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Scientific Journal of Animal ScienceJournal homepage: www.Sjournals.com**Original article****Effect of graded levels of urea treatment on nutrient composition and *in-vitro* digestibility of *Calliandra calothyrsus* (Meissner)****Jenias Ndava^{a,*}, Blessing Timba^b, Christopher Gadzirayi^c**^aDepartment of Biological Sciences, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe.^bDepartment of Animal Production and Technology, School of Agricultural Sciences and Technology Chinhoyi University of Technology, P. Bag 7724, Chinhoyi, Zimbabwe.^cDepartment of Agricultural Economics, Education and Extension, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe.*Corresponding author: sinautajb@gmail.com

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ABSTRACT

Calliandra calothyrsus (Meissner) is a multipurpose forage tree that can improve the nutrition of livestock due to its high crude protein content. However, the shrub has low digestibility. The aim of this study was to determine the effect of graded levels of urea treatment on nutrient composition and *in-vitro* digestibility of *C. calothyrsus* leaves. Mature leaves of *C. calothyrsus* were hand harvested in the first week of August 2016, after the flowering stage. They were air dried under the shed for five days in order to maintain the green colour of the leaves. They were then treated with 0% (control), 3%, 5% and 7% urea solutions for four weeks under anaerobic conditions. Some of the air dried samples were ground and analysed for nutrient composition, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), dry matter (DM) and ash. There was an increase in CP following increase in the level of urea treatment from 210 to 320g/kg DM. A decrease was noted in fibre content with increasing level of urea treatment. Digestibility trial was conducted using the Tilley and Terry two stage method. *In-vitro* dry matter digestibility increased significantly at ($p < 0.05$) with increase in urea treatment noted from 439.7-530.7g/kg DM. The

results show the effectiveness of urea treatment in increasing the crude protein and *in-vitro* digestibility of *C. calothyrsus*.

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1. Introduction

One of the principal limiting factors to ruminant production in the tropics is feed shortages, especially in the dry season. Small holder farmers depend on forages to form the greater part of animal diets. The availability in quantity and quality of these forages decreases due to seasonal fluctuations, overgrazing and increased land use by man (Fennema, 1996). Commercial feeds are expensive and smallholder farmers seek affordable alternatives, which do not compromise the nutrition of the livestock. Multipurpose forage trees are trees that are grown and managed for more than one output. For instance, multipurpose legume trees can provide high-quality feed (Lenné and Thomas, 2006). They may supply firewood, add nitrogen to the soil or supply food in the form of fruits or leaves and some other combination of different output. Forage trees and shrubs are an enormous potential source of protein for ruminants in the tropics. Irrespective of the season, the protein content in the multipurpose forage tree leaves is much higher than that of grasses (Pamo et al., 2006; Datt et al., 2008). They form good quality fodder especially for smallholder farmers with limited land and cash resources.

Opportunities for improving the nutrition of livestock do exist. According to Simbaya (2000), fodder trees/shrub legumes have the potential for alleviating some of the feed shortages and nutritional deficiencies that livestock experience during the dry season. Tree fodders, especially leguminous species are rich in crude protein and minerals, and play an important role where the bulk feed is mainly composed of mature grasses or low nutrient quality. *C. calothyrsus* (Meissner) is a small, thornless, multi-stemmed shrub tropical legume, native to Central America and Mexico (Orwa et al., 2009) and has attracted much interest in recent years as a potential source of protein rich fodder, owing particularly to its fast growth and vigorous re-sprouting ability even on acidic infertile soils (Stewart et al., 2000). Although no toxic substances have been found in *C. calothyrsus* foliage, high concentrations of tannins of up to 11% have been reported by Chamberlain (2001) to reduce digestibility of proteins by about 40%. Reed et al. (1990) point out that digestibility is reduced in animals consuming diets due to condensed tannins, which bind to proteins resulting in most proteins passing undissociated into the faeces. This therefore affects protein utilization by most ruminants.

Several strategies can be used to increase digestibility, which include: Physical treatment, biological treatment and chemical treatment (Sarnkolong et al., 2010; Usha et al., 2001). Urea treatment is a chemical method that has however emerged as a treatment method of choice for use especially on farms in the tropics for smallholder farmers (Ambaye, 2009). It is preferred due to its low cost and ease of handling (Nianogo, 1999). Urea treatment can serve as a delignification process through ammonification and is also a source of nitrogen (Vadiveloo and Fadel, 2009). The effect of urea treatment on chemical composition of forage legumes has not been fully elucidated on *C. calothyrsus*. The tendency has been to extrapolate from known effects on maize stover. This is problematic given the differences between maize stover and leguminous forage trees. A major difference between maize stover and legumes cited by Van Soest (1994) is that whilst legumes often contain a higher content of lignin than grasses, only the xylem vascular tissues are lignified. In general, legumes tend to be higher in lignin and crude protein and lower in cell wall than tropical grasses. In grasses, lignin is distributed throughout the plant tissue (Wilson, 1993). This research therefore sought to investigate the effects of graded levels of urea treatment on nutrient composition and *in-vitro* digestibility of *C. calothyrsus* leaves.



Fig. 1. *C. calothyrsus* shrub (https://topropicals.com/catalog/uid/calliandra_calothyrsus.htm).

2. Materials and methods

The leaves were hand harvested in the first week of August 2016, after the flowering stage, at Grasslands Research Institute, latitude 18° 11' S, longitude 31° 28' E and altitude 1630 m GPS position. The leaves were air dried under the shade in order to maintain the green colour of the leaves for five days. The Completely Randomised Design (CRD) was used in which three replicates were used for the four treatments. Treatments included graded levels of urea treatment to *C. calothyrsus* at the following levels, 0% (control), 3% urea, 5% urea and 7% urea.

2.1. Urea treatment

Some 0.7kg of dried leaf fraction was packed into polythene plastic bags and treated with graded levels of urea solution at (0, 3, 5 and 7%). Incubation period was 5 weeks under room temperature conditions. Bags were kept under anaerobic conditions. Samples, each 5gr were then taken, air dried and ground through a 3mm sieve in preparation for further analysis. Main purpose for aeration was to help in the detection for amount of nitrogen, which was chemically fixed to the *C. calothyrsus* after treatment.

2.2. Chemical analysis

Dry matter (DM) and ash content were determined using the procedures described by the Association of Official Analytical Chemists (A.O.A.C., 2005). Nitrogen (N) was determined using the Kjeldhal procedure and crude protein (CP) was calculated by multiplying percent N by the factor of 6.25. Neutral detergent fibre (NDF), acid detergent fibre (ADF) was determined according to Van Soest and Robertson (1985).

2.3. In-vitro digestibility trial

The digestibility trial was carried out using the Tilley and Terry (1963) two-stage technique. In this technique, 0.5g samples of each treatment were weighed and incubated anaerobically with active rumen liquor inoculum (10ml strained rumen fluid) in a buffered solution (40ml McDougall's artificial saliva solution) for 48 hours at 38°C. The second stage involved digestion with pepsin-HCl for 48 hours at 38°C. The samples were then filtered through a porous alumina crucible before oven drying and weighing to give an end-point measurement. The percentage dry matter digestibility (DMD) was calculated using the formula according to Tilley and Terry (1963):

$$\% \text{ Dry matter digestibility} = \text{Digestible dry matter} / 0.5\text{g Sample} \times 100$$

2.4. Data analysis

GENSTAT statistical package (Version 13) was used to analyse data. Differences among treatments were analyzed using Least Significant Difference (LSD). Confidence interval was set at 95%. The following statistical model was used for the data analysis:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where:

Y_{ij} = Observation of the i^{th} replicate and the j^{th} replicate

μ = Overall mean

α_i = Effect of the i^{th} treatment, $i = 1, 2, \dots, t$

ϵ_{ij} = Random error component

3. Results

3.1. Crude protein (CP) content

Untreated *C. calothyrsus* (Table 1) had a CP value of 210g/kg. The value increased with increase in the level of urea treatment to 320g/kg DM for 7% urea treatment.

3.2. Fiber content

The ADF and NDF values (Table 1) decreased from 324g/kg and 388g/kg to 272g/kg and 220g/kg respectively following the increase in the level of urea treatment to 7%.

Table 1
Chemical composition of *C. calothyrsus*.

Treatment	Chemical composition g/kg				
	CP	ADF	NDF	Ash	DM
0% urea	210	324	388	53	950
3% urea	270	311	238	56	930
5% urea	290	298	233	56	950
7% urea	320	272	220	58	950

3.3. *In-vitro* dry matter digestibility

ANOVA results showed that the dry matter digestibility of *C. calothyrsus* significantly differed ($p < 0.05$) due to the effect of urea treatment levels. There was a significant difference between treatments in the dry matter digestibility ($p < 0.05$) between 0 and 3%, 0 and 5%, 3 and 5% urea treatments of *C. calothyrsus*. However, there was no significant difference between treatments in the dry matter digestibility ($p > 0.05$) between 5% and 7%, urea treatments of *C. calothyrsus*. An increase in the dry matter digestibility was noted with increasing levels of urea treatment.

Table 2
In-vitro digestibility of *C. calothyrsus*.

Treatment	<i>In-vitro</i> dry matter digestibility g/kg DM
0% urea	439.7 ^a
3% urea	448.7 ^{ab}
5% urea	465.7 ^{ab}
7% urea	530.7
*s.e.d.	17.67

*s.e.d. = Standard error of the difference between the means,

^{ab}Means with different superscripts are significantly different at $p < 0.05$

There was a positive linear relationship between the increase in level of urea treatment and digestibility as shown in Fig 2. Digestibility increased from 439.7g/kg DM to 530.7g/kg DM.

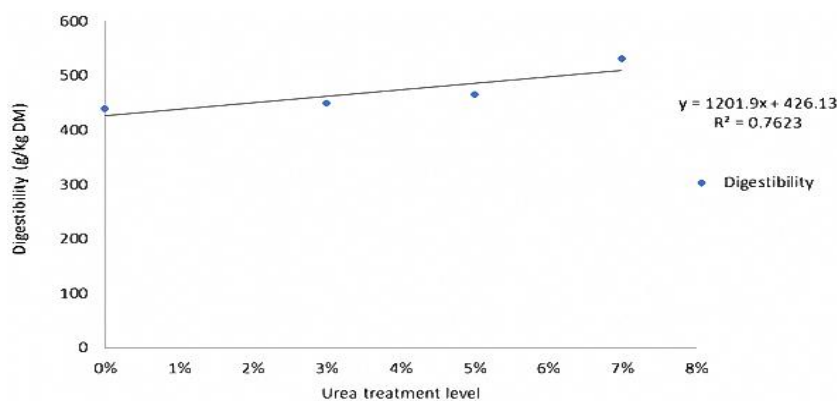


Fig. 2. Linear relationship between graded levels of urea treatment and *in-vitro* digestibility of *C. calothyrsus*.

4. Discussion

4.1. Crude protein

C. calothyrsus had a CP content of 210g/kg. Lopez et al. (2009) reported a CP of 220g/kg which is similar to the CP recorded in this study. The increase in CP of *C. calothyrsus* as a result of increase in the level of urea treatment observed in this study is also consistent with the findings of Ben Salem et al. (2005) who also found that increasing levels of urea and treatment duration increased ash-free neutral detergent fibre content in acacia

leaves. Ramirez (2007) also investigated the CP content in buffelgrass hay, bermuda grass hay, corn stover and corn cobs and found that as urea treatment increased from 0% to 6.5% CP content also increased. Increments in CP level may be attributed to non-protein nitrogen (NPN) addition to the plant particles. CP is directly related to nitrogen. This means that increase in nitrogen due to the addition of NPN resulted in a direct increase in CP with increase in level of urea treatment.

According to Granzin and Dryden (2003) a significant increase in crude protein of Rhodes grass hay was noted when urea treatment was done. Aegheore (2005) also reported that the CP content of corn stover increased from 60-150g/kg DM when stover was treated with 0-7% levels of urea. Abate and Melaku (2009) reported that urea treatment improved the CP content of barley by 110% and this could be attributed to the fixation of urea nitrogen into the barley straw which could be as high as 50% of the nitrogen in urea.

4.2. Fibre

The fibre content of *C. calothyrsus* reported in this study was higher than that recorded by Lopez et al. (2009). Increase in fiber content could be because *C. calothyrsus* was harvested late after the flowering stage in August when the lignin content had increased due to maturity. The reduction in NDF and ADF components as a result of urea treatment observed in this study is in agreement with the findings of Abate and Melaku (2009). Oji et al. (2007) reported decreases in NDF and ADF contents of urea treated wheat straw and maize stalks, husks and cobs. The reduction of NDF and hemicelluloses fraction might have been due to the dissolving effect of urea on the hemicelluloses fraction and subsequent removal from cell contents (Abadale and Melaku, 2009).

4.3. In-vitro digestibility

Urea treatment increases digestibility of forages. The increase in the dry matter digestibility of *C. calothyrsus* as a result of increase in the level of urea treatment has also been reported by some researchers, for example, Despal (2005) who investigated nutritional properties of urea treated cocoa pods. This could be so because urea treatment can break ester bonds between lignin, hemicellulose and cellulose and physically cause structural fibers to swell increasing access of microbes for the breakdown of fiber. Ammonification affects cell wall composition and improves extent of digestibility (Tsfayohannes, 2013).

Tannins are polyphenolic compounds that can form strong H bonds with proteins and carbohydrates rendering them undegradable in the rumen (Kumar and Upadhyaya, 2012). The high alkalinity of urea destabilizes tannin protein complexes and also oxidizes phenols. This tannin deactivation effect of urea was noted on sorghum grains (Ben Salem et al., 2005). This tannin deactivation could have led to the significant increase in digestibility of *C. calothyrsus* in this study, since the bound proteins were released for digestibility.

According to Ramirez et al. (2007) and Oji et al. (2007), the reasons adduced for improvements in digestibility after ammoniation of different roughages evaluated include the collapse of vascular bundle sheath cells and inner cuticular surfaces and separation from adjacent ground parenchyma and alteration in the friability of the rigid cell wall. This increases the ability of ammonia to dissolve parts of hemicellulose-cleaving ester bonds of uronic acids which results in loss of acetyl groups thus releasing acetic and phenolic acids, an effect that may possibly explain the decrease in NDF.

A positive linear relationship between the increase in level of urea and digestibility was noted. There was no optimum level for graded level of urea, since the digestibility was continuously increasing following increase in level of treatment. Optimum level of adding urea results when the ammonia becomes toxic to the animals. Brown and Adjei (1995) also found that *in-vitro* digestibility increased linearly with increasing urea level in *Panicum maximum* hay treated at 0, 4, 6 and 8% of the forage dry matter.

5. Conclusion

Results of this present study indicated that increase in the concentration of urea to *C. calothyrsus* results in an increase in the nutritive value and a positive linear increase in *in-vitro* digestibility. It is recommended that urea treatment should be used to improve the nutritive value and digestibility of *C. calothyrsus*. Farmers should therefore adopt this chemical treatment since it avails protein. This is a potential approach to exploit the uses of crop residues in ruminant livestock production. However, more research work is needed to evaluate the optimum amount of urea, which does not cause toxicity to ruminants.

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