

Forage yield and quality response of annual ryegrass (*Lolium multiflorum*) to different water and nitrogen levels

AB Abraha^{1*}, WF Truter¹, JG Annandale¹ and MK Fessehazion^{1,2}

¹Department of Plant Production and Soil Science, University of Pretoria, Private Bag X20, Hatfield 0028, Pretoria, South Africa

²Agricultural Research Council–Vegetable and Ornamental Plant Institute, Pretoria, South Africa

*Corresponding author, e-mail: senayabraha@gmail.com

Abstract

Water and nitrogen shortages in annual ryegrass (*Lolium multiflorum*) production can be improved by using proper irrigation and nitrogen scheduling methods. The aim of this research was to analyse the effect of water and nitrogen application on dry matter production and forage quality of annual ryegrass. Treatments consisted of three irrigation levels, scheduling application to field capacity once every two weeks (W1), once a week (W2) or twice a week (W3) and three nitrogen levels, top-dressing after each harvest at a rate of 0 (N1), 30 (N2) or 60 (N3) kg N h⁻¹ cycle⁻¹. Yield increased as a function of the amount of water and nitrogen fertiliser applied. The dry matter (DM) content, *in vitro* organic matter digestibility (IVOMD), crude protein (CP) and metabolisable energy (ME) were higher in the treatments being irrigated once every two weeks. These results conclude that higher irrigation coupled with high nitrogen application significantly improved the dry matter yield, while water stress, to some extent, did improve the quality of the pasture by increasing the DM content, IVOMD, CP and ME values.

Key words: pasture production, dry matter yield, crude protein, *in vitro* organic matter digestibility, metabolisable energy

Introduction

In South Africa, annual ryegrass (*Lolium multiflorum*) is one of the most widely grown cool season pasture species under irrigation. It is grown by commercial farmers for intensive dairy, lamb and beef production (de Villiers and van Ryssen 2001). It is best adapted to areas with long seasons of cool, moist weather, and well drained soils but can be tolerant to a wide range of soils and climates. The optimum planting date for irrigated annual ryegrass in South Africa is in February, hence it can be used as a source of feed in late autumn, winter, spring and early summer (Goodenough et al. 1984).

Water (W) and nitrogen (N) are the most important factors determining the growth and yield of annual ryegrass. Despite its high cost, the application of N is still widely recommended for these pastures, although the use of inexpensive sources of N such as manure and/or legumes may become economically viable in the future. Because N plays such a key role in determining the yield and quality of pastures, the timing and amount of N fertilisation are important (Fessehazion et al. 2011; Fessehazion et al. 2014a). The main effect of N fertilisation on grasses is to increase the yield and quality of leaf material. With adequate soil moisture, pastures can make greater use of available N than under dry conditions. As a result, different soil moisture levels are likely to cause high fluctuations in yield and quality of pasture especially at higher rates of N (Akmal and Janssens 2004).

To obtain high levels of milk production, grass quality and dry matter (DM) intake of dairy cows must be high. South African ryegrass cultivars tend to have relatively high moisture contents (Meissner et al. 2000) which affect the DM intake adversely. In order to optimise DM intake of cows, the DM content should be at least 18–20% (Meissner et al. 2000). High levels of neutral detergent fibre (NDF) may limit DM intake and reduce overall digestibility because NDF is degraded slowly in the rumen (Reeves et al. 1996).

It is not always clear how much water and N fertiliser to apply to obtain an optimum yield with acceptable forage quality. Therefore the objectives of this study were focused on quantifying the yield response of annual ryegrass to variable water and N levels, to analyse the effects of different irrigation frequencies in combination with

different N fertiliser applications on the quality of ryegrass and to test the hypotheses that: 1) the DM yield will be positively associated with soil water content and N fertilisation and 2) there is a positive relation between water stress and forage quality.

Materials and methods

Experimental site and design

To exclude rainfall effects on the proposed irrigation treatments, the experiment was conducted under a rain shelter at the Hatfield Experimental Farm of the University of Pretoria (25°45'S and 28°16'E). The area has an elevation of 1327 m above sea level and an average annual rainfall of 670 mm (Fessehazion et al. 2014b). The soil of the experimental site is classified as a silt clay loam of the Hutton form that belongs to the Suurbekom family with a clay content of 26 – 37% (Soil Classification Working Group, 1991). To create suitable conditions for good soil and seed contact, the field was ploughed with a disc plough and rotavated. Prior to the commencement of the study, representative soil samples were taken randomly from the experimental site and the following results were obtained: pH (H₂O) 7.4, NH₄ 1.51 mg kg⁻¹, NO₃ 61.6 mg kg⁻¹, P 118.9 mg kg⁻¹, Ca 8.5 cmol kg⁻¹, K 0.2 cmol kg⁻¹, Mg 8.8 cmol kg⁻¹. P was extracted using Bray I while K, Ca and Mg were extracted using ammonium acetate. Nitrate and ammonium N were determined using KCl extraction. A 149.5 m² (6.5 m x 23.0 m) block was divided into 27 plots of 3.0 m² (1.5 m x 2.0 m) each, with an interspacing of 0.5 m between each plot. Plastic sheeting was inserted to a depth of 1.2 m to limit movement of water between plots. Nine treatment combinations of three water levels and three nitrogen levels were replicated three times. The plots were in a complete randomised block design. In both seasons, superphosphate (9% P) and potassium chloride (50% K) were applied at planting. In the first week of June 2007, annual ryegrass (*Lolium multiflorum* cv. Agriton) was planted at a seeding rate of 30 kg ha⁻¹. Sprinkler irrigation was used for seven weeks until the grass was well established, and thereafter to control the water use more efficiently drip irrigation commenced. In the 2008 season, the grass was planted in April and sprinkler irrigation was used for eight weeks before the commencement of drip irrigation. The lateral spacing between the dripper lines and the distance between drippers in the line was 0.3 m. Irrigation was applied to individual plots depending on the soil water

deficit to field capacity. Weeding was conducted manually during the course of the trial.

Treatments

Three levels of irrigation were applied, namely W1: irrigation of once every two weeks to field capacity, W2: irrigation of once weekly to field capacity and W3: irrigation of twice a week to field capacity. At the beginning of each season, the soil profiles of all the plots were brought to field capacity. In each plot, a neutron probe access tube was installed and the soil water content was calculated using a neutron water meter. Soil water deficit measurements were made using a neutron water meter model 503 DR CPN Hydroprobe (Campbell Pacific Nuclear, California, USA). The water deficit of the profile was calculated over a soil depth of 1.2 m, but irrigation was based on the upper 0.8 m of the soil profile as the roots of the grass were concentrated in the top 0.7 m. Plant available water (PAW), which is the amount of water that a soil can store and is available for plant water use, was calculated as the difference in soil water content between field capacity and permanent wilting point (USDA, 1998). Plant available water was calculated using methods described in Eiasu et al., (2009). Three N treatments, namely N1: 0 kg N ha⁻¹, N2: 30 kg N ha⁻¹ and N3: 60 kg N ha⁻¹ were applied after each cut. The N was applied as a top dressing in the form of limestone ammonium nitrate (28% N).

Dry matter yield, water use and water use efficiency

Yield was measured by sampling plant material from an area of 0.09 m² from each of the 27 plots to a height of 50 mm above the soil surface. After the samples were taken, all plots were cut to a height of 5 cm. In each season the pasture was harvested four times at 28-day intervals. Samples were oven dried for 72 hours at 67°C to a constant mass for dry matter yield determination.

Water use (ET) in mm was calculated using equation 1.

$$ET = I + P - Dr - \Delta S - R \quad \text{eq. 1}$$

where I stands for the applied irrigation in mm, P is precipitation in mm (the value of P is zero because the experiment was under a rain shelter), Dr is drainage in mm

(assumed to be negligible), ΔS is change in soil water storage in mm and R is runoff in mm (assumed to be negligible).

Water use efficiency (WUE) in $\text{kg ha}^{-1} \text{mm}^{-1}$ was calculated using equation 2.

$$WUE = \left(\frac{Y}{ET} \right) \quad \text{eq. 2}$$

where Y is yield in kg ha^{-1} and ET is water use in mm.

Chemical composition

For the quality analyses, samples were dried and milled to pass through a 1 mm sieve and representative samples were stored in airtight containers. Analyses for quality were done for dry matter (DM) content, *in vitro* organic matter digestibility (IVOMD), crude protein (CP), neutral detergent fibre (NDF) and metabolisable energy (ME). The DM content (AOAC 2000, procedure 934.01), IVOMD (Tilley and Terry 1963), using rumen fluid from cannulated sheep, CP (calculated N content using a Leco N analyser, Leco Corporation, St. Joseph, MI, USA, then being multiplied by 6.25), NDF (Robertson and van Soest 1981) and gross energy (GE) (MC – 1000 Modular Calorimeter, Operators Manual) were analysed by their respective procedures. Metabolisable energy (ME) was calculated as:

$$ME = 0.82 \times GE \times IVOMD \quad (\text{Robinson et al. 2004}) \quad \text{eq. 3}$$

Statistical analyses

Cumulative yield, quality variables, water use (WU) and water use efficiency (WUE) were analysed using standard ANOVA of the Statistical Analysis System (SAS) program for Windows v9.2 (SAS Institute, Cary, North Carolina). Least significant differences (LSD) were calculated at the 5% significance level to compare the treatment means using the Student's t-test. Analyses were done for 2007 and 2008 separately as the planting dates were different.

Results

Dry matter yield

The dry matter (DM) yield of the first and second seasons was influenced ($P < 0.05$) by treatment interactions between the amount of water and N fertiliser applied (Table 1). The highest cumulative DM yield for both seasons (Figure 1a and 1b) was obtained from W3N3, the treatment with an irrigation frequency of twice a week and nitrogen application of 60 kg ha^{-1} after each cut. The lowest yield was obtained from the W1N1 treatment. Figure 1a shows that in the first season the highest ($P < 0.05$) cumulative yields of 10.76 t ha^{-1} and 10.26 t ha^{-1} were achieved by W3N3 and W2N3, treatments receiving water twice and once a week with the highest N application, respectively. This trend was also similar in the second season (Figure 1b). In the first season, the lowest cumulative yield of 2.34 t ha^{-1} was produced from W1N1, while a lowest cumulative yield of 3.61 t ha^{-1} was produced from the same treatment in the second season.

Table 1: Summary of ANOVA table on the dry matter yield, water use, water use efficiency and dry matter content of annual ryegrass in 2007 and 2008 (Tukey's studentized range test)

	Source of variation	Degree of freedom	F-probability levels			
			2007		2008	
			F value	Pr > F	F value	Pr > F
Total yield	W	2	180.11	<.0001	987.81	<.0001
	N	2	1896.32	<.0001	7113.00	<.0001
	WxN	4	10.08	0.0003	100.20	<.0001
Water use	W	2	8795.71	<.0001	596.29	<.0001
	N	2	4526.65	<.0001	233.76	<.0001
	WxN	4	70.85	<.0001	13.27	<.0001
Water use efficiency	W	2	13.49	0.0004	8.80	0.0026
	N	2	1383.60	<.0001	536.41	<.0001
	WxN	4	61.59	<.0001	20.85	<.0001
Dry matter content	W	2	383.02	<.0001	858.29	<.0001
	N	2	18.57	<.0001	20.83	<.0001
	WxN	4	0.73	0.5865	0.49	0.7431

W= water treatment, N= nitrogen treatment, WxN= water and nitrogen interaction

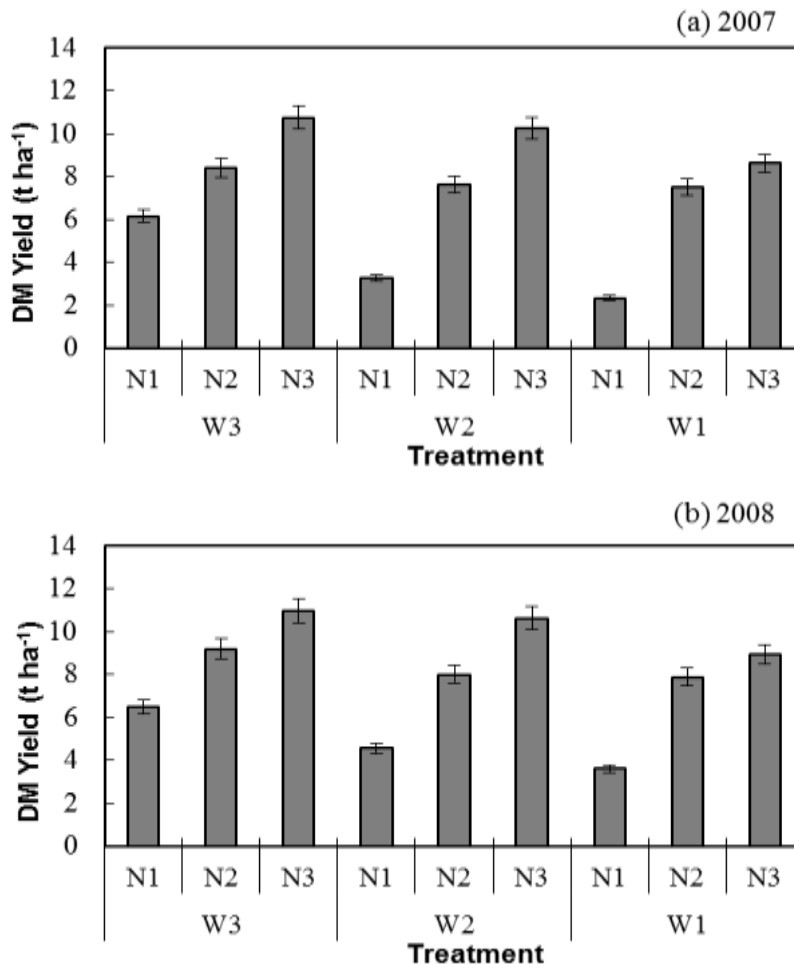


Figure 1: Interaction between water and nitrogen treatments on the DM yield (t ha⁻¹) of annual ryegrass, W= water treatment, N= nitrogen treatment

Water use and water use efficiency

In both seasons, the water use (WU) was influenced by W, N fertiliser and the interaction between WxN (Table 1). Generally, WU increased as the frequency of irrigation increased. Within the same irrigation frequency, WU increased with increasing nitrogen application. As the irrigation interval increased from twice a week (W3) to once every two weeks (W1), the amount per application increased accordingly, but the total amount of water applied throughout the whole season decreased because of the lower irrigation frequency. The treatment that was irrigated twice weekly with the highest nitrogen application, used the most water, a total of 423 mm while the lowest, a total of 282 mm was recorded for the treatment that was irrigated once every two weeks with no nitrogen application (Figure 2).

The soil water depletion levels of different irrigation treatments are presented in Figure 3. For all treatments the soil water deficit was lower at the beginning of the season but increased as the season progressed. Annual ryegrass was irrigated when the 60-85%, 35-55% or 15-30% of PAW was depleted for W1, W2 and W3 respectively (Figure 3).

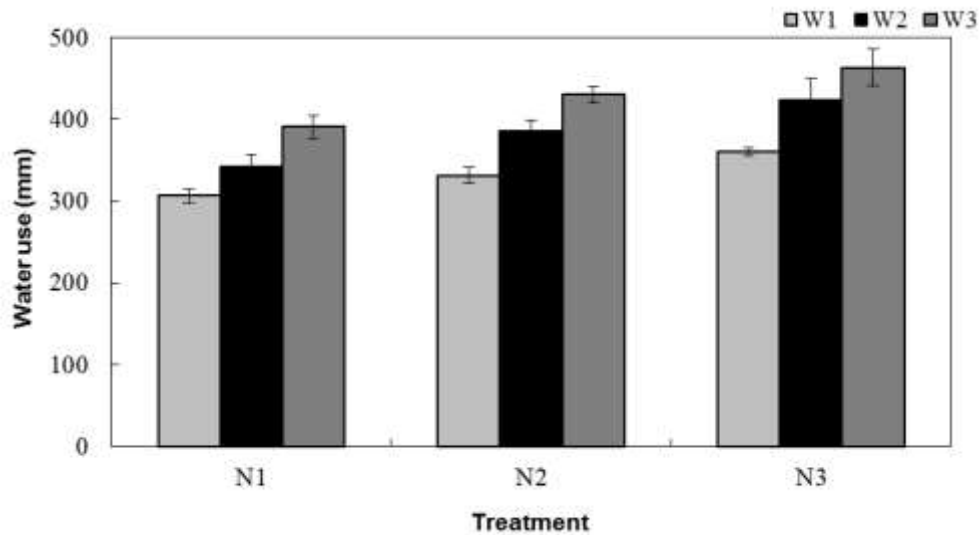


Figure 2: Cumulative water use (mm) of annual ryegrass, W= water treatment, N= nitrogen treatment

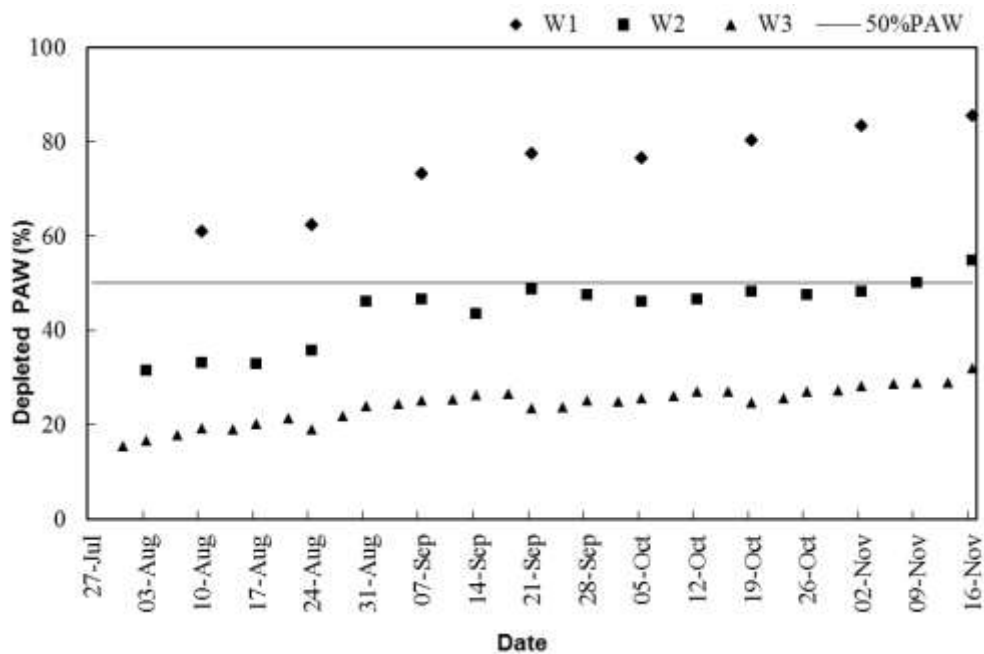


Figure 3: Depleted plant available water (PAW) of annual ryegrass over the growing season, W= water treatment

In both seasons, the water use efficiency (WUE) was influenced by W, N fertiliser and the interaction between WxN (Table 1). There were significant differences ($P<0.05$) in the WUE of the treatments with respect to total yield. Water was used more efficiently in the W1 treatment followed by W2 and W3. Nitrogen fertilisation significantly increased ($P<0.05$) WUE averaged over the irrigation treatment (Figure 4). For the high N treatments, highest WUE of $23.7 \text{ kg ha}^{-1}\text{mm}^{-1}$ was recorded for W1, followed by $22.1 \text{ kg ha}^{-1}\text{mm}^{-1}$ for W2 and $21.0 \text{ kg ha}^{-1}\text{mm}^{-1}$ for W3 (Figure 4).

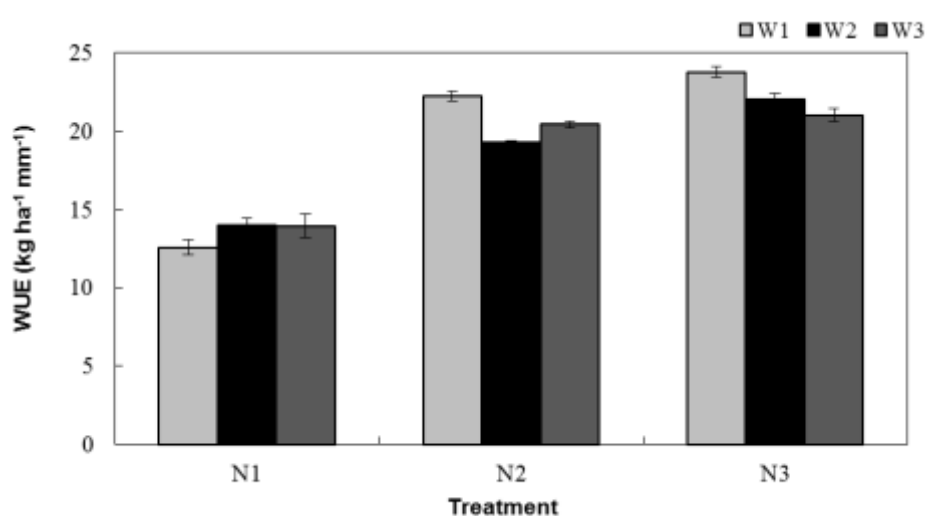


Figure 4: Water use efficiency ($\text{kg ha}^{-1}\text{mm}^{-1}$) of annual ryegrass, W= water treatment, N= nitrogen treatment

Forage quality

In the first and second seasons, the DM content was controlled by W and N fertiliser but there was no interaction (Table 2). With respect to the water treatment, the treatment that was irrigated once every two weeks recorded the highest DM content. With respect to the N treatment, the treatment that had no N application after each cut recorded the highest DM content.

The NDF content was not related to treatment (Table 3). The NDF values obtained from this study ranged from 38.2% DM to 40.9% DM (Table 4). The NDF values remained relatively constant across all treatments. There was a significant positive relation ($P= 0.043$) between NDF and depleted PAW, although the size of the effect was small (Figure 5a).

Table 2: Dry matter content of annual ryegrass in 2007 and 2008

Main effect	2007	2008
W1	15.51a [‡]	15.51a
W2	13.81b	12.42b
W3	11.54c	10.55c
LSD	0.371	0.311
N1	14.08a	13.21a
N2	13.54b	12.83b
N3	13.22b	12.43c
LSD	0.371	0.311
W	**	**
N	**	**
WxN	Ns	Ns

[‡]Values in each column followed by the same letters were not significantly different; **significant at $P<0.01$; Ns= non significant; W= water treatment, N= nitrogen treatment, WxN= water and nitrogen interaction

The CP content was controlled by W and N fertiliser but there was no interaction (Table 3). The CP content from this study ranged from 23.6% to 28.6% (Table 4). The highest ($P<0.05$) CP was recorded for the treatments irrigated once every two weeks (W1), while the lowest ($P<0.05$) was recorded for the treatment with the highest frequency of irrigation (W3). There was a significant positive relation ($P=0.011$) between CP and the depleted PAW (Figure 5b).

The IVOMD was not influenced ($P>0.05$) by WxN treatment interactions or by the level of N fertilisation, but the frequency of irrigation had a significant effect on the IVOMD (Table 4). The range in *in vitro* organic matter digestibility (IVOMD) was between 75.7% DM to 83.2% DM (Table 4). Plots irrigated once every two weeks (W1) and once a week (W2) had a higher ($P<0.05$) IVOMD value than W3. There was a significant positive relation ($P=0.006$) between IVOMD and the depleted plant available water (Figure 5c).

Table 3: Summary of ANOVA table on the chemical composition of annual ryegrass (Tukey's studentized range test)

Source of variation	Degree of freedom	F-probability levels							
		NDF		CP		IVOMD		ME	
		F value	Pr > F	F value	Pr > F	F value	Pr > F	F value	Pr > F
W	2	2.78	0.0923	22.46	<.0001	16.33	<.0001	55.73	<.0001
N	2	1.51	0.2499	15.33	<.0001	0.85	0.4450	10.91	0.0010
WxN	4	0.46	0.7617	1.25	0.3289	0.96	0.4534	3.32	0.0369

W= water treatment; N= nitrogen treatment; WxN= water and nitrogen interaction; NDF= neutral detergent fibre; CP= crude protein; IVOMD= *in-vitro* organic matter digestibility; ME= metabolisable energy

Table 4: Chemical composition of annual ryegrass

Main effect	NDF %	CP %	IVOMD %	ME %
Water (W)				
W1 (61-85%)	40.93a	28.58a	83.18a [‡]	11.76a
W2 (32-55%)	39.71a	26.01b	80.49a	11.33b
W3 (15-32%)	38.21a	23.55c	75.66b	10.67c
LSD	2.986	1.589	3.446	0.271
Nitrogen (N)				
N1 (0 kg)	39.94a	24.12c	80.78a	11.49a
N2 (30 kg)	38.49a	25.77b	79.37a	11.28a
N3 (60 kg)	40.42a	28.24a	79.17a	11.01b
LSD	2.986	1.589	3.446	0.271
W	Ns	**	**	**
N	Ns	**	Ns	*
WxN	Ns	Ns	Ns	*

[‡]Values in each column followed by the same letters were not significantly different; **significant at P<0.01; *significant at P<0.05; Ns= non significant; W= water treatment, values in parenthesis are water deficit from field capacity; N= nitrogen treatment, values in parenthesis are N application rates; WxN= water and nitrogen interaction; IVOMD - *in vitro* organic matter digestibility; NDF=neutral detergent fibre; ME=metabolisable energy; CP=crude protein

The metabolisable energy (ME) value was influenced ($P < 0.05$) by W, N and between WxN treatment interactions (Table 4). Within the same N fertiliser application, irrigating once every two weeks (W1) increased the ME values than irrigating once a week (W2) or twice a week (W3). There was a significant positive relation ($P = 0.0002$) between ME and the depleted plant available water (Figure 5d). The highest ME values were recorded in the W1N1 treatment (Figure 3). The ME concentrations obtained from this study ranged from 10.7 - 11.8 MJ kg⁻¹ DM.

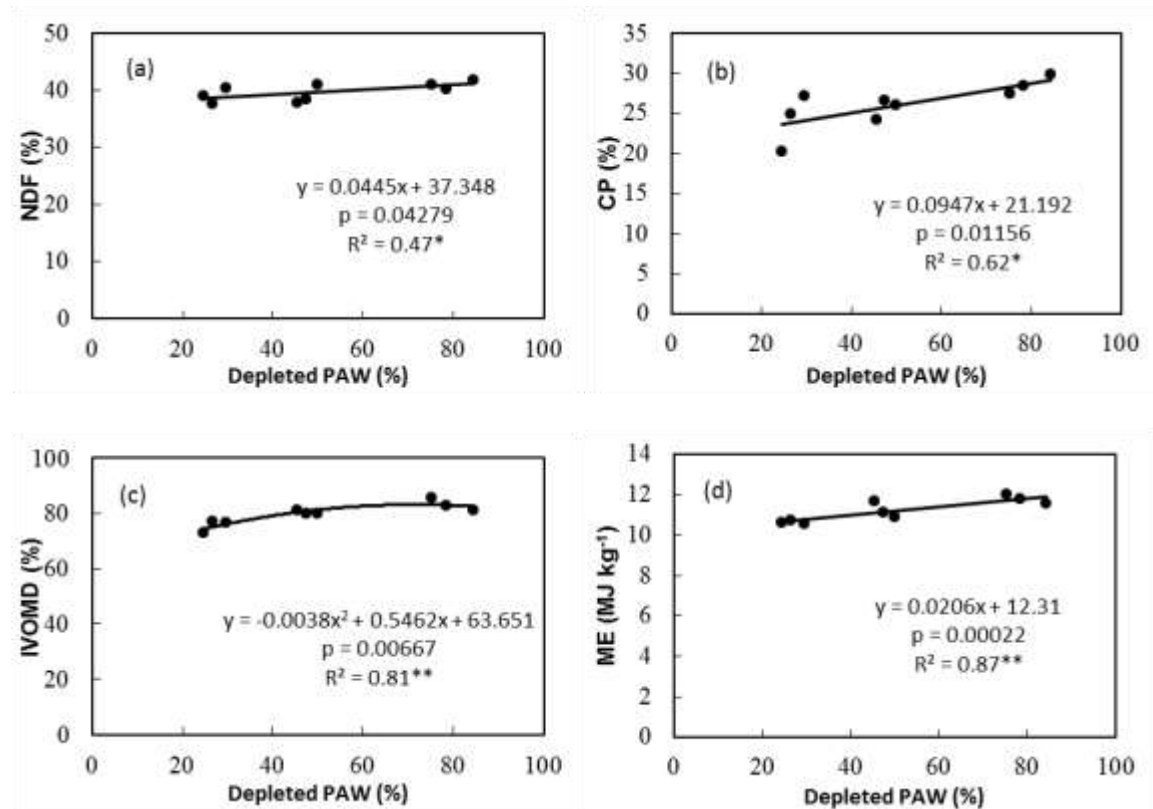


Figure 5: Relation between the depleted plant available water (PAW) and neutral detergent fibre (a), crude protein (b), *in vitro* organic matter digestibility (c) and metabolisable energy (d), p* significant at 0.05 and p** significant at 0.01

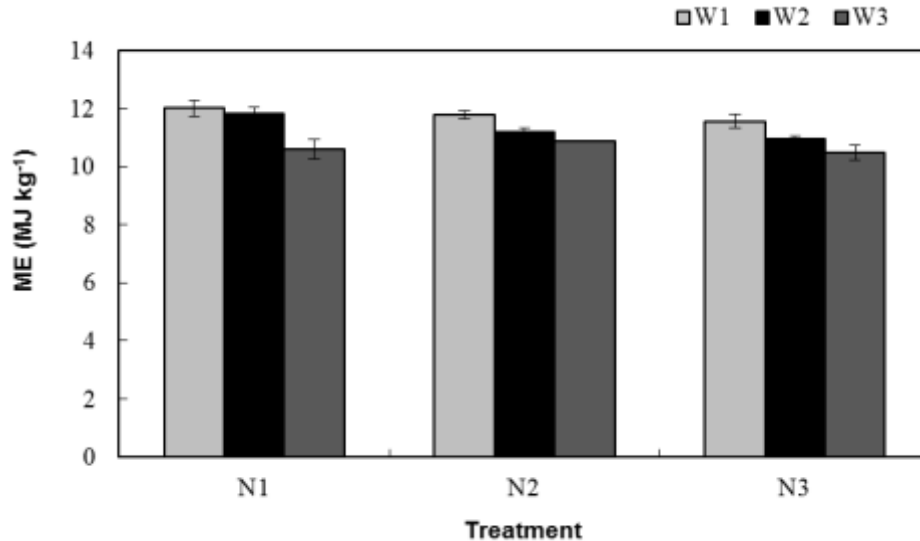


Figure 6: Metabolisable energy (MJ kg^{-1}) of annual ryegrass, W= water treatment, N= nitrogen treatment

Discussion

Lowest DM yield was obtained from the treatment that was irrigated once every two weeks with no nitrogen application. This was expected as there was no fertilisation applied, hence no fertiliser carry-over. Both irrigation and nitrogen had a positive effect on the DM yield ($P < 0.05$), which was consistent across all treatment combinations. The effect of increased irrigation and nitrogen application had a positive effect on the total yield produced. Generally, for the same level of water availability, yield increased with increasing nitrogen application. However, from the unfertilised plots the highest yield was obtained when plots were irrigated twice a week.

The low WUE may be due to the fact that frequently watered treatments had higher evaporation. The treatments that were irrigated once every two weeks recorded the highest WUE for N2 and N3, but for N1, these treatments recorded the lowest WUE, where in these cases N fertiliser was the limiting factor. The reason for this could be because of the very low dry matter production due to water and nitrogen stress.

Dry matter yield was higher in the treatments that were irrigated once or twice a week and top-dressed with 60 kg N ha^{-1} . The reason for this is the favourable

conditions of having more irrigation and N fertilisation. According to Allen et al. (1998), for most crops a soil depletion level of 50% PAW as the highest allowable depletion threshold is used for irrigation scheduling without significant yield reduction. Fessehazion et al. (2014b) used a 50% of plant available water depletion level for developing irrigation calendars of annual ryegrass. The results from this study indicate that the soil was wet enough to fulfil the demand. The range in soil water depletion levels was lower than 50% for W2 and W3 (Figure 3). However, for W1, soil water depletion levels were higher than 50%. This shows that ryegrass in the W1 treatment was water stressed, while W2 and W3 were not stressed for most part of the growing season.

Water stress improved most parameters of forage quality of annual ryegrass (Figures 5a-d). There were significant correlations between the depleted plant available water and most quality parameters considered in this study. The most affected parameters were IVOMD and ME with r^2 of 0.80 and 0.87 respectively (Figure 5). The reason for this is when the frequency of irrigation decreases (water stressed), the grass growth becomes less vigorous, and this increases the leaf:stem ratio, thereby increasing the digestibility. This in turn increases the ME values as there is a positive relationship between digestibility and ME concentration.

A DM content of 10.5% to 15.51% was measured in the current study and similar results were obtained by Meeske et al. (2006). Low frequency of irrigation yielded a higher DM content (Table 2). As the season progressed, the DM content increased probably because the stem of the grass was mature and the grass had entered into a stage of flowering.

Water and nitrogen treatments had no effect on the NDF values obtained in this study. The NDF values obtained were of acceptable levels (NRC, 2001) although slightly lower ranges of results were reported by Meeske et al. (2006). The NDF content of forages grown under higher temperatures are usually less than forages grown under lower temperatures (NRC, 2001) and this explains as to why the results from this experiment have slightly higher NDF concentrations.

Increase in N fertilisation rates resulted in an increase in the CP content. Generally, as the frequency of irrigation increased the CP content decreased ($P < 0.05$). Similar results were also obtained by Meeske et al. (2006). The National Research

Council (NRC 2001) recommended that forage with CP content of 15% and more will maintain high producing dairy cows on grazed pastures. The results show that all treatments have greater than 15% CP content, so practically these can satisfy the CP requirement of high producing dairy cows. These results correspond well with the results of Sumanasena et al. (2004) who reported that the lower CP contents with frequent applications of water were associated with N leaching and the inability of the grass to absorb N in soils with water content near saturation point.

The DM digestibility of annual ryegrass is generally high in the early season of growth, but decreases as the season advances. Digestibility, which determines the relation between contents of nutrients and energy that would be available to animals, as more energy is required to digest feed with high structural content. Theron and Snyman (2004) reported slightly lower (72% to 81%) IVOMD values obtained from this study. W1 and W2 had a higher IVOMD values than W3. However, Marais et al. (2006) found that the whole plant digestibility tended to increase with higher amounts of water applied. In the current study, N fertiliser rate did not influence ($P>0.05$) IVOMD, although Valente et al. (2000) reported that N fertiliser may cause a slight decrease in the digestibility of ryegrass and also the age of the plant at harvest has a more profound effect on the digestibility than does fertilisation.

The ME is one of the first limiting nutrients for dairy cows grazing high quality pasture (Reeves et al. 1996) making it necessary to feed an energy rich supplementation if higher production is to be achieved. The ME concentrations obtained from this study were within typical ranges (10.3 - 11.7 MJ kg⁻¹ DM) of ryegrass reported in literature (Meeske et al. 2006). Generally, as the frequency of irrigation increased, the ME values decreased (Figure 6). Nitrogen applications also had a significant effect on the ME value. As the N fertiliser increased from N2 to N3, the ME values decreased although there was no difference between N1 and N2.

Conclusion

The current study showed that the pasture production was positively associated with the soil water and fertiliser content. Increase in WUE was achieved by reducing the frequency of irrigation from twice a week to once a week without causing significant yield loss. Within the same irrigation frequency, higher WUE was achieved by

alleviating a limiting factor, N fertiliser in this case, through increases in dry matter production. Nutritive value is another aspect that needs to be evaluated with respect to pastures. In this study, the grass recorded a high IVOMD, CP and ME values while the DM content was of acceptable levels. The results highlight that under optimal conditions of growth, the nutritive value of the pasture is able to meet the requirements of high producing dairy cows, provided that animals consume sufficient DM. Results from this study showed that water stress improved the quality of the pastures by increasing the DM content, IVOMD, ME and CP values. Based on the data from this experiment, it can be concluded that by irrigating once a week and fertilising with 60 kg N ha⁻¹ after each harvest, optimum yield can be achieved with a good quality pasture. This in turn can lead to savings in the cost of irrigation and nitrogen fertilisation.

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