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Effect of cultivar, nitrogen application rate and stage of harvesting on yield and nutritive value of perennial ryegrass (*Lolium perenne* L.)

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Abstract

This experiment consisted of a factorial combination of seven perennial ryegrass cultivars, two levels of nitrogen fertilizer (30 and 60 kg ha^{-1}), and three harvesting stages (60, 75 and 90 days after planting; DAP) and arranged in a split-plot randomized complete block design. Harvested herbage was chemically analysed and fermented with bovine rumen fluid using the Reading Pressure Technique and the ANKOM Daisy incubator. All the three factors significantly ($P<0.05$) influenced DM yield. Differences between cultivars were observed in CP and fibre contents as well as in the rate of *in vitro* ruminal gas production from the b fraction (c), which ranged from 6.2 %/h (Bronsyn) to 5.5 %/h (Fitzroy). Ryegrass pastures harvested at 60 DAP had the highest ($P < 0.05$) iDMD (913.5 g/kg DM) and ME (14.0 MJ/kg DM) while those harvested at 90 DAP had the least iDMD (846.7 g/kg DM) and ME (12.9 MJ/kg DM). All cultivars had adequate Ca, Mg, P, Cu and Zn levels to meet the normal animal requirements, however, K, Fe and Mn were found to be in excess. Higher biomass yield is always desirable in low-input production systems where fluctuation in quantity of feed resources is a major limiting factor. Therefore, it was concluded that the high yielding cultivars, Fitzroy (2.8 tons/ha), Samson (2.8 tons/ha), Bealey (2.7 tons/ha) or Bronsyn (2.6 tons/ha) may be preferred for cultivation. A farmer can also choose to harvest at 90 DAP in order to maximize biomass yield at the cost of digestibility.

Keywords: Crude protein, Fibre, Minerals, Nitrogen fertilizer, Perennial ryegrass, Simulated ruminal fermentation

Abbreviations: DAP: Days after planting; **DM**: Dry matter; **iDMD**: *In vitro* ruminal true digestibility; **ME**: Metabolizable energy

Introduction

Lolium perenne (perennial ryegrass) is one of the most commonly cultivated grasses used in dairy and intensive sheep production systems in tropics and sub-tropics because it is economical feed resource compared to silage and concentrates (Smit *et al*., 2005). Several ryegrass cultivars are commercially available, but their genetic variability is likely to influence their nutritive value. Traditionally evaluation trials for perennial ryegrass cultivars have focussed mainly on forage production, disease resistance (Bonthuis *et al*., 2003) and adaptation characteristics (Conaghan *et al*., 2008). Although there is some information on perennial ryegrass in South Africa, not many trials have evaluated the yield and nutritive value of ryegrass cultivars under a low-input farming environment. Also, there are few studies that have investigated the effect of harvesting date and fertilizer application on the nutritive value of ryegrass cultivars. Information on the dynamics of forage quality in response to management practices and plant's growth cycle would help to optimize harvesting or grazing to meet specific animal requirements (Valente *et al*., 2000). The present study was therefore conducted to 1) evaluate seven ryegrass cultivars for their yield, chemical composition and *in vitro* ruminal fermentation characteristics and 2) investigate the response of ryegrass to variations in harvesting period and N fertilizer application.

Materials and Methods

Study site: The experiment was conducted at the University of Fort Hare (UFH) Research Farm in Alice, Eastern Cape province, South Africa. Eastern Cape is the largest province in the country with a suitable climate for dairy and sheep production that have been expanding and thus triggering the search for inexpensive alternatives to commercially available feeds. The study area has a warm temperate climate with an annual rainfall range of 450–600 mm, received mainly during the summer months (November–March). The mean annual temperature in summer and winter are 16–27°C and 6–18.1°C, respectively (Mandiringana *et al.*, 2005).

Plant material: The seven cultivars used in this experiment were Bealey, Bronsyn, Quartet, Fitzroy, Indiana, Pastoral and Samson. Bealey, Pastoral and Quartet are late heading tetraploids. Bealey has high winter and summer growth; good persistence and clover compatibility. Pastoral is bred for improved heat tolerance and rust resistance, while Quartet has been shown to withstand hard grazing. Fitzroy, Indiana and Samson are early maturing diploids with good performance in late winter production. Samson has been currently used at the UFH dairy farm. Bronsyn is a diploid late heading cultivar with improved seasonal growth and excellent performance.

Experimental design and pasture management: The experiment consisted of a factorial combination of seven cultivars, two levels of nitrogen (black urea, 46 % N) fertilizer (30 and 60 kg ha^{-1}), and three harvesting stages (60, 75 and 90 days after planting).The experimental design was a split-plot randomized complete block with the combination of cultivars and fertilizer levels being assigned to the main plots, while harvesting dates were assigned to the sub-plots with three replicates. The main plot had a size of 6 m x 5 m, which were equally divided in to three sub-plots. The space between the main plots was 1 m. The levels of fertilizer applied during establishment were lower than the recommended rates of 130–140 kg ha^{-1} for commercial farms (Pannar, 2013). This approach simulated common practice in low input farming systems that occur in resource-poor communities. No additional fertilizer was applied to the pasture for the rest of the growth period. Seed materials were sown (June 2011) by broadcasting at the rate of 25 kg ha^{-1}. After planting, light irrigation of 20–25 mm water was applied uniformly every four days for four weeks, and every 5–6 days for the remaining growth period. Fertilizer was applied after two weeks of germination.

Harvesting procedure: A total of five 0.25m^2 quadrate samples per sub-plot were harvested each at 60, 75 and 90 DAP when the average plant height was approximately 25 (recommended grazing practice after establishment), 35 and 42 cm, respectively. Plants were cut at a stubble height of 5 cm. The samples were oven dried at 72 °C for 48 hours and weighed. Dried samples were ground to pass through a 1 mm sieve and stored in brown paper bags at room temperature pending for analysis.

Chemical analyses, in vitro ruminal fermentation and true digestibility: Nitrogen content was measured using the standard macro-Kjeldahl method (AOAC 1999). Neutral detergent fibre (NDF) and Acid detergent fibre(ADF) were determined by refluxing 0.45 g ryegrass samples in ANKOM F57 filter bags (ANKOM Technology Corp., Fairport, NY) with neutral detergent and acid detergent solutions, respectively, for 1hour using the $ANKOM^{2000}$ Fibre Analyzer (ANKOM Technology Corp., Fairport, NY) (Van Soest *et al.*, 1991). Phosphorus, K, Ca, Mg, Cu, Zn, Mn and Fe levels were determined using the dry ashing macro and trace minerals methods (AgriLASA, 1998). *In vitro* ruminal gas production was assessed using the Reading Pressure Technique (RPT) described by Mauricio *et al.* (1999). Rumen fluid was collected in the morning prior to feeding from a non-lactating crossbred Holstein cow that was fed a ration comprising of tanner grass (*Brachiaria arrecta*) and a dairy concentrate (Master Mix Feeds LTD., Trinidad and Tobago). Fermentation flasks without samples (blanks) were included to allow correction for gas produced directly from rumen fluid. Incubation of flasks was done at 39°C and head space gas pressure was measured at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36 and 48 h post-incubation using a pressure transducer (Bailey and Mackey Ltd, UK). Gas pressure readings (psi) were converted to gas volume (ml) using the relationship between gas pressure and gas volume pre-determined for the St. Augustine (Trinidad and Tobago) site as:

$\mathrm{Gas}_p = 1.7675 + 2.1244P - 0.0022P^2$

where, Gas_p is the predicted gas volume (ml) and P, the pressure transducer reading (psi). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979): $Y = a + b\,(1 - e^{-c(t-tl)})$

where: y = gas produced at time t (ml/g OM); a = gas production from the immediately soluble fraction (ml/g OM); b = gas production from the insoluble fraction (ml/g OM); c = gas production rate constant for the insoluble fraction (%/h); t = incubation time (h); l = lag time (hours). Effective gas production (*Egas*, ml/g OM) was estimated as:, $a + \frac{b \times c}{k + c}$

where k is the outflow rate of solids, which was assumed to be 5% per hour and a, b, and c are the Ørskov and McDonald (1979) fitted parameters above.

In vitro true DM digestibility (iDMD) of ryegrass substrates was measured after 36 hours of incubation (39°C) with bovine rumen fluid in the ANKOM Daisy^{II} incubator. Ryegrass samples (~ 0.5 g each) were weighed separately into ANKOM F57 bags. The buffer recipe, incubation, and bag processing procedures upon termination of incubation were according to guidelines provided by ANKOM Technology (2005). Rumen fluid for this procedure was obtained from the same donor animal described for the gas production procedure above. Upon termination of incubation after 36 h, ANKOM bags were refluxed with neutral detergent solution for 1 h before being dried at 105 °C and iDMD was calculated as the loss in initial DM weight of ryegrass samples. The metabolizable energy (ME) content (MJ/kg DM) of ryegrass samples was predicted from iDMD (%) according to the following equation for roughage feeds published by CSIRO (2007).

$ME = 0.172 iDMD - 1.707$ ($R2 = 0.93$)

Statistical analysis: Dry matter yield, chemical composition and *in vitro* ruminal fermentation data were analyzed using the general linear models (GLM) procedure of Minitab 16. Initially, a linear model that included all main effects and all possible interaction terms between the 3 factors (cultivar (C), fertilizer (F) and harvesting date (H)) was used. However, the 3-way interaction term (cultivar x harvesting stage x N fertilizer rate) was found to be statistically insignificant for all response variables and was subsequently dropped from the model used. Therefore, the final model used was as follows:

$$Y_{ijk} = \mu + V_i + H_j + F_k + (V \times H)_{ij} + (V \times F)_{ik} + (H \times F)_{jk} + E_{ijk}$$

where Y_{ijk} = dependent variable, μ = population mean, C_i= effect of cultivar (i = 7), H_j = effect of harvesting stage (j = 3), F_k = effect of rate of application of N fertilizer (k = 2), $(C\times H)_{ij}$ = effect of interaction between cultivar and harvesting stage, $(C\times F)_{ik}$ = effect of interaction between cultivar and rate of N fertilizer application , $(H \times F)_{jk}$ = effect of interaction between harvesting stage and N fertilizer application rate, and E_{ijk} = residual error, assumed to be normally and independently distributed. Statistical significance was declared at P<0.05. Least squares means were compared using Tukey's HSD.

Results and Discussion

Dry matter production: Significant differences (P<0.0001) in DM yield occurred among the different cultivars. Mean rankings of DM values (kg ha^{-1}) showed the order of Fitzroy (2830) > Samson (2661) >Bronsyn (2568) > Bealey (2348) > Indiana (2269) > Pastoral (1968) > Quartet (1762). The result showed that diploid cultivars (Fitzroy, Samson and Bronsyn) had generally higher DM yield than all the tetraploid cultivars. This is in contrast to the study of Ahloowalia (1972) who reported that tetraploides out-yielded the diploids by 12-19% when a high nitrogen fertilizer was applied. Other researchers also reported the significant variations in yield among cultivars of rye grasses (Smit *et al.*, 2005). Cultivars with higher DM yield may increase the carrying capacity of the pasture.

Harvesting stage had a significant effect (P<0.001) on DM yield, showing a general order of 90 DAP >70 DAP >60 DAP (Table 1). The increase in DM yield with advance in plant growth was anticipated. Early grazing period produces lower DM yield than late grazing period. Since grazing time is an important management factor, determination of the appropriate time after establishment will benefit both the plants and animals by maximizing the longevity of the pasture, a desired management to make a profitable enterprise. The well-known concept in pasture utilization is that forage yield may be maximised by delaying grazing time, but doing so may lower the quality (e.g. digestibility). However, this study demonstrated that forage yield of ryegrass might be maximized if grazing is delayed, and yet without causing detrimental effects on forage quality. This is because even at late growth stage, forages had acceptable level of nutrients needed for maintenance and production, but whether grazing at this stage has effect on forage intake needs to be further investigated. Fertilizer rate influenced herbage DM yield (P<0.001), but this occurred only at late harvesting period (90 DAP) when all cultivars except Bronsyn and Indiana showed significantly higher yield at 30 kg ha^{-1} fertilizer level than at 60 kg ha^{-1} (Table 1).

Crude protein and fibre content: Cultivar and harvesting stage significantly (P<0.05) affected the values of NDF, ADF and CP contents (Table 2). Cultivar differences in these chemical constituents were not influenced by fertilizer levels and harvest dates as evidenced by the absence of significant C x F and C x H interactions in the combined analysis. Rankings according to CP content showed that cultivar (cv) Pastoral had the highest (P<0.001) value (162.5 g kg^{-1} DM) followed by others. Rankings of harvesting stage according to CP content (g kg^{-1} DM) was in the order of 60 DAP (156) > 75 DAP (145) > 90 DAP (114), while the reverse held true for ADF and NDF. The decline in CP content of herbage with stage of harvesting was much faster at the higher fertilization level (60 kg ha^{-1}). This occurred despite the absence of signi-

-ficant difference in terms of pasture growth rate between the fertilizer rates (mean 0.50 cm day^{-1}), and is therefore difficult to explain. In some cases, as plants grow, they tend to deposit more fibre to ensure structural integrity, but this also leads to a decrease in CP concentration due to the dilution effect of fibre. The decline in CP content and increase in fibre contents with advance in plant growth was reported by workers earlier (Callow *et al*., 2003; Contreras-Govea *et al.,* 2009).Nevertheless, all cultivars showed significantly high total CP yield (kg ha^{-1}) at later growth stages compared to the early growth stages. Low yielding cv pastoral had the highest CP content, while high yielding cv Fitzroy had relatively low CP content, but this did not imply that strong relationship exists between yield and CP content. On the other hand, within each cultivar, it was clear that DM yield increases while CP content declines with maturity. Where early grazing is practiced, Samson and Bronsyn might be chosen because they had relatively higher DM yield and CP content. Samson, Fizroy, Indiana or Bealey might be appropriate if grazing at 75 DAP is planned, because they had better yield and CP content at this growth stage. But if late grazing is planned, Fitzroy, Samson or Bealey might be chosen. Cv Fitzroy is suitable mainly because it had the highest DM yield. Cv Bronsyn had high DM yield at 75 and 90 DAP, but might not be the right cultivar to use because its CP content declined sharply with stage of growth. Crude protein measured in this study was generally lower than that of other cultivars of *L. perenne* from the Netherlands (range: 179–210 g kg^{-1} DM; Smit *et al*., 2005) and Ireland (range: 155–271 g kg^{-1} DM; Conaghan *et al*., 2008). Of the interaction terms investigated, only the H × F interaction significantly affected CP (P<0.001) and NDF (P<0.01) contents (Table 2).

***Macro and micro-elements*:** Cultivar significantly (P<0.05) affected the herbage concentration of all minerals (Table 3 and 4). Overall mean values showed that cv Quartet had the highest K (42.4 g kg^{-1}) and Fe (261.4 mg kg^{-1}) levels, while cv Samson had the highest Mg (2.5 g kg^{-1}), Zn (39.8 mg kg^{-1}) and Mn (66.8 mg kg^{-1}) levels. The highest concentration of Ca was recorded in Indiana, Samson, Bealey, Bronsyn and Fitzroy (range 3.5–3.7 g kg^{-1}). Pastoral had the least Ca (2.8 g kg^{-1} DM), Mg (2.1 g kg^{-1} DM), Fe (190.9 mg kg^{-1} DM), Zn (34.9 mg kg^{-1} DM), and Mn (54.6 mg kg^{-1} DM) concentrations, while Fitzroy had the least K (38.2 g kg^{-1} DM). All cultivars had either adequate mineral levels to meet the normal animal production requirements (Ca, Mg, P, Cu and Zn) or in excess of the requirements (K, Fe and Mn). This suggests that mineral concentration in the seven cultivars is not an important parameter in the selection of ryegrass cultivars. The excess level of K in all the cultivars (range 38.2–42.4 g kg^{-1} DM) is of concern. Excess dietary K may cause grass tetany and hypomagnesia and can also lead to cation: anion imbalances in dry cows and this may contribute to increased milk fever and other metabolic diseases post-partum (Gizachew *et al*., 2002). In this study, for all cultivars, Fe and Mn levels were also above the critical levels of 30–50 and 20–40 mg kg^{-1} DM, but they were still below the maximum tolerable levels of 500 and 2000 mg kg^{-1} DM, respectively (NRC, 2005).

Table 1. Effect of harvesting stage (days after planting) and N fertilizer application on dry matter yield (kg ha^{-1}) of perennial ryegrass cultivars

Cultivar	**N-fertilizer**	**Harvesting period (days after sowing)**		
		60	**75**	**90**
Bealey	30	2135.4^{Ab}	2884.3^{Ab}	3054.0^{Aa}
	60	1689.7^{Aa}	2233.0^{Ba}	2095.0^{Ba}
	Mean	1912	2558	3574
Bronsyn	30	2340^{Ab}	2336.4^{Ab}	3298.7^{Aa}
	60	2169.8^{Aa}	2517.8^{Aa}	2748.2^{Aa}
	Mean	2254	2427	3023
Fitzroy	30	1931.4^{Ab}	2762.3^{Ab}	4540.7^{Aa}
	60	1704.2^{Ab}	2762.8^{Aa}	3280.2^{Ba}
	Mean	1817	2762	3910
Indiana	30	1912.3^{Ab}	2479.6^{Aab}	2786.4^{Aa}
	60	1572.4^{Ab}	2619.3^{Aa}	2241.9^{Aab}
	Mean	1742	2549	2514
Pastoral	30	1557.4^{Ab}	1686.9^{Ab}	2977.8^{Aa}
	60	1307.9^{Ab}	2039.8^{Aa}	2243.2^{Ba}
	Mean	1432	1863	2610
Quartet	30	1881.5^{Ab}	2317.8^{Aab}	2841.9^{Aa}
	60	1288.0^{Ab}	1560.0^{Aab}	1960.5^{Ba}
	Mean	1584	1938	2401
Samson	30	2027.3^{Ab}	2635.8^{Ab}	3660.4^{Aa}
	60	2168.3^{Aa}	2631.0^{Aa}	2846.9^{Ba}
	Mean	2097	2633	3753

^{A,B} In a column, means with different uppercase superscripts differ significantly (P<0.05); ^{a,b,c}In a row, means with different lowercase superscripts differ significantly (P<0.05)

Relative differences between cultivars for P and Cu concentrations were not consistent across harvesting stages as evidenced by a significant (P<0.05) C x H interaction in the combined analysis. As a main factor, harvesting stage significantly (P<0.001) affected the herbage concentration of all minerals but trends were inconsistent.The general decreased trends of P and trace element levels (except Mn) of all cultivars with adva-

Table 2. Effect of harvesting stage and N fertilizer application rate on nutrient contents (g kg^{-1} DM) of perennial ryegrass cultivars

Cultivar	Harvest[1]	CP		NDF		ADF	
		30[2]	60[3]	30	60	30	60
Bealey	60	156.0^{Aa}	175.8^{Aa}	375.2^{Cb}	403.2^{Ba}	229.7^{Ca}	235.6^{Ca}
	75	137.7^{Ba}	145.8^{Ba}	436.4^{Bb}	407.4^{Ba}	243.5^{Ba}	241.2^{Ba}
	90	138.3^{Ba}	120.6^{Ca}	479.8^{Aa}	470.3^{Aa}	283.9^{Aa}	298.7^{Aa}
	Mean	144.0	147.4	430.4	426.9	252.4	258.5
Bronsyn	60	145.2^{Aa}	145.8^{Aa}	369.1^{Cb}	413.7^{Ba}	226.3^{Cb}	252.9^{Ba}
	75	118.3^{Ba}	120.2^{Ba}	410.5^{Ba}	423.2^{Ba}	249.6^{Ba}	227.5^{Cb}
	90	119.2^{Ba}	101.3^{Ca}	458.2^{Aa}	454.4^{Aa}	280.9^{Aa}	279.9^{Aa}
	Mean	127.6	122.4	412.5	430.4	249.7	253.5
Fitzroy	60	153.5^{Aa}	172.3^{Aa}	419.9^{Ca}	423.9^{Ca}	260.2^{Ba}	258.7^{Ba}
	75	138.9^{ABa}	146.5^{Ba}	436.8^{Ba}	437.9^{Ba}	256.7^{Ba}	253.8^{Ba}
	90	125.8^{Ba}	100.8^{Cb}	525.5^{Aa}	471.8^{Ab}	314.6^{Aa}	291.0^{Ab}
	Mean	139.4	139.9	460.7	444.5	277.5	267.8
Indiana	60	152.9^{Aa}	156.7^{Aa}	380.2^{Cb}	417.7^{Ca}	230.4^{Bb}	257.6^{Ba}
	75	146.0^{ABa}	141.7^{Aa}	418.8^{Ba}	397.6^{Bb}	238.5^{Bb}	261.8^{ABa}
	90	134.6^{Ba}	105.4^{Bb}	453.0^{Aa}	454.5^{Aa}	302.6^{Aa}	272.3^{Ab}
	Mean	144.5	136.7	417.3	423.2	257.1	263.2
Pastoral	60	185.2^{Aa}	191.3^{Aa}	343.8^{Cb}	$394.^{Ba}$	203.6^{Cb}	229.2^{Ca}
	75	175.6^{Aa}	171.0^{Ba}	404.6^{Ba}	369.2^{Cb}	228.0^{Bb}	272.2^{Aa}
	90	137.3^{Ba}	116.9^{Cb}	453.3^{Aa}	436.0^{Ab}	256.8^{Aa}	261.5^{Aa}
	Mean	166.0	159.7	400.5	399.8	229.8	254.3
Quartet	60	155.2^{Aa}	182.9^{Ab}	358.2^{Cb}	406.2^{Cb}	288.7^{Ba}	247.2^{Bb}
	75	144.2^{Aa}	141.9^{Ba}	412.7^{Bb}	493.8^{Aa}	240.6^{Ca}	249.9^{Ba}
	90	121.9^{ABa}	117.7^{Ca}	465.6^{Aa}	439.9^{Bb}	280.9^{Aa}	267.2^{Ab}
	Mean	141.0	147.5	412.2	446.6	270.1	254.8
Samson	60	148.5^{Aa}	188.5^{Ab}	422.8^{Bb}	441.9^{Ca}	271.0^{Ca}	274.5^{Aa}
	75	157.5^{Aa}	146.0^{Ba}	386.8^{Ba}	438.1^{Bb}	288.6^{Ba}	256.2^{Bb}
	90	133.5^{Ba}	136.5^{Ca}	471.3^{Aa}	455.7^{Aa}	290.3^{Aa}	266.5^{ABb}
	Mean	146.5	157.0	426.9	445.2	283.3	265.7
SEM		9.50		8.40		7.61	

[1]Harvest = harvesting stage (days after planting); [2]30 = 30 kg ha^{-1} N-fertilizer rate; [3]60 = 60 kg ha^{-1} N fertilizer rate; ^{A,B}In a column, means with different uppercase superscripts differ significantly ($P<0.05$); ^{a,b,c}In a row, means with different lowercase superscripts differ significantly ($P<0.05$)

-ncing growth stage was in agreement with earlier reports for perennial ryegrass (Lindström *et al.*, 2013) and Italian ryegrass (Brink *et al.*, 2006). *Lolium perenne* cultivars harvested at early stages had significantly higher concentration of most minerals but the total pasture yield during this period was low. At later stages of growth, the yield and carrying capacity of the pasture may increase without compromising the mineral nutrition, but the bioavailability of those minerals may be limited. Fertilization rate only affected ($P<0.05$) P, Ca and Mn content of ryegrass herbage but trends were not consistent, and interacted strongly with harvesting stage to influence the mineral levels (Table 3 and 4).

Gas production parameters: *In vitro* ruminal gas production parameters, *a*, *b*, *c*, lag time, and effective gas production (*Egas*) were studied (Table 5). Differences between cultivars were observed in the rate of gas production from the *b* fraction (*c*), which ranged from 6.2 % h^{-1} (Bronsyn) to 5.5 % h^{-1} (Samson and Fitzroy). This variable was influenced by cultivar in opposite direction to ADF. Effective gas production was highest in Bronsyn (781.5 ml g^{-1} OM) and lowest in Fitzroy (682.9 ml g^{-1} OM).When an assumed solid outflow rate of 5% h^{-1} was used to estimate gas production from these substrates, varieties with the highest ADF content had the lowest effective gas production.

In most cases, harvesting stage significantly influenced the size of the *a* fraction, with pastures harvested at 90 DAP producing significantly more gas (140.2 ml g^{-1} OM) from the *a* fraction than at 60 and 75 DAP. This result

Table 3. Effect of harvesting stage and N fertilizer application rate on macro-element content (g kg^{-1} DM) of perennial ryegrass cultivars

Cultivar	Harvest	P		Ca		Mg		K	
		30	60	30	60	30	60	30	60
Bealey	60	4.7^{Ab}	5.4^{Aa}	3.5^{ABa}	3.6^{Ba}	2.0^{Ca}	2.1^{Ba}	38.9^{Aa}	41.9^{Aa}
	75	4.3^{Ba}	4.5^{Ba}	3.1^{Ba}	3.0^{Ca}	2.3^{Ba}	2.0^{Ba}	38.7^{Aa}	40.2^{Aa}
	90	4.2^{Bb}	4.7^{Ba}	3.7^{Ab}	5.4^{Aa}	2.5^{Aa}	2.5^{Aa}	41.9^{Aa}	40.1^{Aa}
	Mean	**4.4**	**4.9**	**3.4**	**4.0**	**2.3**	**2.2**	**39.8**	**50.7**
Bronsyn	60	4.7^{Aa}	4.9^{Aa}	3.3^{Ba}	3.6^{Ba}	2.0^{Ba}	2.1^{Aa}	39.0^{Aa}	39.9^{Aa}
	75	4.2^{Ba}	4.4^{Ba}	3.0^{Ba}	3.3^{Ba}	2.1^{Ba}	2.1^{Aa}	39.8^{Aa}	37.9^{Aa}
	90	4.3^{Ba}	3.9^{Cb}	3.9^{Ab}	4.3^{Aa}	2.5^{Aa}	2.3^{Aa}	40.3^{Aa}	36.0^{Aa}
	Mean	**4.4**	**4.4**	**3.4**	**3.7**	**2.2**	**2.2**	**39.7**	**37.9**
Fitzroy	60	5.6^{Ab}	6.1^{Aa}	3.5^{Aa}	3.5^{Aa}	2.1^{Ba}	2.3^{Aa}	41.9^{Aa}	44.8^{Aa}
	75	4.4^{Bb}	4.7^{Ba}	3.3^{Aa}	3.3^{Aa}	2.1^{Ba}	2.3^{Aa}	36.3^{Ba}	37.0^{Ba}
	90	4.4^{Ba}	3.9^{Cb}	3.6^{Aa}	3.7^{Aa}	2.4^{Aa}	2.0^{Ab}	37.2^{Ba}	32.2^{Cb}
	Mean	**4.8**	**4.9**	**3.5**	**3.5**	**2.2**	**2.2**	**38.4**	**38.0**
Indiana	60	4.9^{Aa}	4.7^{Aa}	3.5^{Aa}	3.1^{Ba}	2.0^{Aa}	2.1^{Aa}	37.5^{Aa}	40.1^{Aa}
	75	4.4^{Ba}	4.4^{Ba}	3.0^{Ba}	3.1^{Ba}	2.3^{ABa}	2.3^{ABa}	40.0^{Aa}	39.8^{Aa}
	90	4.4^{Ba}	3.9^{Cb}	3.9^{Ab}	5.1^{Aa}	2.5^{Aa}	2.5^{Aa}	40.4^{Aa}	35.4^{Bb}
	Mean	**4.5**	**4.3**	**3.5**	**3.8**	**2.3**	**2.3**	**39.2**	**38.4**
Pastoral	60	5.0^{Aa}	5.2^{Aa}	2.7^{Ba}	2.8^{Ba}	1.8^{Aa}	2.0^{Aa}	39.0^{Aa}	41.2^{Aa}
	75	4.4^{Bb}	4.7^{Ba}	2.2^{Ba}	2.4^{Ba}	2.0^{ABa}	2.0^{Aa}	41.1^{Aa}	40.8^{Aa}
	90	4.2^{Bb}	3.8^{Ca}	3.3^{Ab}	4.0^{Aa}	2.3^{Aa}	2.2^{Aa}	38.2^{Aa}	35.2^{Ba}
	Mean	**4.5**	**4.6**	**2.7**	**3.0**	**2.1**	**2.1**	**39.4**	**39.0**
Quartet	60	5.5^{Ab}	6.3^{Aa}	2.5^{Ba}	2.8^{Ba}	1.9^{Ba}	2.2^{Ab}	43.0^{Ab}	47.7^{Aa}
	75	4.5^{Ba}	4.7^{Ba}	2.0^{Ba}	2.2^{Ca}	2.0^{ABa}	2.0^{Aa}	42.3^{Aa}	41.7^{Ba}
	90	4.5^{Ba}	4.5^{Ba}	3.3^{Aa}	3.9^{Aa}	2.4^{Aa}	2.3^{Aa}	42.6^{Aa}	37.4^{Cb}
	Mean	**4.8**	**5.2**	**2.6**	**2.9**	**2.1**	**2.2**	**42.6**	**42.3**
Samson	60	5.2^{Ab}	5.8^{Aa}	3.7^{Aa}	3.1^{Ba}	2.3^{Aa}	2.5^{Ba}	41.4^{Ab}	46.2^{Aa}
	75	4.5^{Ba}	4.7^{Ba}	3.0^{Ba}	3.1^{Ba}	2.4^{ABa}	2.4^{Ba}	40.1^{Aa}	40.5^{Ba}
	90	4.1^{Ca}	4.3^{Ca}	3.9^{Ab}	4.8^{Aa}	2.6^{Aa}	2.7^{Aa}	38.5^{Aa}	35.5^{Ca}
	Mean	**4.6**	**4.9**	**3.5**	**3.6**	**2.4**	**2.5**	**40.0**	**40.7**
SEM		0.23		0.30		0.11		2.2	

^{A,B}In a column, means with different uppercase superscripts differ significantly ($P < 0.05$); ^{a,b,c}In a row, means with different lowercase superscripts differ significantly ($P < 0.05$)

could be explained by differences in the proportion of fibre to cell contents in herbage harvested at different stages of maturity. Younger herbage has greater proportion of cell contents that are highly fermentable but tends to be fermented more efficiently with less gas being produced than when fibre is fermented (Tefera *et al*., 2009). Indeed, ADF content of ryegrass forage steadily increased with harvesting stage. Most ryegrass substrates harvested at 75 DAP produced more gas from the insoluble fraction (*b*) (1202.6 ml g^{-1}OM) than those harvested at 60 and 90 DAP. The slowly fermentable fraction in grass herbage is mostly the fibre fraction thus, as expected, mature forage had higher b fraction. Ryegrass pastures fertilized at 60 kg ha^{-1} had a lower *b* fraction (1139.4 ml g^{-1}OM) compared to those fertilized at 30 kg ha^{-1} (1173.5 ml g^{-1}OM). This was found despite the absence of significant difference in ADF content between fertilization levels and was difficult to explain. At 60 kg ha^{-1}fertilization, rate of gas production from the *b* fraction increased with harvesting stage but the reverse was observed at 30 kg ha^{-1} fertilization rate (Fig. 1).

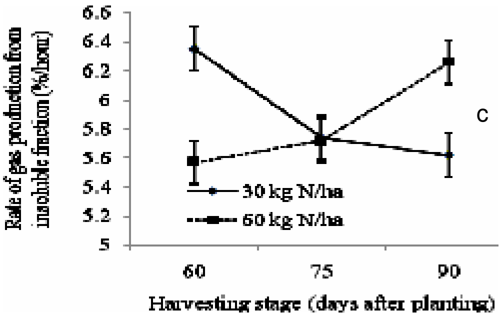

Fig 1. Effects of N fertilizer application rate × harvesting stage interaction on the rate of gas production from the insoluble fraction of ryegrass pastures

Table 4. Effect of harvesting stage and N fertilizer application rate on micro-element content (mg kg^{-1} DM) of perennial ryegrass cultivars

Cultivar	Harvest	Fe		Cu		Zn		Mn	
		30	60	30	60	30	60	30	60
Bealey	60	448.2^{Ab}	230.3^{Ab}	8.9^{Aa}	10.4^{Aa}	41.6^{Aa}	43.7^{Aa}	61.1^{Aa}	63.1^{Ba}
	75	194.4^{Ba}	156.2^{Ca}	7.5^{Ba}	7.7^{Ba}	35.7^{Ba}	34.7^{Ba}	59.3^{ABa}	59.6^{Ba}
	90	190.4^{Ba}	262.3^{Ba}	7.1^{Ba}	7.7^{Ba}	35.8^{Ba}	32.7^{Ba}	52.8^{Bb}	81.2^{Aa}
	Mean	**277.7**	**216.3**	**8.5**	**8.6**	**37.7**	**37.0**	**57.7**	**67.9**
Bronsyn	60	280.9^{Aa}	209.3^{Aa}	8.1^{Aa}	8.1^{Aa}	41.0^{Aa}	40.3^{Aa}	54.8^{Ba}	60.7^{Ca}
	75	162.1^{Ba}	149.^{Ba}	7.0 ^{Ba}	7.1^{Ba}	34.6^{Ba}	35.5^{Ba}	54.8^{Bb}	72.5^{Ba}
	90	209.2^{Ba}	211.3^{Aa}	7.9 ^{Ba}	6.1^{Cb}	36.7^{Ba}	30.1^{Cb}	63.5^{Ab}	87.9^{Aa}
	Mean	**217.4**	**189.9**	**7.6**	**7.1**	**37.4**	**35.3**	**57.7**	**73.7**
Fitzroy	60	312.2^{Aa}	257.8^{Aa}	8.5^{AB}	9.4^{Aa}	42.0^{Ab}	47.0^{Aa}	64.9^{Aa}	62.8^{Aa}
	75	158.8^{Ca}	202.0^{Ba}	6.6^{Ba}	7.1^{Ba}	35.6^{Ba}	35.7^{Ba}	58.5^{Ab}	64.2^{Aa}
	90	229.5^{Ba}	194.9^{Ba}	6.9^{Ba}	5.4^{Cb}	34.6^{Ba}	26.8^{Cb}	63.2^{Aa}	66.3^{Aa}
	Mean	**233.5**	**218.3**	**7.4**	**7.3**	**37.7**	**36.4**	**62.4**	**64.4**
Indiana	60	244.3^{Aa}	232.7 ^{Aa}	8.8^{Aa}	8.1^{Aa}	41.4^{Aa}	38.2^{Aa}	63.2^{Ba}	61.4^{Ba}
	75	174.8^{Ba}	162.7^{Ba}	7.7^{Ba}	7.6^{Ba}	35.2^{Ba}	34.5^{Ba}	60.5^{Ba}	60.4^{Ba}
	90	205.8^{Ba}	205.9^{Ba}	6.4^{Cb}	8.3^{Aa}	33.5^{Bb}	29.4^{Bb}	69.2^{Ab}	84.6^{Aa}
	Mean	**208.3**	**209.4**	**8.0**	**7.6**	**36.7**	**34.0**	**64.3**	**68.8**
Pastoral	60	252.2^{Aa}	213.1^{Aa}	10.5^{Aa}	10.4^{Aa}	42.7^{Aa}	40.6^{Aa}	43.7^{Bb}	56.2^{Ba}
	75	147.2^{Ba}	159.6^{Ba}	9.1^{Ba}	10.3^{Aa}	34.6^{Ba}	34.7^{Ba}	44.7^{Ba}	48.5^{Ba}
	90	177.2^{Ba}	196.3^{ABa}	7.8^{Ca}	6.7^{Ba}	28.8^{Ca}	28.2^{Ca}	62.4^{Ab}	71.9^{Aa}
	Mean	**192.1**	**189.7**	**9.2**	**9.2**	**35.3**	**34.5**	**50.3**	**58.9**
Quartet	60	306.5^{Aa}	327.5^{Aa}	9.0^{Aa}	10.2^{Aa}	41.1^{Ab}	47.6^{Aa}	52.6^{Bb}	61.0^{Ba}
	75	156.4^{Cb}	254.1^{Ba}	7.1^{Ca}	7.0^{Ba}	32.8^{Ba}	34.6^{Ba}	45.9^{Cb}	61.9^{Ba}
	90	239.5^{Ba}	284.2^{Ba}	8.2^{Ba}	6.3^{Cb}	33.2^{Ba}	32.7^{Ba}	63.7^{Ab}	82.4^{Aa}
	Mean	**234.1**	**288.5**	**8.1**	**7.8**	**35.3**	**38.3**	**54.1**	**68.4**
Samson	60	362.4^{Aa}	224.7^{Ab}	9.3^{Ab}	11.2^{Aa}	47.1^{Aa}	48.0^{Aa}	69.1^{Aa}	55.3^{Cb}
	75	160.4^{Ba}	150.1^{Ca}	8.5^{Ba}	8.2^{Ba}	36.4^{Ba}	38.0^{Ba}	56.3^{Bb}	64.2^{Ba}
	90	213.0^{Ba}	223.9^{Ba}	7.0^{Ca}	7.3^{Ca}	34.4^{Ba}	34.9^{Ca}	74.6^{Ab}	81.0^{Aa}
	Mean	**245.3**	**199.6**	**8.2**	**8.9**	**39.3**	**40.3**	**66.7**	**66.8**
SEM		40.3		0.56		2.11		6.50	

^{A,B}In a column, means with different uppercase superscripts differ significantly (P<0.05); ^{a,b,c}In a row, means with different lowercase superscripts differ significantly (P<0.05)

In vitro true digestibility and predicted metabolizable energy: There were no cultivar differences (P > 0.05) in the 36 h true digestibility (iDMD) of ryegrass (866.4 to 897.4 g kg^{-1} DM), however iDMD decreased with stage of harvesting. Ryegrass pastures harvested at 60 DAP had the highest iDMD (913.5 g kg^{-1}DM) while those harvested at 90 DAP had the least iDMD (846.7 gk g^{-1}DM). A similar pattern was observed for predicted ME content of the ryegrass pastures. The ME content (12.9 to14.0 MJ kg^{-1} DM) also declined with harvesting stage. While *in vitro* ruminal gas production is a useful diagnostic tool, it could not be used to rank or select forages since the gas that is produced is considered to be a waste product. As such, it is not prudent to select ryegrass cultivars based on gas production alone. The effect of harvesting stage on *in vitro* ruminal dry matter degradability and predicted metabolizable energy was as expected, with younger forage being more digestible than forage harvested at a later growth stage. This was a consequence of greater fibre accumulation in older plants compared to younger plants, which negatively affects degradability and available ME. Although statistically different, the magnitude of difference between the highest (60 DAP) and lowest (90 DAP) iDMD values was only 76.8 g kg^{-1} DM. This implied that a farmer can choose to harvest at 90 DAP in order to maximize biomass yield at the cost of about 8% digestibility. The lack of rate of fertilizer effect on cultivar parameters evaluated in this study was a critical finding because it suggested that all seven cultivars are suitable pasture plants for this agro-ecological zone. Most importantly, farmers can use the lower fertilizer application rate without compromising the

Table 5. Effect of harvesting stage and N fertilizer application rate on *in vitro* ruminal gas production parameters of perennial ryegrass cultivars

Cultivar	Harvest	a (ml g^{-1} OM)[¥]		b (ml/g OM)[Φ]		c (%/hour) [Ψ]		Lag time (hours)		Egas (ml g^{-1} OM)[*]	
		30	60	30	60	30	60	30	60	30	60
Bealey	60	139^{Ba}	90^{Bb}	1150^{Ba}	1111^{Ab}	6.6^{Aa}	5.5^{BB}	2.0^{Aa}	2.3^{Aa}	794^{Aa}	667^{Cb}
	75	80.5^{Cb}	95.2^{Ba}	1184^{Aa}	1185^{Ba}	5.5^{Ca}	5.5^{Ba}	2.2^{Aa}	1.8^{Aa}	697^{Ba}	717^{Ba}
	90	170.0^{Aa}	131.7^{Ab}	1180^{Ba}	1071^{Cb}	6.0^{Ba}	6.5^{Aa}	1.9^{Aa}	1.9^{Aa}	811^{Aa}	735^{Ab}
	Mean	130	106	1172	1123	6.0	5.8	2.0	2.0	767	706
Bronsyn	60	93.3^{Ba}	90.2^{Ba}	1180^{Ba}	1132^{Cb}	6.6^{Aa}	6.4^{Aa}	2.0^{Aa}	2.0^{Aa}	767^{Ba}	725^{Cb}
	75	88.3^{Bb}	143.5^{Ba}	1260^{Aa}	1223.9^{Ab}	5.9^{Ba}	6.2^{Aa}	2.0^{Aa}	1.8^{Aa}	767^{Bb}	821^{Aa}
	90	179.2^{Aa}	155.1^{Ab}	1168^{Ba}	1170^{Ba}	6.6^{Aa}	5.7^{Bb}	1.9^{Aa}	2.0^{Aa}	837^{Aa}	769^{Bb}
	Mean	120	129	1203	1175	6.4	6.1	2.0	2.0	790	772
Fitzroy	60	64.1^{Ba}	72.3^{Ba}	1117^{Ca}	1120.7^{Ba}	5.6^{Aa}	5.1^{Aa}	2.3^{Aa}	2.7^{Aa}	650^{Ca}	636^{Cb}
	75	70.0^{Ba}	76.0^{Ba}	1184^{Aa}	1193^{Aa}	5.7^{Aa}	5.4^{Aa}	2.1^{Aa}	2.2^{Aa}	702^{Ba}	696^{Aa}
	90	146.4^{Aa}	121.6^{Ab}	1159^{Ba}	1018.0^{Cb}	5.3^{Aa}	5.9^{Aa}	1.9^{Aa}	1.8^{Aa}	740^{Ab}	672^{Ba}
	Mean	93	89	1153	1110	5.5	5.5	2.1	2.2	697	668
Indiana	60	118.6^{Ab}	153.8^{Aa}	1168^{Ba}	1121.1^{Bb}	6.4^{Aa}	6.3^{Aa}	2.0^{Aa}	1.9^{Aa}	773^{Aa}	774^{Ba}
	75	109.7^{Aa}	79.8^{Bb}	1203^{Aa}	1168.5^{Ab}	5.6^{Ba}	5.8^{Ba}	2.2^{Aa}	2.0^{Aa}	744^{Ba}	706^{Cb}
	90	112.5^{Ab}	161.1^{Aa}	1206^{Aa}	1118.5^{Bb}	5.0^{Bb}	6.9^{Aa}	2.3^{Aa}	1.8^{Aa}	716^{Cb}	809^{Aa}
	Mean	113	131	1193	1136	5.7	6.3	2.2	1.9	744	763
Pastoral	60	123.7^{Aa}	109.0^{Bb}	1042^{Cb}	1122^{Ba}	7.1^{Aa}	5.9^{Ab}	1.9^{Aa}	2.3^{Aa}	734^{Ba}	713^{Ab}
	75	128.8^{Aa}	122.6^{Aa}	1206^{Aa}	1191^{Ab}	5.7^{Ba}	5.8^{Aa}	1.9^{Aa}	2.2^{Aa}	771^{Aa}	661^{Bb}
	90	122.5^{Aa}	113.2^{ABa}	1142^{Ba}	1051.7^{Cb}	6.0^{Ba}	6.3^{Aa}	2.0^{Aa}	1.9^{Aa}	747^{Bb}	699^{Aa}
	Mean	125	31	1130	1121	6.3	6.0	1.9	2.1	750	691
Quartet	60	108.2^{Ca}	86.3^{Cb}	1147^{Cb}	1190^{Ba}	6.5^{Aa}	5.1^{Ab}	2.1^{Aa}	3.0^{Aa}	758^{Ba}	685^{Cb}
	75	174.2^{Aa}	136.7^{Bb}	1291^{Aa}	1239.9^{Ab}	6.1^{Aa}	5.9^{Aa}	1.8^{Aa}	2.0^{Aa}	884^{Aa}	804^{Ab}
	90	129.9^{Bb}	157.9^{Aa}	1163^{Ba}	1064^{Cb}	5.9^{Ab}	6.7^{Aa}	2.0^{Aa}	1.7^{Aa}	760^{Ba}	765^{Ba}
	Mean	137	126	1200	1164	6.2	5.9	1.9	2.2	801	751
Samson	60	133.9^{Ba}	106.5^{Bb}	1174^{Aa}	1146^{Ab}	5.6^{Aa}	4.8^{Bb}	2.2^{Aa}	2.7^{Aa}	752^{Aa}	664^{Bb}
	75	61.6^{Cb}	111.2^{Aa}	1154^{Aa}	1148^{Ab}	5.7^{Aa}	5.5^{Aa}	2.2^{Aa}	2.0^{Aa}	677^{Bb}	710^{Aa}
	90	144.3^{Aa}	116.9^{Ab}	1153^{Aa}	1135^{Ab}	5.8^{Aa}	5.9^{Aa}	1.9^{Aa}	2.2^{Aa}	761^{Aa}	725^{Ab}
	Mean	113	131	1160	1143	5.7	5.4	2.1	2.3	730	699
SEM		7.96		8.92		0.15		0.17		10.4	

[¥]a – gas production from the rapidly fermentable fraction of the substrate; [Φ]b – gas production from the slowly fermentable fraction; [Ψ]c – rate of gas production from the slowly fermentable fraction; [*]Egas – Effective gas production (ml/g OM); [A,B]In a column, means with different uppercase superscripts differ significantly ($P<0.05$); [a,b,c]In a row, means with different lowercase superscripts differ significantly ($P<0.05$).

nutritive value of pasture. It is also important to take into account biomass yield when deciding on the fertilization rate to use. Fitzroy, Samson, Bealey and Bronsyn were the top four cultivars in terms of dry matter yield. Higher biomass yield is always desirable in these low-input production systems where fluctuation in quantity of feed is a major limiting factor.

Conclusion

Results indicated cultivars and harvesting stages significantly influenced DM yield and most of the chemical constituents of the rye grass pastures. Cultivar difference depended on harvesting stage and N fertilization rate for most chemical constituents. Higher biomass yield is always desirable in low-input production systems where fluctuation in quantity of feed resources is a major limiting factor. Therefore, it was concluded that when biomass yield is taken into account, the higher-yielding cultivars, Fitzroy, Samson, Bealey or Bronsynmay were found suitable for use as pasture plants in resource-limited farming. A farmer can also choose to harvest at 90 DAP in order to maximize biomass yield at the cost of digestibility.

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