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Original Article

Performance of Italian ryegrass varieties: Effect of cutting stage on dry matter and total non-structural carbohydrates percentages

Patrick N Rakau[1*], Khuliso E Ravhuhali[2], Mpho Nzeru[1]

[1]Agricultural Research Council, Cedara, Animal Production Institute, Forage Breeding, P.O. Box 1055, Hilton 3245, Kwazulu Natal, South Africa

[2]School of Agriculture Sciences, Department of Animal Science, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046 Mmabatho, 2735, South Africa

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**Corresponding author email:*
rakaup@arc.agric.za

Abstract

Plant dry matter (DM) is one of the most limiting factors on the rumen fill. Due to variation in seasons, forages are prone to different stresses ranging from abiotic and biotic factors. The study was done to assess the effect of cutting stage on dry matter yield r and total non-structural carbohydrates of Italian ryegrass varieties (14 varieties). Diploid varieties namely: Supercharge, Sukari, Kigezi, Bartrento, ARC-148 BQX, Maximus and 14 LMD 43 and Tetraploid varieties namely: Green-Spirit, Barmulta II, ARC-214 GQX, Supreme Q, Agriton, AgriBoost and Mona. The trial was run at the Cedara research station in KwaZulu-Natal province (KZN). All 14 varieties were planted in rows which were 150mm apart (at the population rate of 30 kg/ha for both diploid and tetraploid) replicated three times in a laid out and fitted into randomized complete block design (RCBD). Dry matter yield and non-structural carbohydrates were analysed. A consistent effect of cutting stage on DM yield and TNC was not observed on average mean values. The cutting interval did not influence the DM yield and TNC concentration of both on diploid and tetraploid *Lolium multiflorum* varieties. The cutting stages can be reduced to four or five to allow the plant to regain its vigour after cutting /defoliation in order to and enforce the source-sink relationships.

Keywords: Rumen fill, Ruminant, Dairy cattle, Carbohydrates, Linear regression

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Introduction

One of the significant plant's responses to different stresses is changes in carbohydrates reserves such as TNC contents due to defoliation and variations in climatic conditions, which also had an impact on dry matter yield. The total non-structural carbohydrates are known to be translocated within the plant and used for growth, respiration, and they are essential in the recovery of plants from disturbances. Large quantities of soluble carbohydrates, predominantly fructans are stored in the vacuoles of the leaf and crown tissues during cold conditions (Huner et al., 1993). Previous reports indicated an increment in TNC percentages on forages when sheep grazed plants and these commonly influence rumen fermentation thereby enhancing rumen nitrogen degradation and aid in the nitrous oxide emissions reduction in pastures

(Michell, 1973; Jones and Roberts, 1991; Harrison et al., 2011). Fructan polymers are regarded as the principal storage of carbohydrates (Pontis, 1989; Price et al., 2002). To maintain high DM intake by livestock, 18 to 20% of DM should be maintained to maximize the productivity of ruminants (NRC, 2000). According to Regional Climate Development under Global Warming (regclim, 2005) DM intake can vary due to seasonal variations. Cutting is known to influence DM production and quality of plant (Wadi et al., 2004). The amount of residual leaf area must be adequate for continuing photosynthesis to get significant re-growth (Grace et al., 2019). Photosynthesis can be significantly inhibited with the removal of leaf tissue because green tissue is required for photosynthesis. Dry matter yield normally increases with decreasing disturbances (Tessema et al., 2010). Therefore, the objective of this study was to investigate the effect of cutting stage on dry matter, and non-structural carbohydrates of fourteen (14) selected Italian ryegrass varieties (tetraploid and diploid).

Material and Methods

Study site

Experiment was conducted at KwaZulu-Natal Cedara research station, South Africa. The site is located at 29˚32'S 30˚16'E at an altitude of 1075 m above sea level and the average annual rainfall of 885 mm. The average high temperature is 16.2 ˚C, and low temperature in winter was 3.7 ˚C. Frost periods on average, is 75 days per annum. The soil at the experimental farm had 10.5, 97.5, 790, 211, 2.0, 4.5 and 2.45 mg/L for P, K, Ca, Mg, Zn, Mn and Cu, respectively and soil pH (KCL) was 4.47. The soil also recorded 4.0, 0.275 and 50.5% for OC, N and clay, respectively.

Experimental design

The experimental design was laid out and fitted into a randomized complete block design (RCBD) factorial arrangement with three replicates. The experiment consisted of 21 plots, and gross plot size of 2 m × 6 m and 1.4 m × 4.6 m was used at the site. From 36 Italian ryegrass varieties both tetraploid and diploid planted on the 14th March 2018, only 14 varieties were selected (seven diploids and seven tetraploid varieties). The following varieties were used: Tetraploid = ARC-214 GQX, Supreme Q, AgriBoost, Green-Spirit, Agriton, Mona and Barmulta II and Diploid= Kigezi, Supercharge, Sukari, ARC-148 GQX, Bartrinto, 14 LMD 43 and Maximus. All 14 varieties were planted in rows which were 150 mm apart at the sowing rate of 30 kg/ha for both diploid and tetraploid varieties. Twenty-five millimetres per week irrigation was applied. Fertilization of 50 kg N/ha as 1:0:1 was applied when the seedlings were 10 cm height and after each cut. The plots were cut seven times from March to November and were cut at 3-leave stage which represents elongation and ready for grazing. Cutting stages were: Cut 1, 11th May 2018; Cut 2, 13th June 2018; Cut 3, 13th July 2018; Cut 4, 28th August 2018; Cut 5, 29th September 2018; Cut 6, 23rd October 2018 and Cut 7, 26th November 2018. Cutting were done with Agri-mower machine set at 5 cm above ground level.

The following equations were adopted to calculate dry matter (DM) yield and total non-structured carbohydrates (TNC).

Dry Matter Yield $=[PGW\ (SDW/SGW)/1000]\ X\ 10\ 000$, were PGW = Net plot green weight, SGW = sample green weight and SDW = sample dry weight.

TNC (g/kg) = $Absorbance\ sample/absorbance\ standard\ X\ 5555.55\ DM\ \%\ sample$

Total non-structural carbohydrate was analyzed using standard wet chemistry as described by Marais et al. (1966). Chemical Reagents*:* 0.05 M sulfuric acid reagent solution was prepared through slowing adding concentrated H_2SO_4 (2.8 ml) to distilled H_2O (500 ml) and further diluted to 1 L. 0.1 M Sulfuric acid solution reagent was done through slowing adding concentrated H_2SO_4 (0.56 ml) to distilled H_2O (50 ml) and further diluted to 100 ml. Copper reagent solution I was prepared by were dissolving Na_2CO_3 (30 g), $NaHCO_3$ (20 g), $KNaC_4H_4O_6.4H_2O$ (15 g) and Na_2SO_4 (180 g) in distilled H_2O (1 L). Copper reagent solution II was prepared through dissolving Na_2SO_4 (45 g) and $CuSO_4.5H_2O$ (5 g) in distilled H_2O (250 ml). Immediately before use, four volumes of solution I was mixed with 1 volume of solution II. The Arsenomolybdate reagent solution was prepared by dissolving $(NH_4)_6Mo_7O_{24}.4H_2O$ (25 g) in distilled H_2O (400 ml) and concentrated H_2SO_4 (21 ml) was carefully added. The HNa_2O_4. $7H_2O$ (3 g) was dissolved in distilled H_2O (25 ml) and added to the acidic ammonium molybdate solution and made up to 500 ml. The solution was incubated at 37 ˚C for 48 hours and stored in a glass-stoppered brown bottle. For 0.1 M sugar standard, glucose (0.6667 g) was dissolved in distilled H_2O (200 ml).

Procedure

The plant material (0.3 g) samples were weighed into test-tube, 0.05 M H_2SO_4 (10 ml) was added in each test-tube and mixed respectively. Both blank and standard solutions were prepared, blank contained 0.05 M H_2SO_4 (10 ml) without plant material, and the standard solution contained 0.1 M H_2SO_4 (5 ml) and sugar standard (5 ml). Both solutions were heated in a boiling water bath for 30 minutes, cooled to room temperature immediately, transferred quantitatively to a 250 ml volume flask. Both solutions were filtered before analyzing for reducing sugars tests. Samples were filtered to 1 ml volume each and diluted to 3 ml volume with distilled water. Standard and blank test-tube solutions containing 3 ml aliquots of standard sugar solution and 3 ml of distilled water, respectively. Copper mix solution of 3 ml was added to each solution and mixed well, heated in a boiling water bath for 20 minutes and cooled at room temperature. The Arsenomolybdate reagent of 3 ml was added and shaken until bubbles formation ceased. The colour appeared after 1.5 hours, and the solution was transferred to 200 ml volume flask. The absorbance was read at 750 nm against the blank solution.

Statistical analysis

Analysis of variance (SAS, 2010) was used to assess the effect of cutting and variety on DM and TNC data. The least square means were compared using Tukeys test. The least significant difference test was performed at a P<0.05 level of probability to establish the significant difference between treatments means.

Results and Discussion

Results reflecting average TNC and DM values for varieties and the different cutting stages of seven selected Italian ryegrass varieties of diploid and tetraploid are presented in Table 1. Within species, the SuperCharge variety exhibited higher (P<0.05) DM (21%) and TNC (23.1%) as compared to all other varieties in the diploid group. These TNC values in all varieties are comparable with the previous report by Francis et al. (2002). The diploid varieties TNC values different cutting stages from ranged from 12 to 20%. Indeed, different varieties can have variation in TNC and DM yield. Except for Mona variety, GreenSpirit in tetraploid had the same (P>0.05) DM value as all other varieties under the same tetraploid group. SupremeQ in tetraploid had the highest (P<0.05) TNC value (16.7%) when compared to other varieties.

Table-1. Average values on the effect of species and cutting on DM and TNC percentages of 14 selected Italian ryegrass varieties of diploid and tetraploid

Diploid					
Species	**DM %**	**TNC %**	**Cutting**	**DM %**	**TNC %**
148BQX	19.7^{bc}	16.2^{d}	1	17.4^{cd}	17.7^{e}
14LMD43	19.3^{c}	16.4^{d}	2	18.0^{c}	18.9^{d}
Bartento	18.3^{d}	17.2^{b}	3	21.0^{b}	20.3^{b}
Kigezi	20.0^{bc}	14.6^{f}	4	17.4^{cd}	20.6^{a}
Maximus	18.1^{d}	15.7^{e}	5	16.6^{d}	11.7^{g}
Sukari	20.6^{ab}	17.0^{c}	6	17.2^{cd}	18.6^{c}
SuperCharge	21.0^{a}	23.1^{a}	7	29.4^{a}	12.3^{f}
SE	0.329	0.063		0.329	0.063
Tetraploid					
Species	DM %	TNC %	Cutting	DM %	TNC %
214GQX	17.9^{cd}	16.2^{c}	1	16.6^{c}	17.6^{d}
AgriBoost	19.4^{ab}	14.8^{f}	2	16.6^{c}	17.8^{c}
Agriton	19.7^{a}	15.4^{e}	3	20.1^{b}	19.4^{a}
Barmulta	18.5^{bcd}	16.0^{d}	4	17.0^{c}	18.9^{b}
GreenSpirit	18.8^{abc}	15.5^{e}	5	16.4^{c}	9.3^{g}
Mona	17.8^{d}	16.3^{b}	6	16.8^{c}	16.8^{e}
SupremeQ	19.5^{ab}	16.7^{a}	7	28.1^{a}	11.2^{f}
SE	0.328	0.051		0.328	0.051

$^{abcdefg:}$ Means with different superscripts in the same column differ (P<0.05)
SE: standard error
CL: cutting stage, DM: dry matter yield, TNC: total non-structural carbohydrates

At different cutting stages, the seventh cutting had the highest (P<0.05) DM value (28.1%) than all other cutting stages in tetraploid. The third cutting had the highest (P<0.05) concentration (19.4%) as compared to all different cutting stages in the same tetraploid group. The different cutting stages and sampling days influenced the photosynthesis and respiration rate of the plants (Francis et al., 2002; Kagan et al., 2011). Indeed, different species varieties possess different TNC, fructan, glucose, fructose sucrose and starch (Brocklebank and Hendrỳ, 2006; Downing and Gamroth, 2007). Again, stage of plant development can lead to an increase in carbohydrates concentration in the plants (Francis et al., 2002).

Effect of cutting stage and cultivar on average values of DM and TNC percentages of seven selected Italian ryegrass of varieties of diploid

The results of the effect of cutting and variety on average values of DM and TNC (total non-structural carbohydrates) percentages of seven selected Italian ryegrass of diploid are presented in Table 2. Although the significant differences were observed, consistent effect on cutting stage on DM yield and TNC was not observed on average mean values. This can be attributed to the cuttings that reduced tillering that contributes to the dry matter yield of plants and plant normally respond differently after defoliation through tolerance mechanisms as an influence by different morphological and physiological characteristics of those varieties (Wen and Jiang, 2005). Seventh cutting in each variety had higher (P<0.05) DM percentages when compared to all cutting stages within each variety. Within each cutting stage, SuperCharge had higher (P<0.05) DM % than all other species at the first cutting. SuperCharge (23.2%) and Kigezi (23. 2%) has the highest (P<0.05) DM yield at the third cutting. Sukari and SuperCharge varieties had the same (P>0.05) DM % value as Maximus, 14LMD43 and ARC-148BQX varieties at the seventh cutting. Meissner et al. (1992) had similar DM values, as reported in this study. The authors reflected that DM content of 18 to 20% is recommended to maximise the intake of the diets. The feeding value of most Italian ryegrass varieties can be limited by a reduction in intake due to young grass at their vegetative stage (Marais et al., 1993).

Within species, ARC-148BQX at the third (20.0 %) and fourth (20 %) cutting stages had higher (P<0.05) TNC percentages when compared to other cutting stages of the same variety. The third cutting of Bartento had the higher (P<0.05) TNC concentration value (23.2 %) than all other cutting stages from the same variety. SuperCharge at the sixth cutting stage had the highest (P<0.05) TNC value (28.0 %) than all other cutting stages from the same variety.

Within each cutting stage, SuperCharge variety had the highest (P<0.05) TNC concentration than other varieties at the first (24.1 %), second (27.2 %), fourth (27.4 %), fifth (17.5 %), and sixth (28.0 %) cutting stages. As opposed to these results, management such as defoliation or cutting is known to be influencing the concentration of TNC. Formation of sugars, starch, proteins and other foods is dependent on photosynthetic processes of leaves (rarely of stems). If the plant is mowed or grazed, the plant will always utilised the carbohydrates reserved to recover from defoliation, and that will lead to the reduction of TNC concentration in the plants (Nelson, 1995; Prince, 2017).

Table-2. Cutting and variety effect on dry matter and TNC percentages of seven selected Italian ryegrass varieties of diploid

DM %							
CL	148BQX	14LMD43	Bartento	Kigezi	Maximus	Sukari	SuperCharge
1	16.8^{cB}	16.2^{cC}	17.3^{cBC}	17.4^{cBC}	15.5^{bcC}	18.7^{cAB}	20.2^{cA}
2	17.4^{cBC}	17.1^{bcCD}	16.3^{cCD}	22.4^{bA}	14.8^{cD}	18.5^{cBC}	19.8^{cB}
3	20.6^{bBC}	19.2^{bCD}	20.5^{bBC}	23.2^{bA}	17.9^{bD}	22.2^{bAB}	23.2^{bA}
4	17.8^{cA}	18.3^{bcA}	16.6^{cAB}	17.2^{cAB}	15.2^{cB}	18.5^{cA}	18.2^{cdA}
5	16.6^{cA}	17.1^{bcA}	15.9^{cA}	17.0^{cA}	15.3^{cA}	17.5^{cA}	16.8^{dA}
6	17.0^{cAB}	18.5^{bcA}	15.8^{cB}	15.8^{cB}	16.6^{bcAB}	18.4^{cA}	18.4^{cdA}
7	31.5^{aA}	28.5^{aBC}	26.1^{aC}	27.2^{aC}	31.7^{aA}	30.5^{aAB}	30.7^{aAB}
SE	0.868						
TNC %							
CL	148BQX	14LMD43	Bartento	Kigezi	Maximus	Sukari	SuperCharge
1	15.3^{dD}	17.6^{cC}	18.1^{cB}	15.6^{cD}	15.2^{cD}	18.3^{cB}	24.1^{cA}
2	18.5^{bD}	17.5^{cE}	20.2^{bB}	14.4^{dG}	15.5^{cF}	19.2^{bC}	27.2^{bA}
3	20.0^{aC}	17.0^{dF}	23.2^{aA}	18.4^{aE}	21.0^{aB}	19.4^{bD}	23.1^{dA}
4	20.0^{aC}	20.9^{aB}	20.1^{bC}	14.5^{dD}	20.3^{bC}	21.1^{aB}	27.4^{bA}
5	12.3^{eAB}	12.0^{eB}	9.4^{eC}	9.3^{fC}	12.2^{dB}	9.3^{fC}	17.5^{eA}
6	17.0^{cC}	18.4^{bB}	18.6^{cB}	16.2^{bD}	15.4^{cE}	16.5^{dD}	28.0^{aA}
7	10.4^{fF}	11.4^{fD}	10.6^{dE}	13.8^{aC}	10.3^{eF}	15.2^{eA}	14.5^{fB}
SE	0.165						

$^{abcdefg:}$ means in the same column with the different superscripts differ significantly (P<0.05)

$^{ABCDEFG:}$ means in the same row with the different superscripts differ significantly (P<0.05) CL: cutting stage, DM: dry matter yield, TNC: total non-structural carbohydrates

SE: standard error

Effect of cutting and variety on average values of DM and TNC percentages of seven tetraploid Italian rye grass varieties

The results of the impact of cutting and variety on average values of DM and TNC percentages of seven Italian ryegrass varieties selected of tetraploid are presented in Table 3. Even though the consistent effect in cutting was not observed on dry matter yield and TNC percentages, there was a significant difference observed on mean values. Within each variety, the seventh cutting in each variety had higher (P<0.05) DM percentages when compared to all cutting stages within each variety.

Within each cutting stage, Agriton variety has higher (P<0.05) DM percentage (19.5 %) than Mona variety at the first cutting. Agriton has the same (P>0.05) DM % value as AgriBoost, GreenSpirit and SupremeQ varieties at the seventh cutting. Mona variety has the same (P>0.05) DM % value as ARC-214GQX, Barmulta, GreenSpirit varieties at the seventh cutting. Within each variety, ARC-214GQX (21.7 %), AgriBoost (18.3 %) and Barmulta (20.2 %) varieties at the third cutting stage had the highest (P<0.05) TNC percentages (21.7 %) when compared to other cutting stages from the same varieties. Mona and SupremeQ varieties at the fourth cutting stage had the highest (P<0.05) TNC percentages (24.1 and 21.8% respectively) when compared to other cutting stages from their respective same varieties.

Within each cutting stage, ARC-214GQX at the first cutting stage had the highest (P<0.05) TNC concentration (19.9 %) when compared to the other varieties from the same cutting stage. SupremeQ at the seventh cutting stage had the highest (P<0.05) TNC concentration (13.2 %) when compared to the other varieties in the same cutting stage. Comparably the values of TNC in tetraploid are almost similar to those of diploid varieties opposite to what was expected. Many of these TNC values, in general, are higher and also in comparison to those reported by Meeske et al. (2009) on the enhancer varieties. Compared to this study, Hopkins (2000) and Hopkins et al. (2003) reported lower values. Difference between plants is influenced by the fact that plants higher in TNC are unable to convert carbohydrate reserves into structural carbohydrates (Marais et al., 1993). Kellogg and Birchler (1993) highlighted that even though grasses might possess the same shoot anatomy and morphology different sizes of leaves will always be observed or linked with TNC concentration. The broadness of the leaves is known in the prevention of moisture evaporation and assist in the synthesis of carbohydrates when compared to thin or narrow leaves (Murphy et al., 2015; Ravhuhali et al., 2019). Plant species of higher TNC percentages can be selected as they influenced better taste, induce intake and milk production in dairy cows (Miller et al., 1999). The ryegrass is known to have low DM, which may reduce the DM intake of grass by the animal, especially dairy cattle.

Table-3. Cutting and variety effect on DM and TNC percentages of seven Italian ryegrass cultivars of tetraploid.

	DM %						
CL	214GQX	AgriBoost	Agriton	Barmulta	GreenSpirit	Mona	SupremeQ
1	16.6^{cdBC}	15.6^{cBC}	19.5^{bcA}	16.3^{cBC}	16.2^{cBC}	14.3^{eC}	17.8^{cAB}
2	15.9^{cdBC}	16.9^{cAB}	17.4^{cdAB}	16.0^{cBC}	16.6^{cABC}	14.8^{deC}	18.7^{cA}
3	19.7^{bAB}	20.7^{bAB}	20.4^{bAB}	19.4^{bAB}	19.5^{bAB}	18.9^{bB}	21.8^{bA}
4	16.7^{cAB}	16.5^{cAB}	15.3^{dB}	18.3^{bcA}	16.9^{cAB}	17.6^{bcAB}	17.5^{cA}
5	14.4^{dB}	16.8^{cAB}	16.2^{dAB}	16.9^{cA}	16.8^{cAB}	16.9^{bcdA}	16.9^{cdA}
6	17.0^{cAB}	17.4^{cAB}	19.1^{bcA}	16.9^{cAB}	18.1^{bcA}	15.6^{cdeB}	13.4^{dB}
7	25.2^{aD}	32.0^{aA}	29.8^{aAB}	25.8^{aCD}	27.8^{aBC}	26.2^{aCD}	30.3^{aA}
SE	0.868						
	TNC %						
CL	214GQX	AgriBoost	Agriton	Barmulta	GreenSpirit	Mona	SupremeQ
1	19.9^{bA}	14.5^{dG}	19.3^{bB}	17.4^{dE}	18.7^{aC}	15.6^{dF}	18.1^{dD}
2	15.9^{dE}	16.3^{cD}	22.0^{aA}	18.1^{cC}	17.9^{bcC}	15.1^{eF}	19.3^{cB}
3	21.7^{aA}	18.3^{aD}	17.2^{cF}	20.2^{aC}	17.6^{cdE}	20.8^{bB}	19.9^{bC}
4	19.9^{bC}	16.3^{cE}	13.0^{fF}	19.7^{bC}	17.3^{dD}	24.1^{aA}	21.8^{aB}
5	8.2^{fC}	9.9^{fB}	9.7^{gB}	8.1^{fC}	7.8^{fD}	11.6^{fA}	10.0^{gB}
6	18.5^{cA}	17.1^{bC}	14.1^{dD}	17.6^{dB}	18.2^{bA}	17.2^{cC}	14.5^{eD}
7	9.1^{eF}	11.5^{eC}	12.5^{eB}	10.7^{eD}	11.3^{eC}	9.9^{gE}	13.2^{fA}
SE	0.135						

$^{abcdefg:}$ means in the same column with the different superscripts differ significantly (P<0.05)

$^{ABCDEFG:}$ means in the same row with the different superscripts differ significantly (P<0.05) CL: cutting stage, DM: dry matter yield, TNC: non-structural carbohydrates

SE: standard error

Linear regression in establishing the relationship between cutting dates, and varieties on DM and TNC percentage

There was no relationship between cutting interval and the parameter measured on both diploid and tetraploid *Lolium multiflorum* varieties (Figure 1 and 2). The cutting interval did not influence the DM yield and TNC concentration of both on diploid and tetraploid *Lolium multiflorum* varieties. Marais and Goodenough (2000) highlighted that the TNC content is always related to the amount of DM yield, and the improved varieties should have better TNC when compared to the other unimproved varieties. Indeed, a plant responds typically differently to defoliation, wowing or grazing. These results are in similar to those reported by Wen and Jiang (2005) who found no relationship between cutting and dry matter yields or different ryegrass varieties.

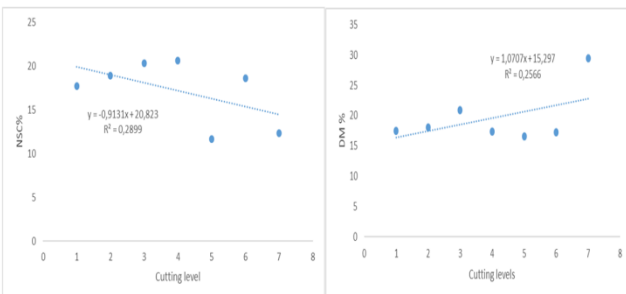

Figure-1. Linear regression in establishing the relationship between cutting dates, and DM and TNC percentage on diploid *Lolium multiflorum* varieties

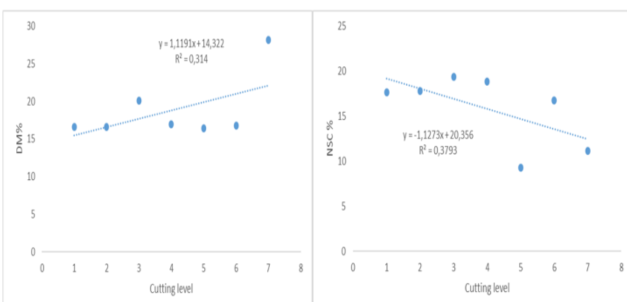

Figure-2. Linear regression in establishing the relationship between cutting dates, and DM and TNC percentage on tetraploid *Lolium multiflorum* varieties

Conclusion

Even though the consistent effect in cutting was not observed on dry matter yield and TNC percentages, there was a significant difference observed on mean values. Cutting had less influence on the DM yield and TNC concentration of both on diploid and tetraploid *Lolium multiflorum* varieties. Fifth cutting produced the DM yields below the recommended rate of 18-20% in both diploid and tetraploid variety groups. Again, fifth cutting had less TNC percentages in both varieties under diploid and tetraploid. The cutting stage can be reduced to four or five so as to allow the plant to regain its vigour after cutting/ defoliation in order to enforce the source-sink relationships.

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Contribution of Authors

Rakau PN: Conceptualization of the study, planting, data collection, Data analysis, Manuscript writing.
Ravhuhali KE: Conceptualization of the study, Data analysis, Manuscript writing.
Nzeru M: Conceptualization of the study, Data analysis.