Research Article

Effects of increasing levels of cow dung application associated with spraying of Light Matrix Organic (probiotic) on the forage production of Tripsacum laxum

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ABSTRACT

Guatemala grass (*Tripsacum laxum*) originating from Tropical America was introduced to Cameroon as an attempt to solve the problem of low forage availability during the dry season. *Tripsacum laxum* forage production under cow dung fertilization associated with the probiotic Light Matrix Organic (LMO) was evaluated at Bambui, North-West Cameroon. The study was carried out during the raining season (July-November) 2011 in plots established in 2010 using a factorial design with 4 cow dung application levels (0, 7.5, 15 and 22.5 t/ha) each with or without LMO (10ml/l water). Growth was evaluated monthly while forage yield and nutritive value evaluation were done after 120 days of regrowth. The results showed that cow dung application appeared to have an ameliorating effect on the growth and fresh yield while the association of cow dung with LMO improved the dry matter yield and nutritive value of the forage. The cow dung application level of 15t/ha associated with LMO recorded the best output in terms of productivity (5.2 ±0.6 DM t/ha) and nutritive value (9% DM Crude Protein).

Key words: *Tripsacum laxum*, organic fertilization, growth, productivity, nutritive value, cow dung, probiotic

RÉSUMÉ

L'herbe de Guatemala (*Tripsacum laxum*) originaire d'Amérique tropicale, a été introduite au Cameroun pour lutter contre le problème de manque de fourrage pendant la saison sèche. La production fourragère de *Tripsacum laxum* fertilisée avec de la bouse de vache associée au probiotique Light Matrix Organic (LMO) a été évaluée à Bambui, Nord-Ouest Cameroun. L'étude a été effectuée pendant la saison de pluie (Juillet - Novembre) 2011 dans des parcelles établies en 2010 selon un dispositif expérimental factoriel avec 4 niveaux de bouse de vache (0, 7.5, 15 et 22.5 t/ha) associée ou pas au LMO (10ml/l d'eau). La croissance a été évaluée mensuellement alors que le rendement et la valeur nutritive du fourrage ont été évalués après récolte à 120 jours de pousse. Les résultats montrent que l'application de bouse de vache apparaît avoir un effet améliorant sur la croissance et le rendement en matière fraîche du fourrage tandis que l'association bouse de vache et LMO améliore le rendement en matière sèche et valeur nutritive du fourrage. L'application de la bouse de vache a 15t/ha associée au LMO a enregistrée la meilleur productivité (5.2 ±0.6 DM t/ha) et qualité (9% DM Protéine Brute) du fourage.

Mots clés : *Tripsacum laxum*, fertilisation organique, croissance, productivité, valeur nutritive, bouse de vache, probiotique.

INTRODUCTION

Forages occupy a significant place in ruminant nutrition as the basic feed for herbivores and ruminants. However, the current increase in crop area coupled with population growth makes less land available for grazing. Indeed, with the current population growth, it is estimated that by 2050, sub-Saharan population will be 1294 million inhabitants (Winrock International, 1992). A rapidly growing population will definitely need more and more food. This will be achieved by increasing the area of cultivated land so as to improve on the food production, and will result in reducing considerably the animal grazing area.

Adequate nutrition is essential to exploit the genetic potential of animals (Chesworth, 1992; Lhoste *et al*., 1993). Possible alternatives to improve on ruminant nutrition have been suggested such as: feed supplementation through the use of agro-industrial wastes, multi-nutritional blocks, sowing legumes in natural pastures and cultivation of forage or its conservation (Fogang, 2010). However, the cost associated to agro-industrial wastes and inputs used in formulation of nutritional blocks do not make them affordable to a great part of the farmer population, especially in the tropics. Farmers may usually possess large land that can be cultivated. In this line, forage can also be cultivated and may stand as main source or a complement of feed for animals especially during periods of scarcity.

Tripsacum laxum was introduced to Cameroon from Tropical America and has a good reputation for its ability to withstand drought and maintain considerable dry matter production during the dry season with a perennial vegetative cycle. The main attributes of *T. laxum* reside in its high ability to produce under good conditions. Also, easy availability of planting material makes it a good candidate for forage cultivation.

Increase in forage production may be carried out using fertilizers. The choice between organic and inorganic fertilizer depends on the resources available. In this regard, organic fertilizers as animal wastes are highly available in animal farms. It has been reported that the application of organic material such as animal manure, plant residue or composted organic matter increases soil organic matter, provides nutrients for plant growth, alleviate aluminium toxicity, and renders phosphorus more available to crops producing high yield and quality food crops (Hue, 1992; Beltran *et al*., 2002). It has also been reported that forage production via an amelioration of soil fertility may increase the herbage production at a particular time (Hnatyszyn and Guais, 1988; Pamo, 1991).

Considering animal manure as potential fertilizers, the application of beneficial microorganisms like probiotics has been considered as an economical and simple way to increase crop yield, alleviate environmental pollution and control diseases. Kamga *et al*. (2006) reported that the use of Light Matrix Organic (LMO) has beneficial effects on crop and animal production: increased soil fertility, plant growth, development and yield, increased livestock appetite and feed consumption, improved feed consumption ratio, and reduced mortality and morbidity rates.

The present study was designed to use cow dung in association with a probiotic (Light Matrix Organic) to assess the production performance of *Tripsacum laxum*.

MATERIALS AND METHODS

Study area

The present study was carried out between June and November 2011 at the experimental farm of the Institute of Agricultural Research for Development (IRAD) Bambui, North-West of Cameroon. IRAD, Bambui, is located at latitude 60 North of the equator and longitude 100 East of the Greenwich meridian. It is situated at an altitude of 1600m above sea level, with a mean minimum temperature of 14^{0}C and mean maximum temperature of 24^{0}C. The average annual rainfall is 2330 mm. The topography is undulated, with hills and valleys intersected with small streams. There are two distinct seasons: the dry season which runs from mid-November to mid-March and rainy season which runs from mid-March to mid-November. The average relative humidity is 52% in the dry season and 70% in the rainy season (Bayemi *et al*., 2010).

Rainfall pattern during the period of experiment is shown in the Fig 1. The baseline data on soil fertility level of the soil in the study area is shown in Table 1 while the characteristic of cow dung used as organic fertilizer in the study is shown in Table 2.

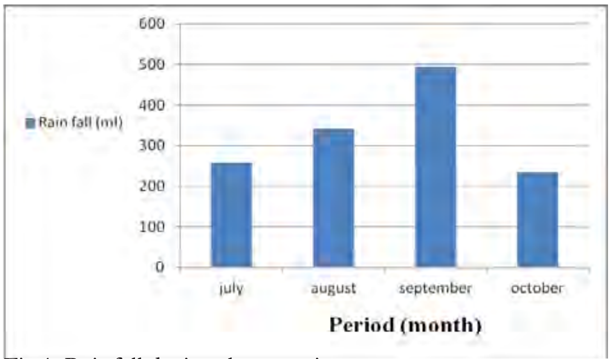

Fig 1: Rainfall during the experiment

Table 1: Baseline soil fertility

Soil Reaction	Value
pH-H_2O	5.64
pH-KCl	4.80
Organic Carbon (%)	2.58
Organic Matter (%)	4.40
Total Nitrogen (g/kg)	2.43
C/N ratio	10.6
Cation exchangeable milliequivalents/100g	
Calcium	5.15
Magnesium	1.48
Potassium	0.15
Sodium	0.02
Sum of bases	6.80
Exchangeable Cationic Capacity in meq/100g	
Effective CEC	4.82
S/CECE	100
CEC pH 7	21.00
Saturation	32.00
Assimilabe Phosphorus (mg/kg)	
Bray II	67.00

Table 2: Cow dung characteristics

Element	Value
Total Nitrogen (g/kg)	38.43
Phosphorus (Bray II mg/kg)	298
Potassium (millequivalent/100g)	6.15
Calcium (millequivalent/100g)	25.15
Sodium (millequivalent/100g)	7.02
Magnesium(millequivalent/100g)	13.48

Experimentation

The trial was a factorial experiment evaluating the effect of four levels of cow dung applications (0, 7.5, 15 and 22.5t/ha) associated with or not to spraying of LMO. The trial was carried out on *T. laxum* pasture established approximately a year prior to this study using cuttings. A total of 32 experimental plots measuring 3x4.5m were identified on four blocks (terraces) with 8 plots per block. The plant density was approximately 1500 plants/ha and the spacing distance between plots was 1.2 m.

Tripsacum laxum plants were cut 15 cm above the ground level using a sharp cutlass. A day following plant levelling, cow dung was applied round each plant and covered with top soil. A volume of 150 ml of LMO was diluted into 15l of rain water and sprayed both on the plants and down on the cow dung. Spraying was done at first a week after levelling and then every two weeks for a total of 16 weeks.

Data collection

Eight plants were randomly selected per plot using random numbers and every four weeks, the height, the number of tillers and the number leaves per plant were assessed for sixteen weeks. The yield per plot was determined at harvest (120 days from levelling) in quadrants of 2.5×3m at the middle of the plot. The plants were cut 15 cm above the ground level and weighed. A representative sample (1.5kg) of forage was collected per treatment for forage quality assessment (AOAC, 1990; Pauwels *et al.*, 1992).

Statistical analysis

Data collected were analyzed using the Statistical Package for Social Sciences (SPSS) Standard Version, Release 17.0. Data within subsets were normally distributed. ANOVA and Turkey Post-Hoc tests were then used to compare groups for significant differences in growth (height, tillers and leaves) and yield (fresh and dry) as affected by replication, plots, blocks and treatment.

Independent-Sample t test was used to compare two independent groups. Pearson pairedcorrelation test was used to assess the influence of individual treatments on the plants whilst regression analysis was used to assess the influence of the interactive effect of two treatments. Multilinear Regression Model, Weight Estimation and General Linear Models were used to evaluate the effect of interaction and the effect of level of each of the factors (Nana, 2012).

The statistical model used was:

$$Yijh = \mu + \alpha i + \beta j + (\alpha\beta) ij + eijh$$

Where:

Yijh = Observation on the plants of the block h having the treatment i and j

μ = general mean

αi = effect of the level of cow dung or factor i

βj = effect of the probiotic or factor j

eijh = residual error on the plant of the block h having received the treatment i and j

(αβ) ij = effect of the interaction between the treatment i and j

RESULTS

Growth of Tripsacum laxum

The effect of cow dung application with or without spraying of LMO on the height of *T. laxum* is shown in Table 3. Treatment combination 22.5 t/ha cow dung application with application of LMO recorded the highest height after 4 months of the study, but no significant difference was observed between the different treatments.

Table 3: Effect of increasing levels of cow dung application (t/ha) with LMO (L) on the height of *T. laxum*

Treatment Cow dung/LMO	Mean increase in height(cm) per day	Std. Error of Mean
0/0	0.80382	0.137783
7.5/0	0.74896	0.057033
15/0	0.72187	0.064431
22.5/0	0.82743	0.055707
0/L	0.77049	0.075730
7.5/L	0.79583	0.125274
15/L	0.71979	0.076194
22.5/L	0.86319	0.076858
Total	0.78142	0.028816
ANOVA (P-Value)	F=0.329, P=0.933	

The number of tillers per plant in the various treatments is shown in Table 4. High cow dung application with or without spraying of LMO appeared to have an ameliorating effect on the production of tillers, with treatment 22.5 t/ha cow dung application without LMO recording the highest increase in number of tillers, followed by no cow dung application with LMO and 22.5 t/ha cow dung application with LMO, but the differences between the treatments were not significant.

Table 4: Effect of increasing levels of cow dung application (t/ha) with LMO (L) on the tiller number of *T. laxum*

Treatment Cow dung/LMO	Mean no. of tillers per day	Std. Error of Mean
0/0	0.04097	0.003989
7.5/0	0.05764	0.016920
15/0	0.06146	0.016938
22.5/0	0.07917	0.027246
0/L	0.07674	0.033383
7.5/L	0.02986	0.007404
15/L	0.05208	0.012481
22.5/L	0.07049	0.033049
Total	0.05855	0.007331
ANOVA (P-Value)	F=0.639;P=0.723	

The number of leaves per plant in the various treatments is shown in Table 5. Cow dung application without spraying of LMO appeared to have an ameliorating effect on the production of leaves. Cow dung application at 22.5 t/ha without LMO recorded the highest number of leaves, followed by 7.5t/ha without LMO and 22.5 t/ha with LMO but the differences between treatments were not significant.

Table 5: Effect of increasing levels of cow dung application (t/ha) with LMO (L) on the number of leaves of *T. laxum*

Treatment Cow dung/LMO	Mean no. of leaves per day	Std. Error of Mean
0/0	0.89236	0.101938
7.5/0	1.20035	0.245074
15/0	1.03438	0.132504
22.5/0	1.18958	0.202060
0/L	1.13646	0.209161
7.5/L	0.77882	0.109827
15/L	0.93611	0.120710
22.5/L	1.05799	0.214202
Total	1.02826	0.059998
ANOVA (P-Value)	**F=0.734; P=0.645**	

Productivity of Tripsacum laxum

The effect of increasing levels of cow dung application and spraying of LMO on the fresh yield is presented in Table 6. The fresh yield ranged between 16484.4 and 24134.4 kg/ha. All the treatment combinations recorded higher yields than the control, but the differences were not significant. When the fresh yield was converted into dry yield, the same pattern was observed (Table 7). An insignificant difference was observed between treatment combinations, with the application of 7.5 t/ha of cow dung with LMO recording the highest yield (6154.6 ±1069.2 kg/ha), followed by the application of 7.5 t/ha without LMO (6127.4 ± 1188.4 kg/ha).

Table 6: Effect of increasing levels of cow dung application (t/ha) with LMO (L) on the fresh yield of *T. laxum*

Treatment Cow dung/LMO	Mean Fresh Yield (Kg/ha)	Std. Error of Mean
0/0	16 484.4	2 323.2
7.5/0	18 371.9	2 810.8
15/0	23 562.5	2 056.8
22.5/0	24 134.4	5 001.3
0/L	18 609.4	1 838.6
7.5/L	20 578.1	3 764.2
15/L	21 125	3 313.1
22.5/L	21 531.3	3 340.2
Total	20 549.61	1 090.5
ANOVA (P-Value)	F=0.671; P=0.695	

Table 7: Effect of increasing levels of cow dung application (t/ha) LMO (L) on the dry yield of *T. laxum*

Treatment Cow dung/LMO	Mean Dry Yield (Kg/ha)	Std. Error of Mean
0/0	5 220.9	422.9
7.5/0	6 127.4	1 188.4
15/0	4 092.3	478.7
22.5/0	3 416	415.7
0/L	4 693.8	795.5
7.5/L	6 154.6	1 069.2
15/L	5 243.6	611.9
22.5/L	5 473.1	472.46
Total	5 052.7	280
ANOVA (P-Value)	F=1.659; P=0.167	

Nutritive value of Tripsacum laxum

The proximate analysis of fresh *T. laxum* is shown in Table 8. The dry matter yield was 25% in the absence of cow dung application and LMO, increasing to 25.1 and 25.2% at 15 t/ha cow dung application without LMO and 22.5 t/ha cow dung application without LMO respectively. The crude protein content varied from 6.9% at 22.5 t/ha cow dung application without LMO to 9.0% at 15 t/ha cow dung application with LMO. The ether extract content varied from 3.0% at 15 t/ha cow dung application with LMO to 3.9 % at 15 t/ha cow dung application with LMO. The crude fibre content varied from 34.0% at 15t/ha cow dung application with LMO to 37.8% at 7.5 t/ha cow dung application without LMO. Metabolisable energy (kcal/kg DM) varied from 443.8 at 22.5 t/ha cow dung application without LMO to 760.8 at 15 t/ha cow dung application with LMO.

Mineral composition of Tripsacum laxum

Calcium, magnesium, potassium, sodium and phosphorus contents of the forage grown under different levels of cow dung application are shown in Table 9. It was observed that the content of calcium, magnesium, potassium and phosphorus generally increased with increasing levels of cow dung application associated with LMO spraying.

The calcium content varied from 1.072% at zero application of cow dung /LMO to 2.0 % at 22.5 t/ha cow dung application with LMO. Magnesium content was 0.437% at no application of cow dung/LMO and increased to 1.196 at 22.5 t/ha cow dung application with LMO. Potassium content varied from 1.410% at zero application of cow dung/LMO to 1.951% at 22.5t/ha cow dung application with LMO. Phosphorus content varied from 0.166% at zero application of cow dung and LMO to 0.233% at 15 t/ha cow dung application with LMO.

DISCUSSION

The heterogeneity observed in the evolution of height of plants with time is similar to the results of Picard *et al.* (1973) who reported a similar pattern in tropical forage behaviour. This may be attributed to the fact that *T. laxum* buds which develop subsequently after cutting are issued from

Table 8: Proximate analysis of *T. laxum* under increasing levels of cow dung application (t/ha) with LMO (L).

Treatment Cow dung /LMO	Proximate analysis							
	DM (%FM)	OM (%DM)	CP (%DM)	EE (%DM)	CF (%DM)	Ash (%DM)	ME (kcal/kg DM)	GE (kcal/kg DM)
0/0	25.38[a]	92.2[a]	7.0[a]	3.7[a]	37.7[a]	7.8[a]	483.6[a]	4436.9[a]
7.5/0	23.75[b]	92.5[b]	7.2[b]	3.5[b]	37.8[a]	7.5[b]	588.9[b]	4493.2[b]
15/0	25.07[a]	93.3[c]	7.4[b]	3.9[c]	37.2[b]	6.7[c]	491.2[a]	4440.0[a]
22.5/0	25.2[a]	90.5[d]	6.9[a]	3.0[d]	37.0[b]	9.5[d]	443.8[d]	4313.3[d]
0/L	24.06[b]	91.2[e]	8.4[c]	3.6[e]	36.4[c]	8.8[e]	556.5[e]	4391.2[b]
7.5/L	23.05[c]	92.6[b]	7.3[b]	3.8[f]	36.6[c]	7.4[b]	603.1[b]	4446.7[a]
15/L	22.5[d]	91.7[f]	9.0[d]	3.0[g]	34.0[d]	8.3[f]	760.8[c]	4370.2[c]
22.5/L	26.87[e]	91.4[e]	8.9[d]	3.3[h]	34.1[d]	8.6[e]	754.7[c]	4371.8[c]
F; P Value	F=64.03 P<0.05	F=64.02 P<0.05	F=403 P<0.05	F=9.49 P<0.05	F=64 P<0.05	F=435 P<0.05	F=1.2e5 P<0.05	F=6.22e8 P<0.05

a, b, c, d, e, f Pairs with the same letter within columns are not significantly different

DM=Dry matter; FM=Fresh matter; OM=Organic matter; CP=Crude protein; EE=Ether extract; CF=Crude fibre; ME=Metabolisable energy; GE=Gross energy

Table 9: Mineral composition of *T. laxum* under increasing levels of cow dung application (t/ha) with LMO (L)

Treatment Cow dung /LMO	Mineral composition (%)				
	Calcium	Magnesium	Potassium	Sodium	Phosphorus
0/0	1.072[a]	0.437[a]	1.410[b]	0.117[a]	0.166[a]
7.5/0	1.456[bc]	0.748[bcd]	1.568[a]	0.124[b]	0.173[b]
15/0	1.450[bd]	0.772[bc]	1.595[c]	0.097[c]	0.201[c]
22.5/0	1.456[cd]	0.554[e]	1.701[d]	0.131[d]	0.207[c]
0/L	1.904[ef]	0.690[bd]	1.648[e]	0.110[e]	0.190[d]
7.5/L	1.840[e]	0.748[bcd]	1.582[a]	0.103[a]	0.193[d]
15/L	1.968[f]	1.001[f]	1.793[f]	0.104[a]	0.233[e]
22.5/L	2[g]	1.196[f]	1.951[g]	0.125[b]	0.230[e]
F; P Value	F=127.3 P<0.05	F=129 P<0.05	F=128.4 P<0.05	F=129.41 P<0.05	F=121.51 P<0.05

a, b, c, d, e, f Pairs with the same letter within columns are not significantly different nodes with great variability. Indeed, older and more fibrous plants delay in shooting after trimming and may instead respond by the production of higher number of tillers. In this line, the apex of some individual plants could have been above the mowing level, inducing possible difficulty in shooting.

The increase in level of cow dung application with or without LMO spraying generally produced an increase in number of tillers and leaves. The increase in production of tillers due to cow dung application agrees with the finding of Miller (2007) who demonstrated an increase in rice tiller following application of poultry manure. However, the structural way in which production of tillers and leaves is affected by the cow dung and spraying of probiotic is not understood. It is probable that cow dung contains tillering and leafing promoting factors. This corroborates Atiyeh *et al* (2001) who suggested possible growth promoting factors from pig manure on tomatoes.

All the treatments in the study recorded higher yields than the control, although the differences were insignificant. These non-significant differences may be attributed to initial soil fertility levels, quality of the environment and climatic conditions. Indeed the base line fertility of the soil used was quite adequate for traditional agriculture and it has been reported that the effect of the addition depends partly on the existing fertility of the soil (Gana, 2009). This may also be attributed to fertilizer type/application as the fertility input in organic farming systems are based on organic matter inputs and only become available to plants after unlocking of nutrients from the solid phase by weathering or mineralization process (Tamm *et al.*, 2007). Their effect could therefore be better appreciated over a longer period of time as residual effect. Furthermore, the soil used for this study was high in organic matter (4.4 %) and of good quality (C/N 10.6), features which probably made nitrogen application responses generally poor (Mengel and Kirkby, 1982).

The relatively high plant response to microbial inoculation (LMO) in dry yield corroborates the observations of Yolcu *et al.* (2010) who reported higher yield in Italian rye grass with the inoculation of particular rhizobacteria. This is also similar to the results of Poonyarit *et al.* (1993) who reported an increase in yield of paddy rice with the application of Effective Microorganisms (EM) plus chemical fertilizer. These authors further reported that microbial inoculation would give more yield when combined with chemical and organic fertilizer.

Crude protein (CP) increased with cow dung application. The increase is similar to the observations of Lanyasunya *et al.* (2007) who reported a higher CP yield after manure application on Columbus grass (*Sorghum almum*). Also, Butler and Muir (2006) reported that CP of tall wheatgrass (*Thinopyrum ponticum Podp*.) was greatest at the two highest rates of dairy manure compost in one of the two years of the experimentation.

The crude fibre level was lower in *Tripsacum laxum* grown on cow dung sprayed with LMO. The lower level may suggest that the forage produced with LMO will be more palatable at this particular growth level. Indeed, Williamson *et al.* (1978) and Boudet (1991) reported that vegetal species are more appreciated if the level of crude fibre (CF) in them is low.

The higher level of phosphorus and magnesium recorded with spraying of LMO is similar to the observations of Çakmakçi *et al.* (2009) who reported that phosphate solubilising and N_2 fixing PGPR increased the uptake of P and Mg in spinach and wheat plants. Microbial inoculation (LMO spraying) provided higher concentrations of calcium and potassium than those of the control. This is different from the results of Yolcu *et al.* (2010) who observed that most of the rhizobacteria (except RC105) provided lower or similar potassium and calcium concentrations than those of control. On the other hand, Ibrahim *et al.* (2008) observed that fertilization of soybean with chicken manure increased considerably Ca and Mg content in the seeds, while the inoculation of seeds with *Bradyrhizobium* caused further increase in Ca and Mg content. The different responses to such treatments could be attributed to differences among cultivars, inoculated microorganisms, as well as to differences in the growing environment.

Correlation analysis between treatment combination and indicators of performance revealed that cow dung could be used alone for optimal fresh yield performance because its correlation coefficient was the same with that of the interactive treatment (cow dung/LMO). The correlation between LMO and fresh yield was negative, showing LMO instead had a negative impact on fresh matter. However, when the yield was converted to dry matter, the situation was reversed. It was the correlation between cow dung and dry matter yield which was negative showing that though cow dung promoted fresh yield, the dry matter yield was promoted by LMO spraying, and cow dung alone had a negative impact on the production of dry matter. Weight Estimation Model confirmed that when correlation between cow dung and yield was weighted by LMO, it dropped from 0.345 to 0.187; weighting correlation between cow dung and dry matter yield by LMO improved on the strength of correlation that moved from -0.216 to 0.111. Cow dung thus seemed to foster growth but at the same time contributed to higher water content in the forage. The explanatory power of the interaction as explained by R-Square was generally very low, indicating that the interactive treatment did not improve the variability of the dependent indicator; this therefore reinforced the assertion that cow dung alone seemed to impact growth and fresh yield but not the dry yield.

There was a strong and significant positive correlation between tillering, leafing and fresh yield. The impact of cow dung on leafing was higher as compared to LMO but, at the same time, cow dung contributed to higher water content resulting in lower yield in dry matter. Cow dung also had higher effect on tillering as compared to LMO during the four months of the study. Growth continued for all the indicators even when the rainfall started reducing, though at a slower rate.

CONCLUSION

The present study indicated that cow dung contributed more to growth and at the same time fostered water content, thus reducing the dry matter content of *Tripsacum laxum*. LMO, on the other hand, increased the dry matter, the nutrient and the mineral content of forage. Considering that the best forage for the animal is obtained with higher dry matter yield and nutritive contents, cow dung cannot be used alone for optimal dry matter yield in improving forage production. Cow dung at the level of 15t/ha in association with LMO gave the best dry matter yield and nutritive value.

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