BOTSWANA UNIVERSITY OF AGRICULTURE AND NATURAL RESOURCES

Comparative assessment of the effects of horse manure and urea as nitrogen sources on seed yield, forage production and nutritional quality of *Cenchrus ciliaris* post seed harvesting.

A dissertation submitted in partial fulfilment of the academic requirements of

Master of Science Degree in Animal Science (Nutrition).

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DECLARATION

I declare that all the work contained in this dissertation is my own independent investigation for the Master of Science Degree in Animal Science which I did at Botswana University of Agriculture and Natural Resources from January 2015 to March 2020. All the sources used have been quoted and acknowledged by means of references. The work has not been previously submitted and shall not be submitted to any other university for the award of any other degree or diploma.

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TABLE OF CONTENTS

PAGE

ABSTRACT

The objective of this research was to evaluate the comparative effects of horse manure and urea, as sources of nitrogen, on seed and biomass yields of *Cenchrus ciliaris* and the nutritional quality of its forage residues after harvesting seeds. Two studies were carried out for the investigation. In the first study, 18 plots of *C. ciliaris*, grown from tussocks under irrigation, were used in a replicated 6 x 3 completely randomized design with 4 months experimental period. Six plots of *C. ciliaris* were assigned to each of the three treatments of no fertilizer, fertilizing with horse manure and fertilizing with urea. After four months, seed yield, forage dry matter yield, number of tillers per crown and lengths of inflorescences of the *C. ciliaris* were measured. Data were analyzed using the General Linear Model Procedures in Statistical Analysis System (**SAS**). Fertilizing *C. ciliaris* with either horse manure or urea caused a **significantly ($P < 0.05$) higher** seed yield, forage biomass yield and number of tillers per crown than the control treatment. There was no **significant ($P > 0.05$) difference** in the length of inflorescence of *C. ciliaris* between the two fertilizer treatments and the control. Fertilizing *C. ciliaris* with horse manure caused a **significantly ($P < 0.05$) higher** seed yield (18.46 ± 1.42 **kg/ha**) than fertilizing with urea (14.51 ± 1.92 **kg/ha**). There was no **significant ($P > 0.05$) difference** in number of tillers per crown between fertilizing with horse manure and fertilizing with urea. Fertilizing the *C. ciliaris* using urea caused a **significantly ($P < 0.05$) higher** forage biomass yield (2739.62 ± 274.42 **kg dry matter/ha**) than fertilizing with horse manure (2237.85 ± 118.99 **kg dry matter/ha**). This research, therefore, showed that fertilizing *C. ciliaris* with horse manure was superior to fertilizing it with urea in terms of increasing the seed yield but fertilizing it with urea was superior in terms of forage dry matter yield. If the objective is to produce seed, horse manure would be the source of nitrogen to use and if the target is to produce higher dry matter yields, urea would be preferable, all other factors held constant. In the second study, the *C. sciliaris* forage residues from the 18 plots in the first study were analyzed for nutritional quality after the seeds were harvested. It involved analyzing the residues for **Crude Protein** (CP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), **Ash**, minerals and **In vitro Dry Matter** digestibility (IVDMD). **Fertilizing *C. ciliaris*** with either horse manure or urea caused **significantly ($P < 0.05$) higher** CP and IVDMD than the control treatment. There was, however, **no significant ($P > 0.05$) difference** between fertilizing with horse manure and fertilizing with urea in terms of the two parameters. Fertilizing the grass with either horse manure or urea caused **significantly ($P < 0.05$) lower** ADF, NDF and ash contents than the control treatment. There was no **significant ($P > 0.05$)**

difference between fertilizing *C. ciliaris* with horse manure and fertilizing with urea in terms of these nutritional quality parameters. In terms of minerals, fertilizing with either horse manure or urea caused no **significant (P > 0.05) difference** from the control treatment, except in sulfur content where fertilizing with urea resulted in **significantly (P < 0.05) higher** levels (**0.435% ± 0.018** dry matter) than the control treatment (**0.230% ± 0.018** dry matter). It became evident from this study that there was no significant difference between fertilizing using horse manure and using urea in terms of increasing the nutritional quality of the forage residues of *C. ciliaris*. The two fertilizer treatments, however, increased the quality of the residues by increasing their CP content. Both fertilizer treatments also reduced the fibrosity of the forage residues and this could have contributed to increased digestibility of the forage residues. The results of this research show that *C. ciliaris* seed production can be done in the semi-arid areas of Botswana using horse manure and be used to establish a seed bank. Such a seed bank could then be used as source of forage propagation material to improve pastures. As further research, on-farm trials may need to be performed beyond the small plots experiment undertaken in this study in order to **validate** the adaptability and productivity of the grass under real field conditions across varied soils, moisture conditions and seasons.

Keywords and phrases: nutritional quality, forage residues, forage dry matter yield, digestibility, forage biomass

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

ADF acid detergent fiber

ANOVA	analysis of variance
AOAC	association of analytical chemists
ATP	adenosine triphosphate
BUAN	**Botswana University of Agriculture and Natural Resources**
Ca^{2+}	calcium ion
CEC	cation exchange capacity
CP	Crude Protein
Cu^{2+}	copper ion
DMD	dry matter digestibility
DM	dry matter
Fe^{2+}	ferric ion
IVDMD	in vitro dry matter digestibility
IVTDMD	in vitro total dry matter digestibility
K^{+}	potassium ion
Mg^{2+}	magnesium ion
NDF	neutral detergent fiber
Zn^{2+}	zinc ion
PDIFF	Piecewise differentiable

CHAPTER 1

1.0 Introduction

1.1 Livestock production on natural pastures in Botswana

Development of the livestock production enterprise as an economic sub-sector in Botswana is hindered by the unavailability of good quality feed at critical stages of the production cycle. The **main sources of livestock feed** in the country are natural pastures and crop residues which are low in quantity and quality for sustainable and profitable livestock production during the dry winter period for about 6 months (**Madibela *et al*., 2002**). In Botswana, goats raised on pasture and mated at the end of the wet season (April) are exposed to adequate nutrition for enhanced sexual activity which results in multiple ovulation and, hence, multiple births (**Madibela and Segwagwe, 2008**). The rest of the pregnancy, however, goes through a period of a shortage of nutrients. This is because most of the native grasses in the rangelands in Botswana are so low in quality that they result in slow growth rates, poor fertility and high mortality rates in ruminant livestock. Walker (2013) was in agreement with this sentiment as she reported that ruminant livestock in Botswana is largely dependent on the communal range or natural pasture and is constrained by fodder scarcity. This natural pasture which consists mainly of grass, is high in fibre but too low in crude protein to meet animal nutritional requirements (Walker, 2013). Most of the rangelands are just large tracts of arid and semi-arid lands that are not suited for rain-fed crop production due to their low crop production potential. The soil has a low humus content which makes it not to retain water long enough for crops to absorb. It also has a low nutrient content due to excessive leaching. The leaching causes the soil to be acidic and cannot promote growth of crops. **Kolawole *et al*. (2013)** reported some results of laboratory analysis of 33 composite soil samples collected from 30 farmers in 3 farming communities of Makalamabedi, Nokaneng and Mohembo, **situated in Ngamiland East, Maun region, in the northern part of Botswana**, which showed that most soils are low in nutrients as well as in cation exchange capacity. The land has, instead, been used for livestock production. **In these areas, it is mainly extensive range management that is practised. Animals graze and browse over large areas that are not fenced**. The productivity of the livestock in these areas has, however, remained relatively low (Kolawole ***et al.***, 2013). This has been attributed to low quantity and poor quality livestock feed in general and forage grasses in particular.

It has been very difficult for the range to be improved through introduction of more valuable forage grasses due to unavailability of planting material in the form of seed. There are no established seed banks for better performing and higher quality forage grasses and this has been the most discouraging factor to farmers. The soils in Botswana are predominantly sandy textured, with very low organic matter content, resulting in deficiencies in essential plant nutrients (Kolawole ***et al.***, 2013). The grasses that grow in such soils inherently show deficiencies in these essential nutrients as well since they can only acquire nutrients that are available in the soil (Koech ***et al.***, 2014). **Most farmers use the extensive and semi-intensive systems of livestock production and fertilization of the pastures is not practised.** Most parts of the country receive below average and quite variable rainfall. The low fertility of the soil and the low rainfall has resulted in the sparse and low quality forage grasses in the natural pastures. If seed material for over-sowing in pastures can be made available, the forage grass quantity can be improved. The seed material should be from high quality grass, such that performance of livestock grazing on such pastures could be enhanced. Some lands, like sand dunes, which occupy large areas in Botswana, abandoned crop lands and degraded rangelands, hardly have any forage to sustain livestock. When the forage grasses in a pasture are sparse and of low quality, large areas of land would be required to sustain few animals. Before even considering the quality of the forage grasses in the pastures, it may be important to be able to increase the quantity of the available fodder through seed bank establishment and making the seed available even in the remote areas where communal farmers rear their livestock. It is important to identify grass species that can be appropriate for the improvement of pasture for livestock in terms of its biomass production and its nutritional quality. It would be an added advantage if the grass species can also be useful for reclamation of wasteland and stabilization of sand dunes. In this context, the grasses would assume importance, not only as livestock feed, but also as soil builders and binders and aid in soil conservation (Parwani, 2013).

Growing demand for pasture grass seed and establishment material in the country and neighbouring countries necessitates researchers to respond in order to create an opportunity for pasture seed supply industry. In Botswana, unfortunately, the distribution of material for propagation, whether it is the seed or vegetative, is seldom a commercial enterprise. On a local basis, a research agency or a farmer may sell the material but usually on a small scale. **The principal issues related to pasture development in Botswana, therefore, can be highlighted as reluctance by government to sponsor research and unavailability of seed banks.** To compound the problem, there is limited data available on the grasses response to fertilizers. The little literature that is available is void of any data on seed production or seed quality responses to fertilizer under conditions prevailing in Botswana.

1.2 Characterization of desirable forage grass

In order to solve the shortage of feed and increase livestock productivity, it is necessary to introduce and cultivate high quality forages with high yielding ability and adaptability to the biotic and abiotic environmental stresses (Hare ***et al.*,** 2009).The suitable grass types would be those that respond very quickly to any availability of moisture, no matter how slight. The selected forage grasses should produce sufficient seed for perpetual reproduction. It should also be of high nutritive value in terms of nutrient content, especially nitrogen and phosphorus. According to Ashraf ***et al.*** (2013) the dry matter crude protein content should be between 6%

and 18% while phosphorus content should be between 0.15% and 0.65%. Ruminants require 6.9% protein for maintenance, 10% for beef production and 11.9% for milk production (Ashraf ***et al.,*** 2013). Ramirez *et al.* (2009) reported that growing beef requires about 0.3% dry matter phosphorus and about 12% dry matter protein. A report from Animal Production and Research Unit (APRU) (1977) showed that phosphorus was always below 0.03% on dry matter basis in natural range forage while crude protein averaged 8.5% in dry seasons and 9.4% in wet seasons in Botswana. This means that without any improvement, the ruminants would not get sufficient crude protein for production.Its digestibility should be reasonably high if it is to be of value to livestock. According to Donaldson and Rootman (2010), the digestibility of forage grass should not be less than 53.8%.

1.3 *C. ciliaris* (Molopo variety) as a forage grass of choice

One very clearly promising forage grass in Botswana is *C. ciliaris*. It can play an important role in providing a significant amount of quality forage both under smallholder farming and intensive livestock production systems (Hassan ***et al.,*** 2015). It is native to Southern Africa and India (Koech *et al.*, 2014). Its drought tolerance and productivity led to its uptake and by the pastoral industry in some countries like Australia, Canada and New Zealand (Parwani, 2013). Unfortunately, its sites in Botswana are usually not more than isolated clusters of one to a few closely spaced individuals. Its populations are extremely fragmented. According to Akiyana ***et al.*** (2005), *C. ciliaris* is always observed growing in dense monotypic stands, small clumps or lone tussocks throughout the landscape. Unprotected, these clusters are heavily and selectively grazed and extremely scarce as the grass is relatively more palatable than most native grasses (Seddi ***et al.,*** 2002). In some countries like Mexico (North-western Mexico), natural desert-scrub and thorn-scrub have been converted to *C. ciliaris* pastures successfully (Seddi ***et al.,*** 2002). *C. ciliaris* is gaining attention in various fields of research, as it is quite competitive under conditions of high temperature, solar radiation and low rainfall (Bulle ***et al.,*** 2011). These are the conditions that are obtaining in Botswana. It is more efficient at gathering carbon dioxide and utilization of recycled nitrogen from the soil (Akiyama ***et al.,*** 2005). The grass has proved useful for pasture and soil retention in a wide range of environments due to its drought tolerance, deep and clustered root system, rapid response to availability of moisture, relative palatability and resistance to overgrazing (Arshadullah ***et al.,*** 2011). The larger and deeper root system makes it capable of providing greater strength against soil erosion than other subtropical grasses (Bulle ***et al.,*** 2011). The swollen stem base accumulates carbohydrates, allowing it to survive drought spells and to sprout after burning and at the commencement of rain (Arshadullah ***et al.,*** 2011). Its drought hardiness makes it an ideal choice for dry communities and for colonization of disturbed sites (Kizima ***et al.,*** 2012). It produces viable seed so that stands can be self-replacing and pastures may not need to be reseeded. According to Akiyama ***et al.*** (2005), *C. ciliaris* is generally apomictic, although some which propagate sexually have been identified. Its seed spreads easily by wind, along water courses and human and animal traffic. It is successfully propagated by vegetative splits, rhizomes and stolons and this makes it quite useful in stabilizing disturbed areas.

According to Bhattarai ***et al.*** (2008), the use of seed for sloping areas like railway batters to control soil erosion is a major concern where the development of good grass cover within a short period of time is required to minimize the risk of damage from storms. This is where vegetative propagation capability of *C. ciliaris* comes into play. Its tufted nature and having buds close to the ground makes it tolerant to heavy grazing and trampling (Arshadullah ***et al.,*** 2011). Its persistence and ease and low cost of establishment add to its competitive advantage. Its vigorous regenerative capacity after a fire is another of its major assets (Martin ***et al.,*** 1999). Its seed germination is inherently poor, normally not exceeding 18% when the seeds are not treated with growth hormones or by scarification and becomes unpredictable, especially in semi-arid regions characterized by low rainfall, due to its extended dormancy

(Bhattarai ***et al.,*** 2008). According to Brummer (2009), however, germination of *Cenchrus* grass seed can be improved by treating it with GA3 growth regulator at **10 ppm** at pH 7.0. It can also be improved by soaking the seeds in 70% sulphuric acid for 5 minutes and washing in running water (Brummer, 2009). The seeds can also be treated with Indole Acetic Acid at **10 ppm** for 8 hours (Brummer, 2009).The grass's regenerative capacity after a fire and commencement of rain is one of its greatest assets (Marshall ***et al.,*** 2012). Its protein content was found to range from 4.0 to 7.9 % of dry matter across different saline levels of soil (Al-Dakheel ***et al.,*** 2015). This means the grass can be grown in many arid areas where vast reserves of saline water exist. This could be important in saving fresh and good quality water sources for other purposes.

1.4 Factors limiting the use of *C. ciliaris* as a forage grass in Botswana at present

Widespread use of forage depends upon the local availability of cheap seed material, which is true to type, viable and would reliably establish good pasture when sown (Parwani, 2013). *Cenchrus* pasture production has been affected by unavailability of forage seeds in Botswana. The seed is crucial in commercial Agriculture as a basic commodity for propagation of the forage grass and also for perpetuation of germplasm (Arshadullah ***et al.,*** 2011). Most farmers who want to improve their natural or sown pastures have no access to commercially processed seed at a nearby retail outlet.

Research and extension services on forage grass seed production and grass management strategies in Botswana are still at rudimentary stages such that the provision of forage seed through supply systems is almost non-existent. The absence of an effective formal seed system reduces the impact of publicly funded forage production. This failure of seed multiplication and distribution translates into negative rate of returns for those progressive farmers who may try to make a living through forage and fodder production. Although the literature relating to its nutritive value (Albu, 2012; Parwani and Rankard, 2012; Donaldson and Rootman, 2010;

Mcdowell, 2003; Ramirez ***et al.,*** 2009; Arshadullah ***et al.,*** 2011), its involvement in feed formulations (Ramirez ***et al.,*** 2009; Mutimura and Everson, 2012; Kumar ***et al.,*** 2005; Hassan ***et al.,*** 2015;During and McNaught, 2012)and its biological invasions (Ogillo, 2010; Osman ***et al.,*** 2008; Marshall ***et al.,*** 2012) has grown significantly, much is lacking in terms of the agronomical techniques needed to establish this grass for various purposes, especially for seed production. There is little published information regarding its multiplication capabilities and agronomical requirements (Bhattarai ***et al.,*** 2008). Plant breeders and agronomists have concentrated on increasing the forage yield and nutritive value, with little attention given to seed production (Tacheba and Moyo, 1985). The fact that it is found in isolated clusters which are extremely fragmented means that its use on a large scale cannot be meaningful. Farmers are also reluctant to take up seed production as a business enterprise because having only seeds as a source of income is not very encouraging.

1.5 Experiences of Cenchrus elsewhere from its centre of domestication

Where it has been successfully introduced like in North-Western Mexico to improve rangelands for cattle production, it has tended to invade adjacent habitats, displacing the native fauna and flora (Bhattaraj ***et al.,*** 2008). Its spread, naturalization and invasion into native plant communities has been recognized as a serious ecological problem threatening biological diversity and ecological function (Gutierrez-Ozuna ***et al.,*** 2009) elsewhere outside Botswana. The grass is seen as a threat to important mesic habitats within the arid zones in Northern Mexico (Bhattaraj ***et al.,*** 2008). These mesic sites are critical parts of the landscape, providing concentrations of water and nutrient resources and refugia for some plants and animals (Gutierrez-Ozuna ***et al.,*** 2009). It threatens the survival of rare species and alters the food supply chain of native animals. Where it spreads into non-target areas, it has become a serious concern to non-pastoral land managers and those responsible for conservation areas and this has been reported in most parts of Northern Mexico where it has been introduced (Gutierrez-

Ozuna *et al.,* 2009). Its invasive and allelopathic effects may also need to be investigated in Botswana. **Environmentalists in Botswana have not yet raised any concerns about it**. This could be due to the fact that the grass has not really been grown on a large scale and it also has not yet been over-sown in natural rangelands and its effects closely monitored. According to Marshall and Ostendorp (2012), the characteristics of *C. ciliaris* which make it versatile and suited to a range of harsh conditions also make it an expert invader of non-target environments and from an environmental point of view, it is important to prevent further spread of the grass. When used temporarily, its eradication is very difficult. Once established, the conversion of a *Cenchrus* pasture to an alternative pasture would be prohibitively expensive. Strategic control of its spread requires knowledge of its physiological characteristics which most farmers, especially rural ones, do not have. Lack of seed, poor seed germination, high fire risk and its invasive and allelopathic nature also contribute to the grass not being used on a large scale in Pakistan (Arshadullah *et al.,* 2011). The seed germination success and allelopathic nature of *C. ciliaris* in Botswana needs to be investigated.

1.6 Opportunities of growing *Cenchrus* as livestock fodder crop

When managed well and its spread restricted to target areas, the grass can play a very crucial role in improving livestock production in Botswana. Large tracts of land may need to be put under the grass so that its impact on livestock production becomes quite significant. The first hurdle to overcome would be to produce seed and make it available on the official market. Farmers may also be encouraged to get involved in seed production if there is more than just seed that would be of value from the whole exercise. The forage residues need to have high nutritional value so that farmers can benefit from them as well, post seed harvesting. The residues can be sold to other farmers to feed their livestock. There is need, therefore, for the use of technologies that can improve seed yield without significantly compromising the quantity and quality of the forage residue. Use of nitrogen-based fertilizers in pasture establishment is one of the technologies that help to replace and maintain soil nutrient levels for quality seed production and increased forage biomass yield (Sahoo ***et al.,*** 2015). According to Kizima ***et al.*** (2011), the application of nitrogenous fertilizer gives a high economic response in seed production enterprises.

In Botswana, limited data is available on *C. ciliaris* forage and seed production responses to nitrogenous fertilizers. The literature that is available (Nsinamwa ***et al.,*** 2005; Koech ***et al.,*** 2014) is void of any data on seed production and seed quality responses to nitrogenous fertilizers, specifically horse manure. The prospects of using horse manure to produce forage grass seed may not interest researchers due to the limited quantity of the horse manure in most areas of Botswana. Seed production, however, does not need to be done on a very large scale which would then require a lot of horse manure. On the whole, the effect of different types of nitrogen fertilizers on *C. ciliaris* productivity need to be investigated. The nutritive value of the residual forage material after harvesting seed also needs to be analysed to find out if the fertilizer that is used can also influence forage grass properties like dry matter digestibility and the levels of major nutrients like nitrogen, phosphorus, potassium, calcium, sulphur and magnesium and also levels of some trace elements like zinc, iron, sodium and copper. According to Marschner (1995), without the right balance of nutrients, the forage residues after seed harvesting have a low feeding potential. When the forage residues after seed harvesting are of little or no value, farmers would not be attracted to the seed production business. The choice of fertilizer to be used when growing forage grass for seed should be such that it is effective, available and affordable. Reliance on vegetative splits for propagation of forage grass in natural rangelands and large pastures is almost unthinkable.

1.7 Statement of problem

At present, farmers in Botswana have no access to commercially processed forage grass seed at their local retail outlets. The capacity of the formal sector is so limited that it is struggling to meet the ever increasing national demand. However, precedence in the form of established cereal and pulses grain seed production system has been made. Emulating this production system for forage seed production will be the way to go. For the seed to act as a catalyst in increasing forage biomass, the seed has to be made available to a broad base of farmers on continuing basis. The absence of an effective formal seed system and failure of seed multiplication and distribution locally, translates into negative perceptions about taking up seed production as an enterprise. Plant breeders and agronomists have concentrated on increasing the forage yield and nutritive value, with little attention given to seed production (Mganga, 2009). As such, limited information is available on pasture seed production of recommended promising grass species under different agro-ecological zones of the country. Most literature is on research done in other African countries like Tunisia (Seddi ***et al.,*** 2002) and Sudan (Burhan and Hago, 2000; Abedelrahman, 2007) where *C. ciliaris* was found to be quite prolific in seed production.

1.8 Justification

Range animal productivity can be increased by the adoption of forage grass species that show rapid and vigorous growth, disease and drought resistance and are digestible. Propagation of such grass would need reliable seed material. The supply of sufficient forage grass seed is determined by the effectiveness and efficiency of the seed production system. The increase in forage productivity can be substantial if farmers are encouraged to be involved in seed multiplication ventures. The seed multiplication of desired forage grass species require modern agricultural practice. Seedbed preparation, planting depth, spacing, use of pesticides and irrigation regimes require modern farming techniques.

The choice of the most appropriate fertilizer, its application rate and timing of application are also technically demanding. On farm trials of forage grass introductions and evaluations to determine seed and forage biomass yield of promising pasture species such as

C. ciliaris, need to be done. The data contained in Animal Production Report Unit (APRU) reports of 1975, 1977 and 1985 is too ancient to be relied upon. According to the APRU (1985), in normal years, only 0.04% of ruminant feed came from improved pastures and forage crops. Just like any other new adoption, the participation of farmers in forage seed production is important. There is, therefore, the need for a centralised and aggressive extension-based push focusing on technological packages that combine fertilizers, improved seed production and better forage management practices in order to improve the forage grass supply side. Once researchers prove that seed can be produced and sold profitably, this can be liberalised, with the emergence of private forage grass seed companies. If the forage residue that remains after seed harvesting is of relatively high nutritive value, farmers can be convinced into taking up seed production, knowing that income will come from both the seed and the forage residues.

Use of nitrogen sources that are relatively cheap, environmentally friendly and effective should be done. This prevents over/under-fertilization, both of which are detrimental to forage seed production. There is need for more published information regarding forage grass multiplication potential using cheap, available and environmentally friendly nutrient sources in Botswana. Developing a nitrogen fertility programme is crucial in terms of increasing forage and seed yield without evoking the negative effects of excess nitrogen. This should be done bearing in mind the implications of the rapidly increasing cost of commercial nitrogen fertilizers and contamination of the environment. A source of nitrogen that promotes seed production without significantly compromising forage yield and quality should be explored.

1.9 The objectives of the study are:

. To determine the effects of horse manure and urea on the length of inflorescence of *C. ciliaris*

. To assess the effects of horse manure and urea on the number of tillers of *C. ciliaris.*

. To measure the effects of horse manure and urea on the seed yield of *C. ciliaris.*

. To assess the effects of horse manure and urea on forage biomass of *C. ciliaris.*

. To evaluate the effects of horse manure and urea on the nutritional quality of the *C. ciliaris* forage residues post seed harvesting.

CHAPTER 2

2.0 Literature Review

2.1 Botswana's natural grassland

Ruminant livestock production in Botswana mainly relies upon natural grassland, cut herbage or crop residues (Nsinamwa ***et al.,*** 2005). **Only a few farmers depend on imported concentrate feeds and baled legumes like Lucerne due to the costs involved**. Most parts of the country are arid or semi-arid and this is why poor quality forage grass species dominate most grazing lands (Nsinamwa ***et al.,*** 2005). The natural pastures, bare land that has been eroded and other wastelands like sand dunes can, however, be rehabilitated through reseeding by promising forage grass species like *C. ciliaris*.

2.2 The desirable characteristics of *C. ciliaris*

C. ciliaris is commonly associated with scattered woody legumes such as ***Prosopis*** species and *Leucaena leucocephala* (Parwani, 2013). It is fast growing, shortly *stoloniferous* and is quick to flower. Individual plants develop as clumps usually with limited lateral spread. Height of flowering culms may range from **15 cm to 1.5 m.** Inflorescences are from **3 cm to 15 cm** long and 1-2 cm wide (Parwani and Mankad, 2012). The appropriateness of this grass is a result of its numerous characteristics that favour local conditions. Parwani (2013) described *C. ciliaris* as a deep-rooted, summer growing and perennial tussock grass with a high herbage yield potential. Its deep and widespread root network makes it very drought resistant and competitive in terms of nutrient uptake. It also makes it ideal for the stabilization of sand dunes and prevention of soil erosion in general. Yossin and Ibrahim (2013) **reported** that the soil binding capacity of *C. ciliaris* is due to its clustered root system in the 8 – 10 cm layer of soil. It is native to Southern Africa and India (Parwani, 2013). It survives extreme and prolonged drought but grows vigorously when favourable conditions set in. The grass has spread successfully from planting in areas having a dry period of 150 to 210 days, mean temperatures of between 24^{o}C and 45^{o}C and the coldest month ranging from 5^{o}C and 15^{o}C. It has a low tolerance to freezing temperatures and does not tolerate waterlogged soils (Yossin and Ibrahim, 2013). Parwani and Mankad (2012) highlighted its rapid response to moisture availability, relative palatability and resistance to trampling and overgrazing as some of its favourable attributes. It produces viable seed that can be used to propagate the grass in other areas. Due to the viable seed it produces, its stands are self-replacing and pastures may not need to be re-seeded. According to Hall (2001), *C. ciliaris* is a highly regarded pasture grass due to its value as pasture for livestock and its soil protection properties. In Australia, it has brought some great financial benefit to many individual producers and companies due to its tolerance to drought, fire and overgrazing (Hall, 2001). It produces more biomass than many native perennial grasses and its high seed yield and fluffy seed allow it to spread readily via wind and water (Seddi ***et al.,*** 2002).

Besides being *apomictic*, *C. ciliaris* is highly polymorphic and variable for several traits of ecological and agronomic importance (Seddi ***et al.,*** 2002). The grass, therefore, is among the species having the greatest potential for forage production in Botswana. It has the potential to produce high yields of high quality hay and pasture for livestock production and ground cover for rehabilitating degraded land and stabilizing sand dunes. According to Redfearn ***et al.*** (1990), the productive potential of this grass is limited by the inadequate soil fertility and limited amount of rainfall. These conditions that limit *C. ciliaris* productivity are generally the ones that are experienced in most parts of Botswana. This is why seed production needs to be done under irrigation and fertilizer has to be applied as well.

Some nutrients are required by forage grasses in very small amounts and are adequately provided from the soil, for example, zinc, cobalt, iron and manganese (Laidlaw, 2005). Others, like nitrogen, phosphorus and potassium are required in greater amounts and are commonly applied as fertilizer amendments (Laidlaw, 2005). . According to Sahoo ***et al.*** (2015), *C. ciliaris* grass has a reputation as a phosphophilic grass. It has a high phosphorus content and a high Phosphorus: Calcium ratio. Effects of fertilisation is not only observed in the forage but it is translated into animal performance.

2.3 The use of nitrogenous fertilizers to improve seed production

Fertilizer, as a mineral nutrient source, is one of the most important technologies that can increase forage grass and seed production. Most soils in Botswana are predominantly sandy-textured, with very low organic matter content, resulting in lack of essential plant nutrients like nitrates and phosphates, which are vital for pasture grass growth and seed maturation (Mudenda and Maeresera, 2009). According to Osman (2008), nitrogen (N) fertilization has been found to typically increase grass dry matter, forage N concentration and seed yield. A number of studies (Hassan ***et al,*** 2015; Ihsan ***et al.,*** 2014; James, 2010; Brummer, 2009; Abedelrahman, 2007 and Ashraf ***et al.,*** 2013) have found that forage yields are increased by fertilization on sandy soils. **N** fertilization is one of the most common practices since the nutrient is the most limiting factors influencing yield and chemical composition of grass pastures. In terms of forage and seed production of ***C. ciliaris,*** **N** is the most limiting nutrient as it does not really stay in the soil for a long time. It is highly mobile and is easily lost from the root zone through leaching, yet it is very crucial (Mujuni and Sibanda, 2007). It is a major factor for increasing the pasture yield and nutritive value of the grasses, including their crude protein content and digestibility (Hassan and Fikru, 2015). According to Donaldson and Rootman (2010), dry matter yield in forage grasses responses primarily to nitrogen fertilizer application. However, the rapidly increasing cost of commercial **N** fertilizer makes it imperative to optimize nitrogen use efficiency.

According Marschner (1995), too little **N** reduces the disease fighting properties in forage grasses and the grasses become susceptible to fungal infections. If over-fertilized with

N, the excess **N** causes the breakdown of grass tissues through sugars and amino acids, making them susceptible to the invasion of fungal spores (Abedelrahman, 2007). In addition, Kizima ***et al.*** (2011) reported that the higher the N fertilization rate, the greater the risk of the nitrate-N exceeding **1000 ppm** and such nitrate levels in forages may affect animal health. Nitrogen fertilization has been found to typically increase grass dry matter, forage N concentration and seed yield (Osman ***et al.,*** 2008). Generally, phosphorus fertilization alone does not increase forage yields sustainably but combined application of phosphorus and N does (Osman ***et al.,*** 2008).

The most rapid spread of *C. ciliaris* occurs in soils of good nutrient status. Kumar ***et al.*** (2007) reported that significant increases in forage N content, digestibility and mean daily live mass gain per sheep were found as **N** fertilization rates increased. Fertilization has also shown to have increased soil water extraction by forages, and improve water use efficiency (Sahoo ***et al.,*** 2015). N application up to 60 kg/ha in two doses at 15 day interval, significantly increased the fodder yield (Sahoo ***et al.,*** 2015).However, nitrogen may be supplied by organic sources such as manure. Application of 10 t/ha of sheep manure increased and sustained the productivity of *C. ciliaris* pasture for 3 years (Sahoo ***et al.,*** 2015).

2.4 Importance of N in forage grasses

N is important as a component of nucleic acids, amino acids, proteins, chlorophyll and enzymes (Taylor ***et al.,*** 1997). It affects shoot growth, shoot and root density, colour, disease resistance and stress tolerance. It has a synergic relationship with phosphorus which is a component of nucleic acids, membranes, ATP, co-enzymes and encourages a dense root network (Taylor ***et al.,*** 1997). When **N** is sufficient, forage grasses are able to significantly absorb potassium. Potassium activates enzymes used in protein, sugar and starch synthesis. It is important in maintaining turgor pressure in plants. It affects drought tolerance, cold hardiness and disease resistance (Taylor ***et al.,*** 1997). Soil sampling and testing are used to determine the amount of nutrients that might need to be added to make up for any deficiencies. According to Ihsan ***et al.*** (2014), a number of studies have found forage yields of *C. ciliaris* to be increased by fertilization in sandy lands. **N** fertilization typically increases grass dry matter, forage **N** concentration and seed yield (Ihsan ***et al.,*** 2014). All *C. ciliaris* varieties respond to a good fertility programme which supplies adequate amounts of **N**, phosphorus and potassium (Redfearn ***et al.,*** 1990). Actively growing *C. ciliaris* removes nutrients from the soil. From a grazing management standpoint, this is of little concern as the majority of the nutrients remain in the pasture and cow dung deposited during grazing recycle back some of the nutrients. As for hay and seed production, the nutrients will have to be replaced as much of the harvested hay will not be fed on the fields where it was harvested from. This causes a loss in nutrients.

N determines the forage and seed yield and is likely the driving factor in irrigated forage production. According to Savoy (1996), for grass pasture to be productive, first priority should be given to meeting nitrogen needs. Burhan and Hago (2000) stated that nitrogen plays an important role in plant growth and physiological processes, as it enters all enzyme composition and enhances vegetative growth and yield. An increase in N increases the leaf: stem ratio. Abedelrahman (2007), however, reported that no significant effect of N fertilizer was detected on mean plant height, inflorescence length and width. This is in agreement with the experimental findings of Yossin and Ibrahim (2013) which showed that growth parameters were not significantly affected by fertilizers but yield parameters. All N fertilizer treatments were found by Yossin and Ibrahim (2013) to have a significant effect on fodder yield and seed yield compared to the control of no N fertilizer. Meena ***et al.*** (2015) reported *C.* seed yield of **98 kg/ha** with organic manure (sheep) compared to **89 kg/ha** without any fertilizer and **110 kg/ha** with NPK compound fertilizer. Kumar ***et al.*** (2005) reported *C. ciliaris* seed yield of **30 kg/ha** with horse manure while Ashraf ***et al.*** (2013) said that the *C. ciliaris* seed yield when using urea was between **21.8 kg/ha** and **150 kg/ha**, depending on the level of fertilizer applied and the irrigation regime followed. Ashraf ***et al.*** (2013) also reported the dry matter yield of *C. ciliaris* as **3.345 t/ha** without any fertilizer and **3.790 t/ha** for sheep manure and **4.21 t/ha** for NPK fertilizer in Rajasthan. Ihsan ***et al.*** (2014) reported *Cenchrus* dry matter yields of between 16.3 t/ha and 30.18 t/ha, depending on the level of the fertilizer applied. Savoy (1996) reported that forage and seed yield response to nitrogen can be very dramatic. Donaldson and Rootman (2010) reported that nitrogen is essential for seed production, with seed yields being raised tenfold up to 150 kg/ha.

Unlike phosphorus and potassium, **N** is not retained in the soil from year to year in a form that grasses can readily use (Taylor ***et al.,*** 1997). N supplied to the soil is rapidly converted to nitrate-nitrogen and is then often incorporated into organic materials, leached out of the rooting zone by soil water or lost back to the atmosphere by denitrification (Savoy, 1996). Denitrification occurs in waterlogged soils due to poor drainage which then causes the nitrate-nitrogen to be converted back to gaseous forms and be lost to the atmosphere (Taylor ***et al.,*** 1997). Grasses respond quickly to **N** when other growing conditions are favourable. The rate of application and the source of the **N** are factors that need to be considered when coming up with an **N** supply programme for forage and seed production. According to Ihsan ***et al.*** (2014), the rate of application and the source of the **N** depend upon the time of the year and weather conditions. Based on these conditions, one source may be better than others in a specific circumstance.

Unlike fertilization for most field crops, pasture fertilization management is more often guided by the purpose of the grass. If the target is to increase forage biomass, higher rates of **N** can be used. If the target is seed production, higher rates may increase herbage yield and compromise seed yield. Developing an **N** fertility programme is an important aspect that can, therefore, affect the seed yield and forage quality of pasture grass. The understanding of how quickly the **N** is released from the source helps to determine the frequency of application and appreciate the dangers therein. This is why pastures must be strategically managed through well-planned fertilization management programmes.

2.5 Possible sources of N for pasture fertilization

The possible sources of N for fertilizing forage grass pastures are legumes, inorganic (chemical/artificial) fertilizers from industry and organic manure from plant and animal material.

2.5.1 Legumes

N can be supplied to forage grasses by over-sowing the pastures with legumes such as clover, *siratro* and alfalfa, which can fix N directly from the atmosphere (Brummer, 2009). These legumes use N fixing bacteria in their root nodules. Intercropping of *Cenchrus* pasture with a legume crop, as a source of N, increased the dry matter yield by 5 times and the same result was obtained when *Dolichos* and cowpeas were used as legumes (Sahoo ***et al.,*** 2015). A combination of 1:1 of grass and legume was found to be most appropriate for establishment of grass-legume pasture (Sahoo ***et al.,*** 2015). The maximum dry forage yield was only obtained under normal rainfall conditions. The only drawback is that the moisture stress-tolerance of the *C. ciliaris* becomes less of an asset as sufficient moisture has to be availed for the legume. The other problem with intercropping C. ciliaris with a legume is when the grass is needed as a pure stand for use in feed formulations or for research experiments. The use of machines to harvest the grass becomes quite difficult if it is intercropped with a legume. In forage systems without legumes like seed production, nitrogen has to be added as a fertilizer material to achieve the best forage grass seed production.

2.5.2 Inorganic fertilizers

Historically, Ammonium Nitrate (33.3% N), Ammonium Sulphate (21% N) and Urea (46% N) have been the major inorganic sources of N for grass pastures. Ammonium Nitrate is no longer used on a large scale due to its explosive nature (James, 2010). Ammonium Sulphate is very expensive on a cost *per* kg of N basis (James, 2010). It also has a relatively high salt index and greater acidification potential *per* unit N applied than other ammonium-containing N sources (Donaldson and Rootman, 2010). It also has a fairly low N content. Urea is one of the generally cheaper sources of N on a cost *per* kg of N basis. When using irrigation, too much water can lead to movement of N beyond the root zone since it is so mobile in the soil. Losses through denitrification can also occur under waterlogged conditions (Brummer, 2009).

These inorganic fertilizers are quick release sources of N. Some of the N that they release is taken up by grasses and stored as non-protein N. Forage grasses are sponges for N and will quickly take up any available N once it moves into the soil (James, 2010).

2.5.3 Organic manures

Organic manures have a long **history of use in farming systems**. The nutrient supplementation through organic sources in pasture is attributed to better availability of nutrients and improved soil bulk density (Monroe, 1996). Organic manures also have solubilising effect on fixed forms of other nutrients and, therefore, improve the soil fertility (Kumar ***et al.*****,** 2007).Chicken manure (broiler litter) results in building of soil phosphorus and potassium levels. There is an increase in soil pH as was observed in the Kentucky studies (Monroe, 1996) and this is attributed to the high base content of the broiler litter. This is said to be desirable at the initial stages but has long term deleterious effects. Hassan ***et al.*** (2005) reported a dry matter forage yield of 5.905 t/ha of *C. ciliaris* fertilized with urea and 5.430 t/ha due to chicken manure fertilization. Goat and cattle manure are so much on demand in arable crop production, which is normally given first priority over pasture fertilization. In this context, horse manure, which is normally regarded as a heap of rubbish that needs to be discarded elsewhere, may be the solution.

Horse manure is a mixture of horse dung, used bedding and urine soaked in the bedding (Hadin ***et al.,*** 2017). It is often regarded as a waste to be disposed of rather than a valuable fertilizer resource and a necessary by-product of the livestock industry. It is the technology involved in the treatment and use of this 'waste' that determines whether it becomes a valuable resource or a costly liability that just needs to be removed from stables as it produces unhealthy ammonia fumes as well as providing a fertile ground for moulds, bacteria and other parasites (Yossin and Ibrahim, 2013). A horse weighing 400-600 kg excretes 19-30 kg dung and urine per day, on average, containing 70-150 g N, 10-30 g P and 20-50 g K (Keskinen ***et al.,*** 2017). The quality of horse manure depends on the feed ration of the horses, amount of litter, bedding or soil included on the stable floor and amount of urine concentrated with the manure. Keskinen ***et al.*** (2017) reported that when pelleted straw is used as bedding material, the horse manure that results has increased ability to retain nitrogen and phosphorus under rainfall. The horse manure will have a carbon: nitrogen ratio of less than 15. In fresh horse manures, carbon: nitrogen ratios normally exceed 30 and this leads to net nitrogen immobilization in the soil (Keskinen ***et al.,*** 2017) Drying the manure concentrates the nutrients in it on a weight basis (Yossin and ibrahim, 2013). When properly dried, horse manure is a value added resource that contains both major and trace elements. The drying should reduce the moisture content from around 80% to about 10% (Yossin and Ibrahim, 2013). This shows how important storage and handling are in quality control.

The digestive system of a horse is such that its hindgut contains an active population of bacteria and protozoa (Jones, 1985). The microbes synthesize amino acids in the large intestines, but essential amino acids are not absorbed in any appreciable quantities from the hindgut (Jones, 1985). This means that, unlike in ruminants, large quantities of microbial protein generated in the large intestines of the horse are wasted because there is no opportunity there for significant absorption of amino acids (Donaldson and Rootman, 2010). It follows then that these amino acids could be lost in the horse droppings. The microbial protein that could be egested can be put to good use by fertilizing pasture grass. Some research indicates that the use of horse manure may result in the build-up of phosphorus and potassium levels (Keskinen ***et al.,*** 2017; Ogren, 2013 and Ogren ***et al.,*** 2014). An increase in soil pH is usually observed and is attributed to the base content of the horse manure (Keskinen ***et al.,*** 2017). For the short term, this is very desirable as many of pasture or hay systems suffer from the effects of soil low pH (Savoy, 1996). The N compounds in horse manure are eventually converted to the available nitrate form (Ogren, 2013). The nitrate is soluble and is moved into the root zone with water (Ogren, 2013). The release of this available N from horse manure during decomposition is very gradual. This slow release of N is the horse manure's greatest asset. The horse droppings are more fibrous in texture compared to goat, sheep and cattle manure. This could explain the slow nutrient release nature of the horse manure as the nutrients are tightly embedded into the fibrous matrix (Monroe, 1996).

According to Savoy (1996), the fibrous nature of the horse manure ensures that the nitrate nutrients are held into the fibre matrix and released slowly. This extends N availability but in measured quantities that reduce the manure's burn potential. The importance of this in sandy soils cannot be overemphasized. The release of N steadily over a long time by the horse manure requires that its application to the pasture is not frequent. This is cost effective. The nutrients from horse manure are less likely to leach into underground water compared to nutrients from fast release fertilizers. Horse manure contains grass and grain fibres, minerals, fat, water and grit (Ogren, 2013). It is not as smelly as that of other non-ruminants. Most people do not find it overly offensive. According to Mudenda and Maeresera (2009), organic manure, of which horse manure is one, increases the water holding capacity of sandy soils. This is important in that the sandy soils would retain the moisture long enough for the forage grasses to use.

Available moisture and available soil N are closely related because both move in the soil. N would be moved to the roots as the plants absorb water. Nutrients are retained in the humus from decomposition of horse manure to the extent that they are not leached out of the rooting zone of pasture grasses. This is important in that it reduces the effect of leached nutrients to the environment through *eutrophication*, which can result in the death of marine organisms (Mudenda and Maeresera, 2009). According to Brady and Weil (2002), horse manure increases the *cation* exchange capacity of the soil. This is the capacity of the soil to hold onto *cations* like Ca^{2+}, Fe^{2+}, Zn^{2+}, Cu^{2+} and others. These *cations* are held by the negatively charged organic matter particles in the soil through electrostatic forces and they become less susceptible to leaching (Yossin and Ibrahim, 2013). Since the N is released steadily over a period of time, there are less chances of N over-supply that can result in reduced seed yield (Keskinen ***et al.,*** 2017).

Horse manure loosens any heavy clay soils and increase their drainage and aeration. This prevents the conversion of nitrate-nitrogen to gaseous forms which get lost to the atmosphere by denitrification (Mudenda and Maeresera, 2009). Horse manure, like most organic manures, moderates soil temperature in the pasture (Mudenda and Maeresera, 2009). The soil temperature should not fluctuate widely between very hot and very cold times of the day. This would not be conducive for microbes involved in processes like N fixation, root nodulation in legumes, ammonification, nitrification and for the process of root respiration (Donaldson and Rootman, 2010). Horse manure result in the proliferation of soil organisms that are involved in decomposition of any new organic matter from leaf fall and the more these organisms in the soil, the richer the soil for forage production (Donaldson and Rootman, 2010). Horse manure promotes the formation of a crumb structure which enhances seed germination and growth of pasture grasses (Abedelrahman, 2007). The larger bulk that must be handled and spread is a definite disadvantage of horse manure. For just a hectare of pastureland, a large quantity of horse manure is required as the nutrient concentration is low. According to Eriksson and Hennessy (2015), the amount nutrients in horse manure depends on whether the horse is sedentary or exercising. On average, horse manure that is from a sedentary horse and managed by composting contains 4.3% K, 3% Mg, 0.3-1.2% Na and 3.8% N (Eriksson and Hennessy, 2015). Decomposing microbes in unmanaged horse manure absorb released N to satisfy their growth requirements, resulting in a high C: N ratio (Trottier ***et al.,*** 2016). Due to N immobilization, horse manure is not a desired fertilizer for forage production but for seed production (Trottier ***et al.,*** 2016). Its fibrous nature makes it attractive to termites that may end up attacking the forage grass seedlings.

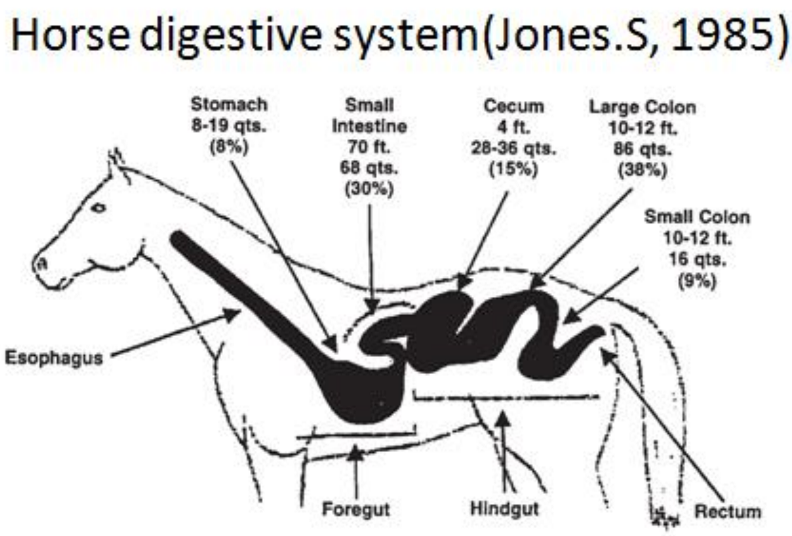

Figure 1: Digestive system of a horse where microbial protein synthesis and digestion occur after the main absorptive region of the small

The digestive system of a horse is typical of a non-ruminant. Microbial fermentation of cellulose occurs in the caecum and colon (Cubitt, 2010). The microbes that are in the caecum are able to use non-protein N to build their bodies. This microbial protein synthesis takes place in the hindgut, as shown in **Figure** 1. Most of this microbial protein is most likely to be flushed out since there is limited absorption of proteins in the hindgut (Cubitt, 2010). Unlike in horses, in ruminant, microbial protein synthesis occurs in the rumen. When the microbes are killed by the acid in the abomasum they are digested and absorbed in the small intestines. This means that more microbial protein would be utilized by a ruminant than that which would be lost in the faeces. Notwithstanding the differences in diets and selectivity, horse manure is expected to contain more nitrogen than ruminant faeces. However, some ruminants like goats are selective browsers which normally feed on highly nutritious and digestible parts of browse shrubs and grasses and this causes an increase in the nitrogen of their faecal matter (Cubitt, 2010).

2.5.4 Urea

Urea (46-0-0) is usually a cheaper source of N on a net N basis compared to other single element N fertilizers (James, 2010. It is non-combustible and non-explosive which make it easy to store. Its high analysis of 46% nitrogen helps reduce handling, storage and transportation costs over other dry N forms. According to Marinari ***et al.*** (2000), urea can be applied as a solid or solution to certain crops as foliar spray. When applied as a foliar spray, its availability to the forage grass is not affected by the soil conditions like pH. During manufacturing of urea, there are less pollutants that are released to the environment (Marinari ***et al.,*** 2010). Urea is a quick release N source which is very soluble, fast acting and gives a rapid green-up response in forage grasses (Marinari ***et al.,*** 2000).

James (2010) reported that when field applied, urea changes to ammonium hydrogen carbonate due to the activity of the enzyme urease. The process releases hydroxide ions which raise the soil pH to as high as 8.5. At this alkaline pH, the ammonium ions $(NH_4)^+$ tend to convert to ammonia gas (NH_3). This is why when urea is applied on the soil surface and not incorporated into the soil, it is subject to volatilization loss as ammonia. Savoy (1996) reported some research findings which showed that the potential for nitrogen loss as ammonia gas increases as temperature, soil pH and moisture increase and as rate of application increases. In fact, Redfearn ***et al.*** (1990) said that N volatilization losses from urea may be as high as 50% under a combination of high humidity, hot temperatures and windy conditions. To reduce volatilization losses from urea, it is best to apply the fertilizer later in the day when the dew has dried. It is also important to apply it no more than seven days to an anticipated precipitation event (Redfearn ***et al.,*** 1990).The urea needs to be incorporated into the soil. James (2010) explained that if the urea – NH_4^+ reaction takes place in the soil, the nitrogen will be captured as exchangeable ammonium on the soil exchange complex and little, if any, ammonia gas would be lost to the air. This, however, could be a challenge when establishing pasture grass like *C. ciliaris*. During pasture grass seedling establishment, a rapid pH increase after urea application caused by hydrolysis of urea can result in ammonia release that can damage seedlings (Redfearn ***et al.,*** 1990). After applying urea, thorough watering is needed as the urea compounds, if not dissolved, remain in the root zone of the grass seedlings, causing burning. Another shortfall of urea is that it does not supply any other nutrients apart from nitrogen. If soil analysis shows that a number of nutrient elements are deficient, the use of urea becomes counter-productive.

2.6 Effect of N fertilization on the nutritional quality of *C. ciliaris* at seed harvesting stage

As far as the chemical composition and digestibility of the grass after seed harvesting are concerned, Hassan ***et al.*** (2015) reported that when N fertilizer was applied to *C. ciliaris*, the hay after seed harvesting produced a range of 8.5-10.6% ash, 0.9-2.5% N, 38.5-45.4% crude fibre and 53.8-70.5% In Vitro digestibility. Unfertilized *C. ciliaris* produced 8.5-10.1% **Ash**, 0.6-0.9% N and 42.9-44.7% fibre. According to Al-Dakheel (2015), at the seed harvesting time, *C. ciliaris* has a CP ranging from 4-6%, ADF of between 36.6% and 47.7%, NDF ranging from 66.5% and 77.6% and **Ash** of between 9.4 and 16.7% dry matter. Ashraf *et al*. (2013) reported that *C. ciliaris* fertilized with horse manure had 37.34% crude fibre, 0.3% sodium, 4.7% potassium and 13-17.5% protein at seed harvesting stage. Donaldson and Rootman (2010) reported results that showed *C. ciliaris* with protein content ranging from 6-16%, crude fibre of 38.5-45.4%, In Vitro digestibility of 53.8-70.5% and NDF of 65% when fertilized with urea. Brady and Weil (2002) said that fertilizing grasses with nitrogen often substantially increases crude protein levels in the forage but has little or no effect on digestibility. In fact, fertilization with phosphorus, potassium or other nutrients that increase yield was said to actually slightly reduce forage quality (Brady and Weil, 2002). Excessive levels of some elements like potassium in some cases were found to decrease the availability of other elements such as magnesium and calcium (Keskinen ***et al.,*** 2017; Ogren ***et al.,*** 2014; Eriksson and Hennessy, 2015).

Maturity at harvest of seeds has the greatest influence on NDF digestibility. The dry matter digestibility of forage grasses decrease with maturity. According to During and McNaught (2012), as forage matures, NDF digestibility can decline significantly. Tailor ***et al.*** (1997) explained that with advancing maturity, plants develop xylem tissue for water transport, accumulate cellulose and other complex carbohydrates which become bound together by a process called lignification. Lignin is more difficult to digest than either cellulose or hemicellulose. Again, as maturity proceeds, leaf- to- stem ratio declines and this causes NDF digestibility to decline. Parwani ***et al.*** (2009) explained that during the forage maturation process, accumulation of the stem mass exceeds leaf mass addition. The stems contain a higher proportion of thick-walled tissues of sclerenchyma, xylem fibre and xylem vessels and less photosynthetic tissues of mesophyll and chlorenchyma than found in leaves (Tailor ***et al.,*** 1997). Lignin concentration in forages has been reported to be negatively correlated with digestibility of forages (Parwani ***et al.,*** 2009). It would, therefore, be interesting to find out the level of lignification that would have taken place in the forage by the seed harvesting time and also the influence of applying either urea or horse manure as inorganic and organic fertilizers respectively. The amount of NDF and ADF can be used to predict the digestibility of the forage and its general utilization potential (During and McNaught, 2012).

CHAPTER 3: COMPARATIVE ASSESSMENT OF THE EFFECTS OF FERTILIZING C. CILIARIS WITH HORSE MANURE VERSUS FERTILIZING WITH UREA ON THE SEED YIELD AND FORAGE BIOMASS PRODUCTION

3.0 Introduction

Livestock production in rural Botswana is **hindered** by low quantities of high quality forage grasses. Most farmers in these rural areas depend on natural rangelands for the grazing of their livestock. For more than 6 months in a year, there is shortage of pasture during the dry period. Strategy by farmers to sustain their livestock during these difficult times is to supplement animals with grass, crop residues, legume forages or commercial energy and protein supplements or concentrates. Most of the time, these supplements are imported from South Africa, especially grass, legume forage and concentrates. Due to the bulkiness of grass, its importation from South African over long distance may not make **economic sense**. Ideally, farmers should be buying bulky roughage feeds locally or grow them to reduce both transport and environmental costs. However, challenges of establishing pastures on a commercial scale are numerous.

According to Nsinamwa ***et al.,*** (2005), it has been very difficult to improve the range through introduction of more valuable and productive forage grasses due to unavailability of planting material in the form of seed. *C. ciliaris* is gaining attention in various fields of research (Akiyana ***et al.,*** 2005; Al-dakheel ***et al.,*** 2015; Arshadullah ***et al.,*** 2011; Bulle ***et al.,*** 2011; Ihsan ***et al.,*** 2014) as **it is quite thriving and productive** under conditions of high temperatures, solar radiation and low moisture. The grass is more efficient **at absorbing, storing and utilizing carbon from the atmosphere** and recycled nitrogen from the soil (Monroe, 1996). The isolated clusters of valuable forage grass like *C. ciliaris* cannot provide sufficient tussocks for vegetative propagation (Monroe, 1996). In natural rangeland, vegetative propagation of the grass would almost be impossible due to **the amount of work that would**

be involved in terms of land preparation and the whole planting process. There are no seed banks for such better performing forage grasses. There is, therefore, the need to research into the possibility of producing forage grass seeds cheaply and abundantly to improve the quality of the grazing areas, even in rural Botswana. These can be achieved by seeding natural pasture or planting the grass on farms. The growing demand for pasture grass seeds locally and in neighbouring countries necessitates researchers to respond by trying to bring the pasture seed industry to a functional level. After having managed to produce seed, the next challenge would be to use it for over-sowing in the rangelands in order to raise the quantity of the available forage. With growing professionalism and experience in seed production by researchers, farmers can then be incorporated.

The use of N fertilizers has been shown to increase the seed and forage biomass yield of *C. ciliaris* (Afzal and Ullah, 2007). N has been found to be the most limiting factor to seed production and biomass yield of *C. ciliaris* (Koech ***et al.,*** 2014). Most soils in Botswana lack **N** mainly due to low levels of organic matter and leaching from the mostly sandy soils (Tacheba and Moyo, 1985). *C. ciliaris* has, however shown to respond well to improved **N** supply. According to Kizima ***et al.,*** (2012), there is need for a constant supply of moderate amounts of **N** for significant increase in seed production to be realized. This is because *Cenchrus* grass stands become unproductive with time as N is tied up in the root system of the forage grass (Bulle ***et al.,*** 2011). According to Burhan and Hago (2000), N enhances the vegetative growth of *C. ciliaris*. It increases the stem: leaf ratio. The increase in vegetative material is, however, detrimental when the aim is to produce seed. When a lot of energy is channelled towards vegetative growth, less is used in seed production. On the other hand, if a plant does not develop sufficient leaves, it would not produce enough food to store in the seed and this compromises the quality of the seed. Establishment of pastures for seed production, therefore, requires enough agronomic information and technical support so that **N** is not under or over-supplied.

The present research seeks to make comparisons between horse manure and urea as sources of **N**, with reference to their effect on the **quantitative parameters** of *C. ciliaris*. This is important in that an increase in the yield of forage grass goes a long way in supporting the livestock sector in the country.

3.1 Specific objectives

To evaluate the effect of the type of N source (horse manure versus urea) on the length (cm) of the inflorescences, the number of above ground tillers per crown (tiller density), seed yield and biomass production of *C. ciliaris*.

3.2 Hypothesis

$H0$: There is no significant difference in the inflorescence length, tiller density, seed yield and forage dry matter yield of *C. ciliaris* produced when using horse manure and urea as fertilizer treatments and no fertilizer as a control.

$\boldsymbol{H0}$: $\mu\ \boldsymbol{horse\ manure/urea\ =\ \mu\ control}$

Ha: There is a significant difference in the inflorescence length, tiller density, seed yield and forage dry matter yield of *C. ciliaris* produced when using horse manure and urea as fertilizer treatments and no fertilizer as a control.

$\boldsymbol{Ha}$: $\mu\ \boldsymbol{horse\ manure/urea\ \neq\ \mu\ control}$

3.3 Materials and Methods

3.3.1 Site description

This **study** was carried out at Livingstone Kolobeng College in Block 8, Gaborone. It is located on **24.6020º S and 25.9067º E**. The site of the experiment was a school garden that measured 60m by 40m.**The average annual rainfall in Gaborone and surrounding areas is 550 mm of which normally falls between November and March (Burgess, 2006). The rainfall is, however, extremely variable from year to year. The mean maximum temperature is 28.6^{0}C in summer and the minimum is 12.8^{0}C in winter** (Burgess, 2006). The soils in the site showed a dominance of the sandy fraction and it was light in colour, showing low levels of organic matter. The study area was a previously cultivated land that just needed to be turned over and levelled.

3.3.2 Soil sampling and testing

At the beginning of the investigation, 8 soil samples were taken randomly from the experimental site at a depth of **10 cm** using an auger. The top soil is the one that is critically important during the root establishing stage of the cuttings. The collected soils were mixed together to make a composite sample. A 300 g subsample of the composite soil was taken to Botswana University of Agriculture and Natural Resources (BUAN) for analysis using procedures of the AOAC **(1996). Total N**, phosphorus, and cation exchange capacity (CEC) were determined. Soil pH was determined using the Universal indicator method and was found to be around 6. The soil was disaggregated using a wood pestle and sieved at 2 mm and was ready for digestion and testing.

3.3.3 Experimental design

A completely randomized design (CRD) with two fertilizer treatments (horse manure and urea) and a control treatment (no fertilizer) was used for this research. **Each treatment and the** control were replicated 6 times, giving a total of 18 plots. Each plot was 3 m long and 1.2 m wide, giving an area of 3.6 m2. The inter-plot distance was 0.6 m. For assigning plots to treatments, 18 pieces of paper were used, with each of the three treatments inscribed on 6 of the pieces of paper. The pieces of paper were put in one box and shuffled around. The plots were numbered 1 to 18. For every plot number, a piece of paper was picked from the box and the treatment inscribed on that paper was the treatment for that plot.

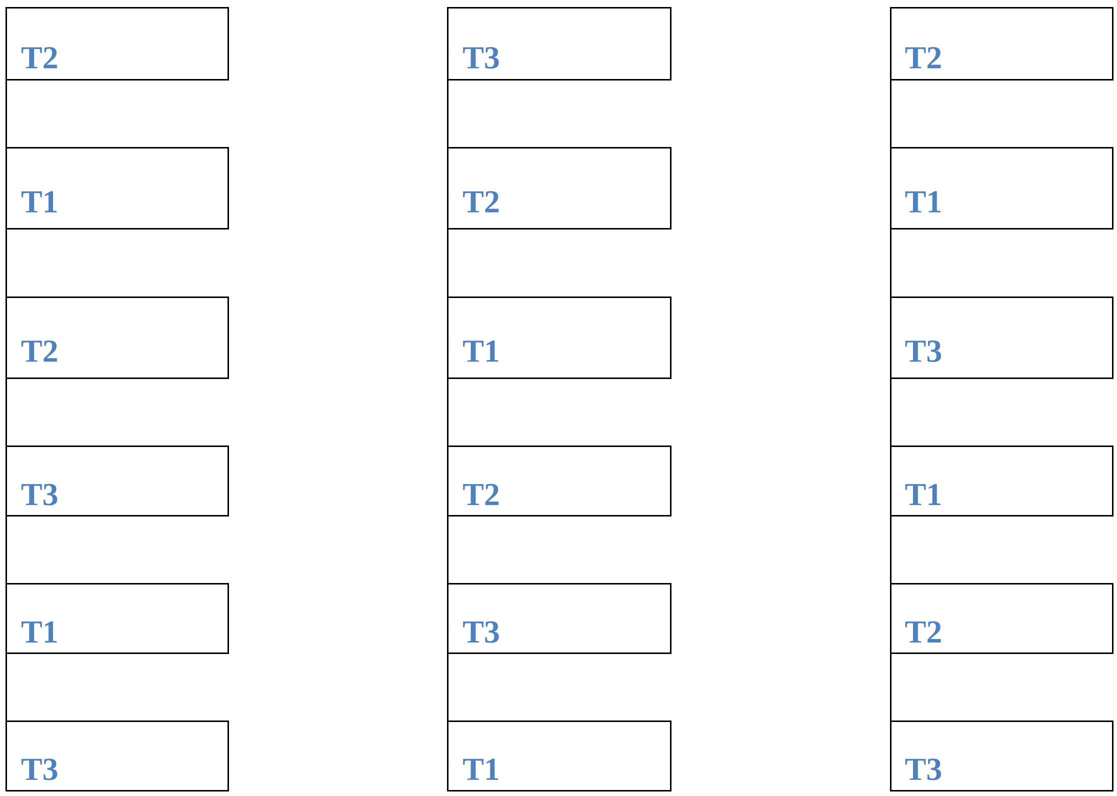

Figure 2: Arrangement of plots in the experimental garden

3.3.4 Land preparation, fertilizer application, planting and management practices

Plots were marked using pegs and a garden line. Ridges were made using a spade and a rake and digging was done to an average depth of 20 cm using a spade. A pick was used for breaking up hard ground. Horse manure was applied to six (6) of the plots at a rate of 12 t/ha and was incorporated into the soil using a digging fork, before planting. Urea was applied to 6 more plots at a rate of 100 kg/ha and was worked into the soil. The fertilizer application rates used in this study for horse manure were as recommended for light textured soils by Mujuni and Sibanda (2007). The remaining six plots did not receive any fertilizer. Thirty (30) well developed tussocks (vegetative splits/cuttings) were planted in each plot in three (3) rows, with ten (10) tussocks *per* row. The seedling tussocks were obtained by separating *C. ciliaris* clumps into individual splits ready for planting. The seedling tussocks were obtained in the bushy area behind Choppies supermarket in Block 8 in Gaborone. The upper parts of the tussocks were cut off to leave them **25 cm** in length. No rooting chemicals were used. The planting depth was **10 cm**, with 3 nodes set into the soil, leaving **15 cm** above the ground. A transplanting trowel was used for planting. The inter-row spacing was **40 cm** and the intra-row spacing was **30 cm**. Routine operations of irrigation, weed control and pest control were carried out. **Irrigation was done at 3 day intervals. Weeds were controlled mainly by hand pulling. Malasol was the main pesticide used for pest control.**

3.3.5 Data collection

Response variables were number of tillers per crown (tiller density), seed yield, length of inflorescence, forage fresh biomass yield and forage dry matter yield. After four months, the number of tillers per crown were counted. The lengths of inflorescences were measured using a ruler. Seed heads were harvested manually, air-dried, put in bags per plot and weighed. All these variables were measured for the middle row and the results were multiplied by three since there were three rows in each plot, to give the results per 3.6 m^2. This was used to extrapolate the seed head yield per hectare. **The middle row was used in order to reduce the edge effect.** The seeds were extracted from crushed seed heads through a combination of sieving and air-blowing until most of the chuff was removed. To estimate the mass of seeds *per* unit weight of seed heads, five samples of seed heads weighing 250 g were crushed by putting them in a sack and thrash them with a log before sieving the chuff off and weighing the seed. The average weight of the five samples was then used as the weight of seed *per* 250 g of seed heads. This was then used to extrapolate the **seed yield in kilograms per hectare**. Soon after seed harvesting, the above ground plant material was clipped at the base of the plant, cut into shorter pieces, put into pre-weighed khaki bags and weighed to obtain the forage yield *per* plot. The result was used to extrapolate the forage yield in kilograms *per* hectare. Forage material from different plots under the same treatment was mixed together and a sub-sample was then taken from the composite sample for laboratory tests. The sub-samples were placed in weighed and labelled khaki bags. After weighing them, they were oven-dried at 60^0C for 48 hours at BUAN Animal Nutrition Laboratory. The oven-dry weights were used to calculate dry matter (DM) yields which were extrapolated to kg/ha.

Figure 3: Inspecting the horse manure upon its delivery.

Figure 4: Fertilizer application

Figure 5: Planting of the vegetative splits

Figure 6: Appearance of tussocks after one week.

Figure 7: Appearance of the grass after 22 days

Figure 8: Top dressing grass with urea after emergence of the first inflorescence

Figure 9: Appearance of the grass after 2 months

Figure 10: Pests of grass seeds

Figure 11: Appearance of C. ciliaris after 3 months

Figure 12: Counting the number of tillers per crown

Figure 13: Measuring the length of the inflorescences

Figure 14: Harvesting seed heads

Figure 15: Weighing the seed heads

Figure 16: Cutting the vegetative material for weighing.

Figure 17: Measuring the forage biomass

Figure 18: Air-dried seed heads

Figure 19: Oven drying the vegetative material

3.4 Statistical Analysis

To compare the significant differences in response variables, ANOVA analysis was done using Procedures for the Generalized Linear Models (PROC GLM) of Statistical Analysis Systems of 2004. Data was analysed as a completely randomized design with three treatments and in a completely randomized design with 18 plots, 6 plots for each of the 3 treatments. The effects of treatments were considered significant at $P < 0.05$. When means were significantly ($P < 0.05$) different, multiple comparison of means was conducted using the least squares means separation which was performed using the PDIFF option (SAS 2004) to evaluate the significance and magnitude of the fixed effects at $P \leq 0.05$.For statistical analysis, the following model was used:

$$\boldsymbol{Yij} = \mu + T_i + \boldsymbol{\varepsilon}ij$$

where:

$\boldsymbol{Yij}$ = *Response variable*

μ = *Population mean*

$\boldsymbol{T}_i$ = $_i$ ***th*** *Treatment effect*

$\boldsymbol{\varepsilon}ij$ = *Random error* $\sim \boldsymbol{N}$ $(\mathbf{0}, \sigma\mathbf{2})$ (*population mean, variance*)

3.5 Results

Table 3.1: Soil analysis results

Soil analysis results are presented in Table 3.1 below. The results showed that the soil N was lower than the requirements for most forage grasses.

Parameter	**%**
Nitrogen	**3.92**
Phosphorus	**0.36**
Cation Exchange Capacity	**8**

Table 3.1 above shows the results from analyzing the soil from the experimental site.

3.5.1 Length of inflorescence

There was no **significant ($P > 0.05$) difference** in the average length of inflorescence between horse manure or urea and the control treatment

3.5.2 Tiller density

The results of this study showed that tiller density did not **significantly ($P > 0.05$) differ** between the two fertilizer treatments, but both fertilizer sources resulted in **significantly ($P < 0.05$) higher** tiller density than the control treatment

3.5.3 Seed yield

Both horse manure and urea resulted in a **significantly ($P < 0.05$) higher** seed yield than the control treatment. Generally, horse manure yielded a **significantly ($P < 0.05$)** higher seed yield than urea fertilizer.

3.5.4 Forage biomass yield

Urea produced a **significantly ($P < 0.05$) higher** fresh biomass and dry matter yield than both horse manure and the control. Horse manure produced a **significantly ($P< 0.05$) higher** dry matter yield than the control.

Table 3.2: Effects of fertilizer treatments on agronomic parameters of *C. ciliaris.*

	Parameter				
Treatment	Tillers per crown (n)	Seed Yield (kg/ha)	Fresh Biomass Yield (kg/ha)	Forage Dry Matter Yield (kg/ha)	Length of Inflorescence (cm)
No fertilizer (Control)	23.67^{b}	11.07^{c}	4346.14^{c}	1627.82^{c}	10.96^{a}
Horse Manure	30.00^{a}	18.46^{a}	5447.44^{b}	2237.85^{b}	11.23^{a}
Urea	31.67^{a}	14.51^{b}	6398.53^{a}	2739.62^{a}	11.02^{a}
P-value	0.0013	0.0001	0.0002	< 0.0001	0.65

Treatment (column) means with different superscripts significantly ($P < 0.05$) differ.

3.6 Discussion

3.6.1 Length of inflorescence

There was **no significant ($P < 0.05$) difference** between either of the two fertilizer treatments and the control treatment in average length of inflorescence. There was also **no significant ($P < 0.05$)** difference between horse manure and urea. This could be explained in terms of the genotypic effect. Since one variety (Molopo) was used, the genetic expression of inflorescence length could not show significant differences between fertilizer treatments and the control and between the two treatments themselves. Even the nutrients that were inherently present in the soil (control treatment) were sufficient for the inflorescences to fully grow. Although the differences were insignificant, the average length of inflorescence from the use of horse manure was slightly longer than the use of urea and the control. The inflorescence length has an influence on seed yield. This could have contributed to the differences that were observed in this study in seed yield where fertilizing with horse manure produced a higher average seed yield than urea.

3.6.2 Number of tillers per crown (tiller density)

There was a **significant ($P < 0.05$) difference** between horse manure and the two fertilizer treatments and the control in terms of number of tillers per crown of *C. ciliaris*. This could have been due to the high N concentration in urea (46% N) and the sustained supply of N from the slow releasing horse manure. This concurred with the findings of Laidlaw (2005) where tiller densities varied with fertilizer application. High levels of nitrogen cause vigorous sprouting of vegetative material (Mganga, 2009). A sufficient supply of N makes the grass to recruit more tillers. In addition to adding N to the soil, urea can also increase the vegetative sprouting of grasses through its effect on soil pH. When urea dissolves in soil water, it raises the pH of the soil and this is crucial in sandy soils which are usually acidic. According to

Donaldson and Rootman (2015), soil pH values of between 5.5 and 6.5 enhance tillering of forage grasses. This is important because tillering is an important attribute of forage grasses as it increases the chances of survival and the amount of available forage for livestock (Laidlaw, 2005). When forage grass produces a large number of tillers, such grass normally attains maximum growth at an earlier age and recovers fast after defoliation (Laidlaw, 2005). Tillering also has an influence on leaf-area production and dry matter yield (Kumar ***et al.,*** 2005; Tailor ***et al.,*** 1993; Kizima ***et al.,*** 2012). In this regard, it determines photosynthetic rates and act as food reserves. According to Mganga (2009), a high rate of tillering complements both forage yield and resilience of a grass under defoliation. It also contributes to the effectiveness of the grass towards soil conservation through increased ground cover. This may be important for forage grasses that have to be grazed in-situ and those used for soil conservation and land rehabilitation but not so important for grasses that are being produced for seed production. Ordinarily, the more the tillers the more the inflorescences and hence the expected seed yield as tillers produce inflorescences which bear the seed.

This study, however, produced results to the contrary. Fertilizing with horse manure, which produced less tillers than urea, yielded more seed by weight, though the weights did not differ significantly. It would seem as if too much vegetative material lowered the seed yield. This is in agreement with the findings of Hassan and Fikru (2015) who reported a highly negative correlation between seed yield and tiller density. Such a correlation agrees with the classical trade-off between reproductive and vegetative allocation of resources. The overall treatment mean of 28.44 tillers *per* crown in this study was higher than 13.30 tillers *per* crown reported by Meena (2015) for *C. ciliaris* and this could have been due to the fact that the results reported by Meena (2015) were for a research carried out under rain-fed treatments while this study used irrigation. Lower tiller numbers under rain-fed treatments could be attributed to the response of the grass to some water stress during the growing season. Moisture stress reduces tillering as a way of reducing transpiration losses (Hassan, 2015). Tillering also has an influence on forage digestibility. According to Kumar ***et al.*** (2005), tillers contain leaves which have more easily digestible nutrients and less structural components, especially from newly developed tillers with young leaves. Typically, however, a tiller will have both old and new leaves. The old leaves would be lower in quality but would also contribute to biomass yield. There was no significant ($P > 0.05$) difference between the use of horse manure and the use of urea in terms of tillering. This could mean that fertilizing with either horse manure or urea provided sufficient levels of N in the soil for the tillering of the grass. This means that where tillering is the positive attribute being sought from the forage grasses, a farmer can use either of the nitrogen sources without significantly affecting the tillering. The issue of cost may then need to be considered.

3.6.3 Seed Yield

The future survival and general propagation of forage grass are determined by the ability of the forage grass to produce seed (Ogillo, 2010). This study recorded a **significant ($P < 0.05$) difference** in the mean seed yields between fertilizing with either horse manure or urea and the control treatment and between the fertilizer treatments themselves. The amount of N supplied by the two fertilizers could have been sufficient for causing a significant increase from the control treatment. N is a nutrient that is highly mobile and does not stay long in the soil. The length of time from the previous application of a N fertilizer and the carrying out of this research could have been long enough to result in insufficient amounts of N in the soil. This experiment was carried out 5 months after the last N fertilizer application in the area. Loss of nitrogen from the soil could have been due to leaching, denitrification, absorption by the previous crop or volatilization (Donaldson and Rootman, 2010). When the fertilizers were added, they raised the N content to levels that caused a significant difference in seed yield. The significant difference in seed yield between fertilizing with horse manure and using urea could be attributed to the slow and steady release of nitrogen from horse manure. Nitrogen, a very important nutrient for seed formation, would be available to the grass throughout its growing and seed formation stages and in measured amounts (Koech ***et al.,*** 2014). Due to its fibrous nature, horse manure decomposes slowly and releases its nutrients steadily over a long time. Sufficient N in grass enhances the process of photosynthesis which builds carbohydrates that would then be stored in seeds (Tailor ***et al.,*** 1997).

The slow release means that there is no N over-supply during the seed formation stage. Such an over-supply of N at this stage would result in grass having a more vigorous vegetative sprouting at the expense of seed formation (Hassan and Fikru, 2015). When there is a lot of vegetative growth, there is often a corresponding vigorous growth and development of roots so that absorption of water and nutrients can sustain the above ground material. This means that a lot of energy would be expended on root growth rather than on seed formation. Well established root systems do not guarantee high seed yields but vigorous sprouting during regeneration (Bulle ***et al.,*** 2009).

Urea, on the other hand, supplies N in surges and the N quickly disappears from the soil through absorption, volatilization or leaching. Such losses may cause N deficiencies at critical times in the formation of seeds, resulting in reduced seed yields. Fertilizing with horse manure avails other nutrients like potassium which facilitate formation of starch and its storage in the seeds, while urea only avails N. As an organic fertilizer, horse manure improves the structure of the soil, raising the water holding capacity of the characteristically sandy soils as were the soils in this study. This could have ensured the availability of moisture for a reasonable amount of time after irrigation. Seed formation requires a lot of moisture at the initial stages as the seed itself would be about 90% water by composition at that stage. A similar study by Kizima ***et al.*** (2012) recorded an average seed yield of **77.5 kg/ha**, which was way higher than the average of 14.68 kg recorded in this study. The difference could be explained in terms of higher levels of N that were used than in this study. The different agro-ecological zones in which the experiments were carried out could also be a factor. The results of this research were, however, close to the findings of Ashraf ***et al.*** (2013) of 21.8 kg/ha. Kumar ***et al.*** (2005) reported seed yields of 75 kg/ha at wider spacing of grasses. This could have been because of reduced competition for moisture, nutrients and sunlight. Wider spacing results in better transmission of light to the lower canopy, which results in greater tillering of grass. The seed yield in this study could have been lowered by wild birds. Despite erecting scare-crows and using cassette tape to scare wild birds away, the birds were a constant bother after seed setting.

3.6.4 Forage Biomass Yield

Fertilizing *C. ciliaris* with either horse manure or urea caused a **significantly (P < 0.05) higher** forage fresh biomass yield than the control treatment. This could have been due to the fact that nitrogen makes plants to be hydrophilic (Tailor ***et al.,*** 1993). According to Tailor ***et al.*** (1993), high levels of N in a plant make the plant to absorb a lot of potassium ions from the soil. This lowers the osmotic potential in the plant root hair cells, causing the soil to get into the root hair cells from the soil by osmosis. Due to increased leaf growth, absorption of water from the soil is increased to cater for losses due to transpiration. This results in plant cells to be turgid. The forage fresh biomass may be important in areas where water for livestock consumption is scarce and the grass is fed fresh to the animals.

Ruminant livestock production is directly and overly influenced by the dry matter productivity of forage plants (Hare ***et al.,*** 2009). Fertilizer regimes that yield the highest dry matter should be used when forage production is for livestock feed but a compromise may need to be reached when the aim is to produce seed. This study showed a **significant (P < 0.05) difference** in forage dry matter yield between the two fertilizer treatments (horse manure and urea) and the control treatment. This could have been because of the N added to the soil through the fertilizers. Application of N to the soil increases vegetative (above ground) growth of forage grasses. According to Guiot and Melendez (2003), high N content in the soil results in large sized leaves and thick but soft stems in forage grasses. This causes an increase in forage dry matter. When there is sufficient N, chlorophyll formation is enhanced and more photosynthesis takes place. The presence of carbohydrates (from photosynthesis) and N (absorbed from the soil) in a plant results in the formation of amino acids which are precursors for protein synthesis which contribute to the increase in the dry matter yield. Some of the proteins formed would be growth hormones such as auxin. Auxin causes an increase in the above ground growth (shoots), hence contributing to an increase in forage dry matter yield. The leafy nature of forage grasses might, however, be retrogressive in dry areas where water supply is limited, as it speeds up water loss through transpiration (Guiot and Melendez, 2003).

This study recorded a **significantly ($P < 0.05$) higher** forage dry matter yield when fertilizing with urea than using horse manure. This could be attributed to the high concentration of N in urea (46%). The N in the horse manure could have been released slowly and steadily over a long time and as such, may not have been sufficient at certain crucial times. Fertilizing with horse manure produced a lower forage dry matter yield probably because of weed pressure. There were always a problem of weeds on plots that were treated with horse manure. This could be due to weed seeds that were ingested by the horses, passed through the digestive system intact and were present in the horse droppings. As far as the results of this study are concerned, fertilizing with urea would be more preferable as a source of N for forage grasses if dry matter yield is the most desirable attribute and not the seed yield. A very high N concentration in the soil due to urea application can, however, cause a greater risk of nitrate-nitrogen concentrations exceeding 1000 ppm. Such high nitrate levels may affect animal health (Donaldson and Rootman, 2010). As such, a steady supply of moderate levels of nitrogen, such as from horse manure, would be preferable. The mean forage dry matter yields of 2237.85 kg/ha when fertilized with horse manure and 2739.62 kg/ha for urea in this study were higher than the 1913.30 kg/ha reported by Sawal ***et al.*** (2009) when the *C. ciliaris* was fertilized with **75 kg N/ha**. The DM yield of this study was, however, less than an average of 3790 kg/ha when the grass was fertilized with sheep manure and 4210 kg/ha for NPK fertilizer as reported by Meena (2011). This could have been due to differences in soil, climatic and management differences. The control treatment in the results in Meena's report averaged 3345 kg/ha while in this research, it averaged 1627.82 kg/ha. This points to the inherent soil and climatic conditions. The DM yield of this study was, however, quite comparable with the 2420 kg/ha that was reported by Donaldson and Rootman (2010), albeit, without any nitrogen applied. The soil that was used could have been rich in nitrogen from the fertilizers applied for the previous crops.

3.7 Conclusion and recommendations

The findings of this study show that fertilizing with horse manure or using urea as N sources, significantly increases the seed yield, tiller density and forage dry matter yield of *C. ciliaris*. This is important as farmers who may want to venture into seed production can have income from both seed production and forage production from residue after seed harvesting. The results also show that fertilizing with horse manure, which is a cheap resource, causes a significantly higher seed yield than fertilizing with urea. The use of horse manure is, therefore, of greater importance when the objective is to produce seed for the establishment of seed banks.

Fertilizing with urea causes the production of a significantly higher forage dry matter yield than using horse manure. This means that the fertilizer can be of higher value when harvesting seed is not part of the objectives. This is because urea encourages more vegetative growth which compromises seed production. The slow N release by horse manure ensures its measured availability which moderates vegetative growth and enhances seed production. Considering the cost implications and how easily the fertilizer can be obtained, horse manure would be more appropriate for seed production, especially for large scale and rural livestock farmers who may want to propagate *C. ciliaris* in their pastures or establish planted pastures through seed. Propagation of the forage grass using vegetative splits would be quite costly in terms of labour and time. The slow nutrient release by horse manure may also mean that there would be reduced leaching and, hence, less chances of eutrophication in water bodies.

CHAPTER 4: STUDY TWO

Chemical composition and in vitro digestibility of ***C. ciliaris*** var. Molopo forage grass post-harvest residues fertilized with horse manure or urea.

4.0 Introduction

Botswana usually has short rain seasons which make the country experience prolonged dry winters. This poses a constraint to livestock farmers in their management of fodder flow. This can be overcome by using forage grasses that are resilient to dry conditions and do not lose their quality drastically with advanced maturity. When allowed to grow and set seeds, the nutritional value of the grass should not deteriorate to levels that cannot, at least, provide sufficient nutrients for maintenance. Soils in Botswana are predominantly sandy-textured, with very low organic matter content, which results in deficiencies of essential nutrients. According to Joshua (1988), most of the soils in the southern parts of Botswana are predominantly sub-desert soils with low levels of carbon (< 0.5%) and low clay proportion (< 40%). Van Waveren (1988) also reported that the soils in Botswana were generally light textured with low phosphorus content. Grasses that grow in such soils lack the essential nutrients as they can only contain what they absorb from the soil, in terms of mineral nutrients. The low fertility of the soil results in forage grasses of low quality.

N fertilization influences the nutritional value of forage grasses (Hassan ***et al.,*** 2015). According to Ramirez ***et al.*** (2009), the crude protein concentration in forage grasses is influenced mainly by the supply of available N in the soil and the state of maturity of the grass. Nitrogen fertilization has also been shown to increase the dry matter digestibility of *C. ciliaris*. However, there are different sources of nitrogen which may elicit different responses depending on the supply or release of nitrogen to the soil and plants. Information regarding the comparative effects of different sources of nitrogen on the nutritional quality of forage grasses is important. Minerals are required to meet the needs of livestock for optimum development, health and productivity (Monroe, 1996). According to Albu (2012), calcium and phosphorus play a very important role in the growth and development of animals. These two macro-elements should be analysed in combination because the dietary levels of calcium and phosphorus should be balanced to increase their availability and utilization Ogren ***et al.*** (2014). Ogren ***et al.*** (2014) explained that if forage supplies more phosphorus than calcium to a livestock, calcium absorption can be impaired and skeletal malformations, poor growth and muscle disorders can occur. Even if the total diet contains adequate calcium, excessive phosphorus intake may cause abnormalities (Ogren ***et al.,*** 2014).

Even when the objective is seed production, forage residues that remain after harvesting seeds can be a useful feed resource for ruminants and hence the mineral composition in these residues needs to be known. The source of N that results in the increased nutritional quality of *C. ciliaris* after seed harvesting, needs to be found. With the concept of sustainable Agriculture taking traction in Botswana, organic fertilizer will become more important. However, a possible source of organic fertilizer; manure from horses, have not been researched adequately except a few studies in the 1980s by Department of Agriculture Research (APRU, 1985). The two possible sources of nitrogen that this study seeks to compare are horse manure and urea.

4.1Specific objectives

-To determine the chemical composition of *C. ciliaris* forage residues after seed harvesting.

-To determine the in vitro dry matter digestibility of *C. ciliaris* forage residues after seed harvesting.

4.2 Hypothesis

$H0$: There is no significant difference in chemical composition and digestibility (forage quality) of *C. ciliaris* forage grass residue after the use of horse manure or urea as fertilizer treatments and no fertilizer (control treatment).

$\boldsymbol{H0}$: $\mu\, \boldsymbol{horse\ manure/urea\ =\ \mu\ control}$

Ha: There is a significant difference in chemical composition and digestibility (forage quality) of *C. ciliaris* forage grass residue after the use of horse manure or urea as fertilizer treatments and no fertilizer (control treatment).

$\boldsymbol{Ha}$: $\mu\, \boldsymbol{horse\ manure/urea\ \neq\ \mu\ control}$

4.3 Materials and methods

For details of the methods, see sections 4.3.1.1 up to 4.3.2.2

4.3.1 Determination of chemical composition of the forage grass residue

After determination of the dry matter yield for the control, horse manure treated and urea treated, the dried *C. ciliaris* forage biomass samples from the same treatment were mixed together and a subsample extracted from each composite sample for chemical analysis. The subsamples were ground through a 1mm sieve Wiley mill and stored in air-tight plastic bottles in preparation for the analysis.

4.3.1.1 Ash determination

Samples were analyzed for Ash by combustion in the muffle furnace. Ash content was determined using the formula:

$$\%\ Ash = \frac{(weight\ of\ crucible + ash - weight\ of\ crucible) * 100}{weight\ of\ oven\ dry\ sample}$$

4.3.1.2 Determination of phosphorus content and mineral cations

For the determination of phosphorus and mineral cations, the 6 samples for each treatment were mixed together and subsamples taken for testing, in duplicates. The phosphorus content was determined using a spectro-photometer using the Molybdenum Blue Method of Dickman and Bray (1940). Plant sample solutions were prepared by digesting 1.25 g of each of the *C. ciliaris* samples. The blank did not contain any *Cenchrus* material and was used as a correction factor. One ml of plant digest solution was put into a 50 ml glass beaker using a pipette. Thirty ml of chloromolybdic acid working solution were added and gently mixed. One ml of stannous chloride working solution was also added and mixed gently. The spectrophotometer was calibrated and the wavelength set to 670 nm. The absorbance of the blanks and samples were measured at 10 minutes intervals. A possible source of error is the presence of contaminants in water, reagents or glassware. For this reason, a blank was run at the same time as the samples. This meant that if the samples contained the same concentrations of contaminants as the blank then the concentration of the mineral in the blank was subtracted from the value determined for the samples. Mineral cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Cu^{2+}, Zn^{2+} and Fe^{2+}) were determined as per the standard methods described in AOAC (2005) using a Perkin Elmer ICP-Optical Emission Spectrometer Optima 7300 DV Series.

4.3.1.3 Determination of nitrogen content

Digestion, neutralization and titration were all done manually.

4.3.1.3.1 Digestion

The nitrogen of protein was transformed into ammonium sulfate by acid digestion with boiling sulfuric acid. **1.25 g** of ground sample material was weighed onto a piece of lens tissue. The lens tissue was folded carefully and dropped into digestion tube. This was done for all the samples. Two blanks of only lens tissue were also put into their own digestion tubes. 20ml of

98% sulfuric acid and some selenium solution were added to the digestion tubes using dispensation. The tubes were inserted into the digestion blocks and rubbers inserted. The block was switched on and temperature was controlled as follows; 150°C for 1 hour, 250°C for the following hour and 330°C for the last two hours. After a total of four hours of digestion, the block was switched off and was left to cool for 60 minutes. 4 ml of hydrogen peroxide were added to each tube. The tubes were inserted onto the digestion block, the scrubber unit put and the digestion block switched on for 2 hours at 330°C. After 2 hours, the tubes were removed from the block and allowed to cool in the fume cupboard for 50 minutes. The contents of each tube were transferred into a volumetric flask of **250 ml** capacity and 100 ml of distilled water were added. After 24 hours the contents were then made up to volume with distilled water.

4.3.1.3.2 Neutralization and titration

Twenty-five ml of each solution was added into a distillation tube using a pipette and 40ml of 3% sodium hydroxide was automatically added by the distillation unit. The distillation was carried out for 4 minutes. The distillate was collected in 1% boric acid solution and then titrated with standard sulfuric acid. The blank samples were treated in the same way as samples. The nitrogen percentage in the sample was determined using the formula

$$\%N = \frac{\%\,[volume\ of\ standard\ acid\ (cm^3) - volume\ of\ blank(cm^3)]\ x\ Molarity\ of\ acid\ (N)\ x\ 1.4}{Weight\ of\ sample(g)}$$

The conversion of nitrogen percentage to protein percentage was done by multiplying the percentage nitrogen by 6.25 as follows:

$$Protein\ \% = Nitrogen\ \%\ x\ 6.25$$

4.3.1.4 Determination of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF)

The NDF and ADF determinations were done in duplicates. Twelve empty fiber bags were weighed. Two fiber bags were used for each of the three treatments. A sample amount of 0.5 g was placed in each of the two bags per treatment and weighed. This gave a total of 12 sample bags. Two empty fiber bags were included to determine the NDF and ADF in the blank bags for correction. The bags were heat sealed. The NDF and ADF were then determined according to the *AOAC* procedures of 2009 and Van Soest's proximate analysis (1994)

4.3.2 In vitro digestibility determination

4.3.2.1 Animal care and ethics

Animals that donated rumen fluid and those which nylon bags were incubated in were cared according to international guidelines for biomedical research involving animals (Council for International Organization of Medical Science-CIOMS, 1985).

4.3.2.2 Animal handling, feeding, collection and processing of rumen liquor for in vitro dry matter digestibility

A rumen cannulated ox that was kept at the Department of Agricultural Research kraals was used as the source of rumen liquor for in vitro digestibility determination. The rumen liquor was collected and kept in thermos flasks pre-heated to 39^0C with hot water and flashed with carbon dioxide in order to maintain anaerobic conditions inside. This is important as oxygen is toxic to rumen bacteria.The ground forage samples were put in fiber bags which were heat sealed and placed in individual flasks and incubated with rumen liquor containing rumen microbes. The flasks also contained buffers, macro-minerals, trace-minerals, nitrogen sources and reducing agents to maintain pH and provide nutrients required for growth of rumen bacteria. The bags were incubated in the flasks for 96 hours. At the end of the incubation period, the bags were rinsed **four times. Buffer** solution consisted of two solutions prepared according to Ankom Daisy Incubator digestibility procedure (solution A and solution B; Ankom Technology Corporation, Fairport, NY, USA). Solution A was made of 20 g of KH_2PO_4, 1.0 g $MgSO_4.7H_2$, 1.0 g NaCl, 0.2 g $CaCl_2.H_2O$ and 1.0 g Urea (reagent grade) in 2 litres of distilled water while buffer Solution B consisted of 15 g Na_2CO_3 and 1 g $Na_2S.9H_2O$ in 1 litter of distilled water. Both buffer solutions (A and B) were pre-warmed to 39°C by placing them in a water bath set at 39°C before use. In separate containers, 266 **ml** of solution B was added to 1330 **ml** of solution A (1:5 ratio). The exact amount of solution A to B was adjusted to obtain a final pH of 6.8 at 39°C. Four hundred millilitres (400 ml) of rumen inoculum in a graduated cylinder was added to the mixture of solution A and B (1:4 ratio) with distilled water, dried, weighed and placed in an ANKOM fiber analyzer using reagents specified in AOAC (2005) for NDF and refluxed for 60 minutes in neutral detergent solution. In vitro total dry matter digestibility (IVTDMD) was computed as the difference between dry matter incubated and the residue after the neutral detergent analysis. The following formula was used:

$$\%IVTDMD = [100 - (final\ weight\ after\ NDF\ extraction - blank) / initial\ weight\ x100]$$

4.4 Statistical analyses

Data were analysed using the General Linear Model in Statistical Analysis Systems (**SAS, 2004**) and simple correlation using Pearson's correlation analysis (SAS, 2004). Data was considered as a completely randomized design with two treatments and a control, using 18 plots, 6 plots for each treatment. The following model was used:

The treatment effects were considered significant at $P < 0.05$. When means were significantly different at $P < 0.05$, multiple comparison of means was conducted using the least squares means separation which was performed using the PDIFF option (SAS, 2004) to evaluate the significance and magnitude of the fixed effects at $P \leq 0.05$

$$Y_{ij} = \mu + T_i + \beta_j + \varepsilon_{ij}$$

where:

Y_{ij} = Response variable

μ = population mean

T_i = ith treatment effect

β_j = jth blocking effect

ε_{ij} = Random error $\sim N(0, \sigma^2)$ (population mean, variance).

4.5 Results

The post-harvest nutritional quality of the *C. ciliaris* forage residues determines how useful the residues can be **as animal feed sources** and their contribution to the overall income to the farmer.

4.5.1 Nutritional quality of the forage residue

The results in Table 4.1 show that fertilizing *C. ciliaris* with either horse manure or urea caused a **significantly ($P < 0.05$) higher** crude protein content in forage residues than the control treatment. The control treatment caused a **significantly ($P < 0.05$) higher** ADF than the two fertilizer treatments. The NDF was also **significantly ($P < 0.05$) higher** for the control treatment than for either horse manure or urea. The findings of this study also show that *C. ciliaris* from the control treatment had the highest Ash content (12.86%), which was **significantly ($P < 0.05$) higher** than when fertilized with horse manure (11.74%) or urea (12.22%). The IVDMD of the forage when the *C. ciliaris* was fertilized with horse manure or urea was **significantly ($P < 0.05$) higher** than the control treatment.

Table 4.1: Effects of fertilizer treatments on chemical composition and DM digestibility (%DM) of *C. ciliaris* after seed harvesting

Treatment	CP	ADF	NDF	Ash	IVDMD
No fertilizer (control)	10.11^{a}	43.82^{b}	37.45^{b}	12.86^{b}	33.73^{a}
Horse manure	12.29^{b}	39.53^{a}	34.60^{a}	11.74^{a}	44.14^{b}
Urea	12.43^{b}	39.62^{a}	35.94^{a}	12.22^{a}	37.81^{b}
P – value	0.0001	0.0113	0.0290	0.0438	0.0001

Treatment (column) means with similar superscripts do not significantly ($P < 0.05$) differ

CP= Crude Protein; ADF= Acid Detergent fiber; NDF=neutral; IVDMD= In Vitro Dry Matter Digestibility.

4.6 Pearson's Correlation Coefficients between crude protein and ADF, NDF and IVDMD

This study showed that there was a generally moderate negative linear association (r = -0.52146) between ADF and crude protein as shown in Table 4.2. As the ADF increases, the CP generally decreases but this relationship between the two variables was, however, statistically insignificant ($P > 0.05$). There is no probability of less than 0.05 that r = -0.52146, with n=6, could occur if there is no relationship between the two variables. Table 4.2 also shows a generally negative association (r = -0.57744) between NDF and CP. This relationship was significant ($P < 0.05$). There was a relatively large positive relationship (r = 0.70639) between crude protein and dry matter digestibility. For larger values of CP, the values of IVDMD are also larger and there is conclusive evidence about the significance of the association between these two variables (P < 0.05).

Table 4.2: Correlation coefficients between ADF, NDF, IVDMD and CP. It shows a positive correlation between CP and Dry Matter digestibility.

	Crude Protein
ADF	-0.52146 0.2887
NDF	-0.57744 0.0121*
IVDMD	0.70639 0.001*

Values with a * denote significant levels

The mineral content analysis results for the forage residues are shown in Table 4.3. They showed that, except for sulphur, there was no **significant (P > 0.05) difference** in mineral content between fertilizer treatments and the control treatment. There was no **significant (P > 0.05)** difference between fertilizing with urea and using horse manure. For sulphur, fertilizing with horse manure or urea caused a **significantly (P < 0.05) higher** content of the mineral than the control treatment.

Table 4.3: Mineral content (mg/kg DM) of *C. ciliaris* forage residue after seed harvesting

Treatments	P	Mg	Ca	K	Na	Cu	Zn	Fe	
No fertilizer (control)	0.49^{a}	0.40^{a}	0.49^{a}	1.55^{a}	0.35^{a}	0.95^{a}	1.49^{a}	1.11^{a}	0.23^{a}
Horse manure	0.52^{a}	0.43^{a}	0.56^{a}	1.72^{a}	0.37^{a}	0.97^{a}	1.36^{a}	1.30^{a}	0.40^{b}
Urea	0.55^{a}	0.46^{a}	0.57^{a}	1.89^{a}	0.37^{a}	0.91^{a}	1.41^{a}	1.32^{a}	0.44^{b}
P – value	0.33	0.73	0.47	0.06	0.95	0.45	0.24	0.42	0.01

Treatment means with different superscripts differ significantly ($P < 0.05$)

4.7 Discussion

4.7.1 Crude Protein

Crude protein and digestible dry matter are the most important components of a feed (Afzal and Ullah, 2007). In the present study, fertilizing with horse manure or urea resulted in significantly higher crude protein levels in the forage residues than the control treatment. The N applied to the soil through horse manure or urea was absorbed and may have been rapidly converted to nitrate-nitrogen and was then incorporated into organic materials (Abedelrahman, 2007). The crude protein contents of *C. ciliaris* in the present study (12.59% when fertilized with horse manure and 12.43% with urea) were significantly higher than the 7.9% reported by Al- Dakheel (2015) when no fertilizer was used. Walker (2013) reported a protein content of *C. ciliaris* of 10.2% in the wet season and 4.2% in the dry season, which were lower than the findings of the current study. Yossin and Yassin (2012) reported that the crude protein content of grass depends on soil nitrogen availability and that fertilization increases it.

The result of the current study, with an average of 11.71% crude protein was comparable to the 12% reported by Ramirez ***et al.*** (2009). Donaldson and Rootman (2010) reported a 15.63% crude protein content in *C. ciliaris* while Mcdowell (2003) reported a 17.5% and the differences between these two sets of results and those of the current study could be because of the differences in the supply of available N in the soil and the state of maturity of the grass. According to Ramirez ***et al.*** (2009), the crude protein content of forage grass markedly declines with maturity, possibly because of the relative increase in cell wall and decrease in cytoplasm. The crude protein content of the *C. ciliaris* across treatments, in the current research, was higher than the minimum requirements for ruminants (Mcdowell, 2003; 6.9% for maintenance, 10% for beef production and 11.9% for milk production). It was the control treatment **(10.11%)** that resulted in the grass having less crude protein than the minimum required for milk production. This means that if the forage grass residues after seed harvesting are meant to be fed to lactating cows, grass needs to be fertilized. It also means that soon after harvesting seed, the forage residues need to be quickly harvested before further decline in crude protein content. In the context of this study, very high levels of nitrogen would not be recommended due to the negative correlation between seed yield and vegetative biomass yield.

Significant increase in forage N content has shown to increase the soil water extraction by forages and improve water use efficiency (Yassin and Yossin, 2013). There was no difference in crude protein content between fertilizing with horse manure and fertilizing with urea. Since there is no difference between fertilizing using the two, the source of nitrogen that would be economically beneficial and provide a profit would be ideal. All other factors held constant, horse manure would be preferable in that regard since its costs are likely to be less. Despite being insignificantly a difference, fertilizing with horse manure produced a higher crude protein content in the forage than urea. This could be because when using irrigation as was the case in the current study, the water can lead to movement of nitrates beyond the root zone since it is very mobile in the soil. This would be more pronounced when urea is used than when horse manure is used as the horse manure increases the water retention capacity of the soil **(Mudenda and Maeresera, 2009).**

Apart from losses through leaching, N in urea can also be lost through volatilization (Yossin and Yassin, 2013). The level of phosphorus in the soil also affects the efficiency with which forages utilize N. According to Mcdowell (2003), low levels of phosphorus in the soil reduces the efficiency of nitrogen utilization. This could explain the slightly higher crude protein content in forage due to application of horse manure (12.59%) than due to urea (12.43%) as horse manure supplies phosphorus and other nutrients while urea only supplies N (Albu, 2012; Erikssen ***et al.,*** 2009; Keskinen ***et al.,*** 2017). Despite the fact that for forage grass to be productive, the first priority should be given to nitrogen, the grass responds to nitrogen rapidly and vigorously when phosphorus and potassium are adequate (Arshadullah ***et al.,*** 2011). The fact that the difference between the effect of horse manure and urea is not significant in this study, however, shows that the soil used in this research had relatively sufficient phosphorus.

4.7.2 Dry Matter Digestibility

Fertilizing *C. ciliaris* with horse manure or urea resulted in significantly higher dry matter digestibility than the control treatment in the current study. This could have been because the N supplied by these two fertilizers stimulated the growth of new tillers, shoots and leaves and accelerated the rate of stem development and accumulation of dead materials which were low in cell wall and lignin content, leading to higher digestibility (Ros Barcelo, 1997). This is contrary to what was reported by During and McNaught (2012) that fertilization has usually little or no effect on forage digestibility. There was no difference in dry matter digestibility between fertilizing with horse manure and with urea indicating that the two fertilizers similarly enhance digestibility of the forage residues by effectively reducing the proportion of ADF and increasing the protein content as shown in the current study.

Fertilization with phosphorus, potassium or other nutrients that increase yield may slightly reduce forage quality when growth is rapid (During and McNaught, 2012), followed immediately by maturation of tissues. According to During and McNaught (2012), maturity at harvest of seeds has the greatest influence on forage digestibility. As forages mature, their digestibility declines significantly. It is at the flowering stage that accumulation of stem mass starts to exceed leaf mass addition and stems contain a higher proportion of thick walled xylem tissue and less photosynthetic mesophyll tissue (During and McNaught, 2012). Overall herbage cell wall concentration increases as the leaf: stem ratio shifts towards a greater proportion of stem. Ashraf ***et al.*** (2013) reported that if a tissue begins to deposit lignin in its cell wall due to advanced maturity, its digestibility rapidly declines. Lignin in plant cells is more difficult for rumen bacteria to digest than cellulose and hemicellulose. The *in vitro* dry matter digestibility of 37.81% when fertilized with urea and 44.14% when fertilized with horse manure found in the current study, were below the 50% that Hassan *et al.* (2015) reported as the critical threshold level for ruminant efficient digestion in the rumen. Values lower than that would limit intake through delayed dry matter degradation resulting from inefficient microbial environment.

The digestibility of the forage grass after seed harvesting found in the current study was also lower than the 53.8% - 70.5% range for *C. ciliaris* that was reported by Donaldson and Rootman (2010). It was also lower than the 67.5% reported by Waramit and Moore (2006). This could be attributed to the differences in the stage of maturity at seed harvesting. According to Hassan ***et al.*** (2015), if *C. ciliaris* is grown in a season with a lower environmental temperature, its quality could be higher. During the first two months of the current study (December and January), the temperatures were quite high. Higher environmental temperatures encourage lignification, rapid physiological development and metabolic activity, resulting in the decline of forage quality (Hassan ***et al.,*** 2015). The findings of this research could therefore, be made more meaningful if it is carried out across seasons. The grinding of the forage grass to 1mm before the *in vitro* dry matter digestibility determination increases the proportion of the cell wall which is immediately accessible to the microbes in the rumen liquor. This may result in higher digestibility indication than what actually happens in reality.

4.7.3 Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF)

Fibrosity indicates the extent to which the grass can be degraded by rumen micro-organisms (Topps, 1996). The NDF and ADF content of *C. ciliaris* with no fertilizer was higher than when the grass was fertilized with either horse manure or urea. There was, however, no difference between the effect of horse manure and urea on both ADF and NDF content of the *C. ciliaris*. Without addition of nutrients, especially nitrogen to the soil, forage grass tends to mature, flower and set seed early. This could be in a bid to successfully reproduce in an environment that is not quite conducive and would, otherwise, shorten the life span of the grass. Since the forage grasses were harvested at the same time in the current study, the grass from the control may have been more mature, more lignified and hence more fibrous than the grass fertilized with horse manure and urea. According to McNaught (2012), the flowering stage heralds the start of the accumulation of stem mass exceeding leaf mass addition. This increases the fibrosity of the grass.

The ADF findings of the current study (40.99%) was within the range of36.60% - 47.70% reported by Al-Dakheel (2015) and higher than the 38.50% reported by Donaldson and Rootman (2010). NRC (1989) recommended a minimum dietary ADF content of 19-21% DM for lactating cows. Lu ***et al.*** (2005) recommended dietary ADF of 18-20% DM for goats. The ADF in the current study could have been higher due to the fact that the grass was analysed after seed harvesting and this shows that their maturity was advanced. Nsinamwa ***et al.*** (2005) concurred as they reported that the fibre content of forage increases with age. The NDF of 36% found in this study was lower than the 66.5% reported by Donaldson and Rootman (2010) and the 70.2% reported by Al-Dakheel (2015). It was lower than the NDF content of 25-28% DM recommended by NRC (1989) for lactating cows and 41% for lactating goats (Lu ***et al,*** 2005). **The** fibre content of the *C. ciliaris* in the current study was comparable to the 37.34% reported by Ashraf ***et al.*** (2013) for *C. ciliaris* grown in Cholistan desert, Pakistan. Koech ***et al.*** (2014) qualified the forage grass with crude fibre of less than 50% to be of high quality and that with more than 60% to be low quality. This means that the forage residues were still of high quality even after seed harvesting. This can be attributed to the fact that the grass was irrigated and hence did not get moisture stressed. According to Topps (1996), moisture stress causes grass to mature early and become more fibrous.

4.7.4 Minerals

Minerals are vital for normal growth, reproduction, health and proper functioning of the animal body. They protect and maintain the structural components of the body, organs and tissues and are constituents of body fluids and tissues as electrolytes (Taylor, 1997). They catalyse several enzymatic processes and hormone systems and maintain the acid-base balance, water balance and osmotic pressure in the blood and cerebral spinal fluid (Soni ***et al.,*** 2014). Both deficiencies and excessiveness of minerals have adverse effects on the animal body and production (Mcdonald ***et al.,*** 2011). It is, therefore, important to know the mineral composition of forage feed if it is to be used to feed livestock. The results of this study show that there was no difference in concentrations of most minerals between fertilizer treatments and the control. The concentrations of these minerals were within the sufficient range requirements for livestock (Soni ***et al.,*** 2014). This means that livestock that could be fed on the forage residues after seed harvesting would not have deficiencies.

The average phosphorus content of the *C. ciliaris* of 0.518% in this study was in the same range as the 0.53% reported by Sawal ***et al***. (2009) and the 0.15% to 0.65% range reported by Ramirez ***et al***. (2009). It was above 0.25% DM critical level for maintenance of a dry cow as reported by Soni ***et al***. (2014). Phosphorus is critical because forage grasses respond to nitrogen rapidly when they also absorb sufficient phosphorus from the soil. The phosphorus content of *C. ciliaris* recorded in the current study across treatments, was lower than the 0.8% reported by Mutimura and Everson (2012). It was higher than the 0.26% reported by Juma ***et al***. (2006) for most tropical grasses at their prime harvesting time. This could be because *C. ciliaris* has a reputation of being a phosphophilic grass. It efficiently absorbs phosphorus from the soil (Donaldson and Rootman, 2010). The calcium content of an average of 0.54% of dry matter was higher than the 0.3% for *C. ciliaris* reported by Soni ***et al.*** (2014). This could have been due to the liming that could have been done for the previous crop since the sandy soils in the area were generally acidic. The calcium: phosphorus ratio of 1:1 could result in some mineral imbalances that could lower their bioavailability (Soni ***et al.,*** 2014). Excessiveness of one mineral may cause antagonistic effects for other elements and causing mineral imbalances (Soni ***et al.,*** 2014).

In the current study, there were higher potassium and sulphur contents caused by fertilizer treatments than the control. When forage grasses have sufficient N supply, they absorb more sulphur in order to make sulphur containing amino acids. The N from urea and horse manure may have caused an increase in sulphur uptake from the soil. As the forage produces more seed, as was the case with forage grass fertilized with both horse manure and urea, the demand for potassium increases. This is because potassium is essential for flower and seed formation and it is also required for the formation of starch.

4.7.5 Conclusion

The results of the current study reveal that fertilizing *C. ciliaris* with either horse manure or urea cause a significantly higher crude protein content of the grass at seed harvesting stage of maturity than when no fertilizer is applied. The fact that there was no significant difference between fertilizing the *C. ciliaris* with horse manure and fertilizing it with urea in terms of protein content means that they can be interchangeably used. Cost implications and availability become the real factors to consider. The results show that the protein content of *C. ciliaris* after seed harvesting is way above the maintenance level of livestock. This means that the forage residues after seed harvesting can actually be used to feed livestock for production. The forage residues can be baled and be sold by seed producing farmers. This would widen the sources of income. Seeds can be produced without significantly compromising the quality of feed produced from a piece of land.

The results of this study also show that the ADF and the NDF contents of the *C. ciliaris* forage residues are significantly reduced by application of nitrogen fertilizers. There was a negative correlation between the crude protein content and the crude fibre of the forage residue. Fertilization *C. ciliaris* with nitrogen increases the quality of the forage residue as it becomes less fibrous and hence easier to digest even at seed harvesting stage. Fertilizing with either horse manure or urea significantly increased the dry matter digestibility of *C. ciliaris* forage residue. Since there was no significant difference between fertilizing *C. ciliaris* with horse manure and fertilizing with urea in terms of forage dry matter digestibility, it would be advisable to use horse manure if it is available as it is applied once in three years (Mujuni and Sibanda, 2007). This reduces production costs and also limits the effect of excess nutrients on the surrounding open water bodies as well as underground water.

Chapter 5

General conclusions, limitations and future research

From the findings of the current research, it can be concluded that horse manure is a superior source of nitrogen to urea in terms of seed yield and quality of the forage residue. This was shown by the horse manure causing significantly higher seed yield, crude protein content and dry matter digestibility. Urea is superior to horse manure in terms of the forage dry matter yield. This means that when producing seed, fertilizing with horse manure adds more value than using urea but when the aim is just to produce high herbage yields, fertilizing with urea would be a more suitable choice. The limitations to the reliability of the results of this study include the fact that it has not been replicated both in space and in time. This means that it cannot represent what is expected from all areas of Botswana. The experiment may need to be carried out on various soil types, as opposed to on station experiments as was in this study. The experiment needs to be done across seasons as well. Since the current study was conducted under irrigation, the results may not be directly applicable to rain-fed conditions, which are prevalent under normal farming conditions in Botswana. N use efficiency is limited by water stress, a condition normally encountered under rain-fed situation.

The quality of the forage residue reported in the current study was only from laboratory analysis. Studies on feeding trials by animals would be necessary to find out if the quality is mirrored by the animal performance in terms of body weight gain or milk production. This would help to authenticate the forage residue's nutritional value to livestock. There is little published information specifically relating to its management in situations where it is not wanted. This is pertinent as seeds are bound to be dispersed from seed production fields to other places. It is not enough to just label it as one of the most notorious weeds without finding out how best to control its propagation. A similar research can be carried out with varying amounts of urea. It could be possible that a lower urea application rate could increase seed yield by reducing vegetative growth. The grass is reported to be fairly high on oxalate levels that may cause poisoning of young sheep and big head condition in horses. A research could be carried out to find out how the oxalate levels vary with stage of maturity. It may be possible that the forage residues obtained after harvesting seed could be very safe for feeding the sheep and horses.

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APPENDICES

Agronomic Parameters

Sample	Seed yield (kg/ha)	Number of tillers /crown	Inflorescence length (cm)	Forage dry matter yield (kg/ha)	Fresh mass yield (kg/ha)
C1	10.25	29	11.6	1525.92	4833.32
C2	12.18	19	10.98	1748.33	4583.51
C3	11.73	23	11.2	1498.76	4166.25
C4	10.87	25	11.5	1387.39	3333.76
C5	12.17	20	10.66	1581.84	3750.43
C6	9.22	26	9.8	2024.67	5409.57
Mean	11.07	23.67	10.96	1627.81	4346.14
H1	16.33	32	10.7	2409.44	6111.11
H2	18.95	28	11.6	2265.41	5000.12
H3	17.55	27	11.2	2123.67	4986.76
H4	18.12	31	11.7	2256.64	5334.85
H5	20.22	30	10.8	2081.52	5416.67
H6	19.56	32	11.4	2290.43	5835.13
Mean	18.46	30	11.2	2237.85	5447.44
U1	16.27	28	11.2	2918.11	6250.06
U2	13.46	37	10.2	2940.25	6283.33
U3	16.22	32	11.6	2415.81	5833.82
U4	14.12	34	11.2	3081.67	7500.34

U5	11.36	29	10.6	2583.52	6666.73
U6	15.62	30	11.3	2498.33	5856.92
Mean	14.51	31.67	11	2739.59	6398.53

Effects of fertilizer treatments on agronomic parameters of *C. ciliaris.*

Data analysis results

Parameter	Treatment	Means ± s.e	General Mean	F value	Pr > F
Number of tillers per crown	Control*	23.67 ± 3.78[a]	28.44	10.64	0.0013
	Horse manure	30.00 ± 2.10[b]			
	Urea	31.67 ± 3.39[b]			
Seed yield (kg/ha)	Control	11.07 ± 1.18[a]	14.68	34.65	<0.0001
	Horse manure	18.46 ± 1.42[b]			
	Urea	14.51 ± 1.92[c]			
Fresh biomass yield (kg/ha)	Control	4346.14 ± 753.22[a]	5397.37	16.41	0.0002
	Horse manure	5447.44 ± 450.93[b]			
	Urea	6398.53 ± 621.85[c]			
Forage Dry Matter Yield (kg/ha)	Control	1627.82 ± 227.58[a]	2201.76	39.50	<0.0001
	Horse manure	2237.85 ± 118.99[b]			
	Urea	2739.62 ± 274.42[c]			
Length of inflorescence (cm)	Control	10.96 ± 0.66[a]	11.07	0.44	0.65
	Horse manure	11.23 ± 0.41[a]			
	Urea	11.02 ± 0.52[a]			

[ab] Means in the same column within a parameter with different superscripts differ significantly; P <0.05; means ± s.e;*No fertilizer.

Chemical Composition and Dry Matter Digestibility Raw data

Sample	ADF (%)	NDF (%)	Ash (%)	In vitro Dry Matter Digestibility (%)	Protein (%)
C1	46.2	35.43	12.67	34.46	10.4
C2	43.4	37.9	12.54	37.4	9.46
C3	43.63	38.35	15.17	36.6	10.61
C4	43.2	38.7	12.13	31.94	9.82
C5	43.51	36.03	12.44	33	10.15
C6	43	38.3	12.18	29	10.21
Mean	43.82	37.45	12.86	33.73	10.11
H1	37.13	37.35	11.64	41.4	12.71
H2	37.6	32.9	11.49	41	12.38
H3	47.5	38.1	11.88	43	12.32
H4	38.52	32.9	11.4	45.4	13.14
H5	37.4	33.1	12.09	45.8	12.58
H6	39	33.23	11.93	48.21	12.42
Mean	39.53	34.6	11.74	44.14	12.59
U1	40.2	36.1	12.09	37	12.44
U2	38.72	34.89	11.98	40.2	12.48
U3	39.08	36.23	12.19	38.54	13.12

U4	40.6	35.9	12.05	35.8	12.2
U5	39.92	36.43	12.59	38.2	12.02
U6	39.17	36.1	12.39	37.1	12.34
Mean	39.62	35.94	12.22	37.81	12.43

Data analysis results for chemical composition and digestibility

Parameter	Treatment	%DM Means ± s.e	General Mean	F value	Pr > F
Crude Protein	Control*	10.11±0.41^{a}	11.71	86.00	<0.0001
	Horse manure	12.59±0.30^{b}			
	Urea	12.43±0.38^{b}			
ADF	Control	43.82±1.19^{a}	40.99	6.13	0.0113
	Horse manure	39.53±3.97^{b}			
	Urea	^{a} 39.62±0.73^{b}			
NDF	Control	37.45±1.37^{a}	36.00	4.52	0.0290
	Horse manure	34.60±2.44^{b}			
	Urea	35.94±0.23^{b}			
Ash	Control	12.86±1.15^{a}	12.27	3.88	0.0438
	Horse manure	11.74±0.27^{b}			
	Urea	12.22±0.23^{b}			
IVDMD	Control	33.73±3.11^{a}	38.56	24.84	<0.0001
	Horse manure	44.14±2.81^{b}			
	Urea	37.81±1.52^{b}			

[ab] Means in the same column within a parameter with different superscripts differ significantly; Pr<0.05; means ± s.e;*No fertilizer.

Mineral Composition

Sample	Phosphorus	Magnesium	Calcium	Potassium	Sodium	Copper	Zinc	Iron	Sulphur
C1	0.46	0.38	0.55	1.53	0.33	0.97	1.54	0.96	0.24
C2	0.51	0.42	0.43	1.57	0.37	0.92	1.43	1.25	0.22
H1	0.55	0.45	0.6	1.81	0.31	0.95	1.33	1.36	0.41
H2	0.49	0.41	0.52	1.62	0.42	0.99	1.39	1.23	0.38
U1	0.53	0.53	0.55	1.92	0.39	0.95	1.44	1.41	0.46
U2	0.57	0.38	0.58	1.86	0.34	0.87	1.37	1.22	0.41

Data analysis results for minerals

	Mineral content (mg/kg)								
Treatment	P	Mg	Ca	K	Na	Cu	Zn	Fe	S
No fertilizer (control)	0.485	0.400	0.490	1.550	0.350	0.945	1.485	1.105	0.230
Horse manure	0.520	0.430	0.560	1.715	0.365	0.970	1.360	1.295	0.395
Urea	0.550	0.455	0.565	1.890	0.365	0.910	1.405	1.315	0.435
General mean	0.518	0.428	0.538	1.718	0.360	0.942	1.417	1.238	0.3533
P-value	0.3287	0.7277	0.4726	0.0590	0.9469	0.4543	0.2446	0.4197	0.0076