloom stage before inflorescence. There are bud development, leaf development of main shoot, formation and elongation of side stem, and shoots, and development of harvestable vegetative plant parts before appearance of inflorescence stages (Meier, 2001). The early-bloom TL develops for six to a year before establishing. The survival ratio of young shrubs is 86% of seedling planting and 26% in direct seedling during the first years and become resilient to environment constraints by the second years (Assefa, 1998). Following the non-availability of the early-bloom, only post-bloom was selected for rumen degradability studies based on high availability of browse during this period.

OBJECTIVES OF THIS STUDY

The aim of this study was to assess effects of drying on forage value of *Chamaecytisus palmensis* leaves, twigs, and stems harvested at different growth stages

The objectives of this study were to:

1. Evaluate effects of stage of growth (early-bloom and post-bloom) and postharvest drying method on chemical composition, structural carbohydrate fractions and relative forage value of browseable leafy and woody components of tree Lucerne;
2. Evaluate drying mature herbage (post-bloom) on rumen degradability of edible components

BENEFITS OF THE STUDY

This study plans to evaluate the quality of dried browseable TL versus fresh browseable TL materials as an ecological and valuable fodder to supplement ruminants. It will promote the recognition of this exotic TL among conventional fodder crops of South Africa. The results might promote the cultivation of TL as fodder in South Africa to improve animal performance.

CHAPTER 2

LITERATURE REVIEW ON CHAMAECYTISUS PALMENSIS BROWSE AND DRYING PLANTS

2.1 INTRODUCTION ON CHAMAECYTISUS PALMENSIS BROWSE

Chamaecytisus palmensis (tree Lucerne; TL) known as Tagasaste (Townsend & Radcliffe, 1987; Kitaw *et al.,* 2012), originated from the Canary Islands (Dann &Trimmer, 1986) invaded heavy rain regions (Wambugu *et al.,* 2006). The TL naturally was reported as fast-growing, frost and drought tolerant fodder (van den Berg, 2010). They endure extremely lower temperature of -9 ºC in the steamy plateaux and efficiently grow at the altitude of 3 000 m (Cook *et al.,* 2005). Tree Lucerne was identified as intolerant to alumina acidic soils (Francisco-Ortega *et al.,* 1991; Aronson *et al.,* 2002) and waterlogged conditions that predisposed to root rot bacteria and silver leaf fungus called *Chondostereum purpureum.*

The tree Lucerne forage is classified in the *Fabaceae* family and *Papilionoideae* subfamily (Borens & Poppi, 1990). It is a trifoliate bulky leaflet forage tree (Dann & Trimmer, 1986; Wambugu *et al.,* 2006) that could produce creamy white flowers during spring and none in winter. The maturity is reached at six months of age and its life span is approximately 20 to 30 years (Aronson *et al.,* 2002). The three-subspecies identified are the white tree Lucerne, the escobón (‘broom’) tree Lucerne and Tagasaste (the only forage) (Lefroy, 2002). In South Africa, commercial cross-pollinating species was also developed with an aim of ensuring, an effective rate of its establishment over fluctuating climatic conditions (Malanot, 2013).

2.2 TREE LUCERNE COMPOSITION AND QUALITY

Tree Lucerne has a high-quality forage despite the presence of minor levels of secondary compounds (Dann & Trimmer, 1986; Douglas *et al.,* 1996). The foliage was free from poisonous substances. The proteins, vitamins and mineral contents were deficient in poor quality forage and poor quality of TL is well described by Ventura *et al.* (2002). According to Wanapat (1996), poor forage quality is characterized by fibrous feeds at very advanced vegetation stage and harvested during the dry season. Calcium (Ca) and phosphorus (P) deteriorated in the growing foliage on poor quality soils. The TL was declared a potential animal fodder and a livestock supplement forage in scarcity periods (Wambugu *et al.,* 2006).

During springtime in well-fertilized soil, TL leaves could contain approximately 25% CP on a dry matter basis and 75% digestible matter (Douglas *et al.,* 1996). The chemical composition of TL leaf ranged between 21-24% CP DM and 30-37% NDF DM (Borens & Poppi, 1990). According to Marques *et al.* (2008), stem materials contained 9.5% CP on DM basis and 66% NDF DM. Oldham *et al.* (1994) and Stokes (2008) observed approximately 9% CP and 46% digestible matter in twig materials. The average of 11.5% dry matter was estimated on various TL leaves (Assefa *et al.,* 2008ab). The regrowth of fresh leaf materials could contain 25-29% of CP (DM) and 16-19% crude fibre with high digestibility of 77-82%.

The studies of Snook (1986 and 1996) on fragmented browseable materials such as leaves and stems (< 5 mm) had 16-25% of crude fibre over a period of a year and 17-21% CP quantity in wet. According to Borens and Poppi (1990) crude protein of browseable leaves of TL varied between 17-22%. The TL was highly valued in both late dry and early wet seasons as well as its quality started to regress over late wet and early dry seasons but could be used for animal maintenance (Lambert *et al.,* 1989ab).

Table 2.1 Comparison of TL foliage with other common forages (% DM)

Constituents	Tree Lucerne	Lucerne rye	Grass	Wheat grain
CP % DM	17.31	21	16	11
Carbohydrate % DM	38-58	40	46	82
CF % DM	2-6	3	4	2
CF % DM	14-30	26	24	3
Total ash % DM	4-10	10	10	2
Ca % DM	0.48-1.62	2.2	1.0	0.04
P % DM	0.12-0.41	0.33	0.26	0.24

(Source: Snook, 1986)

CP: crude protein, CF: crude fat, CF; crude fibre, Ca: calcium and P: phosphorus

Table 2.2 Nutritive value of TL leaves (% DM) by seasons

Season	% DM	OM % DM	CP % DM	NDF % DM	ADF % DM	ADL % DM	EE % DM	NSC % DM
Spring	33.3	93.9	19.8	46.7	33.9	7.9		24.5
Summer	37.5	92.2	14.7	40.0	32.5	10.4		34.7
Autumn	28.0	91.7	22.0	40.7	30.7	8.0		26.1
Winter	33.5	90.4	13.1	47.9	23.8	4.6		26.5
Mean	33.1±3.4	91.9±1.3	17.4±3.6	43.8±4.3	30.2±4	7.7±21	2.9	27.9±4

(Source: Ventura *et al., 2002)*

DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, ADL: acid detergent lignin, EE: ether extract, NSC: Non-structural carbohydrates

Table 2.3 Nutritive value of TL leaves and twigs

	Leaves %	**Twigs %**
IVDOM (in vitro dry matter)	69	56
CP	20	9
ADF	24	47
NDF	39	61
ADL	10	11
Cellulose (ADF-ADL)	14	36
Hemicellulose (NDF-ADF)	15	14

(Source: Lindeque & Rethman, 1998)

2.2.1 Macro and micronutrient composition of tree Lucerne

Douglas *et al.* (1996) measured the concentration of macro minerals (% DM) and micro minerals (mg/kg DM content) in browseable TL materials sampled in moist sites. The concentration recorded were phosphate (P) 0.24% DM, potassium 1.65% DM, sulphur (S) 0.16% DM, Ca 0.47% DM, magnesium 0.13% DM and sodium (Na) 0.02% DM for macro minerals (Douglas *et al.* 1996). The levels for micro minerals were 6 mg/kg DM for copper (Cu), 74 mg/kg DM for iron (Fe), 139 mg/kg DM for manganese, and 47 mg/kg DM for zinc (Zn). In overall, the mineral concentrations of leaves were quite acceptable though Borens and Poppi (1990) indicated that TL leaves, contained little amount of Na (0.03-0.049% DM) an, P (0.14-0.15% DM). The sulphur content was 1.93 g kg^{-1}(1.7-2.17 g kg^{-1}) in the study of Assefa

et al, (2015). Poppi (1982) and Borens (1986) additionally stipulated that Ca and P forage content could decline in quantity on poor mineral soils. Therefore, mineral supplement was recommended in livestock while feeding on TL (Wambugu *et al.,* 2006).

2.2.2 Phytochemical compounds in tree Lucerne

The major phytochemicals in TL were phenolics, mostly flavones (5-15% DM: Lowry *et al.,* 1984), luteolin and apigenin. Muzquiz *et al.* (1996). Ventura *et al.,* (2000) noted that TL contained sparteine at 90% as the main alkaloid. The aerial parts of TL were reported to contain sparteine while calycotomine was found in their seeds (Edwards *et al.,* 1997a). The bitter sparteine could reduce feed intake (Hill & Pastuszewska, 1993) due to unpalatability. Therefore, Muzquiz *et al.* (1996) declared that lower TL alkaloid content of 0.05% was an accepted toxicity limit, suitable to animal as healthy fodder. Higher alkaloid content was recorded mostly in springtime than in autumn samples (Seaman, 1987). Sparteine used alone or with other compounds has pharmacological virtue.

Further studies on various forage species reported negative effects of variability of alkaloid such as pyrrolizidine alkaloid susceptible to intoxication on sheep (Seaman, 1987). The indolizidine alkaloid castanospermine in the seeds was reported to cause gastroenteritis symptoms correlated to death in ruminants (McKenzie *et al.,* 1988). Tree Lucerne fed exclusively on ruminants for long periods did not present adverse effects compared on horses (van den Berg, 2010). Fortune and Baily (1993) reported high concentrations of total phenolic compounds in leafy TL. Oldham (1993) reported lower than 7% of total phenolic.

Tannins are phenolic group compounds simply existing as hydrolysable and condensed tannins (CT). They are measured as lignin. Condensed tannins were the principal tannin existing in woody parts of TL (Assefa *et al.,*2008b). Levels of tannins were detected in diverse species of TL such as hydrolysable tannins (6.6-176 g/kg) and condensed tannins (3.3-18.4 abs/g NDF) (Kaitho *et al.,* 1998b). The CT level could increase with maturity (Assefa *et al.,* 2008b) to affect the nutrient utilization and reduce the animal performance. Sheep feeding on forage with high CT levels developed a symptom of hepatomegaly as a sign of detoxification process in liver. The presence of tannins in forage could adhere to proteins and affect its digestibility (Assefa *et al.,* 2008b). The fast outflow proportion of TL in rumen dropped in degradability (Assefa, *et al,* 2008a). Furthermore, tannins were associated with the faecal nitrogen (N) increasing levels and urinary N decreasing levels. The high tannin forage consumption

produced a poor quality of carcass (Morales & Ungerfeld, 2015). Additionally, low level of condensed tannins in forage (2- 4%) was valuable in the feed protein shield that augmented the amount of essential amino acids in the blood circulation (Makkar, 2003).

2.2.3 Food value of tree Lucerne

Tree Lucerne foliage is highly nutritious and rich in vitamin A (Wambugu *et al.,* 2006). It is a free methane-producer forage and free from antioxidant compounds. According to Borens and Poppi (1990), no toxicity was reported on livestock fed TL and unlike with Lucerne such that ruminants fed TL do not bloat (Myles & Esterhuizen, 2012). Borens and Poppi (1990) found that lambs nourished on TL leaves produced approximately 95 g/day live weight lower than 150 g/day *(Bromus willdernowii*) and 265 g/day *(Medicago sativa*). In contrast, Becholie *et al*. (2005) observed that lambs fed on TL (178 g/day) gained 11.2-52.0 grams daily in body weight. Tree Lucerne protein substituted to concentrate mixed with wheat bran (three units) and *Guizotia abyssinica* seed block (one unit) amended the body weight of male Menz sheep (Assefa *et al.,* 2008a). The findings by Mengesha *et al.* (2016) were aligned the report of Assefa *et al.* (2008a). Steers browsed on TL mixed with lupins [*lupinus spp*] gained body weight during autumn and had also their rumen physiology healthier (Milton *et al.,* 2000). A mixture sample of browseable TL composed of leafy and woody materials (stem < 5mm) had high nutrient content (16.2% CP/DM and 74.8% DMD (Milton *et al.,* 2000). The *in sacco* DM digestibility of TL leaves ranged between 60% DM disappearing in 4h and 85% in 24h (Borens and Poppi, 1990).

2.2.4 Carbohydrates in tree Lucerne

Carbohydrates in forages are the main energy supplier of ruminants and consists of two distinctive groups that is non-structural and structural (Das *et al.,* 2015). Under the non-structural, there are cell contents that comprise of sugars, starch, pectin and some cellulose. For example, the grain of barley is highly energetic set for biochemistry reactions (Newman *et al.,* 2009; Ball *et al.,* 2001). According to Snook (1986), tree Lucerne contained 38-58% DM of carbohydrate and 14-30% DM of crude fibre. Fibrous cellulose, hemicellulose and lignin are part of structural components that build the cell walls, for example straw (Ball *et al.,* 2001).

Structural and non-structural contents, and livestock utilization

Cell wall structure and composition influence utilization of plant structural carbohydrates. The biosynthesis of the secondary plant cell wall is guided mainly by polysaccharides including cellulose, hemicellulose, lignin, proteins in cell wall and small amounts of flavonoids, tannins, terpenoids and stilbenes and insoluble pectins (Kumar *et al.*, 2015). The cross-linkages between cell wall polymers of grasses are mostly created by ferulic acid and p-coumaric acid (pCA). Ferulic acid interconnects with hemicellulose and cellulose via ester and ether bonds. With pCA esters are formed with S-lignin (Hatfield *et al.,* 2017). Phenolic compounds and lignin affect cell wall degradability. In woody plants such as browseable spp. e.g. cultivated and non-cultivated shrubs, cellulose accounts for 40-50% of stem part, 25% hemicellulose and 25-35% lignin. Lignin infers rigidity and structure to leaves, stems, and enables water transportation in plants. However, the relative proportions of cell in plant walls determine extent of degradation of plant materials. The amounts of xylose: arabinose and p-coumaric: ferulic acid tend to be less in parenchyma than sclerenchyma cells; and the concentrations are higher in stems (Grabber *et al.,* 1991). Sclerenchyma and parenchyma of grass stems tend to degrade in rumen fluid after extended incubation (Jung & Casler, 2006; Grabber *et al.,* 1991). Méchin *et al.* (2005) stated that high degradability of maize silage was related to low levels of lignin.

The leafy dry matter digestibility of TL is ranged between (75-80%) comparable to tree Lucerne content (Wills, 2008). The *in vitro* OMD (DM) recorded ranged between 77.0-85.0%, whereas the total nitrogen content (DM) fluctuated between 26-40 g/kg. The digestibility in edible material (TL leaves and edible stems) and N content were respectively 82.0% DM and 32 in N (Dann & Trimmer, 1986; Douglas *et al.,* 1996). Thus, the *in vivo* digestion average taking place in ruminant paunches was 55% for DM and 80% for NDF (Borens and Poppi, 1990). The leafy DMD was 71-78% compared to stem DMD of 46%. Borens and Poppi (1986) reported that 77-82% vs. 59% IVDMD leaves and stems, respectively comparable to Pande (1990). Mean IVDMD CP was 73%.

The *in sacco* degradability of TL in rumen performed on cannulated crossbred steers resulted in 21.5% CP, 22.1% ADF, 6.9% ADL and 35.1% NDF (Assefa *et al.*, 2008a). The *in sacco* digestibility of *Sululta* hay supplemented with various leaves of multipurpose trees (MPT in

mg g $^{-1}$) resulted in 204.3 (12h), 243.3 (24h), 458.4 (48h), 548.8 (72h), 597.3 (96h); 104.0(a) 651.1(b) 755.1 potential digestibility (PD) 0.0162(c) -1.120(TL) for TL (Odenyo *et al.*, 1997). Poppi, (1982) and Borens (1986) for *in vivo* DMD recorded TL (70.4±1.4%) and Lucerne hay chaff (66.9±1.4%). The *in vitro* DMD recorded 68% for TL (Kaitho *et al.*, 1998a; Heuzé *et al.*, 2016) vs 62.1% for the Lucerne hay (Del Razo *et al.* 2015). According to Pande (1990), the *in vitro* DMD value of TL was higher in leaves (69.3%) compared to stems (47.5%). Dry matter consumptions were 33.4±1.6 (g/kg BW/ day) for TL foliage and 26.5 (g/kg BW/ day) for Lucerne hay (Pande, 1990). The literature shows that there is very limited scope on TL utilization and hence the need to further evaluation to improve uptake as fibre and protein source.

Table 2.4 Comparison of TL fractions supplied to goats as an exclusive diet (% DM)

	Tree Lucerne	
In vitro	**Leaves**	**Stems**
DMD %	69.3	47.5
OMD %	74.3	58.2
DOMD %	67.2	47.4

(Source: Pande, 1990)

Table 2.5 Protein content and digestibility of TL

	CP (%)	**Digestibility (%)**
Leaf	20-30	70-80
Thin Stem (Twig)	9	50-60
Large stem	6	40-50
Wood	3	No data
Bark	13	No data

(*Source: Wiley, 2005)*

2.3 DISTRIBUTION AND PREVALENCE OF TREE LUCERNE IN SOUTH AFRICA

Tree Lucerne was introduced in the Republic of South Africa in the ninetieth century (Esterhuyse, 1989; Francisco-Ortega & Jackson, 1991). The growing of TL was favourably predicted in western, southern and Eastern Cape (Esterhuyse, 1989; South African Agricultural Journal, 1913). The cultural field of TL in South Africa has expanded to natural environments (Royal Botanic Gardens, Kew, 1891; D.M, 1893; Francisco-Ortega & Jackson, 1991). The early South African unsuccessful experience on planting seeds was reported by Hutchinson (1918). Nowadays, the plant is grown in home gardens for esthetical purposes (Lindeque & Rethman, 1998; Esterhuyse, 1989; Wambugu *et al.,* 2006; Gutteridge & Shelton, 1994; Marques *et al.,* 2008). Regardless of the presence of some sporadic cultivation in some provinces (Western Cape, Limpopo and Gauteng) which are still at the experimental phases of commercializing of seeds and seedlings (Farmer's weekly, 2012), the prevalence of TL fodder is poorly and unequally distributed within South Africa since three decades. The propagation level of this fodder is very low and could be due to a lack of stimulus when there is no added value attached the crop (LDA, 2012; Wiley, 2005) as confirmed in Table 2.6.

Table 2.6 The establishment of tree Lucerne seedlings in diverse location in South Africa

Location	Number of seedlings	Seedling mortality rate	Planting date
Mara Research Station	98	35 (36%)	November 2012
Toowoomba Research Station	198	71 (36%)	October 2012
Madzivhandila ATC	92	66 (72%)	November 2012
Tompi Seleka ATC	98	96 (98%)	October 2012
ARC/API/ Pretoria	100	74 (74%)	September 2013

(Source: LDA, 2012; ARC, 2013)

2.4 FORAGE CONSERVATION

Drying is the oldest preservation method for foodstuff involving dehydration by heat (Maisnam *et al.,* 2017). Reduction in water content inhibits enzyme activity and hence reduce spoilage, phytochemical compounds are detoxified, and sometimes food taste is improved (Hui *et al.,* 2006). There are many methods for conventional drying of forages (McDonald *et al.,* 2010). Plant moisture plays a major role in how forages are preserved; forages for silage are preserved when moisture content is high while hay making is based on dried materials. (Li *et al.,* 2014), (Zheng *et al.,* 2005). Plant part and fractions also play key roles in selection and preservation of forages. Leafy fractions tend to dry faster than woody twigs and stems (Dongmei *et al.,* 2009). Pelletier *et al.* (2010) and Das *et al.* (2015) indicated that non-fibre carbohydrate depletion occurs much quick in leaves than in woody materials during oven drying. Nutrient losses, however, occur during drying (Zheng *et al.,* 2005) regardless of heat or light intensity.

2.4.1 Drying treatment and nutritional values of forage

Heat and sunlight exposure affect forage quality. Denaturation of proteins in forage often takes place during sun drying (Zheng *et al.,* 2005) due to Maillard reactions when proteins and carbohydrates form complexes and denature (Deinum & Maassen, 1994). Crude protein content is reduced especially during direct sunlight drying (Zheng *et al.,* 2005). The NSC are complexed to form part of the structural materials (Moore & Jung, 2001; Pelletier *et al.,* 2010; Das *et al.,* 2015). Bastos *et al.* (2012) noted that dehydration process of forage by heat impacted on crystallisation of glucose content in twig and stem fractions. According to Wanapat (2003), sun-drying improved palatability of cassava hay (*Manihot utilissima*), as 90% of hydro-cyanic acid was destroyed by sunlight to improve cattle feed value. Oni *et al*. (2015), however, reported that air-dried and freeze-dried vegetables were highly ranked in protein and fibre content compared to sun and oven-dried materials. Oven and sun drying results in forage that is free of phlobatannin compared to air and freeze-drying that had high saponin content. Whilst, sun drying reduced the phytochemical compounds for dried vegetables, their nutritional value was affected (Oni *et al.,* 2015).

Maillard reactions known as the non-enzymatic reaction occurs at higher temperature above 80°C between carbohydrate and protein units. This reaction over carbohydrate contents seems to be not significant with storage, apart from accumulating reducing sugar and sucrose at one side and condensing starch on the other side. Maillard products are made from high temperature

reaction. They turn into brown colour and lysine content is damaged (McDonald *et al.*, 2010). In contrast, respiration (enzymatic reaction) occurs at lower temperature than that of oven drying (Smith, 1973). Rapid drying at 70°C prevents Maillard reaction formation and protein breakdown. However, temperature over 90°C destroys digestible components (Deinum & Maassen, 1994). Vitamin A (Vit A) is affected by light, heat and oxidizing agents due to the existence of unsaturated side-chain: synthetic Vit A are enveloped by enclosed sacs to prevent the oxidation reaction (Ballet *et al.*, 2000).

2.4.2 Drying treatment and forage kinetics

Forage digestibility is influenced by cellulose and lignin contents that compose ADF fractions (Belyea & Ricketts, 2018). Farnham *et al.* (1997), found that CP degradability of switchgrass (*Panicum virgatum)* was affected by drying. The bypass protein in freeze-dried switchgrass was negligible, but that of oven drying (71°C) increased exponentially as temperature increased. McDonald *et al.* (2010), stated that overheating is directly related to amount of bypass CP. On the positive side condensed tannin (CT) content decline with drying (Ahn *et al.*, 1989; Stewart *et al.*, 2000). Norton and Ahn (1997) noted high digestibility of structural carbohydrate content in sheep diets fed on dried *Calliandra calothyrsus* leaves. The increase in fibre ratio negatively influenced the energy content and the digestibility of dried forage (McDonald *et al.*, 2010).

2.4.3 Drying treatment and forage energy

Visagie and van de Vyver (2010), Khajali and Slominski (2012) and Masi *et al.* (2015) indicated that fibre is inversely associated with food ingestion and energy ranking. Belyea and Ricketts (1993) stated that fibre concentration is in reverse proportional to energy level and both vary along with forage fractions and growth periods. Therefore, the estimation of energy content of forage is estimated on the lignin, cellulose and ADF contents (Belyea & Ricketts, 1993). *Crotalaria* was affected by sun-drying compared to shade drying (Yashim *et al*. 2012) affecting the energy density of the forage.

2.5 SUMMARY OF REVIEW

Protein deficiency is a major limitation on animal production especially herbivores. Production of high quality fodder crops is therefore critical, browse such as TL have more resilience

against harsh climatic conditions. The deeper root system enables cultivated browse to produce more high-quality biomass; high in protein content and digestible carbohydrates. This study evaluates nutrient quality of early-bloom and post-bloom browseable components and shows how post-harvest processes impact available proteins and carbohydrate fractions.

CHAPTER 3

MATERIALS AND METHODS

Study site

The study was done at ARC-Irene, Pretoria (25º 53' 59.6" S; 28º 12' 51.6" E and 1785 m **a.s.l.**). Tree Lucerne was harvested in orchards at Bela Bela, Limpopo province (24º 54' 0"S; 8º 19' 60" E and 1 109m **a.s.l**.). The average annual rainfall in this region ranges from 530-800 mm. Temperature of 15.7-29 °C and 6.7-24.0 °C are recorded respectively during wet period (December to February) and dry period (June to August) (SAWS, 2016). The edible early-bloom sampling was harvested during spring and post-bloom was gathered after flower fall. About 2 grams dry matter was assessed by overnight drying at 105 °C.

The biomass of both early-bloom and post-bloom samples was partitioned into three fractions: leaves, twigs (< 4 mm diameter) and stems (< 8 mm diameter). Each plant fraction was further subdivided into three portions which were subjected to air-drying, sun-drying or no drying (control) treatment in a complete randomised design. Six treatments were defined for each plant fraction according to the design in Table 3.1.

Table 3.1 Tree Lucerne fractions and treatments

Plant fraction		**LEAVES**		**TWIGS**		**STEMS**	
Harvesting stage		Early-bloom	Post-bloom	Early-bloom	Post-bloom	Early-bloom	Post-bloom
Post- harvest management	Fresh	EBLf	PBLf	EBTf	PBTf	EBSf	PBSf
	Air-dried	EBLa	PBLa	EBTa	PBTa	EBSa	PBSa
	Sun-dried	EBLs	PBLs	EBTs	PBTs	EBSs	PBSs

EBLf: Early-bloom fresh leaves; **EBLa:** Early-bloom air-dried leaves; **EBLs:** Early-bloom sun-dried leaves; **PBLf:** Post-bloom fresh leaves; **PBLa**: Post-bloom air-dried leaves; **PBLs**: Post-bloom sun-dried leaves; **EBTf**: Early-bloom fresh twigs; **EBTa**: Early-bloom air-dried twigs; **EBTs**: Early-bloom sun-dried twigs; **PBTf**: Post-bloom fresh twigs; **PBTa**: Post-bloom air-dried twigs; **PBTs**: Post-bloom sun-dried twigs; **EBSf**: Early-bloom fresh stems; **EBSa**: Early-bloom air-dried stems; **EBLs**: Early-bloom sun-dried stems; **PBSf**: Post-bloom fresh stems; **PBSa**: Post-bloom air-dried stems; **PBLs**: Post-bloom sun-dried stems.

Fresh harvested plant materials (control) were chopped finely and refrigerated at -4 °C pending proximate analyses. Sub-samples of leaves, stems and twigs (EB or PB) were oven-dried at 63 °C for 48h to estimate DM (AOAC method 934.0). Air-drying was done on a clean concrete surface and under shed for 14 days and sun-drying was done for 7 days (from 09:00 to 15:00). Tree Lucerne samples were milled through a 2-mm sieve of a Willey Mill.

3.1 Experiment 1: ANALYSIS FOR CARBOHYDRATE AND PROTEIN FRACTIONS

Samples were analysed for ash and ether extract (EE) according to AOAC, (2002) procedures (methods 942.05 and 920.37, respectively). Ash corrected neutral detergent fibre (aNDF, AOAC method 2002.04), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991). Cellulose and hemicellulose were estimated using equations of Van Soest *et al.* (1991). Crude protein (CP) was determined using Kjeldahl method (AOAC 2002, procedure 954.01). Non-fibre carbohydrates were calculated as NFC = [100- (NDF + percentage CP + %Fat + Ash)]. Energy content was determined using the oxygen bomb calorimeter (AOAC, 2002).

The equations of Traxler *et al.* (1998), Weiss (2004) and Van Soest (1994) were used in estimating available carbohydrates and indigestible fractions as shown below:
U = 100 - [147.3 - 78.9 log10 ´ (Lig/ADF)] Van Soest
U = 2.4 ´ (Lig/NDF) Chandler
Non-structural carbohydrate (**NSCHO)** = 100 - (crude protein + fat + (NDF protein) + ash)
Starch, pectin, glucans, volatile fatty acids = NSCHO - sugar
sugars (component of NSCHO)
B2=NDF – (NDFN x 6.25) - C
Fraction C= 2.4 x lignin

Forage value was calculated as given by Fox *et al.*, (2003):
Cellulose = %ADF - %ADL
Hemicellulose = %NDF - %ADL
Total digestible nutrients (TDN) = (NFC*0.98) + (CP*0.87) + (FA*0.97*2.25) + TDN = (NFC*.98) + (CP*.93) + (FA*.97*2.25) + (NDFn *(NDFD/100) - 7 (NDF*NDFD/100) - 10); TDN = (NFC*.98) + (CP*.93) + (FA*.97*2.25) + (NDFn *(NDFD/100) – 7

For forages, based on Tedeschi (2001, Ch. 2):

dTDN = 0.53 + 0.99 × TDN1x - 0.009 × NDF + 0.00005 × TDN1x × NDF + 8.96 × DMIFactor - 0.1 × TDN1x × DMIFactor - 0.13 × NDF × DMIFactor + 0.0005 × TDN1x × NDF × DMIFactor

For concentrates, based on Tedeschi (2001, Ch. 2):

dTDN = 1.01 × TDN1x - 1.77 × DMIFactor - 0.99

TDN1x = TDN discount = DMIFactor× (0.033 + 0.132×NDF - 0.033×TDN1x)/100

ADFICP = NDFICP - 0.38

IADICP = 0.4* ADFICP

NDFn = %NDF - (NDFICP + IADICP)

Non-fibre CHO (NFC) = 100- (%NDF + CP + Fat + Ash)

Lig = (%ADF/ 100) * %NDF

Available CHO: B2 = %NDF - (2.5 + %ADL)

Indigestible fibre CHO C= %ADL * 2.4

Unavailable CHO CC = %NDF * 0.01 * %ADL * 2.4

Equations of Fox *et al.* (2003) were applied in estimating digestible Total digestible nutrients **(dTDN),** Digestible energy **(DE),** Metabolizable Energy **(ME),** and Net Energy **(NE).**

According to Ward (2008), Digestible dry matter **(DDM),** Dry matter Intake **(DMI)** and Relative Feed Value **(RFV)** were calculated as follows:

DDM=88.9 - (0.779 * %ADF)

DMI=120 / %NDF

RFV=DDM * DMI / 1.29

3.2 Experiment 2: IN SACCO RUMINAL DEGRADABILITY

The *in sacco* ruminal degradability was determined by factorial design of 1*3*3. Post-bloom TL fractions (leaves, twigs, stems) were used (period of high biomass production). Approximately 5 grams of dried samples were set separately in various labelled nylon bags and then sealed. Samples were incubated in duplication at 0h, 6h, 12h, 18h, 24h, 30h, 36h and 48h

inside the rumen according to McDonald (1981). At completion of the incubation period, samples were removed and cleaned with running tap water to interrupt the enzymatic action of rumen microbial ecosystem over the feedstuff. Ash corrected neutral detergent fibre digestibility (aNDFD) (aNDF, AOAC method 2002.04), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al*. (1991). Cellulose and hemicellulose digestibility were estimated using the equations of Van Soest *et al.* (1991). The neutral detergent fibre digestible (NDFD) values of the *in sacco* were deducted after calculation as follows:

The DM degradation was estimated in terms of Ørskov and McDonald's (1979) equation **(PD = a + b (1 − e^{-ct}),** where **PD** = potential degradability at time t; **a** = rapidly degradable fraction at time zero; **b** = slowly degradable fraction; **c** = fractional rate constant at which the fraction described by **b** will be degraded per h; and **t** = time of incubation.
As ruminal retention time affects the extent of degradation, a fractional outflow rate of undegraded protein from the rumen (kp) was considered when the effective degradability (Deff) was calculated as Deff = a + bc / (c + kp), where kp was assigned at 0.01 and 0.002 respectively.

Effective Degradability (ED %) assumed fractional passage rate (kp) of 5%/h, McDonald (1981):

$$ED = a + bc/(c + k)$$

3.3. STATISTICAL ANALYSES

Data was subjected to one-way analyses of variance in Minitab 18.1.0.0 version using the model below.

Model: Y = u + ai + bj + (abij) + error

Y= is mean; u = population mean; ai = harvest time; bj = plant components, and abij = interactions.

The posthoc, Tukey's' test on Minitab 18.1.0.0 version was used to compare the mean treatments and significant differences confirmed at $p<0.05$.

CHAPTER 4

RESULTS

Findings of this study are projected to compare drying treatment results for each TL fraction separately within an exclusive growth stage (early-bloom or post-bloom). It is not intended to compare drying treatment results between different growth stages neither nor across TL fractions (leaves, twigs and stems). Tables 4.1 to 4.3 show the chemical composition and forage value of edible leafy and woody components of TL harvested in early-bloom and post-bloom periods.

4. 1 Chemical composition and forage value of edible leaves

Early-bloom

The mean crude protein content of leaves was 25% and did vary within treatment (21-28% DM) with higher level in EBLa and lower in sun-dried leaves (EBLs). Fat content was lower in EBLs (2.1% DM) but higher in EBLf (5.2% DM). Ash contents in EB were relatively low (<1% DM) in all treatments. Leaf NDF varied between 21.1-28.7% DM and was lower in air-dried EBLa and higher in EBLf contents, respectively. Lignin and indigestive CP were both valued less than 2% and 5%, respectively. Non-fibre carbohydrates (NFC) content were high and ranged between 45.0-52.6% DM with highest level in sun-dried EBLs and lowest in EBLf. Total digestible nutrients (TDN1x) were between 84.6-90.5% DM with high digestible TDN ranging from 82.0-88.1% DM. Resultant digestible energy density (DE Mcal/kg) of leaves was above 3.5 Mcal/kg, yielding highest metabolizable energy (ME) value in EBLa (3.5 ME Mcal/kg) and least in EBLf (3.0 ME Mcal/kg). The leaves supplied approximately 1.9 Mcal/kg NE_m and 1.2 Mcal/kg NE_g. Based on the above estimates predicted DMI of EBLf was lower (4.2kg /dairy cow/day) relative to other treatments. The digestible dry matter (DDM) was high (84.5%) and did not vary ($p>0.05$) within period and drying method. Post-harvest treatments affected nutrient composition and forage value of leaves. Leaves averaged overall 87.1% TDN, (3.0-3.5) Mcal/kg ME with estimated dry matter intake (5.7%) and relative feed value/ index (372). Air-dried leaves had the highest forage quality (Table 4.1

Post-bloom

The CP content of leaves ranged between 21.0-25.4% DM (averaged 23.2% CP DM) and statistic difference was observed within treatments (Table 4.1). Fat content was below 5.1%

across all treatments and did vary between 4.1-5.1% DM, while ash content was relatively low (<1% DM) in all treatments. Leaf NDF slightly varied between (23.2-36.6% DM) in reverse proportion with the increase in NFC contents (45.7- 49.1%) in hays vs. (38.6%) in fresh, respectively. However, PBLs and PBLa of NFC content did not vary compared to fresh content. Lignin content and indigestive CP were both less than 3%. Non-fibre CHO were high and ranged between 38.6-49.1% DM within treatments in PB. Forage value in PB leaves varied between 81.5-89.4% TDN, 2.8-3.4 Mcal/kg ME, 3.3-5.2% DMI and 213.1-339.3 of RFV index. The leaves supplied approximately 1.9 Mcal/kg NE_m and 1.2 Mcal/kg NE_g. Based on the above estimates predicted DMI of PBLf and PBLa were lower (3.3-5.2 kg /dairy cow/day, respectively) relative to other treatments. Although, difference was observed within treatments, PBLa have indicated as well to be good quality forage.

Table 4.1 Chemical composition and forage value of fresh and dried tree Lucerne leaves harvested in early bloom and post-bloom

Growth stage	**EARLY BLOOM**				**POST BLOOM**			
Drying	Fresh	Air	Sun		Fresh	Air	Sun	
	EBLf	EBLa	EBLs		PBLf	PBLa	PBLs	
% DM	**Lsmeans**			**sem**	**Lsmeans**			**sem**
DM	22.0				26.3			
CP	22.2^{b}	27.8^{a}	21.0^{b}	1.90	22.2	25.4	21.0	2.20
Fat	5.2^{a}	5.1^{a}	2.1^{b}	0.33	4.2^{b}	5.1^{a}	4.1^{b}	0.13
Ash	0.4^{b}	0.4^{b}	0.7^{a}	0.73	0.4	0.7	0.4	0.33
	Structural and non-structural carbohydrates (% DM)							
NDF	28.7^{a}	21.1^{b}	23.0^{b}	0.43	36.6^{a}	23.2^{c}	28.1^{b}	0.67
ADF	7.0^{a}	5.7^{b}	5.9^{ab}	0.57	6.6^{a}	5.7^{b}	7.1^{a}	0.30
ADL	6.3	5.2	5.4	0.73	5.9	5.2	6.5	0.37
Cellulose	0.8	0.5	0.5	0.23	0.7^{a}	0.5^{b}	0.6^{a}	0.07
Hemi cellulose	21.7^{a}	15.5^{b}	17.1^{b}	0.87	30.1^{a}	17.5^{c}	21.0^{b}	0.50
ADFICP	0.1^{c}	2.2^{b}	2.8^{a}	0.17	0.4^{c}	2.2^{b}	2.7^{a}	0.20
NDFICP	0.5^{c}	2.6^{b}	3.2^{a}	0.17	0.8^{b}	2.6^{a}	3.0^{a}	0.20
IADICP	0.0^{b}	0.9^{a}	1.1^{a}	0.07	0.2^{b}	0.9^{a}	1.1^{a}	0.10
NDFn	28.2^{a}	17.7^{b}	18.7^{b}	0.60	35.7^{a}	19.7^{c}	24.0^{b}	0.70
non-fibre CHO (NFC)	45.0^{b}	46.2^{b}	52.6^{a}	2.13	38.6^{b}	45.7^{a}	49.1^{a}	2.63
lignin	1.8^{a}	1.1^{b}	1.2^{b}	0.17	2.1^{a}	1.2^{b}	1.8^{a}	0.13
available CHO: B2-	19.9^{a}	13.4^{b}	15.1^{b}	1.00	28.3^{a}	15.4^{c}	19.1^{b}	0.47
Indigestible fibre CHO C	15.0	12.4	13.0	1.77	14.0	12.6	15.5	0.83
unavailable CHO CC	4.3^{a}	2.6^{b}	3.0^{b}	0.40	5.1^{a}	2.9^{b}	4.4^{a}	0.37
	Forage Value							
TDN1x %	84.6^{b}	90.5^{a}	86.2^{a}	1.13	81.5^{c}	89.4^{a}	84.6^{b}	0.73
dTDN %	82.0^{b}	88.1^{a}	84.0^{b}	1.00	78.4^{c}	86.9^{a}	82.0^{b}	0.70
DE Mcal/kg (beef & dairy cattle)	3.6^{c}	3.9^{a}	3.7^{b}	0.03	3.5^{c}	3.8^{a}	3.6^{b}	0.00
ME Mcal/kg (dry and lactating dairy)	3.2^{c}	3.5^{a}	3.3^{b}	0.03	3.0^{c}	3.4^{a}	3.2^{b}	0.00
ME Mcal/kg (beef and growing dairy)	3.0^{b}	3.2^{a}	3.0^{b}	0.03	2.8^{c}	3.1^{a}	3.0^{b}	0.00
NEm Mcal/kg (beef & dairy cattle)	1.8^{b}	1.9^{a}	1.9^{a}	0.00	1.7^{c}	1.9^{a}	1.8^{b}	0.00
NEg Mcal/kg (beef & dairy cattle)	1.1^{b}	1.2^{a}	1.2^{a}	0.00	1.1^{b}	1.2^{a}	1.1^{b}	0.00
Digestible Dry Matter %	83.4	84.5	84.3	0.50	83.8	84.5	83.4	0.27
Dry matter intake %	4.2^{b}	5.7^{a}	5.2^{a}	0.07	3.3^{c}	5.2^{a}	4.3^{b}	0.07
RFV	270.6^{c}	372.1^{a}	341.1^{b}	5.13	213.1^{c}	339.3^{a}	276.3^{b}	6.20

Means with different superscript ($^{a, b}$ and ^{c}) in the similar row are significantly different.

CP- Crude protein; **NDF**- Neutral detergent fibre; **ADF**-Acid detergent fibre; **ADL**-Acid detergent lignin; **ADFICP**-ADF indigestible crude protein; **NDFICP**-NDF indigestible crude protein; **IADICP**-Indigestible acid detergent insoluble crude protein; **NDFn**-NDF corrected for nitrogen; **TDN1x**- Total digestible nutrients on intake at maintenance level; **dTDN**- Digestible Total digestible nutrients; **DE**-Digestible energy; **ME** -Metabolizable Energy; **NEm -** Net Energy maintenance**; NEg**- Net Energy gain; **RFV**- Relative Feed Value; **Lsmeans**-Least Square Means

4. 2 Chemical composition and forage value of edible twigs

Early-bloom

Composition and forage value of fresh and dried twigs are shown in Table 4.2. Although, CP content of twigs varied between 9.8-17.1% DM, no significant difference was observed. Fat content in early-bloom was less than 1.1% DM. Ash content was higher in EBTs (24.0% DM) and lower in EBTf (12.1%). Neutral detergent fibre of twigs was between 40.7-48.7% DM in EB; presented higher ($p<0.05$) for EBTf and lower in EBTa. Lignin content ranged between 5.1-8.7% DM in EB materials. Indigestible CP (ADFICP) and NDFICP values were minor and less than 2% DM. Mean of non-fibre CHO (NFC) was 26.7% DM and the indigestible fibre CHO was higher in EBTf (42.6%) and lower in EBTa (29.8%). Total digestible nutrients (TDN1x) was higher in EBTa 56.2% DM. The DE varied between 2.0-2.4 Mcal/kg DM in EB. Drying improved net energy density NE which had a positive effect on the expected dry matter intake (DMI) as noted in EBTa (about 2.9 kg DM /dairy cow/day). The highest RFV of twigs was reported in the EBTa (176.7) followed by EBTs (156.3) and least for EBTf (140.3).

Post-bloom

Although, PB of twigs ranged between 48.3-51.5% NDF DM, no variation was observed within treatments. Ash content was high in PBTs (21.4% DM) and less in PBTf (13.3% DM). Fat content in PB was less than 1.1% DM; PBTf, however, had the least fat content. The CP of twigs varied 5.7-16.7% in PB whereas fresh was greater than hay materials. The estimated indigestible CP (ADFICP) was below 2% DM. The NFC were between 19.4-24.0% DM and indigestible fibre CHO was 37.3-46.1%. However, both NFC and indigestible fibre CHO did not statically vary. Total digestible nutrients (TDN1x) had the greatest value in fresh (51.7%) and the least for PBTs (39.3%). The RFV values were between 131 and 139. The post-bloom RFV index did not showed variation across treatments (Table 4.2) and was higher in PBTa (139.1) but below the minimum RFV for good quality forage.

Table 4.2 Chemical composition and forage value of fresh and dried tree Lucerne twigs harvested in early-bloom and post-bloom

Growth stage	**EARLY BLOOM**				**POST BLOOM**			
Drying	Fresh	Air	Sun		Fresh	Air	Sun	
	EBTf	EBTa	EBTs		PBTf	PBTa	PBTs	
%DM	**Lsmeans**			**sem**	**Lsmeans**			**sem**
DM	28.2				30.2			
CP %	17.1	12.6	9.8	1.97	16.7[a]	9.7[b]	5.7[b]	2.20
Fat %	0.4[b]	1.1[a]	0.8[a]	0.33	0.1[b]	0.8[a]	0.5[a]	0.13
Ash %	12.1[c]	16.1[b]	24.0[a]	0.73	13.3[c]	14.8[b]	21.4[a]	0.33
Structural and non-structural carbohydrates (%DM)								
NDF %	48.7[a]	40.7[c]	44.6[b]	1.30	51.1	48.3	51.5	3.93
ADF %	20.0[a]	14.8[c]	17.9[b]	0.60	17.5[b]	22.0[a]	21.4[a]	0.67
ADL %	17.7[a]	12.4[c]	15.6[b]	0.60	15.5	17.4	19.2[a]	0.97
Cellulose %	2.2	2.4	2.3	0.13	2.0[b]	4.7[a]	2.2[b]	0.67
Hemicellulose %	28.7[a]	25.9[b]	26.8[b]	0.77	33.6	26.3	30.1	3.90
ADFICP %	0.7[b]	1.3[a]	1.2[a]	0.23	0.8	1.3	1.4	0.27
NDFICP %	1.1[b]	1.6[a]	1.6[a]	0.23	1.1[b]	1.7[a]	1.8[a]	0.27
IADICP %	0.3	0.5	0.5	0.10	0.3	0.5	0.6	0.10
NDFn %	47.3[a]	38.6[c]	42.6[b]	1.30	49.7	46.1	49.2	3.90
non-fibre CHO (NFC) %	23.0[b]	32.1[a]	21.2[b]	2.80	21.9	24.0	19.4	2.90
lignin %	8.7[a]	5.1[c]	7.0[b]	0.43	7.9	8.4	9.9	0.93
available CHO: B2-%	28.5[a]	25.8[b]	26.6[b]	0.93	33.1	28.4	29.8	3.47
Indigestible fibre CHO C %	42.6[a]	29.8[c]	37.4[b]	1.47	37.3	41.7	46.1	2.27
unavailable CHO CC %	20.8[a]	12.1[c]	16.7[b]	1.07	19.1	20.3	23.8	2.23
Forage Value								
TDN1x %	52.8[a]	56.2[a]	45.7[b]	1.97	51.7[a]	48.7[a]	39.3[b]	2.27
dTDN %	50.7[a]	54.7[a]	44.5[b]	1.87	49.4[a]	47.0[a]	38.0[b]	2.33
DE Mcal/kg (beef & dairy cattle)	2.2	2.4	2.0	0.67	2.2[a]	2.1[a]	1.7[b]	0.10
ME Mcal/kg (dry and lactating dairy)	1.8	2.0	1.5	0.67	1.8[a]	1.6[a]	1.2[b]	0.10
ME Mcal/kg (beef and growing dairy)	1.8	2.0	1.6	0.67	1.8[a]	1.7[a]	1.4[b]	0.10
NEm Mcal/kg (beef & dairy cattle)	1.0	1.1	0.8	0.67	0.9[a]	0.8[a]	0.5[b]	0.10
NEg Mcal/kg (beef & dairy cattle)	0.4	0.5	0.2	0.67	0.4[a]	0.3[a]	0.0[b]	0.10
Digestible Dry Matter %	73.3[c]	77.4[a]	75.0[b]	0.47	75.2[a]	71.7[b]	72.2[b]	0.53
Dry matter intake %	2.5	2.9	2.7	0.10	2.4	2.5	2.3	0.17
RFV	140.3[c]	176.7[a]	156.3[b]	5.10	138.0	139.1	130.5	10.97

Means with different superscript ([a, b] and [c]) in the similar row are significantly different.

CP- Crude protein; **NDF**- Neutral detergent fibre; **ADF**-Acid detergent fibre; **ADL**-Acid detergent lignin; **ADFICP**-ADF indigestible crude protein; **NDFICP**-NDF indigestible crude protein; **IADICP**-Indigestible acid detergent insoluble crude protein; **NDFn**-NDF corrected for nitrogen; **TDN1x**- Total digestible nutrients on intake at maintenance level; **dTDN**- Digestible Total digestible nutrients; **DE**-Digestible energy; **ME** -Metabolizable Energy; **NEm -** Net Energy maintenance**; NEg**- Net Energy gain; **RFV**- Relative Feed Value; **Lsmeans**-Least Square Means

4. 3 Chemical composition and forage value of edible stems

Early-bloom

The CP content of stems varied between 9.6-13.3% DM. Mean CP of stems approximated 11.5% DM, however, was not affected within treatments (Table 4.3). Ash content of EBSs was higher (29.0% DM) compared to fresh and air-dried stems (9.0-11.2% DM). Fat content was less than 2.1% DM in EB. Neutral detergent fibre was greater in EBSf (54.9% DM NDF) compared to EBSa (35.7% NDF DM). Lignin content was low in EB hays, but 11% in fresh stems associated with higher indigestible CHO C content (46% in EBSf vs 23.7% in EBSa). Air-dried stems had high NFC content (37.4% DM) and lower in EBSs (7.4% DM). Indigestible CP (ADFICP) was high, about 10% CP, indicating a high level of unavailable CP. Indigestible carbohydrates for stems were high up to 50% DM. Total digestible nutrients (TDN1x) varied between 34.5-65.9% DM. The EBSa materials had the highest levels compared to other two treatments. Digestible energy content ranged between 1.5-2.8 Mcal/kg, whereas the estimated supply of ME to dairy and beef cattle ranged between 1.0 (EBSs) and 2.4 Mcal/kg for EBSa. Drying method affected both NEm and NEg supply in EB and estimated dry matter intake was 2.0-3.4% of animal body weight with higher intake prediction in EBSa. The estimated DMI was less than 1.4% of animal body weight, with higher intake prediction in EBSa. Air-dried stems yielded better-quality forage (208.3 RFV) compared to EBSs (137.1 RFV) and EBSf (122.3 RFV), which resulted in nutrients loss of indigestible carbohydrates.

Post-bloom

Drying treatment in PB growth stage did not affect crude protein contents, which varied between 8.3-11.6% DM. Ash content of PBSs was higher (29.0% DM) compared to fresh (12.3% DM) and air-dried stems (12.0% DM). Fat content was less than 1.5% DM in PB. However, post-bloom NDF varied widely (57.5-60.1%) and was about 60% DM. Lignin content was increased (> 10%) in this growth stage in association to the decline with maturity of the indigestible CP (0.3-1.7% DM). Non-fibre carbohydrates in PB showed less variation (3.1-18% DM) across all treatments. Total digestible nutrients (varied between 27.9-46.9% DM, PBSa materials had the highest levels to PBSs and PBSf. Digestible energy content ranged between 1.2-1.9 Mcal/kg, whereas the estimated supply of ME to dairy and beef cattle ranged between 0.7 (PBSs) and 1.6 Mcal/kg for PBSa. Drying method affected net energy supply

(NEm and NEg), which was less than 1% for the animal body weight. The estimated DMI and RFV index (<115), however, did not change across all treatments in PB (Table 4.3).

Table 4.3 Chemical composition and forage value of fresh and dried tree Lucerne stems harvested in early bloom and post-bloom.

Growth stage	EARLY BLOOM				POST BLOOM			
Drying	Fresh	Air	Sun		Fresh	Air	Sun	
	EBSf`	EBSa	EBSs		PBSf	PBSa	PBSs	
%DM	**Lsmeans**			**sem**	**Lsmeans**			**sem**
DM	24.5				31.7			
CP	13.3	11.2	9.6	1.97	11.6	8.3	10.3	2.20
Fat	0.5^{b}	2.1^{a}	0.6^{b}	0.33	1.2^{a}	1.2^{a}	0.3^{b}	0.13
Ash	9.0^{c}	11.2^{b}	29.0^{a}	0.80	12.3^{b}	12.0^{b}	29.0^{a}	0.33
Structural and non-structural carbohydrates (%DM)								
NDF	54.9^{a}	35.7^{b}	50.3^{a}	2.63	57.6	60.1	57.5	2.03
ADF	21.7^{a}	11.8^{b}	19.4^{a}	1.00	23.9	22.4	23.3	1.60
ADL	19.2^{a}	9.9^{b}	16.7^{a}	0.77	21.4	19.1	20.1	1.20
Cellulose	2.5	2.0	2.7	0.30	2.5	3.2	3.3	0.80
Hemi cellulose	33.2^{a}	23.9^{b}	30.9^{a}	1.97	33.7	37.8	34.2	2.40
ADFICP	0.6	0.8	1.2	0.20	0.7	0.9	1.3	0.17
NDFICP	0.9	1.2	1.6	0.20	1.0^{b}	1.2^{b}	1.7^{a}	0.17
IADICP	0.2	0.3	0.5	0.07	0.3^{b}	0.3^{b}	0.5^{a}	0.03
NDFn	53.7^{a}	34.1^{b}	48.2^{a}	2.47	56.3	58.5	55.3	2.17
non-fibre CHO (NFC)	19.9^{b}	37.4^{a}	7.4^{c}	3.87	16.4^{a}	18.0^{a}	3.1^{b}	3.00
lignin	10.5^{a}	3.5^{c}	8.4^{b}	0.80	12.3	11.5	11.5	0.73
available CHO: B2-	33.2^{a}	23.3^{b}	31.1^{a}	2.10	33.8	38.5	35.0	2.23
Indigestible fibre CHO C	46.0^{a}	23.7^{c}	40.1^{b}	1.77	51.2	45.9	48.1	2.90
unavailable CHO CC	25.3^{a}	8.4^{c}	20.2^{b}	1.87	29.6	27.6	27.7	1.80
Forage Value								
TDN1x %	47.5^{b}	65.9^{a}	34.5^{c}	1.47	43.6^{a}	46.9^{a}	27.9^{b}	1.50
dTDN %	45.2^{b}	64.1^{a}	33.6^{c}	1.53	41.4^{a}	44.2^{a}	26.9^{b}	1.57
DE Mcal/kg (beef & dairy cattle)	2.0^{b}	2.8^{a}	1.5^{c}	0.03	1.8^{a}	1.9^{a}	1.2^{b}	0.07
ME Mcal/kg (dry and lactating dairy)	1.6^{b}	2.4^{a}	1.0^{c}	0.03	1.4^{a}	1.5^{a}	0.7^{b}	0.07
ME Mcal/kg (beef and growing dairy)	1.6^{b}	2.3^{a}	1.2^{c}	0.03	1.5^{a}	1.6^{a}	1.0^{b}	0.03
NEm Mcal/kg (beef & dairy cattle)	0.8^{b}	1.4^{a}	0.4^{c}	0.03	0.6^{a}	0.7^{a}	0.1^{b}	0.03
NEg Mcal/kg (beef & dairy cattle)	0.2^{b}	0.8^{a}	0.0^{c}	0.03	0.1^{b}	0.2^{a}	0.0^{c}	0.03
Digestible Dry Matter %	72.0^{b}	79.7^{a}	73.8^{b}	0.80	70.3	71.5	70.7	1.27
Dry matter intake %	2.2^{b}	3.4^{a}	2.4^{b}	0.17	2.1	2.0	2.1	0.07
RFV	122.3^{b}	208.3^{a}	137.1^{b}	10.17	113.6	110.7	114.5	4.13

Means with different superscript ($^{a, b}$ and ^{c}) in the similar row are significantly different.

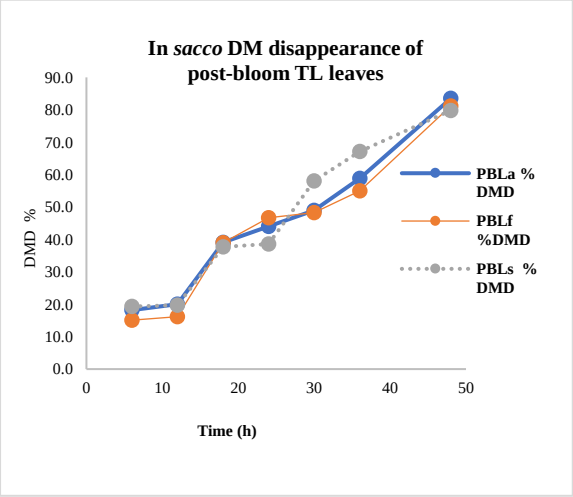
CP- Crude protein; **NDF**- Neutral detergent fibre; **ADF**-Acid detergent fibre; **ADL**-Acid detergent lignin; **ADFICP**-ADF indigestible crude protein; **NDFICP**-NDF indigestible crude protein; **IADICP**-Indigestible acid detergent insoluble crude protein; **NDFn**-NDF corrected for nitrogen; **TDN1x**- Total digestible nutrients on intake at maintenance level; **dTDN**- Digestible Total digestible nutrients; **DE**-Digestible energy; **ME** -Metabolizable Energy; **NEm** - Net Energy maintenance**; NEg**- Net Energy gain; **RFV**- Relative Feed Value; **Lsmeans**-Least Square Means

4.4 The *in sacco* degradation of leafy components

The *in sacco* disappearance of fresh and preserved edible leaves are in Figure 4.1 and Table 4.4. Dry matter and NDF disappearance of leaf components were the highest in PBLa and PBLs, respectively. The leaves degraded slowly before 12h and increased through to 48h of incubation. The DMD and NDFD of leaves ranged between 79.8-83.5% DM and 60.1-72.9% DM respectively at 48h. Air-dried leaves (PBLa) had the highest DMD of 83.5% while PBLs had the least 79.8%. The NDF disappearance was higher in PBLs (72.9%) compared to PBLf (70.3%) and PBLa (60.1%). However, there were no differences within all treatments. The leafy degradation rate (c) was the highest ($p<0.05$) for PBLf (3.7%/ h) and the least was for PBLs (2.7%/h). Post-bloom leaves (PBLs) had the highest slow degraded fraction (b) of 84.5% and PBLf had the least (60.8%). Effective degradability was the highest in PBLs and least was for PBLf, 64.9% and 53.9%, respectively.

4.5 The *in sacco* degradation of twig components

Figure 4.2 and Table 4.5 show the *in sacco* DM and NDF disappearance of PBTf, PBTa and PBTs. Dry matter and NDF disappearance of twigs was rapid after 12h of incubation through to 48h of incubation. Dry matter disappearance ranged from 53.9-63.2% and 10 units lower for NDF (46.6-49.4%). The PBTa had the highest (63.2% DMD) and highest NDFD (49.4%) and PBTf had the least DMD and NDFD. However, differences were not significant. The "a" component was between 6-8% and did not vary among treatments and no differences were noted in "b" rate of PBTf degradation was 3.4%/h and higher than for both PBTa and PBTs. Effective degradability was similarly higher in PBTs compared to PBTf and PBTa.



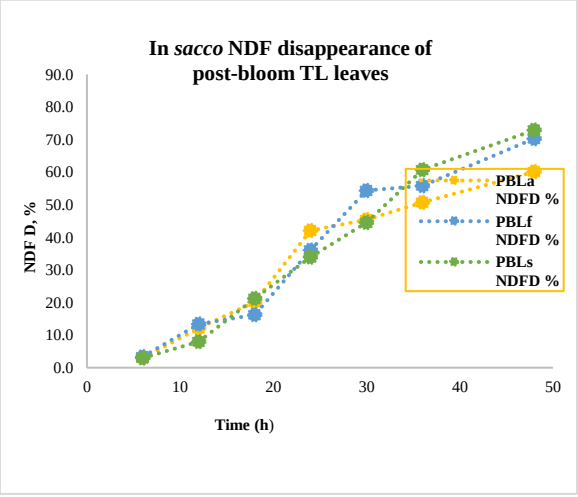
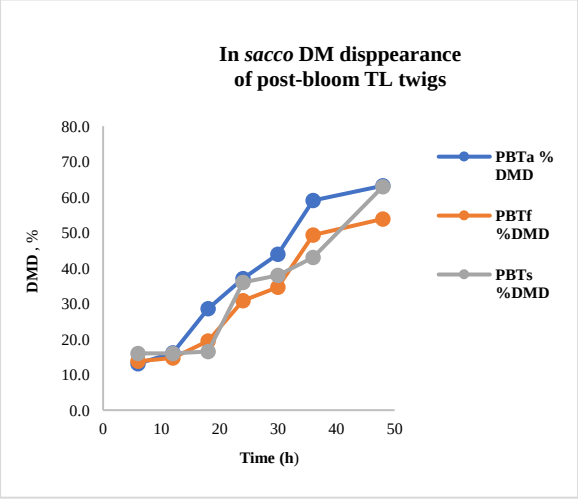


Figure 4.1 *In sacco* DMD and NDF disappearance of post-bloom TL leaves
PBLf: Post-bloom fresh leaves; **PBLa**: Post-bloom air-dried leaves; **PBLs**: Post-bloom sun-dried leaves;
DMD: Dry matter degradability; **NDF D**: Neutral detergent fibre degradability

Table 4.4 The *in sacco* degradation characteristics and effective dry matter degradability (ED) of tree Lucerne post-bloom leaves

Parameter	PBLf	PBLa	PBLs	sem	p
Degradation characteristics					
a (%)	7.5	9.1	9.7	0.43	ns
b (%)	60.8^{b}	66.3^{ab}	84.5^{a}	10.67	***
c (%)	0.037^{a}	0.032^{ab}	0.027^{b}	0.0023	*
a + b (%)	68.4^{b}	75.4^{b}	94.1^{a}	11.32	**
Effective degradability (%)					
kp = 0.002	64.9^{b}	71.0^{b}	87.6^{a}	6.55	*
kp = 0.01	53.9^{b}	57.5^{b}	68.7^{a}	7.22	**

a=rapidly degraded fraction (soluble fraction), **b**= slowly degraded fraction (but potentially degradable);
c=rate of degradation of" fraction" b"; **(a+b)** = Total degradability; **kp**=passage rate.
Means with different superscript ($^{a–b}$) in the similar row are significantly different
Overlapping superscript ($^{a, b}$ and ^{ab}) in the similar row mean no significant difference
sem=Standard Error of the Mean; indicate means in the same row with different superscripts differ at $p<0.05$
ns=$p>0.05$; ***p*<0.05*= Significant; ****p*<0.01*= highly significant; *****p*<0.001*= highly significant



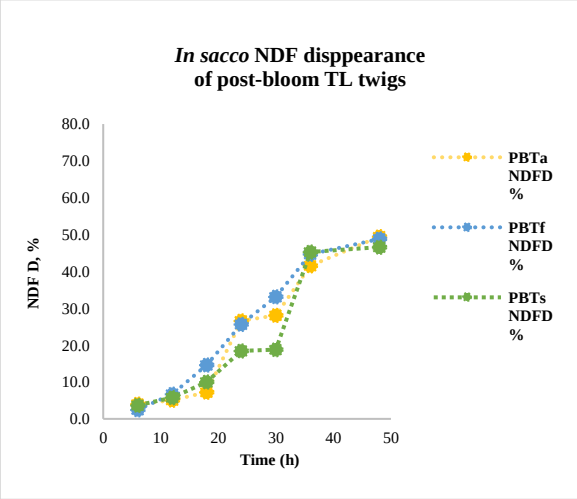


Figure 4.2 *In sacco* DMD and NDF disappearance of post-bloom TL twigs
PBTf: Post-bloom fresh twigs; **PBTa**: Post-bloom air-dried twigs; **PBTs**: Post-bloom sun-dried twigs.
DMD: Dry matter degradability; **NDF D**: Neutral detergent fibre degradability

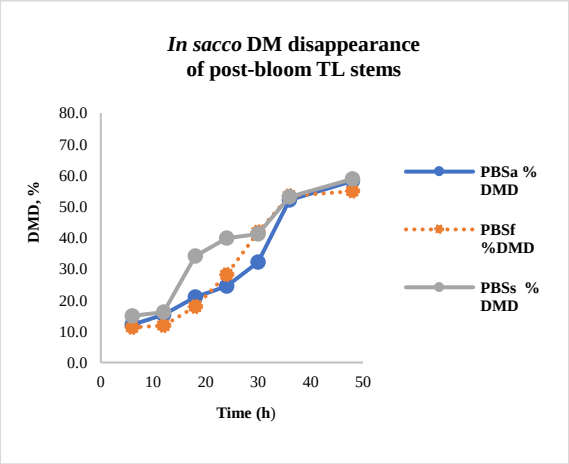
Table 4.5 The *in sacco* degradation characteristics and effective dry matter degradability (ED) of tree Lucerne post-bloom twigs

Parameter	PBTf	PBTa	PBTs	sem	p
Degradation characteristics					
a (%)	6.9	6.6	8.0	1.13	Ns
b (%)	43.6^{b}	67.5^{a}	56.1^{ab}	10.33	*
c (%)	0.034^{a}	0.024^{b}	0.024^{b}	0.002	*
a + b (%)	50.5^{b}	74.1^{a}	64.0^{ab}	11.76	**
Effective degradability (%)					
kp = 0.002	47.7^{b}	68.4^{a}	59.1^{ab}	3.44	**
kp = 0.01	39.0^{b}	52.4^{a}	45.2^{ab}	3.12	**

a=rapidly degraded fraction (soluble fraction), **b**= slowly degraded fraction (but potentially degradable).
c=rate of degradation of" fraction" b"; **(a+b)** = Total degradability; **kp**=passage rate.
Means with different superscript ($^{a–b}$) in the similar row are significantly different
Overlapping superscript ($^{a, b}$ and ^{ab}) in the similar row mean no significant difference
sem=Standard Error of the Mean; indicate means in the same row with different superscripts differ at $p<0.05$
ns=$p>0.05$; ***p*<0.05= Significant; ****p*<0.01= highly significant; *****p*<0.001= highly significant

4.6 The *in sacco* degradation of stem components

I*n sacco* disappearance of stem samples are displayed in Figure 4.3 and Table 4.7. At 48h, DMD was about 56% DM, in the order PBSs< PBSa< PBSf. The NDFD component was between 38-51% NDF. Potentially degradable "b" was high in PBSf and PBSa was lowest (42.1%). Effective degradability was greater ($p<0.05$) in PBSf at both kp (61.0% and 47.4%).



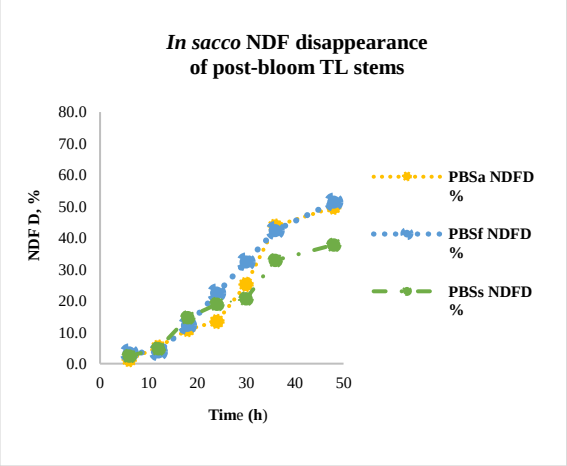


Figure 4.3 *In sacco* DMD and NDF disappearance of post-bloom TL stems
PBSf: Post-bloom fresh stems; **PBSa**: Post-bloom air-dried stems; **PBLs**: Post-bloom sun-dried stems
DMD: Dry matter degradability; **NDF D**: Neutral detergent fibre degradability

Table 4.6 The *in sacco* degradation characteristics and effective dry matter degradability (ED) of tree Lucerne post-bloom stems

Parameter	PBSf	PBSa	PBSs	sem	p
Degradation characteristics					
a (%)	5.6^{b}	6.1^{ab}	7.5a	1.22	*
b (%)	60.0^{a}	42.1^{b}	45.5^{ab}	9.67	*
c (%)	0.026^{b}	0.026^{b}	0.037^{a}	0.002	**
a + b (%)	65.6^{a}	48.1^{b}	53.0^{ab}	8.54	*
Effective degradability (%)					
kp = 0.002	61.0^{a}	44.7^{b}	50.2^{ab}	7.31	*
kp = 0.01	47.4^{a}	34.8^{b}	41.6^{ab}	6.33	*

a=rapidly degraded fraction (soluble fraction), **b**= slowly degraded fraction (but potentially degradable).
c=rate of degradation of" fraction" b"; **(a+b)** = Total degradability; **kp**=passage rate.
Means with different superscript ($^{a–b}$) in the similar row are significantly different
Overlapping superscript ($^{a, b}$ and ^{ab}) (a, b and ab) in the similar row mean no significant difference.
sem=Standard Error of the Mean; indicate means in the same row with different superscripts differ at $p<0.05$
ns=$p>0.05$; ***p*<*0.05*= Significant; ****p*<*0.01*= highly significant; ***$p<0.001$= highly significant

CHAPTER 5

DISCUSSION

5.1 Chemical composition, forage value and degradability of leafy components

Crude protein of TL leaves in this study is equivalented to the findings by Oldham *et al.* (1994). This value was more than what is reported by Borens and Poppi (1990) on TL and higher than findings by Marumo *et al.* (2016) on Lucerne leaf-meal (LLM). Although, lower ash content was reported in both growth stages, minor differences were observed within treatments as mineral content was not affected by preservation method. Tree Lucerne has low fat content, which is a positive attribute as materials high in fat tend to affect rumen degradation.

Generally, cell wall contents are composed by structural carbohydrates (SCHO) which varied according to the plant fractions. The variations were less in leafy components and wider variation was noted in woody parts especially after flowering. The fibre composition of leafy materials is related to lignification, and their amounts are inversely associated with variation in food ingestion and energy ranking (Visagie & van de Vyver, 2010). Fresh TL materials contain more soluble fractions and drying tends to cause breakdown and complexing of the materials into insoluble fractions. Leaf contents in both EB and PB had high NFC contents (> 50% NFC DM in some samples), aligned with observation by Snook (1986) on carbohydrate content and by Nocek and Russell (1988). It was indicated values ranged between 30-40% NFC tree Lucerne as a benchmark diet to support optimal lactating cows. Observation of an inverse relationship between NSCHO component and fibre composition in the current study was noted by Jafari (2012). Lignin and indigestible CP were less than 10%, indicate that the forage has higher potential to yield energy as less structural materials were lignified. Drying did not seem to reduce feed value but rather nutrient were concentrated.

The high CP and NSCHO contents are critical energy and protein sources for rumen microbes for fermentation, the high levels of *in sacco* NDF and DDM attest to that. Plant carbohydrates which constitute the main energy source in ruminant diets, are fermented into acetate, propionate and butyrate during glycolysis (Freer & Dove, 2002). These are metabolised for energy production and utilized in synthesis of body tissue and milk. These short-chain fatty acids (SCFA) are absorbed and metabolized into the form of adenosine triphosphate (ATP) to furnish energy to cells. The molecule of ATP is disintegrated into the molecule of adenosine diphosphate (ADP) to release phosphate and avail energy for cell metabolisms (O'Leary &

Plaxton, 2016). Forage energy values, with exception to DDM were affected in both growth stages across drying treatment. Belyea and Ricketts (1993 and 2018) stated that fibre content increases inversely with the cell energy level within growth periods.

Air-drying of EB and PB yielded high DE density (DE Mcal/kg) which was equivalent to that of grain crops (3.8 DE Mcal/kg) and greater to tree Lucerne (8.3 MJ/kg =2.01 Mcal/kg) according to McDonald *et al.* (2010). The estimated supply of ME for dairy and beef animals extended between 2.8-3.5 Mcal/kg (11.7-13.4 MJ) relative to the average value recommended by NRC (2001) which is 11.3 MJ ME/ kg DM and 2.5-3.4 Mcal/kg ME. The predicted NE values for dairy and beef cattle were higher in EBLa and EBLs compared to EBLf. The same trend was observed in post-bloom with dried materials compared to fresh in NEm, but NEg only of PBLa was the highest. Nevertheless, the difference in NE (m and g) was minor (0.1% unit in NEm and NEg). Moreover, EBLa and PBLa were ranked first when forage energy values were considered.

High nutrient contents were observed in EB and PB air-dried leaves compared to both sun-dried and fresh in both growth stages. Similar results were reported by Martin-Garcia & Molina-Alcaide (2008) in olive and Abioye *et al.* (2014) of baobab leaves (*Adansonia digitate*). In addition, Satwase *et al.* (2013) ranked air-drying in second position after cabinet drying. These two treatments (air-drying and cabinet drying) applied on drumstick leaves (*Moringa oleifera*) retained higher nutrients compared to sun-drying and oven drying. Sun-drying was reported efficient to reduce anti-nutritional factors in forage and phytochemical compounds. (Wanapat *et al.*, 2000ab and 2003; Oni *et al.* (2015).

Tannin, saponin, oxalate and phlobatannin were reduced by this treatment according to the report by Oni *et al.* (2015). Edwards *et al.* (1997a) indicated that low tannin content forages are more rapidly degraded to support better animal performance. Growing lambs fed on TL showed good weight performance (Becholie *et al.*, 2005). Palatability and digestibility of PBLs was improved probably due to Maillard reactions that sweetened the forage subsequently to NSCHO condensation (Bastos *et al.*, 2012). Raghavan *et al.* (1997) declared that sun-dried forage has their flavour ameliorated. Maillard reaction additionally produces brown and highly digestible food (Hui *et al.*, 2006) manufactured from the chemical reaction between amino-acid groups and reducing group (Lund & Ray, 2017).

The process of degradation by the rumen microbial flora intervened after 12h of incubation which shows that microbes get adapted to TL and reach the total degradation at 48h period of

incubation. The *in sacco* digestibility of leaves in this study ranged between (79.8-83.5% DM), which are much greater than the findings by Wiley (2005: 70-80% DM of digestibility in TL leaves). Oldham *et al*. (1994) reported 25% CP content and above 80% DDM in young TL herbage which is similar with the results of the current study. In contrast, Borens and Poppi (1990) showed that incubation values of 60 and 85 % DM at 4h and 24h of TL disappearance, respectively. These values are not aligned with our findings. According to McDonald *et al.* (2010), digestibility is merely function of passage rate, particle size, digestion time and forage nature. Breaking down of hydrogen bonds in indigested nutrients may have made them digestible and available component for microbial growth. About 12.4-15.0% DM of indigestible fibre CHO C was recorded as residues. Across treatment, no difference was found, although air-drying treatment in EB was much affected by drying treatment, and leaf components were the most digested in post-harvest period. Mean DMD was above 74.8% similar with the results of Milton *et al.* (2000). Air-dried leaves (PBLa) had the highest DMD, but PBLs produced higher NDFD associated with ED and degradable fractions (b). Foodstuff of all PB materials with regards to the treatment, were fast absorbed at kp (0.2%/h) than at kp (1%/h). The mean ED was highly consistent at kp (0.2%/h) and lower at kp (1%/h). At elevated kp, the value of ED was reduced and vice versa and associated with the value of fraction "b". Slowly degraded fraction (b) of TL was correlated with soluble fraction (a), but inversely connected with the degradation rate (c), greater in PBLf (0.037). The explanation is that leafy materials are composed with high CP and low fibre contents which is related with high DMD and low indigestible fibre CHO C retained in rumen. This is aligned with the reports by Valderrama and Anrique (2011). Lignin matrix interferes with access to the fibre matrix and subsequently reduced the cell wall digestibility of fibre (Moore & Jung, 2001). Vermaak *et al.* (2011), however, noted that cell wall contents and the degradation rate of nutrients influenced the degradation of soluble plant fractions in rumen. Leafy fibre components were less than 2% to negatively affect degradation. Furthermore, PD is inversely related to degradation rate (Belachew *et al*., 2013). However, differences were not observed within treatment in term of NDFD, DMD, total degradability and ED contents.

In general, air-drying and sun-drying were more effective in preserving and improving forage value as noted by the high RFV during both EB and PB. Air-drying was the most effective method for preserving leafy components and improving both intake and nutrient digestibility, although differences in *in sacco* degradability were not significant. Feeding fresh materials has the advantage of supplying non-fibre carbohydrates, vitamins and antioxidants, however,

nutrient concentration is lower hence the lower density of energy supply. It is therefore advisable to air-drying or sun-drying to enable better utilization of TL leaves. Post-bloom most nutrients are locked up in the fibre matrix hence the RFV are lower (33 units than EBLa). However, all drying leaves and fresh materials fall under the RFV requirement for high-quality forage as reported by Undersander (2003ab). No difference between PB leaves within drying methods was observed. Overall, degradation parameters and effective degradability of leaves in PB growth stage were significantly varied across treatments (Table 4.4).

5.2 Chemical composition, forage value and degradability of twigs components

According to our findings on twigs, CP values were much greater in leafy than in twig materials within drying treatments as expected. Meanwhile, EB and PB fresh twig materials were estimated at 16% CP DM, which is much greater compared to the results of previous studies on twigs (9% CP) (Lindeque & Rethman, 1998; Stokes, 2008 and Marques *et al.*, 2008). In contrast, the study by Oldham *et al.* (1994) found 9% CP in edible stems. Mean CP of twigs in the early-bloom was above the threshold recommended by NRC (2001) essential to sustain rumen fauna of lactating calves. Borens *et al.* (1990) and Assefa *et al.* (2008) confirmed that premature twig materials can be used as supplementary protein for livestock.

Cellulose and lignin increased in twigs compared to leaves. Lignified twigs prevent cell invasion by microorganisms (Wang & McAllister, 2002) which is a negative attribute. However, lignin content of PB materials did not change with drying compared to EB as most structural materials had already been incorporated into the structural matrix. Wang and McAllister (2002) stipulated that degradation rate is limited by both lignin structure and phenolic compounds. As stated by Belyea and Ricketts (1993 and 2018), the digestibility of forage is related to ADF fractions which are linked to lignin. The amount of lignin in ADF would therefore be equal to the fragments of cellulose being bound to the digestible ADF (Belyea & Ricketts, 1993 and 2018). In addition, Redfearn and Zhang (2011) indicated that, forage with CP > 25%, ADF < 25%, as well as NDF < 35% is valuable for dairy milk production. Lower rumen digestibility may, however, entail high bypass protein (Wanapat *et al.*, 2000ab) which is an advantage as there is less N loss.

Tree Lucerne twigs could contribute to energy needs of livestock. Newman *et al.* (2009) indicated that high RFV index (164) indicates good-quality forage; the EBTa would be considered as good forage while EBTs and EBTf were of low value. Mean RFV index of twigs

was 170, hence twigs have potential value as livestock forage (Ball *et al.,* 2001; Undersander, 2003). The DE density (DE Mcal/kg) of twigs was 2 Mcal/kg which is less equivalent to that of grain crops (McDonald *et al.,* 2010), however, much of that energy is locked up in the lignin matrix.

Air-drying preserved nutrients in leaves and twigs; and sun-drying resulted in locking up or loss nutrients due to thermal processes. The variation was, however, not observable in degradation kinetics of the components. (Bastos *et al.,* 2012) reported that the accumulation of glucose in twig tissues during the thermal processing development of taste changes due to Maillard reactions. The study of Oni *et al.* (2015) showed decline on protein, fibre, ash and carbohydrate contents subsequently to oven and sun-drying treatments. This confirms how heat or overheating can cause deterioration of forage quality. Wang and McAllister (2002) noted that breaking down of hydrogen and esterified bonds in polysaccharides by fibrolytic microbes and enzymes result in accumulation of simple sugars.

Air-dried twigs were better degraded compared to fresh and sun-dried materials in both growth stages. Degradation was influenced by drying probably due to reduced solubility of protein and carbohydrates. Products of heat treatment affect palatability of the forage (Wanapat *et al.,* 2000ab and 2003) and reduce binding sites hence lowering rumen digestibility (Bastos *et al.,* 2012). Degradation rate was great in PBTf like reports by Valderrama and Anrique (2011). Degradation rate is higher in low NDFD material. Twigs have higher structural material which accords strength to shrubs, however, the higher the tensile strength the lesser the degradability. McDonald *et al.* (2010) reported benchmark for dried tree Lucerne of 20% CP DM, 5% sugar, 36% ADF and 2.3-2.4 Mcal/kg DE. Twigs of TL were within this range and therefore, suitable for supporting ruminant livestock even as sole diets. This observation needs further testing.

5.3 Chemical composition, and forage value and degradability of stems components

Ash content of post bloom dried materials was high which could be an indicator of higher accumulation of minerals as the plant matures. Crude protein was comparable to that of veld forage grasses, and therefore valuable nutrient source for ruminants. Borens and Poppi (1990) reported similar values which was reported also by Wiley (2005). Moreover, mean TL stem had a better nutrient profile than that of *Panicum maximum* (guinea grass: 5.0-5.5% CP DM) (Aganga & Tshwenyane, 2004). Tedeschi *et al.* (2015) stated that stem bound proteins may supply bypass minimising protein loss to ammonia in the rumen.

Pelletier *et al.* (2010) and Das *et al.* (2015) indicated that plant ageing was associated with lower non-structural carbohydrates (NSCHO), NFC and CP contents. McDonald *et al.* (2010) observed that air-drying treatment was more effective in preserving nutrient integrity of plants. Nherera-Chokuda *et al.* (2017), reported that drying lowered NFC, which reduced forage digestibility as also noted by Rotz and Muck (1994).

The predicted ME of TL stems was lower than of tree Lucerne hay (2.38 Mcal/kg, respectively) and less than that of maize (3.7 Mcal/kg). Ward (2011) indicated that forage ADF was 41% and 53% NDF resulted in lower RFV index (100); which was noted in this study that stems NDF exceeded 57% DM as also noted by Moore and Jung (2001) and Newman *et al.* (2006).

Buxton (1991) stated that structural integrity was due to formation of cellulose (long unbranched linear chain of glucose), hemicellulose (long chains of glucose: xyloglucans, xylans) and pectins (cellulose and hemicellulose embedded in lignified old cells) (Smith *et al.*, 2013). Collenchyma cells in stems solidify the primary cell wall due to the presence of cellulose, no cellulosic components and water (Chen *et al.*, 2017). Lignin binds together to cellulose and hemicellulose (HC) (Jung & Engels, 2002; Smith *et al.*, 2013). According to Hatfield *et al.* (2017), hemicellulose is covalently and strongly bound to lignin through ester linkages; but softly bound to cellulose by hydrogen linkages that prevent recycling of most SCHO compounds to occur in rumen. Lignin content and hydrogen linkage are the main limiting factors of digestibility of polysaccharides in plant cell walls (Hatfield *et al.*, 2017). Lignified stems are digested in rumen by the synergetic interaction of microorganisms (fibrolytic bacteria, fungi and protozoa) coupled with the complex enzymatic action of hydrolyse, polymerase, cellulase, hemicellulose, and xylanase (Selinger *et al.*, 1996). The fibrolytic enzymes are therefore necessary to stimulate degradation of these materials. The lignin matrix interferes with access to the fibre matrix and reduces digestibility. In this study, lignin was high and such interference was evident to happen. Findings of the current study align with the results by Ngodigha and Oji, (2009), indicating stems as the least important and poor protein supplier fractions in ruminant feeding. Drying treatment influenced lignin content compared to the rest of treatment within both growth stages.

Overall, the EBSa yielded in high-quality forage, which is associated with high NFC, TDN, ME, NE, DDM, DMI and RFV forage and inversely related with low NDF, ADF, ADL, cellulose, lignin, indigestible fibre and unavailable CHO C forage values. According to Moore

and Jung (2001), CP content was depleted with lignification process that is associated with the accumulation of deleterious nutrient of SCHO. This will influence negatively on N supply to microorganisms and NPN supplementation required to feed livestock (Demeyer, 1981).

5.4 SUMMARY

1. Evaluate effects of stage of growth (early-bloom and post-bloom) and postharvest drying method on chemical composition, structural carbohydrate fractions and relative forage value of browseable leafy and woody components of tree Lucerne

Sun-drying exposes plant materials to thermal effects of variable intensity; and the combination of high moisture content in freshly harvested materials and heat activates plant enzymes initiating nutrient breakdown. The reactions are uncontrolled and hence a myriad of compounds is formed that may not be hydrolysed in the rumen. The effects are more detrimental in leafy components which has small membranes for screening UV light and are damaged instantly. Stems and twigs have lesser damage as moisture content is less and stems have more dead tissue (xylem vessels) and the outer bark. Findings of this study consistently revealed that there were less variations in loss of NSCHO components, which are essential sources of nutrients. Fresh TL materials have higher levels of vitamins and minerals, lost upon air or sun-drying TL, however, fresh material is only useful for a shorter period unless preserved as hay or silage or pelleted. Hay making is the cheapest form of preservation and therefore the choice between sun-drying which often occurs for large fodder banks and air-drying is critical. Air-drying has limitation regarding labour, but the end-products are preferred as noted in this study. It is essential that separation of twigs and leaves from the woodier stem be done and the latter be dried under shed while the stems can be left in the field. Once the plant has flowered, most materials are matrixed in the SCHO fractions; the variation in enzymatic effects and heat damage is less observed. This entails that if post flowering harvest is preferred all components can be left in the field; separation is only critical before flowering.

2. Evaluate of drying mature herbage (post-bloom) on rumen degradability of edible components

Overall, degradation parameters and effective degradability of leaves in PB growth stage significantly varied across treatments. Twigs have higher structural material which accords strength to shrubs however, the higher the tensile strength the lesser the degradability. Fresh

stems would have contained much non-degradable and indigestible cell wall nutrients compared to hay due to the complexity of anatomic structure. The thickness of cell walls changes considerably with age and type of cells. Young cell walls are immature while old cells are composed of xylem, phloem, lignin and dead tissue contents. Hay was affected by post-harvest treatment compared to fresh samples. However, drying methods could not affect degradation characteristics and effective DMD of stems. On degradability point, sun-drying produced on highly digested leaves with no different to air-drying. Air-dried twigs (PBTa) were better degraded compared to PBTf and PBTs. However, feeding fresh materials has the advantage of supplying for a short period of time moisturized forage, highly content of non-fibre carbohydrates, vitamins and antioxidants. However, nutrient concentration is lower hence the lower density of energy supply.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

This study has confirmed that browseable tree Lucerne hay is highly nutritious with high amounts of digestible structural carbohydrates that provides energy. Air-drying was the most beneficial compared to sun-drying and fresh TL; both leafy and woody components retained higher levels of nutrients and RFV. Sun-dried TL materials will supplement livestock as the second-choice forage next to air-drying. Utilization of the components will be varied based on intensity of production. The high input systems of dairy milk production would profit from the early-bloom harvest that is air-dried while extensive systems such as growing heifers and small stock could be fed shredded woody components. There is a need for further research to assess how these fractions can be best utilised in ruminant production systems.

This study provided in-depth analysis of carbohydrate and protein fractions and the metabolizable energy of both leafy as well as woody fractions. In addition, it provided an explanation on how various components are affected by post-harvest management. However, early-bloom growth stage and leafy samples have shown to be much sensible and affected by drying treatment. Therefore, separating leaves with woody parts in early-bloom must be recommended to highly conserve nutrient content through silage, pelleted or hays. Overall, post-harvest management with young and immature TL fractions to make hay is advised. The regulating effective temperature and air velocity factors of hay making to prevent nutrient content depletion, while storing hay and covering should be utilized to prevent oxidation and Maillard reactions factors. None of the previous studies has full analysis of carbohydrate fractions and forage value, which this study has expounded. It is therefore critical that the information be submitted for incorporation into the FAO-STAT database.

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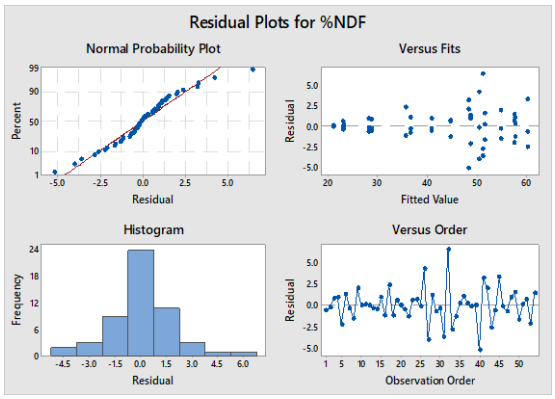
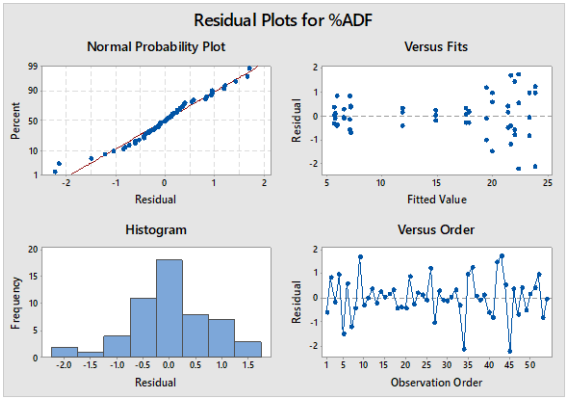
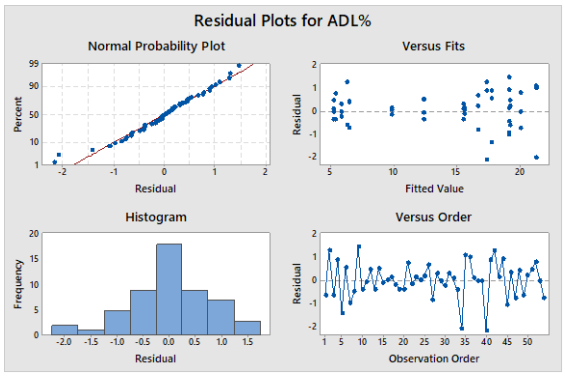
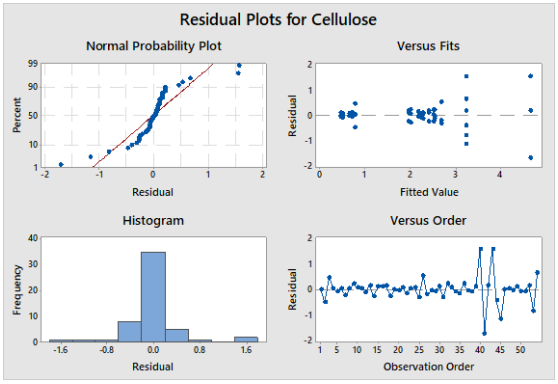
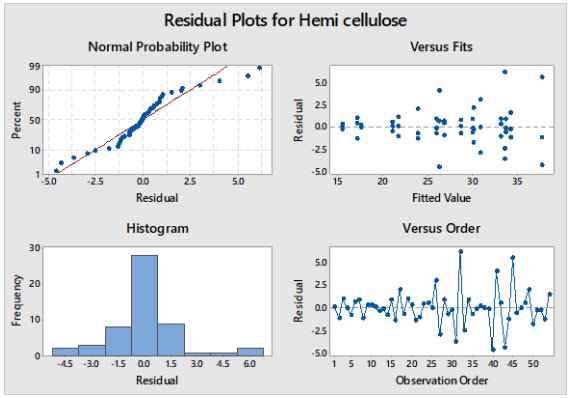
ANNEXURE A: DESCRIPTIVE STATISTICS

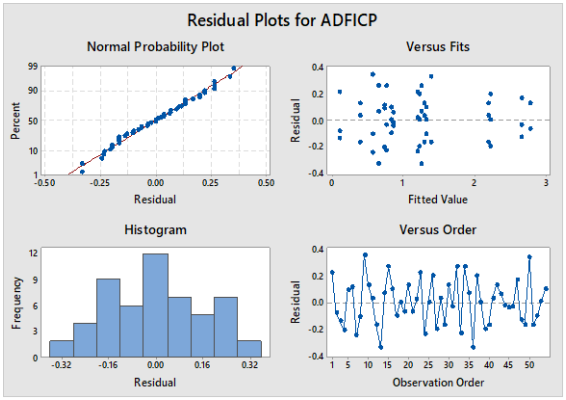


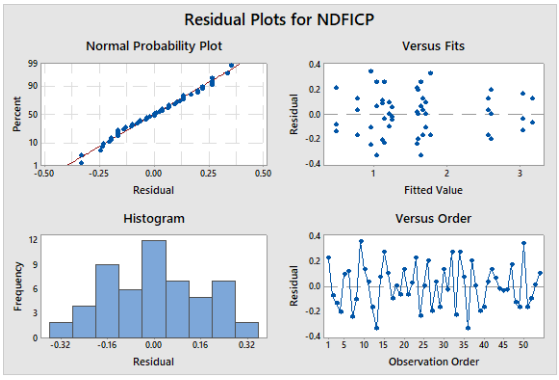


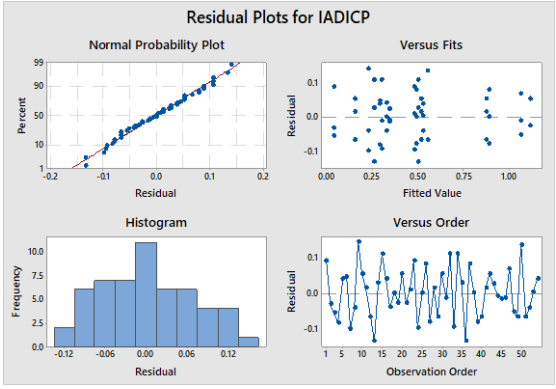


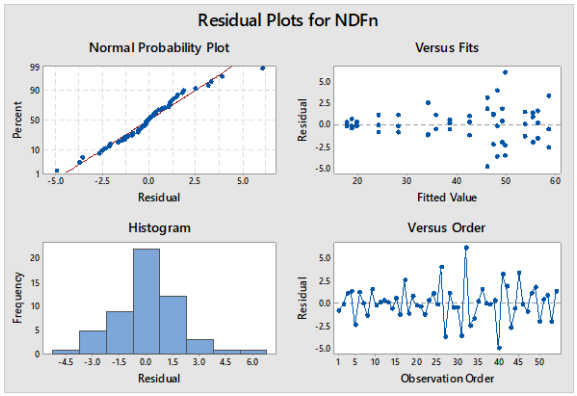








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Versus Fits
Histogram
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Fitted Value
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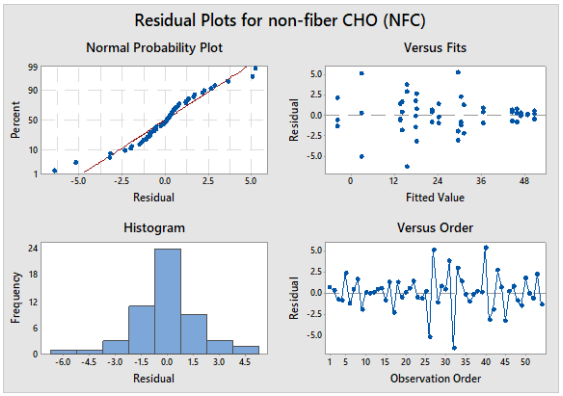
Observation Order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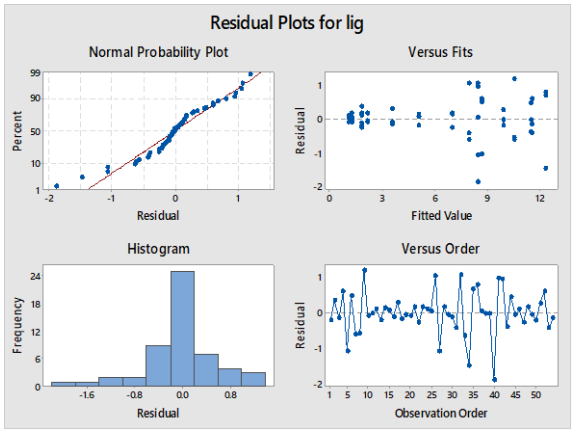
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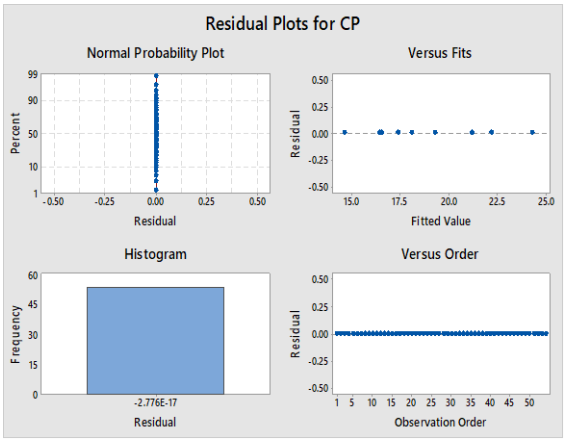
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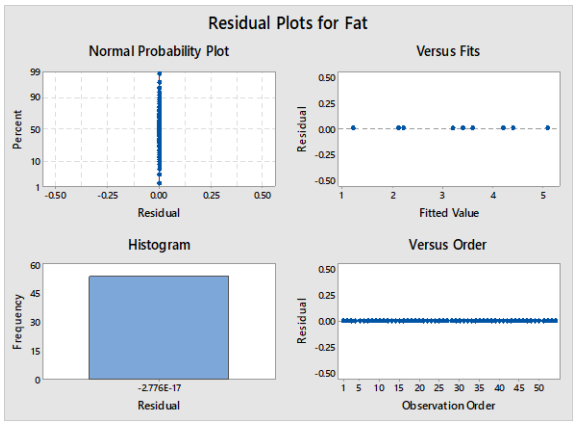
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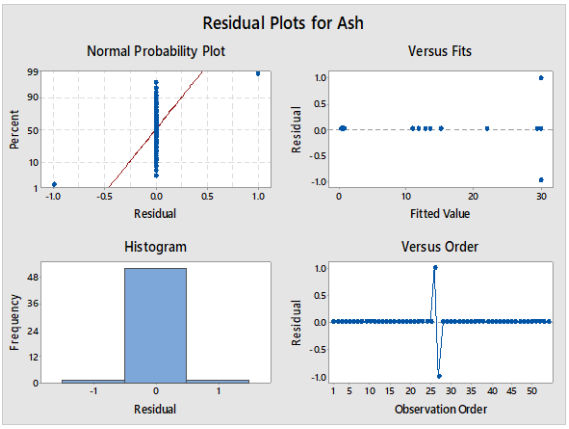
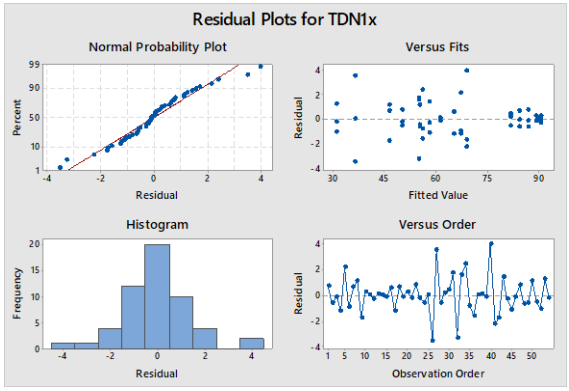
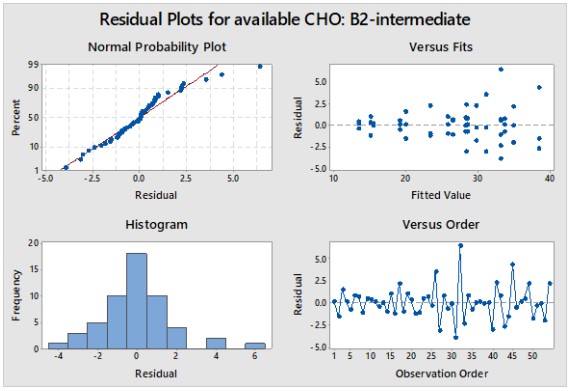
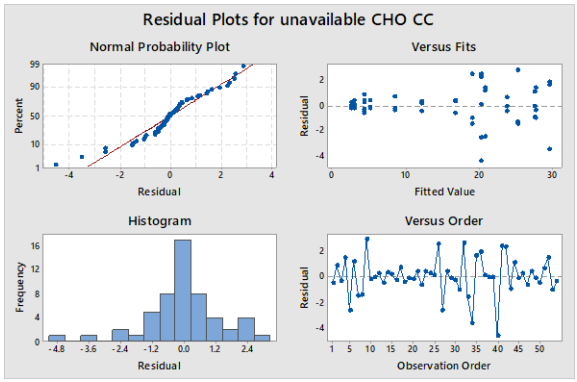
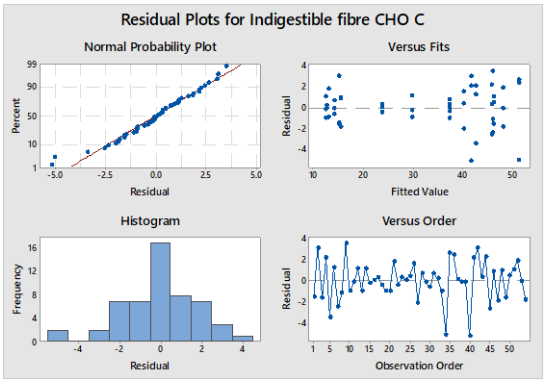
Observation Order

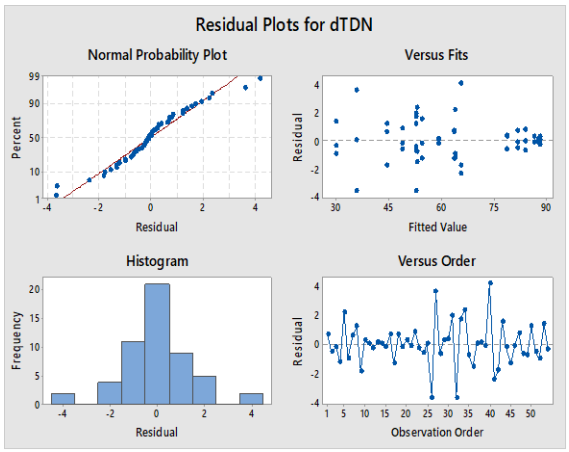

Residual Plots for Ash
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

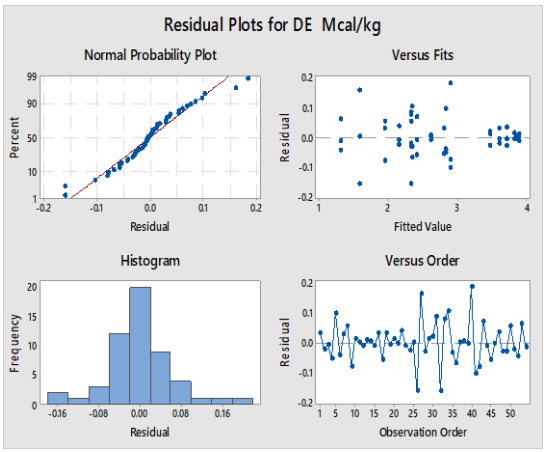

Residual Plots for TDN1x
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

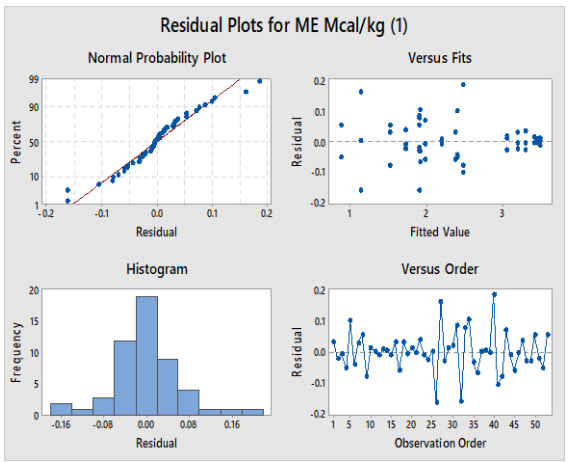

Residual Plots for available CHO: B2-intermediate
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

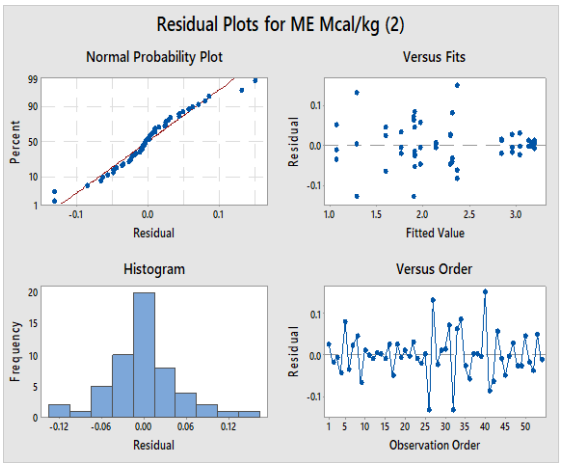

Residual Plots for unavailable CHO CC
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

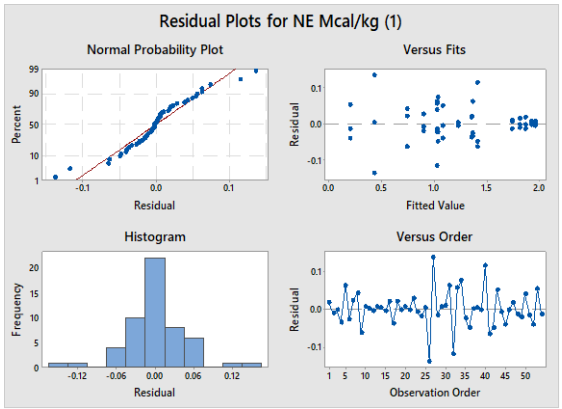

Residual Plots for Indigestible fibre CHO C
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

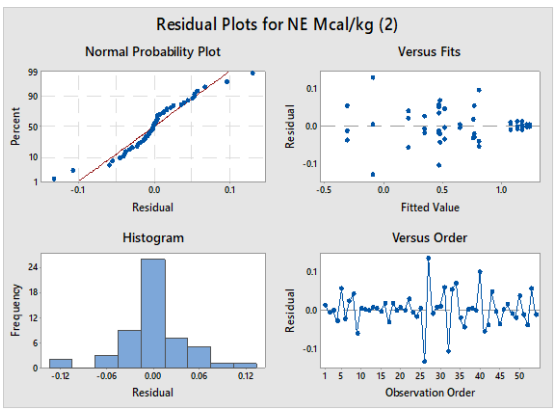

Residual Plots for dTDN
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

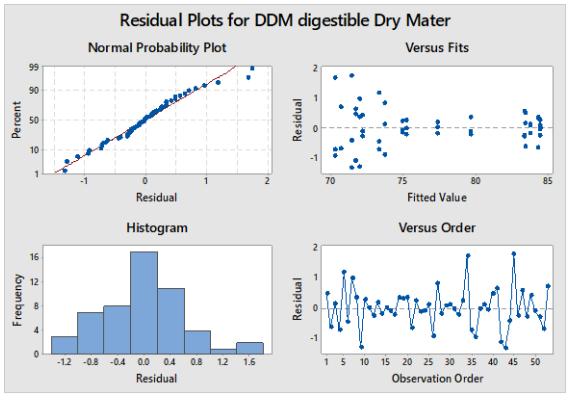

Residual Plots for DE Mcal/kg
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

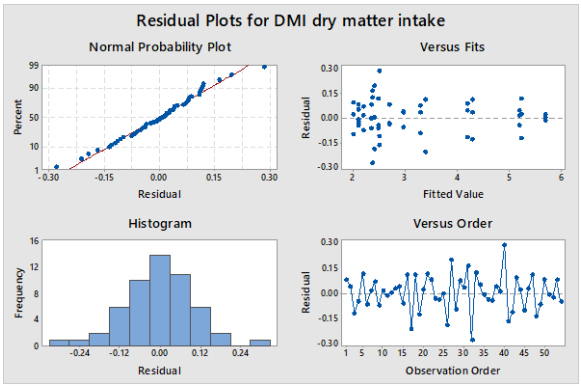

Residual Plots for ME Mcal/kg (1)
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order

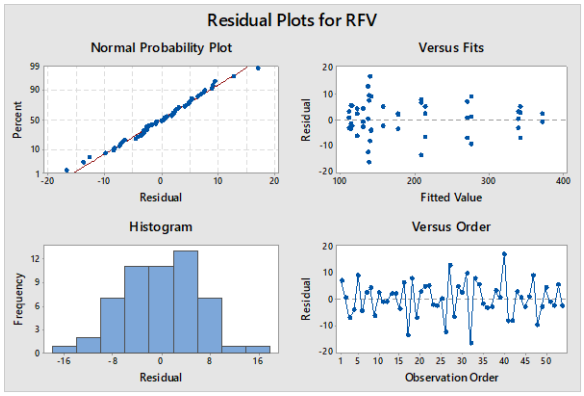

Residual Plots for ME Mcal/kg (2)
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order


Residual Plots for NE Mcal/kg (1)
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order


Residual Plots for NE Mcal/kg (2)
Normal Probability Plot
Versus Fits
Histogram
Versus Order
Percent
Residual
Fitted Value
Frequency
Observation Order


Residual Plots for DDM digestible Dry Mater
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Residual Plots for DMI dry matter intake
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order


Residual Plots for RFV
Normal Probability Plot
Percent
Residual
Versus Fits
Residual
Fitted Value
Histogram
Frequency
Residual
Versus Order
Residual
Observation Order

ANNEXURE B: TREE LUCERNE FIGURES

Fig 1-a

Fig 1-b

Fig 1-c

Fig 1-d

Fig 1-e

Fig 1-f

Fig 1 (a-b-c) Post-bloom tree Lucerne at the flowering stage, and - (d-e-f) Tree Lucerne pods

Fig 2-a

Fig 2-b

Fig 2-c

Fig 2-d

Fig 2 (a-b-c-d) Tree Lucerne conservation

Fig 3-a

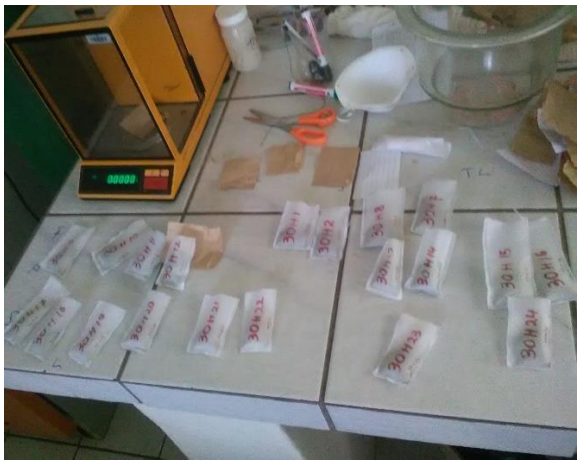


Fig 3-b

Fig 3 (a-b) Tree Lucerne sampling