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***Research Paper***

# Performance of young Nellore bulls on guineagrass pastures under rotational stocking in the Brazilian Cerrado

## *Ganancia de peso de toretes Nellore en pasturas de guinea bajo pastoreo rotacional en el Cerrado brasileño*

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**Abstract**

New highly productive guineagrass (*Megathyrsus maximus* syn. *Panicum maximum*) cultivars have been released in Brazil and grazing trials are necessary to evaluate their carrying capacity and forage quality. The objective of this study was to evaluate the liveweight gains of young Nellore bulls grazing 3 guineagrass cultivars under rotational stocking. The experiment was carried out in Planaltina (Federal District, Brazil) during a single rainy (November–April) and dry (May–August) season. Treatments were Massai (control), BRS Tamani and BRS Zuri cultivars. Zuri and Tamani pastures provided greater average daily liveweight gains (ADG) (0.38 and 0.42 kg/head, respectively) over the experimental period than Massai (0.28 kg/head). For all cultivars liveweight gains decreased markedly from May onwards at the beginning of the dry season. Nevertheless, bulls grazing Tamani and Zuri pastures still gained 0.20 kg/hd/d until late August, while those on Massai pastures gained only 0.08 kg/hd/d. The differences in ADGs can be explained to some extent by differences in quality of available forage. In vitro dry matter digestibility of plucked samples of Massai was 555 g/kg, compared with 621 g/kg for Tamani and 590 g/kg for Zuri. Crude protein concentration in plucked samples was also greater for Tamani and Zuri (71.9 and 74.2 g/kg, respectively) than for Massai (62.2 g/kg). As feed wastage was particularly high in Massai, further studies are needed to verify if higher stocking rates during the wet season could result in greater production of live weight per ha on this cultivar, assuming that ADG does not decrease further with the increased stocking rate.

**Keywords**: Crude protein, digestibility, grazing, stocking rate, tropical grass.

**Resumen**

En Brasil se han liberado nuevos cultivares del pasto guinea (*Megathyrsus maximus* sin. *Panicum maximum*) altamente productivos y es necesario evaluar su capacidad de carga y la calidad del forraje en condiciones de pastoreo. El objetivo de este estudio fue evaluar las ganancias de peso vivo de toretes Nellore en tres pasturas de guinea bajo pastoreo rotacional. El experimento se llevó a cabo en Planaltina (Distrito Federal), Brasil, durante una temporada lluviosa (noviembre–abril) y una temporada seca (mayo–agosto). Los tratamientos fueron los cultivares Massai (testigo), BRS Tamani y BRS Zuri. Zuri y Tamani proporcionaron mayores ganancias de peso vivo diarias (0.38 y 0.42 kg/animal, respectivamente) durante el período experimental que Massai (0.28 kg/animal). Para los tres cultivares, las ganancias de peso vivo animal disminuyeron notablemente a partir de mayo (comienzo de la estación seca). No obstante, los toretes pastoreando Tamani y Zuri ganaron 0.20 kg/animal/día hasta fines de agosto, mientras que los animales en Massai ganaron solo 0.08 kg/animal/día. Estos resultados se pueden explicar por las diferencias en la calidad del forraje disponible, determinada con base en un muestreo de simulación de pastoreo (‘hand-plucking’). La digestibilidad in vitro

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de la materia seca de Massai fue de 555 g/kg, en comparación con 621 g/kg para Tamani y 590 g/kg para Zuri. La concentración de proteína cruda también fue mayor para Tamani y Zuri (71.9 y 74.2 g/kg, respectivamente) que para Massai (62.2 g/kg). Como el desperdicio de forraje fue particularmente alto en Massai, se necesitan más estudios para determinar si en este cultivar eventuales cargas animal más altas durante la temporada lluviosa podrían resultar en una mayor producción de peso vivo por hectárea sin afectar la ganancia de peso diaria por animal.

**Palabras clave**: Carga animal, digestibilidad, gramínea tropical, proteína cruda.

## Introduction

There is an increasing demand for highly productive forage species for the Brazilian savannas ('Cerrados'), owing to the growing intensification of livestock and agricultural systems. Guineagrass (*Megathyrsus maximus* syn. *Panicum maximum*) is recommended for regions with annual rainfall of 800–1,800 mm on well-drained soils with medium-high fertility (Muir and Jank 2004). This species produces around 21 t DM/ha of forage per year in the Cerrados (Fernandes et al. 2014), almost all during the rainy season. As a result, these pastures usually require rotational stocking management to provide more efficient grazing by minimizing forage losses, especially for the high-tufted cultivars.

Massai and Tamani are small leafy guineagrass cultivars released by Embrapa (Brazilian Agricultural Research Corporation). Since its release in 2001, Massai has been increasingly cultivated in Brazil because it will produce well on less-fertile soils in comparison with other guineagrass cultivars (Volpe et al. 2008), but it has below-average nutritive value (Brâncio et al. 2003). The hybrid Tamani (intraspecific cross between sexual S12 and apomictic T60), released in 2015, has thin leaves and stems and higher digestibility than Massai (Fernandes et al. 2014). The cultivar Zuri was released by Embrapa in 2014, and in contrast with Massai and Tamani, is a tall tufted guineagrass, and is resistant to leaf spot disease (*Bipolaris maydis*). Zuri and Massai were developed from accessions collected by ORSTOM (Office de la recherche scientifique et technique outre-mer, France) in East African savannas during the 1960s, and designated at the time as T65 and T21, respectively.

In general, guineagrass grows vegetatively until autumn (April–May), when reproductive tillers emerge and nutritive value declines (Euclides et al. 2014). This growth pattern limits its use during the dry season as stand-over forage grown during the rainy season (i.e. stockpiling). The small-sized and early-flowering cultivar Tamani could be an option to prolong the use of guineagrass in the rainy-dry season transition.

The objective of this work was to compare the liveweight gains of young Nellore (*Bos indicus*) bulls in pastures of 3 guineagrass cultivars (Massai, Tamani and Zuri) in the Brazilian Cerrados under a rotational grazing system.

## Materials and Methods

### *Experimental site*

The study was carried out at the Embrapa Cerrados Research Center in Planaltina, Federal District, Brazil (15°35' S, 47°42' W; 993 masl) during a single production cycle from December 2013 to August 2014. The climate at the site is Aw (tropical savanna) according to the Köppen-Geiger classification (Peel et al. 2007). Daily rainfall and maximum and minimum daily air temperatures were recorded 1,400 m away from the experimental site (Table 1). The study was conducted on a clay soil (Rhodic Haplustox) with $pH_{(H2O)}$ 5.5, organic matter concentration 29 g/kg and P concentration of 4.2 mg/kg (Mehlich-I) in the 0–20 cm soil horizon.

In August 2010, 3 t/ha of dolomitic lime was broadcast onto a 5-yr-old *Brachiaria* spp. pasture (16 ha). On 3 February 2011, the area was plowed and disked, was divided into 6 blocks of 2.6 ha and seed of the 3 guineagrass cultivars was sown (2 blocks per cultivar) with a Semeato® machine into a prepared seedbed at a rate of 3 kg/ha. Row spacing was 0.25 m. A commercial granular fertilizer was applied at seeding to supply 14, 39 and 67 kg/ha of N, P and K, respectively. During the establishment phase annual applications of 100 kg N/ha (as urea) were made to paddocks and 35 kg P/ha (as simple superphosphate) was applied in October 2012. From planting until 2014, pastures were rotationally grazed for 28 days followed by 28 days rest during the rainy season (19 December–7 May) and 56 days grazing and 56 days rest during the dry season (8 May–28 August) (Maciel et al. 2018).

### *Experimental design and grazing management*

Treatments were 3 guineagrass cultivars (Massai, Zuri and Tamani) distributed in a completely randomized design with 2 replications. From commencement of the grazing study, pastures were rotationally grazed at a variable stocking rate. Each experimental unit (2.6 ha)

**Table 1**. Monthly rainfall and mean monthly temperatures during the experimental period (2013–2014), and medium-term (1973–2003) mean values for Planaltina, Federal District, Brazil.

| Month | 2013 | 2014 | Av.[1] | 2013 | 2014 | Av. | 2013 | 2014 | Av. |
|---|---|---|---|---|---|---|---|---|---|
| | Rainfall (mm) | | | Maximum temperature (ºC) | | | Minimum temperature (ºC) | | |
| Jan | 319 | 123 | 254 | 27 | 29 | 27 | 18 | 17 | 18 |
| Feb | 96 | 91 | 184 | 30 | 28 | 28 | 17 | 17 | 18 |
| Mar | 143 | 300 | 214 | 29 | 28 | 28 | 18 | 18 | 18 |
| Apr | 97 | 116 | 93 | 28 | 28 | 28 | 17 | 17 | 17 |
| May | 19 | 7 | 27 | 28 | 28 | 27 | 16 | 15 | 15 |
| Jun | 51 | 21 | 5 | 27 | 27 | 27 | 15 | 13 | 14 |
| Jul | 0 | 2 | 5 | 28 | 27 | 27 | 13 | 13 | 13 |
| Aug | 2 | 0 | 16 | 29 | 30 | 28 | 14 | 13 | 15 |
| Sep | 56 | 9 | 41 | 30 | 32 | 30 | 17 | 17 | 17 |
| Oct | 126 | 87 | 137 | 29 | 32 | 29 | 17 | 17 | 18 |
| Nov | 188 | 257 | 191 | 28 | 29 | 28 | 17 | 17 | 18 |
| Dec | 221 | 338 | 230 | 27 | 27 | 27 | 18 | 17 | 18 |
| Total | 1318 | 1351 | 1397 | - | - | - | - | - | - |

[1]Average data for 1973–2003.

was divided into 4 paddocks (0.65 ha each). The rest and grazing periods were 21 and 7 d, respectively, in the rainy season (19 December–7 May; 5 grazing cycles in 140 d) and 42 and 14 d, respectively, in the dry season (8 May–28 August; 2 grazing cycles in 112 d). N fertilizer of 100 kg N/ha was applied as urea, as equal dressings following grazing in the first 2 grazing cycles.

*Animal measurements and herbage allowance*

Five young (12-month-old) Nellore bulls per experimental unit were assigned as testers for animal performance evaluation. Mean initial weight was 216 ± 19.1 kg. A mineral mix (Minerthal®) was supplied ad libitum to animals throughout the experimental period. Other Nellore bulls, similar in weight to the testers, were added to or removed from the experimental units during each grazing cycle to ensure a mean daily herbage allowance (HA) of around 12 kg DM/100 kg LW (Hodgson 1990; Herling et al. 2011). These adjustments occurred at the beginning of the grazing cycle based on pre-grazing herbage mass and calculated as LW = [(HM × 0.65)/DG]/HA × 100, where LW is the live weight (kg/ha), HM is the pre-grazing herbage mass including dead material (kg DM/ha), DG is the days of grazing and HA is the daily herbage allowance (kg DM/100 kg LW). Bulls were weighed 'unshrunk' at the end of each grazing cycle in order to evaluate the average daily weight gain (ADG) and assess how many bulls to introduce for the next cycle. Herbage allowance was recalculated based on average live weight observed during each grazing cycle. Bulls were weighed 'shrunk' (i.e. after 16 h without feed or water) at the beginning and end of the experimental period.

In addition to calculating ADG, the stocking rate (SR – calculated as an animal unit of 450 kg/ha) and liveweight gain per unit area (GA) were also estimated.

*Pasture parameters*

Pre-grazing herbage mass (HM) was evaluated at the beginning of each grazing cycle in the control paddock (1 of 4 rotated paddocks) by destructive samplings (at soil level) along 3 transects (12 quadrats of 2.0 × 0.5 m). Three subsamples (each containing 4 original pre-graze HM samples) were obtained and separated into green leaf blades and green stems (true stem plus leaf sheaths) and dead material. Dead material was visually defined as senescent leaves and stems with ≥50% of the area as yellow or dry tissue. All samples were dried in an air-forced oven at 55 ºC for 72 h.

*Nutritive value*

Crude protein (CP) (AOAC 1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) (as described in Van Soest et al. 1991) and in vitro dry matter digestibility (IVDMD) (as described by Tilley and Terry 1963 and modified by Moore and Mott 1974) were evaluated on dried 1 mm milled forage samples (Wiley mill). They were collected in the control paddock by the hand-plucking method (Sollenberger and Cherney 1995) on day 4 of the grazing period (7 days) for the second to the fifth grazing cycle and on day 7 for the sixth grazing cycle (14 days). No quality sampling was conducted in the first and seventh grazing cycles.

*Data analysis*

Data were analyzed using Proc Mixed (SAS 2002). For herbage mass components, nutritive value and SR, the effects of cultivar, grazing cycle and their interactions were assigned as fixed. Grazing cycle was analyzed as repeated measures and the covariance structure was chosen based on the parameters of Akaike information criterion. Mean ADG and GA of the entire experimental period were analyzed considering the effects of cultivar. For ADG, each tester was considered a subsample assigned as a random effect. The interactions not presented in the Results section were not significant (P>0.05) and treatments were considered different when P<0.05 by t test. Means reported were Least Square Means and were compared using PDIFF option. The accumulated weight gain of bulls over time (unshrunk weight) was analyzed as covariance analysis testing for polynomial effect of days using Proc Mixed.

## Results

While adjustments in SR were pre-established based on a HA of 12 kg DM/100 kg LW, greater allowances were provided, mainly in the first and fifth grazing cycles, when extra bulls were not available to increase stocking pressure (Figure 1). The variation in HA was relatively uniform among treatments, except for Massai pastures in the dry season (last grazing cycle) when weight losses in bulls appeared imminent so only the 5 testers were retained on each experimental unit for all treatments, increasing Massai HA to ~21 kg DM/100 kg LW (Figure 1).

Average daily gains of bulls (ADG) were affected by cultivar (P = 0.0005), with Tamani and Zuri supporting higher values than Massai over the experimental period (0.42 and 0.38 vs. 0.28 kg/hd/d, respectively). Stocking rates for the different cultivars were not significantly different (P = 0.0816), although stocking rates on Tamani were consistently lower than on Massai and Zuri, regardless of grazing cycle, because of lower forage yields (Table 2). Stocking rate was affected by grazing cycle (P<0.0001) and after an initial increase from December to January, it declined steadily until the end of the experimental period, regardless of cultivar (Figure 2). There was an effect of grazing cycle (P<0.0001) on leaf allowance, but no effect of cultivar (P = 0.3195). Leaf allowance closely followed herbage allowance by increasing in early April and decreasing again with the beginning of the dry season in May (Figure 2). GA for the 3 cultivars was 310 (Massai), 300 (Tamani) and 362 (Zuri) kg LW/ha but differences were not significantly different (P = 0.5315) (mean 324 ± standard error of mean 37.5 kg LW/ha).

The accumulated weight gains of the young bulls over time fitted the quadratic model, and the slope for Massai pastures was lower than that for Tamani and Zuri (P = 0.0134) (Figure 3). According to the quadratic effect, bulls stopped gaining weight at 250 days (August 26) for Tamani and Zuri and at 219 days (July 26) for Massai.

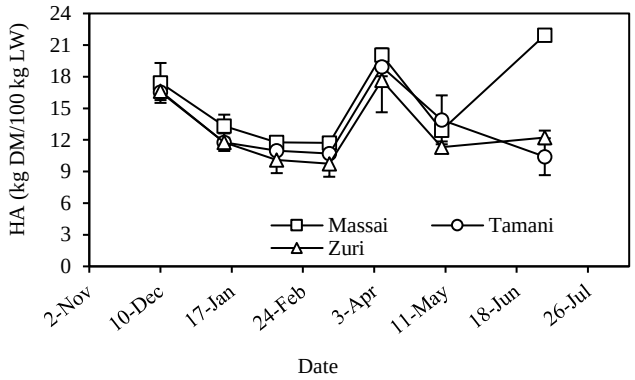


**Figure 1.** Herbage allowance (HA) in guineagrass pastures (cvv. Massai, Tamani and Zuri) during the experimental period in Planaltina, Federal District, Brazil. Each bar represents 2 standard deviations.

Cultivar had significant effects on herbage mass (HM) (P = 0.041), leaf mass (P = 0.045) and stem mass (P<0.0001) at the commencement of grazing. Massai presented greater HM than Tamani, while Zuri was intermediate (Table 2) and Tamani presented less leaf blade and stem than Massai and Zuri, regardless of grazing cycle. Amount of dead material was highest in Massai and lowest in Zuri, with significant (P<0.0001) differences between all cultivars. Forage quality was also affected by cultivar with crude protein concentration being highest in Zuri (74.2 g/kg) and lowest in Massai (62.2 g/kg; Table 2) (P = 0.039). IVDMD was highest in Tamani (621 g/kg) and lowest in Massai (555 g/kg) (P = 0.008).

Effects of grazing cycle on HM, leaf, stem and dead material are shown in Figure 4. In general HM and leaf yields peaked in April and declined steadily thereafter, while stem yields peaked in May. On the other hand dead material peaked in July.

Effects of grazing cycle on nutrient concentrations of forage on offer at the start of grazing are shown in Figure 5. Crude protein concentration and IVDMD were at their highest levels in March and April and declined subsequently and were lower in Massai than in Tamani and Zuri. On the other hand NDF concentration in available feed peaked in May, while ADF concentration continued to rise until July.

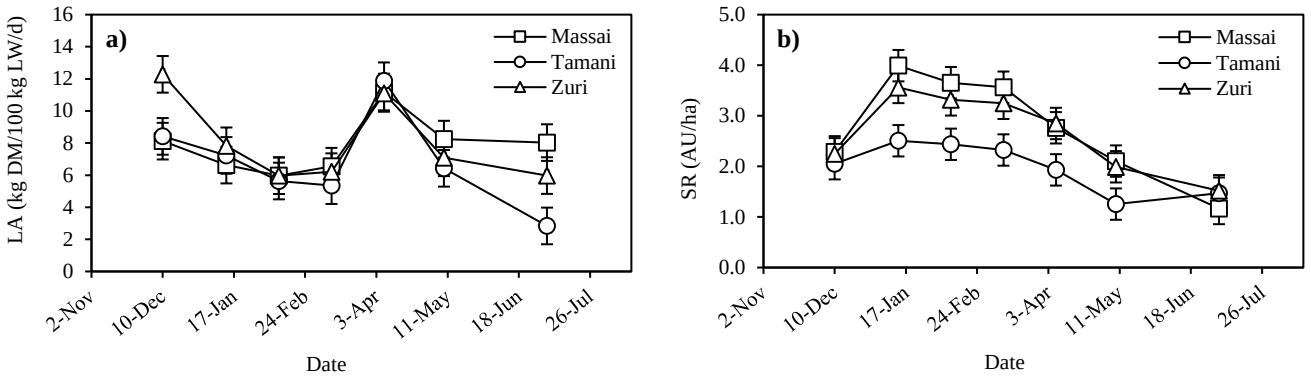


**Figure 2. a)** Mean leaf allowance (LA) and **b)** stocking rate (SR) in guineagrass pastures (cvv. Massai, Tamani and Zuri) during the experimental period in Planaltina, Federal District, Brazil. Each bar represents 2 mean square errors (MSE).

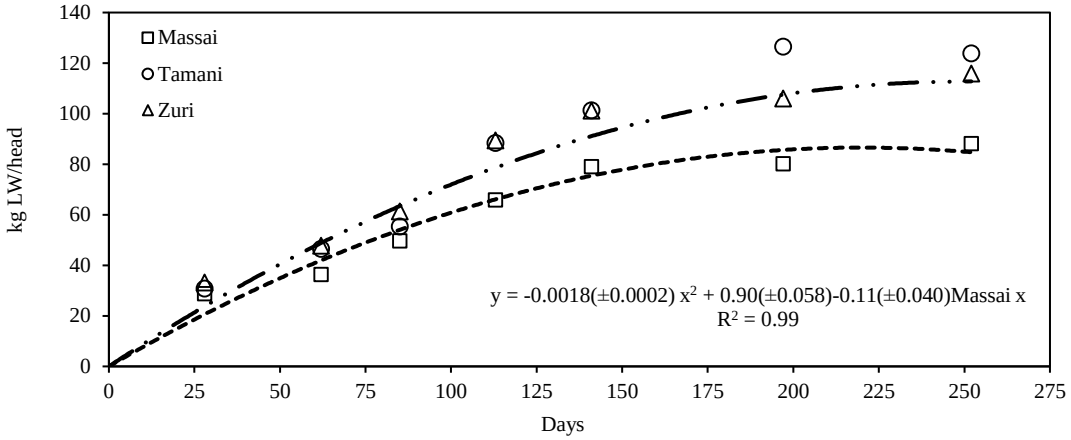


**Figure 3.** Accumulated liveweight (unshrunk) gain of young Nellore (*Bos indicus*) bulls on guineagrass pastures (cvv. Massai, Tamani and Zuri) in Planaltina, Federal District, Brazil. Each point represents 2 replications. The 2 quadratic curves present data for Tamani and Zuri (-..-) and Massai (---) cultivars, respectively.

**Table 2.** Mean stocking rate (SR), leaf allowance, pre-grazing herbage mass (HM), green leaf blade, green stem, dead material, in vitro dry matter digestibility (IVDMD) and crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations of Massai, Tamani and Zuri cultivars in Planaltina, Federal District, Brazil.

| Parameter | Cultivar | | | P | m.s.e.[3] |
|---|---|---|---|---|---|
| | Massai | Tamani | Zuri | | |
| SR (AU/ha) | 2.79 | 2.00 | 2.68 | 0.08 | 0.20 |
| Leaf allowance (kg/100 kg LW/d) | 7.82 | 6.82 | 8.06 | 0.32 | 0.54 |
| | | | | | |
| HM (kg/ha) | 6,093a[1] | 3,900b | 4,937ab | 0.04 | 329 |
| Green leaf (kg/ha) | 2,840a | 1,930b | 2,826a | 0.045 | 162 |
| Green stem (kg/ha) | 1,192a | 595b | 1,292a | <0.0001 | 86 |
| Dead material (kg/ha) | 2,060a | 1,374b | 819c | <0.0001 | 135 |
| | | | | | |
| IVDMD (g/kg)[2] | 555b | 621a | 590ab | 0.008 | 12.6 |
| CP (g/kg) | 62.2b | 71.9a | 74.2a | 0.039 | 3.2 |
| NDF (g/kg) | 716 | 686 | 696 | 0.106 | 6.5 |
| ADF (g/kg) | 415a | 390c | 401b | 0.0005 | 3.5 |

[1]Means for cultivars followed by the same letter within rows do not differ (P>0.05) by t test.
[2]Quality parameters are for hand-plucked samples.
[3]Mean square error.

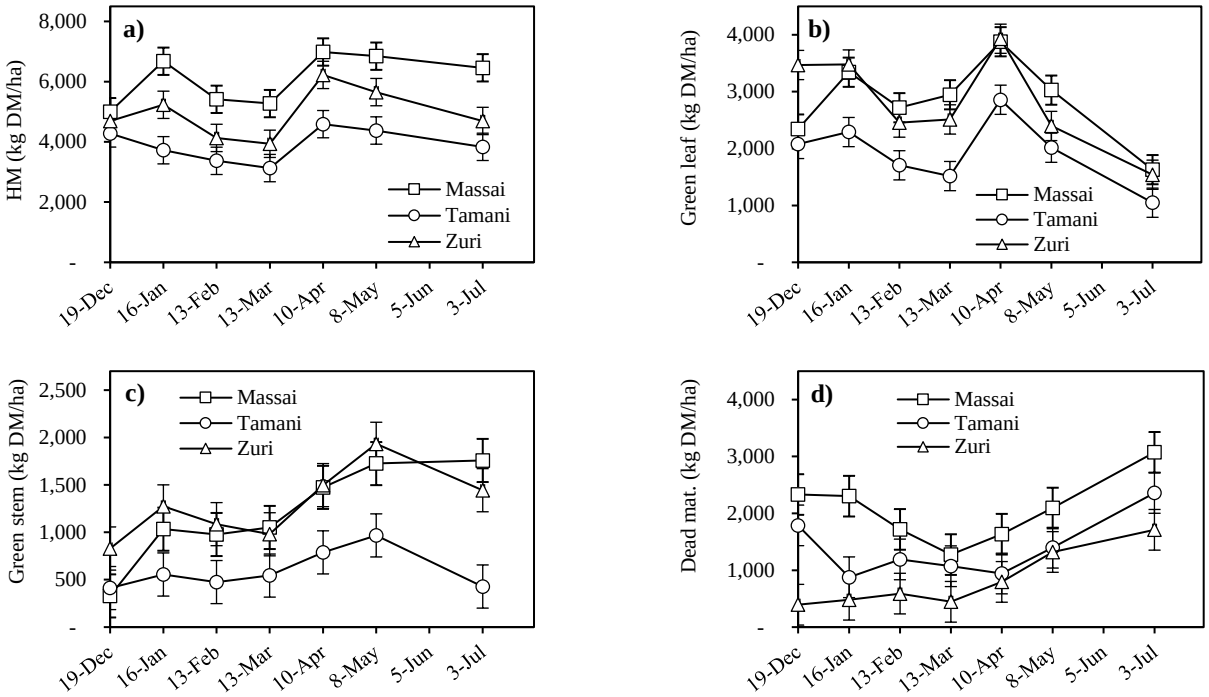


**Figure 4**. Pre-graze **a)** herbage mass (HM), **b)** green leaf blade, **c)** green stem and **d)** dead material in guineagrass pastures (cvv. Massai, Tamani and Zuri) in Planaltina, Federal District, Brazil. Each point represents 2 replications. Each bar represents 2 mean square errors (mse).

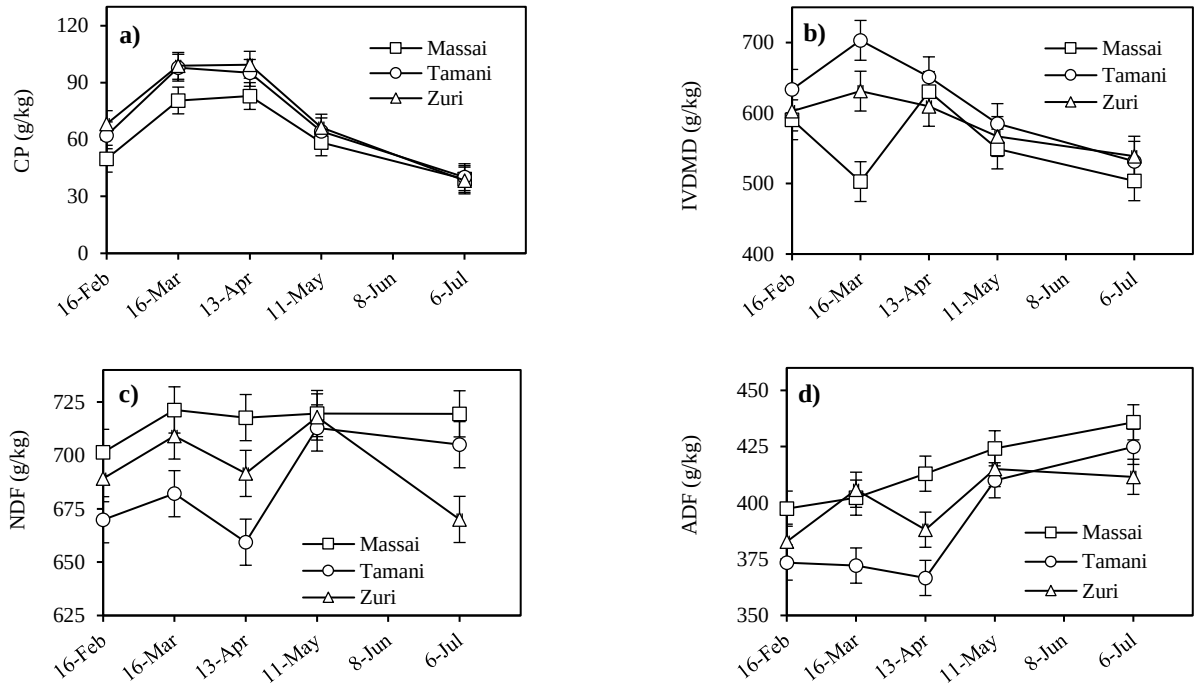


**Figure 5.** Variation in nutritive value of guineagrass cultivars in Planaltina, Federal District, Brazil with time: **a)** crude protein (CP); **b)** in vitro dry matter digestibility (IVDMD); **c)** neutral detergent fiber (NDF); and **d)** acid detergent fiber (ADF). Each point represents 2 replications. Each bar represents 2 mean square errors (mse).

## Discussion

Although some variation occurred during the experimental period, overall we were successful in maintaining HA at similar levels among treatments throughout the study (Figure 1). The variations from the desired target levels which did occur were a result of simultaneous increases in forage accumulation and non-availability of extra bulls, as observed in early April, which in theory could be an opportunity to harvest the extra forage before flowering for haymaking. Towards the end of the experimental period, in order to avoid severe weight loss of the bulls, only the 5 testers were retained on the pastures, resulting in marked increases in HA in Massai pastures for the last grazing cycle. Despite this, leaf allowances remained similar between cultivars, and most importantly, at levels considered non-restrictive to forage intake and weight gain.

Using the alternate stocking method (2 rotated paddocks) in the same area as the current study, Maciel et al. (2018) also observed greater ADGs in the rainy season for bulls grazing Tamani and Zuri pastures than for those grazing Massai, but no differences were observed in the dry season. The overall mean ADG observed in the current study (0.36 kg/hd) was much lower than that observed by Maciel et al. (2018) in the rainy and dry seasons (0.54 kg/hd), at a mean stocking rate of 2.1 AU/ha. One can also speculate that the alternate stocking system provided a better opportunity for forage selection than the 4-paddock rotational system in the current study.

Average daily gain for steers grazing Massai in our study was similar to that observed in Campo Grande, MS, where Nellore steers gained 0.30 kg/hd/d over a full year on pastures managed under rotational stocking (Euclides et al. 2008). In the current study, the superiority in ADG of Tamani and Zuri over Massai was 49 and 36%, respectively. The quadratic response of accumulated liveweight gain/head confirmed the advantage of Tamani and Zuri over cultivar Massai (Figure 3), in both rainy and dry seasons. The estimated final individual unshrunk live weights of bulls (initial weight plus the liveweight gain for the period) were 310 kg/head for Massai and 340 kg/head for both Tamani and Zuri. This liveweight advantage on Tamani and Zuri pastures can result in more animals reaching the desired finishing weight, which can provide an economic benefit. According to the quadratic model, rate of liveweight gain from May onwards (beginning of dry season) decreased markedly, with the accumulated maximum gains occurring by July 26 (219 d) for Massai and August 26 (250 d) for Tamani and Zuri. In the dry season (May–August) bulls grazing Tamani and Zuri pastures still gained 0.20 kg/hd/d, while bulls on Massai pastures gained just 0.08 kg/hd/d. As rate of liveweight gain decreased steadily throughout the grazing period, even with HA maintained at a high level (~12 kg DM/100 kg LW), feeding a nitrogen supplement or giving access to a legume stand (e.g. protein bank of *Stylosanthes* spp. or *Cajanus cajan*) would become an option to maintain satisfactory levels of gain. Since the decline in rate of weight gain commenced earlier on Massai pastures than on Tamani and Zuri pastures, protein supplementation should start at least 30 days earlier on Massai pastures than on Tamani and Zuri pastures. On the other hand, the greater HM on Massai pastures would enable animals to be supported for a longer period or more animals to be supported for the same period in the dry season than on Tamani and Zuri, as long as nitrogen supplements are fed. Stocking Massai pastures more heavily early in the growing season could mean more of the DM produced was consumed rather than becoming senescent and possibly restricting access to new leaf. This hypothesis should be tested.

During the experimental period, the onset of flowering promoted a peak in stem percentage in the canopy and a concomitant decline in percentage of leaf, resulting in contrasting mean leaf blade:stem ratios of ~5.0 and 1.6 in December and May, respectively (Figure 4). Stockpiling of guineagrass forage for grazing from this time of the year onwards is considered counterproductive because flowering accentuates the decline in nutritive value and boosts the growth of thick stems that suppress intake (Benvenutti et al. 2008), although this effect could be less pronounced in small-sized Massai and Tamani due to thin stems and greater green leaf blade:green stem ratio (2.38 and 3.24, respectively) than Zuri (2.19). An earlier and prolonged flowering (starting in February) for Tamani could also contribute to lessening the negative effects of concentrated flowering at the end of the rainy season on plant morphology (i.e. stem increase) making it more useful during the rainy-dry season transition. Interestingly, the lower leaf blade:stem ratio for the late-flowering Zuri was not sufficient to affect ADG in comparison with leafy Massai and Tamani cultivars, even in the rainy-dry season transition. Measurements of non-compressed canopy height (considering the newest leaf insertion at pre-grazing) in the current study revealed mean values of 0.45, 0.39 and 0.56 m in the rainy season and 0.72, 0.61 and 0.89 m in the rainy-dry season transition for the cultivars Massai, Tamani and Zuri, respectively. This canopy height observed for Zuri was far from that observed when it was allowed to grow uninterruptedly (1.8 m), while Massai and Tamani heights were closer (0.60–0.70 m) (data not reported). Euclides et al. (2008) observed mean leaf blade:stem ratios of 4.2 and 2.1 for available forage of Massai and the high-tufted cultivar Mombaça of the same species, respectively, in rainy and dry seasons, a similar trend to that observed between Massai and Zuri in the current study. The mean dead material in Massai

pastures was around 0.5 and 1.5-fold greater than in Tamani and Zuri pastures, respectively. The greater forage accumulation in Massai pastures associated with the largely unrestricted HA in March and during June-July would have contributed to the high levels of dead material in these pastures as a consequence of leaf senescence, probably leading to a large amount of forage that will be wasted, not consumed.

The IVDMD and CP of Tamani were 12 and 16% greater than those of Massai. Although Massai pastures produced a leafy canopy like Tamani, the nutritive value of Massai forage was usually lower, which ultimately was reflected in poorer animal performance as shown by Maciel et al. (2018). According to Maciel et al. (2018), digestibility of both Tamani and Zuri was 5 units higher than that of Massai, while CP was similar among cultivars. While our findings support these findings on digestibility, we found that CP was significantly lower in Massai than in Tamani and Zuri. The superior nutritive value of Tamani (formerly PM45) was also observed in a cutting trial, where leaf blade digestibility (in vitro digestibility of organic matter) and CP were 5 and 12% greater than for Massai, respectively (Fernandes et al. 2014). The CP concentration of Massai in our study (62 g/kg) was close to that observed by Brâncio et al. (2003) (~70 g/kg) and much lower than those observed by Gama et al. (2014) and Euclides et al. (2008) (81 and 89 g/kg, respectively), including both rainy and dry seasons.

In low latitudes of the Brazilian savanna region the dry season (April–October) receives about 15% of the annual rainfall, most of which falls in October. The water deficit is responsible for the marked decline in plant growth and reduction in nutritive value of forage, affecting grazing animal performance as a whole, as can be observed for the results obtained during the dry season in this study. According to Euclides et al. (2008), there was a decrease of 9% in IVDMD and 20% in CP from the rainy to the dry season in guineagrass pastures, with no changes in NDF. In the current study, IVDMD decreased 15% and CP decreased by 50% compared with rainy season mean values, probably affected by the absence of N fertilization in a prolonged period with low rainfall in Federal District (Table 1). In contrast with ADF, NDF remained relatively constant during the experimental period, including the transition between the rainy and dry seasons (April and May).

Individual cattle liveweight gains on Tamani and Zuri pastures were superior to those on Massai under rotational stocking management, due to their greater nutritive value (i.e. IVDMD and CP). However, Massai and Zuri pastures supported higher SRs than Tamani, which compensated for the lower ADG on Massai pastures and resulted in similar GA for Massai and Tamani with higher levels on Zuri. Feed wastage was particularly high in Massai, and further studies are needed to verify if higher stocking rates would increase GA on this cultivar by changing its canopy structure, without exacerbating the decrease in ADG. Zuri revealed greater productive potential and nutritive value than the other cultivars with positive effects on SR and ADG, simultaneously, even showing lesser leaf blade:stem ratio than Massai and Tamani. Rotational stocking is always a suitable option for managing highly productive guineagrass, avoiding forage losses and reduced grazing efficiency. However, for cultivars like Massai, and especially for Tamani, continuous stocking would be an option owing to favorable leaf blade:stem ratios and high nutritive value. Considering its great forage production potential but lesser nutritive value, Massai becomes an option for exclusive cow-calf farming systems, while Tamani and especially Zuri can be recommended for more demanding cattle categories (i.e. growing and fattening animals) in grass-fed systems by combining high carrying capacity and good forage quality. The high yields of Massai present possibilities for conservation as hay or silage for feeding back during periods of feed shortage.

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***Research Paper***

# Between-year variation in the effects of phosphorus deficiency in breeder cows grazing tropical pastures in northern Australia

## *Variación interanual de los efectos de la deficiencia de fósforo en vacas reproductoras en pasturas tropicales del norte de Australia*

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**Abstract**

Breeder herd productivity can be severely reduced by dietary phosphorus (P) deficiency. The performance of small groups of P-deficient ($P_{defic}$) or P-supplemented ($P_{supp}$) breeder cows was studied over 5 annual cycles while grazing C4 grass-*Stylosanthes* pastures at a site in the seasonally dry tropics of northern Australia. Soils contained c. 4 ppm of bicarbonate extractable P. Plasma inorganic P concentrations (PIP) during the wet season indicated that the $P_{defic}$ cows were deficient in P in 4 years and marginal in one year. Annual liveweight (LW) changes ranged widely between annual cycles from −71 to +13 kg in $P_{defic}$ cows and from +4 to +44 kg/head in $P_{supp}$ cows. The LW responses to increased dietary P ranged from −9 to +115 kg, were greatest in years when LW losses by the $P_{defic}$ cows were greatest, and were associated with low-rainfall years. LW gains of calves suckling $P_{supp}$ cows (mean 0.86 kg/d) tended to be higher (range 0.01–0.17 kg/d; mean 0.09 kg/d) than those of calves suckling $P_{defic}$ cows, but were significantly (P = 0.03) higher in only one year. Reconception appeared to be higher in $P_{supp}$ than $P_{defic}$ cows during the 2 years of lower rainfall. Overall, the results indicated that responses to P supplementation by breeders grazing P-deficient pastures can vary widely between years. Therefore, the response in any one year may not reliably indicate responses in the longer term.

**Keywords**: Cattle, mineral deficiency, phosphorus responses, phosphorus supplements, seasonal variation.

**Resumen**

La deficiencia de fósforo (P) en la dieta puede reducir severamente la productividad de las vacas reproductoras. En una zona de clima tropical estacional seco en el norte de Australia, durante cinco ciclos anuales se estudió el rendimiento de este tipo de vacas deficientes en P ($P_{defic}$) o suplementadas con P ($P_{supl}$), en pasturas de *Stylosanthes* y gramíneas C4. El suelo tenía una concentración aproximada de 4 ppm de P soluble (bicarbonato). Las concentraciones de P inorgánico en el plasma (PIP) determinadas en época lluviosa indicaron que las vacas $P_{defic}$ presentaron deficiencia de P durante 4 años y suficiencia marginal de P en un año. El cambio anual de peso vivo varió ampliamente entre 4 y 44 kg/animal en las vacas $P_{supl}$ y entre −71 y +13 kg en las vacas $P_{defic}$. En los años con pocas lluvias la respuesta a la dieta con $P_{supl}$ varió entre −9 y +115 kg, y fue mayor en las vacas $P_{defic}$. El crecimiento de los terneros amamantados por las vacas $P_{supl}$ (0.86 kg/dia) tendió a ser mayor (rango 0.01–0.17, promedio 0.09 kg/dia) que en aquellos de las vacas $P_{defic,}$ pero esta diferencia fue significativa (P = 0.03) solo en uno de los cinco años experimentales. La reconcepción fue más alta en las vacas $P_{supl}$ que en las $P_{defic}$ durante los dos años de menor precipitación. La amplia variación entre años en las concentraciones de PIP y los efectos de la deficiencia de P estuvieron ligadas a una alta variación en la lluvia anual y estacional. Los resultados mostraron que la respuesta de las vacas en pasturas

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deficientes en P a suplemento de P puede variar ampliamente entre años. Por lo tanto, la respuesta en un período determinado no garantiza la misma tendencia en el largo plazo.

**Palabras clave**: Bovinos, deficiencia de P, respuestas de P, suplementación, variación estacional.

## Introduction

Phosphorus (P) deficiency is one of the most widespread and economically important mineral deficiencies affecting grazing livestock. For example, based on soil P concentrations, some 70% of northern Australian rangeland pastures are expected to be deficient in P for grazing cattle (McCosker and Winks 1994; CSIRO 2007). P deficiency reduces voluntary pasture intake, leading to adverse effects on liveweight (LW) change and hence live weight in both growing and breeder cattle, lower calving rates and calf growth, and increased breeder mortality (Winks 1990; McCosker and Winks 1994; Suttle 2010). The economic benefits of P supplementation (Dixon et al. 2011) obviously depend on the magnitude and consistency of animal responses in specific regions and paddocks and across years.

Large between-year variability in the amount and distribution of rainfall is typical of many regions in the seasonally dry tropics, and particularly in northern Australia (Weston 1988; Cook and Heerdegen 2001). As a consequence there is generally high between-year variability in pasture quantity and quality (McLennan et al. 1988) and in annual LW gains of growing cattle (McCown 1981; Winks 1984; Jones et al. 1990). The performance of breeder cows in terms of LW, fertility and mortality also varies between years in association with climatic factors. However, the effects of environmental variability on responses to P supplementation in LW and productivity of breeder herds grazing pastures growing on P-deficient soils, and the economic implications of these variable responses, have received little attention. Furthermore, between-year variability in responses by breeder cows to P supplementation is often complicated by carry-over effects through successive annual cycles associated with cow body condition, time of calving and skeletal P reserves (Dixon et al. 2017). The present study examined the magnitude of the variation between years in performance and the responses to providing adequate dietary P in groups of breeder cows grazing pastures growing on severely P-deficient soil at a site in the seasonally dry tropics.

## Materials and Methods

### *General outline and animal cohorts*

The study reports results from experiments with breeder cows at the Lansdown Pasture Research Station (19º41' S, 146º51' E), approximately 50 km south of Townsville in north Queensland, Australia. Droughtmaster breeders (*Bos indicus* × *Bos taurus*) grazed an area of pasture growing on soils very low in available P through 4 annual cycles in Experiment 1 (Expt 1), and during an additional fifth annual cycle, when cows grazed similar low-P pasture in an adjacent area (Expt 2). The soils of the experimental site comprised a mixture of yellow earth (Gn 2.64, Northcote 1971), solidic (Dy 3.43) and solodic/solonized-solonetz (Dy 3.43) types (Murtha and Crack 1966; Coates 1994). The low dietary-P treatment ($P_{defic}$) was imposed by grazing the cattle on the P-deficient pasture without any additional dietary P. The P-supplemented treatment ($P_{supp}$) was achieved by grazing the cattle on the same P-deficient pasture and providing a P supplement during Expt 1 or by grazing pasture fertilized with P in Expt 2. Since each new group of heifers or cows used in the study (i.e. Expts 1a, 1b and 2) had grazed pastures expected to be adequate in P for at least 2 years before introduction to the experimental treatments, it was expected that the animals would initially have had replete body P reserves. Rainfall was recorded at the Lansdown weather station (Bureau of Meteorology Station #33226) c. 2 km from the experimental site.

### *Experiment 1*

The trial paddocks were in an area of P-deficient soil with bicarbonate extractable P ($P_B$, Colwell 1963) of c. 4 ppm in the top 100 mm of soil and comprised part of the P-deficient treatment paddock described by Coates et al. (2018). The pasture was a mixture of native grasses (*Heteropogon contortus, Chrysopogon fallax, Aristida* spp. and naturalized *Bothriochloa pertusa*) plus sown sabi grass (*Urochloa mosambicensis* cv. Nixon) and sown legumes (*Stylosanthes hamata* cv. Verano and *S. scabra* cv. Seca). The 16 ha trial area was divided into 2 paddocks of 8 ha. Each was grazed by a group of 4 breeders, which were allocated to the treatments by stratified randomization according to LW. One group ($P_{supp}$ group) received a P supplement via medication of the drinking water with Monophos ($NaH_2PO_4$) to provide 0.2 g P/L water. The $P_{defic}$ treatment received no P supplement and both groups had access to salt blocks (sodium chloride). The treatment groups were alternated between the 2 paddocks fortnightly to minimize between-paddock effects. Access to water was arranged to maintain the integrity of the treatments.

Measurements were made throughout 4 annual grazing cycles (Expts 1a, 1b, 1c and 1d). Expt 1a commenced in September 1986 with 3.5-year-old cows pregnant with their second calf (second-calf cows, SCC; calving October–December) and continued until June 1987 when the paddocks were destocked for animal welfare reasons owing to severe drought. The paddocks remained de-stocked until August 1988, when a new draft of 8 pregnant heifers, initially c. 2.5-year-old, (first-calf cows, FCC; calving October–December) was allocated to the 2 treatments ($P_{supp}$ and $P_{defic}$) by stratified randomization according to LW. These groups of animals remained in the study and received the same treatments through 3 annual cycles: 1988-89 (Expt 1b, FCC), 1989-90 (Expt 1c, SCC; calving December-January) and 1990-91 (Expt 1d, mature cows; calving December-January). The only exception was that one non-pregnant cow in the $P_{supp}$ group was replaced by a P-replete pregnant cow at the end of the Expt 1c grazing cycle. In Expts 1a, 1b and 1c the cows were joined for at least 3 months with 1 physically sound bull per treatment group after calving was complete. Calves were weaned in early to mid-June of each grazing cycle.

*Experiment 2*

In Expt 2 measurements were made on $P_{defic}$ and $P_{supp}$ groups of FCC which initially were pregnant heifers c. 2.5 years old in April 1994. The $P_{defic}$ heifers (n = 10) grazed a 20 ha paddock that comprised the area used in Expt 1 plus an adjoining 4 ha of similar low-P pasture. The $P_{supp}$ heifers (n = 10) received no P supplement directly but grazed a nearby 16 ha sabi grass-stylo pasture which had been fertilized annually for >10 years with super-phosphate at 10 kg P/ha so that improved dietary P was provided by the pasture. This experiment has been described previously (Coates et al. 2018), where the focus was primarily on the mineral density of tail bone. The heifers calved in November-December 1994 and the calves were weaned on 11 May 1995, when the study was terminated. Both groups of breeders were joined with one bull per group for 3 months after calving was completed.

*Measurements*

Cows and calves were weighed regularly following an overnight fast, usually at about 4 week intervals. Jugular blood samples from the cows were collected on most weigh days and centrifuged to separate plasma, which was retained for subsequent measurement of plasma inorganic phosphorus (PIP) concentrations. PIP was measured at 4–8 week intervals in Expt 1a, predominantly at 4 week intervals in Expts 1b, 1c and 1d, and at about 8 week intervals in Expt 2, and analyses were as described by Murphy and Riley (1962). Samples of feces were obtained from each cow per rectum when the cows were weighed during Expts 1b, 1c and 1d. The $\delta^{13}C$ in the individual samples of feces was measured by mass spectrometry (LeFeuvre and Jones 1988) and used to calculate the proportion of C3 plants (i.e. non-grass components, comprising predominantly *Stylosanthes* spp.) and C4 tropical grasses in the diet (Jones et al. 1979). In addition the concentrations of total P and total N were measured in feces sampled during Expt 1b and part of Expt 1c. Pregnancy rates were determined at weaning by manual palpation of the uterus via the rectum by an experienced operator to select pregnant animals for each new group, and also of the cows at weaning at the end of each annual cycle.

*Animal welfare*

All experimental procedures involving the animals were carried out according to the Code of Practice for the Care and Use of Animals for Scientific Purposes and with the approval of the relevant Animal Ethics Committees operating when the experiments were conducted.

*Calculations and statistical analyses*

Statistical analysis within each grazing cycle was restricted to cows that reared a calf to weaning because of the effects of pregnancy and lactation on cow LW and PIP. Statistical analysis was conducted using GENSTAT (release 16.1 9VSN International Ltd, Hemel Hemstead, UK). There was no paddock replication in either experiment and the differences in measured parameters between the $P_{defic}$ and $P_{supp}$ groups of cows at each measurement date were examined using ANOVA with individual animals considered as the experimental units. As the $P_{defic}$ and $P_{supp}$ treatment groups were rotated between paddocks each fortnight in Expt 1, we considered the effects of any paddock differences on response parameters were minimized. The LW of the cows at the beginning of each grazing cycle, and the individual calving dates, were examined as covariates in the analyses of response variables, and included when there was a greater than 90% probability that the covariates did affect the responses.

## Results

*Seasonal conditions*

Monthly rainfall for the grazing years 1986-87 to 1990-91 (Expt 1) and for 1994-95 (Expt 2) and the dates of the seasonal breaks (the first 3 day interval after June 30 when

≥50 mm rain was received) are shown in Table 1. These data highlight the summer-dominant rainfall pattern and the extreme variability in both total and effective annual rainfall in this environment. The 1986-87 grazing year (Expt 1a) ended in severe drought with insufficient rainfall during any 3 day interval to constitute a seasonal break as defined herein. Rainfall during the 6 months January–June 1987 was only 45% of the long-term mean for that interval and the trial area was destocked during the 1987-88 grazing cycle. In contrast, rainfall for 1988-89 and 1989-90 was high at 128 and 129% of the long-term annual average, while that for 1990-91 was excessive (197% of the long-term average) with particularly high rainfall during January-February 1991. In 1994-95 (Expt 2) rainfall was only 36% of the long-term annual average.

*Cow LW and LW change*

Mean LWs of cows through each grazing cycle are presented in Figure 1. Differences between grazing cycles in the effects of P treatment on cow LWs and patterns of LW change were unexpectedly large due most likely to the large differences between grazing cycles in the amounts and distribution of rainfall. In Expt 1a, when rainfall was much lower than average (Table 1), the differences in LW between $P_{defic}$ and $P_{supp}$ cows increased progressively from the end of November ($P<0.05$). By the end of the annual cycle the $P_{supp}$ cows had gained 30 kg whereas $P_{defic}$ cows had lost 62 kg, i.e. a difference of 92 kg, which was significant ($P<0.01$). Treatment effects on cow LWs during Expt 1b (1988-89) were in marked contrast to those in Expt 1a. Rainfall was well above average from March to June resulting in an extended green season. In addition, on average, the $P_{supp}$ cows calved 28 days earlier than the $P_{defic}$ cows so were lactating for longer during the grazing cycle; this is the likely reason for the lower mean LW of the $P_{supp}$ cows than that of the $P_{defic}$ cows, although these differences failed to reach significance ($P>0.05$). In Expt 1c (1989-90) rainfall was comparable with that in Expt 1a until the end of February 1990, but there was abnormally high rainfall in March and April (346 and 337 mm, respectively). The differences in mean LW between $P_{defic}$ and $P_{supp}$ cows became progressively greater until mid-January 1990, when $P_{supp}$ cows were 59 kg heavier than $P_{defic}$ cows. From mid-January until the end of the grazing cycle in June 1990, LW changes in both groups were similar and the final LW advantage of $P_{supp}$ cows was 49 kg. When initial LW was used as a covariate in the analysis, treatment differences were significant on all weighing dates from August 1989 to March 1990 ($P<0.05$ to $<0.001$). When calf birth date was used as a covariate, treatment differences in cow LWs were significant from January to June 1990 ($P<0.05$ to $<0.01$). During the full annual cycle $P_{supp}$ cows gained 43 kg and $P_{defic}$ cows lost 5 kg with the difference approaching significance ($P=0.07$). There was a carryover effect on cow LW (Figure 1) from Expt 1c to Expt 1d such that the average LW of $P_{supp}$ cows was initially 33 kg greater than that of $P_{defic}$ cows. Changes in LWs during the Expt 1d grazing cycle were similar for the 2 treatment groups. When initial

**Table 1.** Rainfall (mm) during each of the annual cycles for Expts 1 and 2. In Experiment 1 the area was destocked from 10 June 1987 until 4 August 1988 (1987–88). The long-term (1891–1990) average rainfall (BoM 2019) is also given. The seasonal break was calculated as the first 3 day interval after June 30 when ≥50 mm rain was received.

| Month | 1986-87 Expt 1a | 1987-88 Destocked | 1988-89 Expt 1b | 1989-90 Expt 1c | 1990-91 Expt 1d | 1994-95 Expt 2 | 100 year mean |
|---|---|---|---|---|---|---|---|
| Jul | 8 | 9 | 48 | 55 | 38 | 0 | 16 |
| Aug | 19 | 14 | 12 | 9 | 0 | 0 | 13 |
| Sep | 4 | 3 | 0 | 0 | 3 | 0 | 11 |
| Oct | 105 | 74 | 41 | 7 | 29 | 16 | 21 |
| Nov | 32 | 65 | 52 | 131 | 0 | 0 | 41 |
| Dec | 21 | 144 | 224 | 68 | 297 | 29 | 96 |
| Jan | 102 | 19 | 114 | 28 | 616 | 53 | 227 |
| Feb | 73 | 162 | 186 | 23 | 727 | 129 | 221 |
| Mar | 79 | 76 | 151 | 346 | 24 | 46 | 148 |
| Apr | 35 | 52 | 179 | 337 | 9 | 8 | 51 |
| May | 7 | 47 | 111 | 79 | 17 | 40 | 31 |
| Jun | 23 | 1 | 41 | 88 | 18 | 5 | 29 |
| Total | 507 | 667 | 1,157 | 1,171 | 1,780 | 326 | 906 |
| Seasonal break | None[1] | 30–31 Dec | 10–12 Dec | 21–23 Nov | 25–27 Dec | 10–12 Feb | |

[1]There was not sufficient rain during any 3 day interval to meet the designated criterion for a seasonal break.

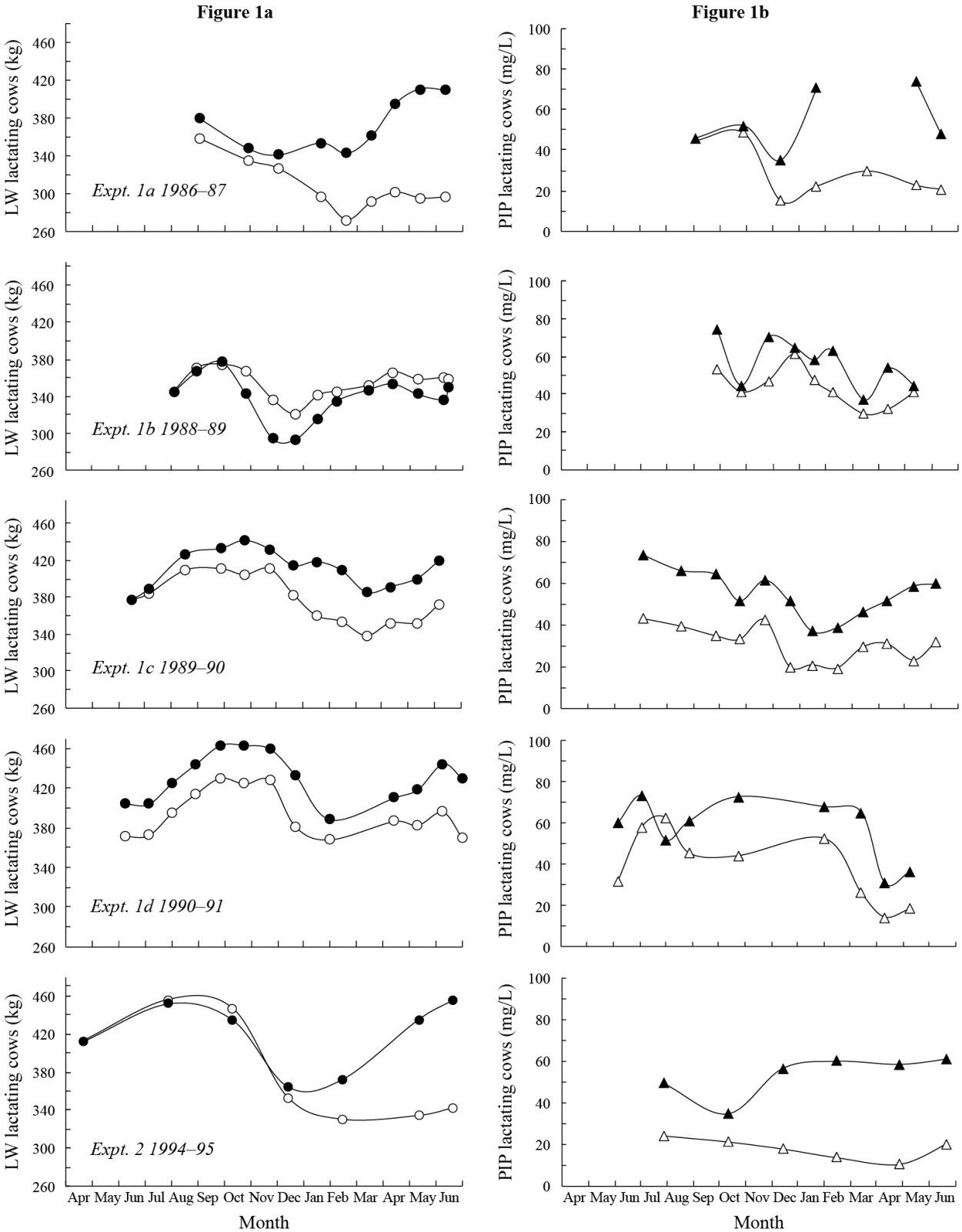


**Figure 1a.** Treatment mean live weights (LW) of cows grazing P-deficient pasture without (○) or with (●) P supplement through 4 annual cycles (1986–87, 1988–89, 1989–90 and 1990–91), or grazing P-deficient or P-fertilized pasture in 1994-95. **Figure 1b.** Plasma inorganic phosphorus (PIP) concentrations of cows without (Δ) or with (▲) P supplementation or P-fertilizer application through the same annual cycles. Cows in Expt 1 calved in November–December and were weaned in June, while those in Expt 2 calved in December–January and were weaned in mid-May.

LW was included as a covariate in the analysis, differences were not significant (P=0.14 to 0.89) except at the end of the grazing cycle in June and July 1991 (P=0.03). Through the entire grazing cycle, $P_{supp}$ cows gained 17 kg, while $P_{defic}$ cows lost 5 kg and the difference (22 kg) was significant (P=0.01).

In Expt 2 there were no significant differences in LWs between $P_{defic}$ and $P_{supp}$ treatment groups between April and December 1994 by which time calving was complete. Thereafter $P_{supp}$ cows gained LW rapidly while $P_{defic}$ cows virtually maintained LW. The differences between mean LWs of the 2 groups became increasingly greater (P<0.01 in February 1995 and P<0.001 thereafter). At the end of the annual cycle $P_{defic}$ cows had lost 71 kg LW compared with a 44 kg gain in the $P_{supp}$ group (i.e. a difference of 115 kg LW).

*Plasma inorganic phosphorus (PIP) profiles*

There were large differences between annual cycles in the effects of treatment on PIP profiles (Figure 1b), with differences apparently related to seasonal rainfall patterns in a similar manner to the differences in the LW profiles. In Expt 1a there were large treatment differences after calving (P<0.01) when all cows were lactating. Mean PIP of the $P_{defic}$ cows was only 22 mg/L from December to June, reflecting the effects of low soil P, probably exacerbated by the effects of low rainfall on forage P concentration and the high demand for P during lactation. Over the same interval PIP of $P_{supp}$ cows averaged 57 mg/L, reflecting the increased P intake in this group through supplementation and likely also increased pasture intake. In Expt 1b calving was complete in both groups by the end of December. The PIP of $P_{defic}$ cows for the 6 sampling occasions from December to May averaged 42 mg/L compared with 54 mg/L for the $P_{supp}$ cows, but on no sampling occasion was the difference between groups significant (P>0.10). Mean PIP concentrations for all sampling dates in Expt 1c were consistently higher in the $P_{supp}$ treatment than in the $P_{defic}$ group, the differences being significant on all 12 occasions (P<0.05 to P<0.001). During lactation (December–June), PIP of $P_{defic}$ cows averaged only 26 mg/L, half that of $P_{supp}$ cows (50 mg/L). In Expt 1d, fewer samplings were carried out and PIP in $P_{defic}$ cows remained appreciably lower than in $P_{supp}$ cows for most of the grazing cycle; differences between mean values for the 2 groups between August 1990 and April 1991 were significant on 3 occasions (P=0.002 to 0.01) and approached significance on 2 occasions (P=0.08). For the 3 sampling occasions conducted during late lactation (March–May 1991) mean PIP of cows in the $P_{defic}$ treatment (20 mg/L) was less than half that of cows in the $P_{supp}$ treatment (44 mg/L). In Expt 2 the PIP in $P_{defic}$ cows for the period July 1994–June 1995 averaged only one-third of the PIP of $P_{supp}$ cows (14 vs. 53 mg/L; P<0.01; Figure 1b) and indicated severe and prolonged deficiency of dietary P.

*Contribution of* Stylosanthes *to the diet and fecal concentrations of P and N*

During the early to mid-wet season (November–January) in Expts 1b, 1c and 1d, when measurements were made, stylo comprised c. 10–30% of the diet selected by both treatment groups of cows (Figure 2). However the proportion of stylo increased progressively from the mid-wet season through to the mid-dry season (February to July), and remained as a high proportion through to the late dry season (August–September). Furthermore, during these latter intervals the $P_{supp}$ cows increased their selection of stylo to a greater extent (to c. 70–85%) than the $P_{defic}$ cows (c. 40–60%).

During the interval from 30 August 1988 to 13 February 1990 (Expt 1b and part of Expt 1c), when measurements were made, the concentration of P in feces of $P_{defic}$ cows averaged 1.93 g P/kg DM, whereas the concentration in $P_{supp}$ cows was consistently higher and averaged 2.41 g P/kg DM (Figure 3a). During Expt 1b fecal P concentration was higher during the wet season (December–April; >2.2 g P/kg DM) than during the following dry season (May–November; 1.5–2.1 g P/kg DM). During the late dry season fecal N concentration was generally 12–14 g N/kg DM in both treatment groups (Figure 3b). However fecal N was generally 14–20 g N/kg DM during the wet season through to the mid dry season (December–July) of Expt 1b, and on average tended to be higher for $P_{supp}$ cows (mean 17.2 g N/kg DM) than for $P_{defic}$ cows (mean 15.1 g N/kg DM).

*Calf growth rates and reconception in the cows*

Calf growth rates (Table 2) averaged 0.77 kg/d in the $P_{defic}$ treatment groups across the 5 grazing cycles. Calf growth rate was on average 0.09 kg/day higher in the $P_{supp}$ treatment groups, but the difference was significant (P<0.05) only for Expt 1a. In Expt 1a, none of the $P_{defic}$ cows, but 3 of the 4 $P_{supp}$ cows, conceived. In Expt 1b, all $P_{defic}$ cows and 3 of the 4 $P_{supp}$ cows conceived. The cow that failed to conceive in the $P_{supp}$ group was comparable in LW and condition with the cows that conceived. All $P_{defic}$ and $P_{supp}$ cows conceived during Expt 1c. Cows were not joined in Expt 1d. In Expt 2, 9 of the 10 $P_{supp}$ cows conceived, while only 1 of the 10 $P_{defic}$ cows conceived.

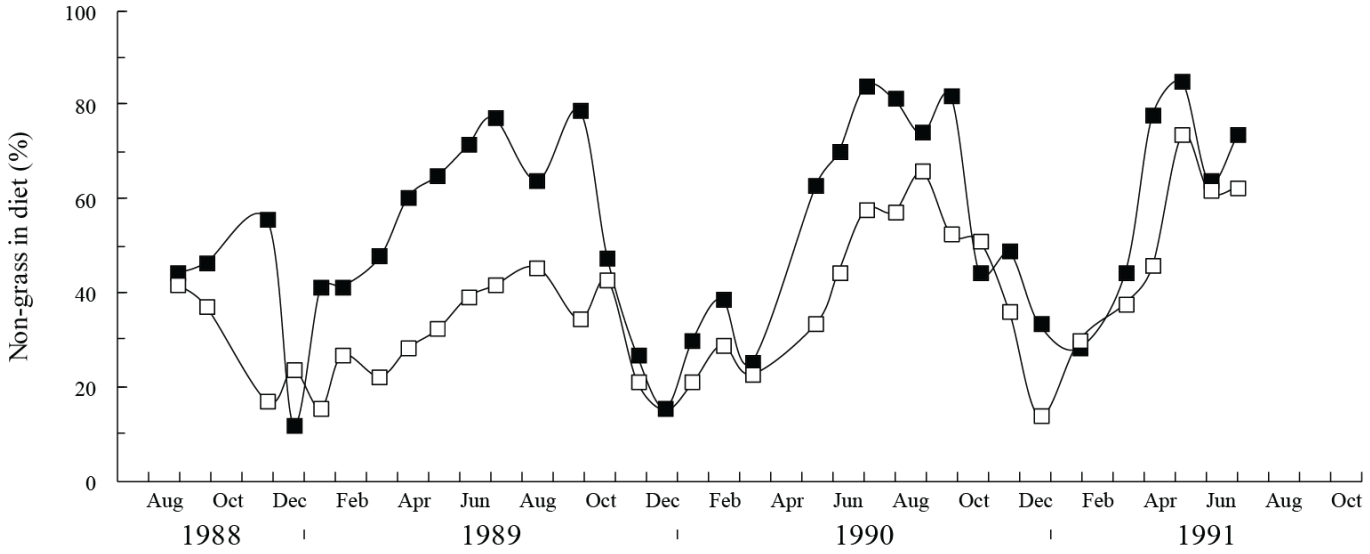


**Figure 2.** The percentage of non-grass (i.e. principally *Stylosanthes* spp.) in the diet selected by cows not fed P supplement (□) or provided with P supplement (■) from 30 August 1988 until 2 July 1991 during Expts 1b, 1c and 1d. The non-grass in the diet was calculated from the measured $^{13}\delta C$ of feces.

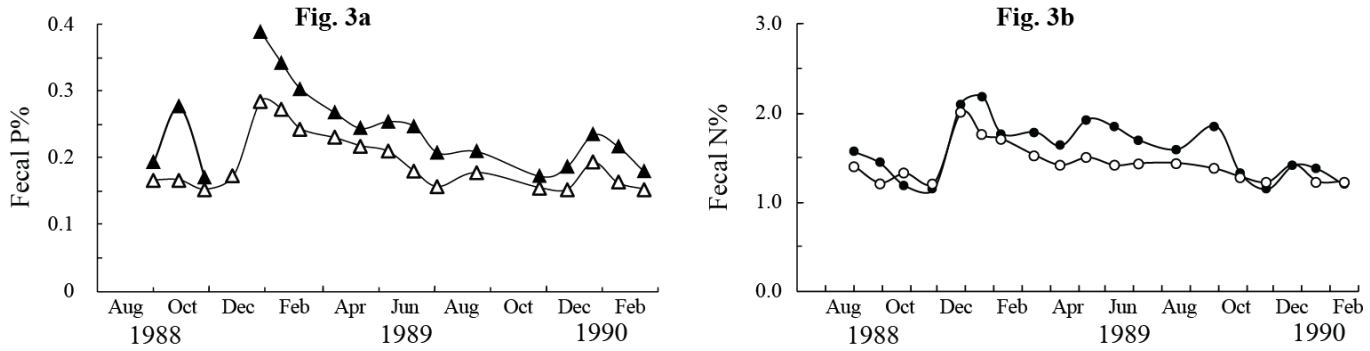


**Figure 3a.** The concentration of P in feces of cows not fed a P supplement (Δ) or provided with P supplement (▲) from 30 August 1988 until 13 February 1990 during Expts 1b and 1c. **Figure 3b**. The concentration of N in feces of cows not fed a P supplement (○) or provided with a P supplement (●) during the same interval.

**Table 2.** Calf growth rates for Expts 1 and 2. Interval dates and days represent the time from the first weighing after all calves were born through to weaning. Values in parenthesis indicate the number of calves.

| Expt | Grazing cycle | Calf growth interval | Days | Calf LW gain (kg/d) | | s.e.m. | P |
|---|---|---|---|---|---|---|---|
| | | | | $P_{defic}$ | $P_{supp}$ | | |
| 1a | 1986-87 | 2.12.86–10.6.87 | 190 | 0.76 (3) | 0.86 (4) | 0.026 | 0.03 |
| 1b | 1988-89 | 21.12.88–14.6.89 | 175 | 0.73 (3) | 0.90 (3) | 0.076 | 0.18 |
| 1c | 1989-90 | 16.1.90–6.6.90 | 141 | 0.71 (4) | 0.74 (4) | 0.061 | 0.48 |
| 1d | 1990-91 | 31.1.91–4.6.91 | 124 | 0.77 (4) | 0.92 (2) | 0.051 | 0.07 |
| 2 | 1994-95 | 13.12.94–11.5.95 | 149 | 0.88 (8) | 0.89 (9) | 0.026 | 0.78 |

## Discussion

The most important findings from the study were the large variations between annual cycles in: (i) the performance of breeder cows grazing P-deficient pastures; and (ii) the responses of the breeder cows to the provision of additional dietary P via supplement or fertilizer. The differences between annual cycles in the responses to improved P intake were observed in cow LWs and LW change profiles, PIP profiles, cow conception rates and to some extent calf growth rate, and were associated with large variation in the amount and pattern of rainfall during each annual cycle as

shown in Table 1. However, in most inland regions of northern Australia the dry seasons are usually longer, and there is a lower probability of any significant winter rainfall events than at the experimental site. This suggests that the results observed in the present study during the years with the lower rainfall but typical rainfall distribution (Expt 1a and Expt 2) are likely to be most representative of the northern Australian cattle industry.

The large variation between years in the present study was especially apparent with respect to cow LW change. Responses in PIP to improved P intake (Figure 1b) were apparent in 4 of the 5 grazing cycles but were most marked in the 2 years of lower rainfall (Expts 1a and 2). The between-year differences in PIP profiles of the $P_{defic}$ treatment indicated that the severity of dietary P deficiency was not constant across grazing cycles, although PIP concentrations did decline to <20 mg P/L during some intervals in 4 of the 5 annual cycles (the exception being Expt 1b, 1988-89). Thus the breeders in the $P_{defic}$ treatment were often severely deficient in dietary P (Wadsworth et al. 1990; Coates 1994; Anderson et al. 2017), while the PIP concentrations of 50–60 mg P/L in the $P_{supp}$ treatment established that these breeders were ingesting adequate dietary P for their level of production.

The poor LW performance of the $P_{defic}$ cows compared with the $P_{supp}$ cows was most marked in Expts 1a and 2, with mean differences between treatments in annual LW change of 92 and 115 kg, respectively. These large responses to improved dietary P were comparable with large LW responses to P supplement of 62 and 88 kg in breeder cows also grazing grass-stylo pastures during 2 annual cycles in another experiment at a P-deficient site at Springmount, Mareeba, in the seasonally dry tropics of northern Australia (Miller et al. 1998; Dixon et al. 2016; Coates et al. 2018). In Expt 2 additional dietary P was provided via applied P fertilizer, which potentially had additional dietary advantages besides improved P nutrition. However, previous work with similar stylo-grass pastures and low-P soil at the same location showed that annual LW gain was the same for yearling heifers grazing either pasture fertilized annually with superphosphate or grazing unfertilized pasture but supplemented with P (Coates 1994). It therefore appears unlikely that in the present study factors other than dietary P contributed in any substantial way to differences in productivity between the $P_{supp}$ and $P_{defic}$ treatments in Expt 2.

We hypothesize that the variation in responses to additional dietary P between annual cycles was due primarily to variation in the amounts and distribution of rainfall (i.e. seasonal conditions) with consequences for P concentration in the diets selected. The absence of treatment effects on cow LW changes during Expt 1b was most likely due to the favorable seasonal conditions throughout Expt 1b with well-above-average rainfall and an extended period of pasture growth through March and April, i.e. extended green season as defined by McCown (1981). This was in marked contrast to the responses during Expt 1a and Expt 2. Since P concentration is highest in young green leaf and lowest in mature, dry forage, an extended green season will promote higher P intakes through the grazing cycle (Coates 1994). Therefore, favorable seasonal conditions during Expt 1b probably resulted in better-than-average dietary P intakes, especially during lactation. This was supported by the higher PIP concentrations of the $P_{defic}$ cows during Expt 1b, i.e. mean PIP concentration during the December–June lactation interval (41 mg P/L) was appreciably higher than the mean concentrations of 22, 26 and 14 mg P/L for Expts 1a, 1c and Expt 2, respectively (Figure 1b). In Expt 1d the PIP concentrations for the $P_{defic}$ treatment cows remained high (>40 mg P/L, mean 52 mg P/L from July to January) for much of the grazing cycle and declined to much lower levels only late in the grazing cycle. This may have been a consequence of the high rainfall from December 1990 to February 1991 in this experiment (Table 1).

The observation in the present study that the adverse effects of P deficiency in breeder cows were much greater in low-rainfall years is in accord with past observations in the northern Australian rangelands with low-P soils. The symptoms of acute P deficiency such as 'peg-leg' and reduced breeder productivity have been observed to occur most often during droughts and low-rainfall years (Turner et al. 1935; Rose 1954; Barnes and Jephcott 1955, 1959; Ferguson and Sklan 2005). Similarly, a 5-year experiment in semi-arid New Mexico, USA, examining effects of P supplementation on breeder cow performance, reported that the absence of P supplementation had a detrimental effect only when coupled with the effects of drought (Judkins et al. 1985). In 2 studies reported by Holroyd et al. (1977; 1983) with breeders grazing native grass pasture or native grass-stylo pastures, there was relatively low variation among years in the LW responses of breeders to P supplements fed during the wet and early dry seasons at a site in the seasonally dry tropics of northern Australia similar to that at Lansdown. However this was associated with low variation in both total rainfall and the rainfall pattern. We suggest, therefore, that the present study is in accord with these other reported studies in demonstrating a large impact of variable seasonal conditions on the LWs and fertility of breeder cows grazing pastures on low-P soils.

The magnitude of the responses in $P_{defic}$ cows to the provision of additional dietary P in the present study was directly related to the extent of the annual LW losses in the $P_{defic}$ cows (Figure 4). The largest LW responses to

additional dietary P occurred during those annual cycles when the $P_{defic}$ cows lost the greatest LW. Furthermore, large LW responses of $P_{defic}$ cows to additional dietary P in 2 drafts of another comparable study with similar genotype breeders in northern Queensland (Miller et al. 1998; Dixon et al. 2016; Coates et al. 2018) were consistent with this relationship. Presumably the annual LW gain of $P_{supp}$ cows in the present study was constrained by the availability of other nutrients such as protein and energy rather than a lack of P through the annual cycle. The magnitude of the cow LW responses to P supplement was also related to the PIP of the $P_{defic}$ cows during lactation; these PIP concentrations were, except for Expt 1b, in the range 14–26 mg P/L indicating severe P deficiency (Wadsworth et al. 1990; Coates 1994; Anderson et al. 2017). The high PIP concentrations (50–59 mg P/L) in the $P_{supp}$ cows in all annual cycles established that the breeders in this treatment were ingesting adequate dietary P. Thus the variations in the responses to P supplement were closely linked to the amount and pattern of rainfall during each annual cycle and to the performance of the $P_{defic}$ breeders. However, the magnitude of the variations in the responses of $P_{defic}$ breeders to P supplements in the present study and in that of Miller et al. (1998), where the breeders also grazed stylo-grass pastures growing on P-deficient soils, may have been greater than would occur with breeders grazing native grass pastures containing little or no legume. Stylo-grass pastures growing on low-P soils are usually higher in protein and metabolizable energy relative to P concentration than grass pastures, and also have a high Ca:P ratio (e.g. often >10:1 and up to 25:1), which reduces bone-P mobilization (Minson 1990; Coates 1994). Consequently, the responses of growing cattle to supplementary P will often be much higher for stylo-grass pastures than for grass pastures. This may also occur with breeders grazing grass-stylo pastures. A lesser variation between annual cycles in the LW response of breeders grazing grass pastures to P supplements would presumably lead to a lesser variation in other measures of breeder herd productivity.

An important observation in the present study was that calf growth was generally similar in both unsupplemented and supplemented groups of breeders. Thus the breeders must have largely maintained milk production, regardless of the dietary P deficiency, even when undergoing substantial LW loss through annual cycles. The LW gain of calves suckling $P_{defic}$ cows was on average only 0.09 kg/day (or c. 10%) lower than that of calves suckling $P_{supp}$ cows, a difference of generally lower economic importance than cow fertility and mortality in rangeland production systems (M. Bowen and F. Chudleigh pers. comm.). The *Bos indicus* × *Bos taurus* genotype cows used in the present study must have been able to mobilize body reserves of both energy and P to maintain lactational output when nutritional intake was insufficient for LW maintenance. However, such mobilization of body reserves by lactating cows must clearly depend on availability of sufficient body reserves for this purpose. The $P_{defic}$ cows in the present study apparently had sufficient body reserves for this purpose but obviously P mobilization can continue only until body P reserves become depleted. The adverse effects of exhaustion of body P reserves with large and severe effects in the second and third years of ongoing P deficiency on intake, LW and milk production have been shown by Read et al. (1986) for Bonsmara crossbred beef cows and by Valk and Sebek (1999) for dairy cows. If the cows used in Expt 1a or Expt 2 had become pregnant and continued for another annual cycle of severe P deficiency, then poor lactational performance and/or cow mortality would probably have occurred. When cows mobilize body P and body energy reserves (as LW and body condition) during early lactation it is obviously essential that the reserves be replaced in late lactation or post-weaning for annual calving in harsh and P-deficient seasonally dry tropical environments (Dixon et al. 2017).

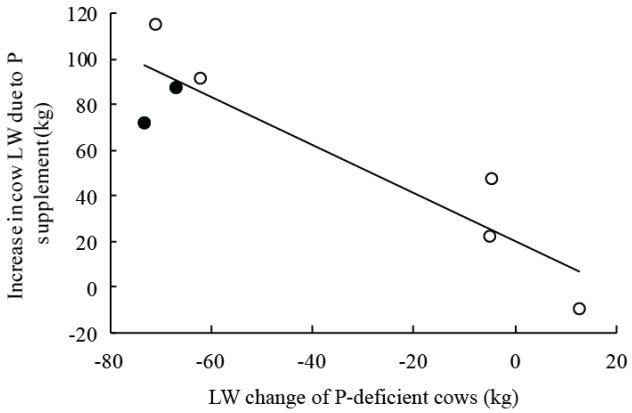


**Figure 4.** The relationship in paddock groups of cows between the increase in cow LW due to the provision of P supplements and the LW change of the $P_{defic}$ cows during the annual cycle for groups of cows in the present study (o) and for 2 annual drafts of an experiment (●) at Mareeba, north Queensland, Australia, where breeder cows of similar genotype grazed P-deficient pasture and received no supplement or were supplemented with P (Miller et al. 1998). The regression of the results from the present study was: $Y = 19.9 - 1.29\ X$ ($R^2 = 0.93$; $P<0.01$). The regression of the pooled results was: $Y = 20.3 - 1.06\ X$ ($R^2 = 0.84$; $P<0.01$).

Although the number of cows in each treatment during each annual cycle was small in the present study, the

pronounced effects of $P_{defic}$ on pregnancy rates in cows in Expts 1a and 2, but the absence of differences in Expts 1b and 1c (not measured in Expt 1d), indicate the differences likely to occur in commercial herds. The very poor re-conception rates in the $P_{defic}$ cows in Expts 1a and 2 were associated with low cow LW and body condition throughout the mating period. Breeder cows grazing P-deficient pastures are often prone to lactational anoestrus and fail to conceive while lactating, resulting in patterns of either delays in re-conception in consecutive years (i.e. 'slippage') or calving every second year (e.g. Read et al. 1986; McGowan et al. 2014; Dixon et al. 2017). Such a calving pattern was not observed in the $P_{defic}$ cows during the grazing cycles of Expts 1b, 1c and 1d but was probably a consequence of the sequence of annual cycles with well-above-average rainfall (Table 1).

## Conclusions

In breeders grazing P-deficient *Stylosanthes*-grass pastures at a site in a seasonally dry environment, there was wide variation among annual cycles in the severity of P deficiency and the LW performance and LW responses of reproducing breeders to provision of adequate dietary P. This was apparently associated with variation in seasonal conditions and dietary P intakes of un-supplemented animals. Thus the assessment of the severity and economic consequences of P deficiency on breeder performance within a given paddock requires cognizance of potential between-year differences and may require diagnostic measurements over a number of annual grazing cycles that encompass a range of seasonal conditions.

## Acknowledgments

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*(Note of the editors: All hyperlinks were verified 11 April 2019.)*

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***Research Paper***

# Perennial ryegrass and novel festulolium forage grasses in the tropical highlands of Central Kenya: Preliminary assessment

## *Nuevos pastos de ryegrass y festulolium en tierras altas del trópico de Kenia central: Evaluación preliminar*

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**Abstract**

Two perennial ryegrass (*Lolium perenne*) varieties and 5 festulolium hybrids (*L. perenne* × *Festuca* spp.) were evaluated on-farm for their performance over one growing season on clay loam soils at Ol-joro-Orok in the central highlands of Kenya at about 2,600–2,800 masl. Seed was sown in May 2015 and fertilizer (90 kg N + 90 kg P/ha) was applied at planting. The study continued for 8 months with harvests after 113, 99 and 32 days (3 growth cycles). Growth attributes assessed included dry matter yield (DMY) and plant height, while forage nutritive value was measured in terms of crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations. At the end of the first growth cycle, 61 local dairy farmers rated the grasses on criteria they nominated as being important, including DMY, growth rate, height, frost tolerance, disease tolerance and leafiness. Total herbage yields for the whole study period (8 months) ranged from 14.6 to 18.0 t DM/ha for perennial ryegrass and 14.3 to 20.9 t DM/ha for festulolium with very poor growth in the third growth cycle. All perennial ryegrass and festulolium lines contained similar (P>0.05) concentrations of CP (163–190 g/kg DM), ADF (264–281 g/kg DM) and NDF (448–493 g/kg DM). For perennial ryegrass, farmers gave a minimum weighted score of 6.7 and for festulolium, 7.9. Based on herbage production, forage nutritive value and farmers' assessments, we conclude that all perennial ryegrass and festulolium lines tested have the potential to contribute to improving the forage resource base in this and other similar areas, especially for farmers whose land sizes allow grazing instead of stall-based feeding only. Further studies with N applications after each harvest would determine whether yields can be maintained at high levels for longer than in this study, while grazing and feeding studies would determine how well the pastures support weight gains and milk yields. Studies over a number of years are needed to assess how persistent these varieties/hybrids are in this and other environments.

**Keywords**: Fodder, participatory research, rainfall-use-efficiency, tropical grass.

**Resumen**

En Ol-joro-Orok, zona alta de Kenia central (2,600–2,800 msnm), en suelos franco-arcillosos se evaluó la producción de forraje de dos variedades de ryegrass perenne (*Lolium perenne*) y cinco híbridos de festulolium (*L. perenne* × *Festuca* spp.). La siembras se efectuaron en dos fincas en mayo de 2015 con la aplicación de 90 kg N + 90 kg P/ha. El estudio se realizó durante 8 meses con mediciones después de 32, 99 y 113 días de crecimiento. En cada medición se determinaron el rendimiento de materia seca (MS), la altura de planta y las concentraciones de proteína cruda (PC), fibra detergente ácida (FDA) y fibra detergente neutra (FDN). Al final del primer ciclo de crecimiento, 61 productores de leche locales calificaron los pastos según los criterios que consideraron importantes: rendimiento de MS; tasa de crecimiento; altura de la planta; tolerancia a heladas; resistencia a enfermedades; y frondosidad. El rendimiento total de forraje para el período de 8 meses varió entre 14.6 y 18.0 t MS/ha para ryegrass perenne y entre 14.3 y 20.9 t MS/ha para festulolium; durante el tercer ciclo

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el crecimiento fue muy pobre. Todas las líneas de ryegrass perenne y festulolium contenían concentraciones similares (P>0.05) de PC (163–190 g/kg MS), FDA (264–281 g/kg MS) y FDN (448–493 g/kg MS). Tanto el ryegrass perenne como festulolium fueron ampliamente aceptados por los productores de la zona y tienen alto potencial de contribuir a mejorar la base del recurso forrajero en esta y otras áreas similares, especialmente para los productores con suficiente tierra para poder practicar el pastoreo en vez de alimentación del ganado únicamente en confinamiento. Se señalan una serie de estudios que se consideran necesarios para avanzar el desarrollo de estos pastos.

**Palabras clave**: Eficiencia en el uso de agua de lluvia, forraje, gramínea tropical, investigación participativa.

## Introduction

In recent times, the dairy industry in Kenya has been growing because of improving milk markets coupled with increasing per capita milk consumption (Auma et al. 2017). Predominantly, dairy is concentrated in the wet highlands and is practiced by smallholder farmers who keep either improved and/or crosses of Friesian or Ayrshire cattle (Muia et al. 2011). Due to decreasing land holdings because of land subdivision over generations, available grazing land has also been reduced, leading to dairy intensification, where cattle are kept in confinement under cut-and-carry systems of feeding. However, inadequate fodder in terms of both quantity and quality, especially during the dry season (Lukuyu et al. 2011; Njarui et al. 2012), leads to low milk yields that fall in the range 1,300–4,575 kg/lactation (Omore et al. 1999; Mugambi et al. 2015). Tropical grasses, e.g. Napier grass (*Cenchrus purpureus* syn. *Pennisetum purpureum*) and Rhodes grass (*Chloris gayana*), have been used successfully to support milk production but perform poorly at elevations above 2,000 masl (Boonman 1993). Forages adapted to temperate environments could possibly be preferable under such conditions.

Recently temperate grass breeding has focused on increasing the water-soluble carbohydrate (WSC) fraction to improve forage intake by ruminants and increase grassland productivity in terms of meat and milk (Humphreys et al. 2014a). These breeding efforts have resulted in new and novel high-sugar grasses (HSG) that combine good agronomic performance with high WSC (Humphreys and Theodorou 2001). Research shows that feeding HSG increases milk and meat production and decreases greenhouse gas emissions. These HSG include both *Lolium* species and festulolium hybrids (Humphreys and Theodorou 2001). Perennial ryegrass (*Lolium perenne*) is the most important grass used in grazing dairy systems in temperate environments worldwide (Wims et al. 2013; Humphreys et al. 2014a), especially in developed countries. Festuloliums (*Lolium* spp. × *Festuca* spp.), on the other hand, are natural or bred hybrids between any *Festuca* (fescue) and *Lolium* (ryegrass) species, designed for their combined complementary characteristics (Humphreys et al. 2014a; 2014b). As festuloliums are more tolerant of drought and cold than perennial ryegrass (Humphreys et al. 2014b), they are now attracting more interest for livestock production. Approximately 6,000 t of HSG seed has been sown in the UK since 2005, covering an area of 175,000 ha, and the impact on the livestock sector has been significant. The benefits to the dairy sector over the period 2005–2010 have been estimated at up to £78.2 (USD 90.2) million (Farming Futures 2013).

We hypothesized that HSG would help overcome forage shortages in winter in tropical highlands, e.g. the foothills of Mt. Kilimanjaro, Mt. Kenya, the Aberdare ranges in Central Kenya and especially in Nyandarua region (Muia et al. 2011). In the former sites, relatively low temperatures (5.5–10.7 °C mean minimums) create conditions similar to temperate climates, and dairy production is a common enterprise, as it generally is in Kenya (Njarui et al. 2012). Temperate grasses would withstand low temperatures better than tropical pastures (Larcher 2003) and would be more nutritious, more digestible and capable of recuperating after grazing (Lee et al. 2010; Humphreys et al. 2014a, 2014b). Weller and Cooper (2001) reported maximum crude protein (CP) concentration of 239 g/kg DM in a 2-year-old perennial ryegrass pasture with monthly yields in the range 1.68–2.34 t DM/ha, while Dierking et al. (2008) observed 200 g CP/kg DM in festulolium. By comparison, Napier grass in the tropics and subtropics, has crude protein concentrations in the range 80–130 g CP/kg DM (Wijitphan et al. 2009; Tessema et al. 2010), in addition to performing poorly in cold areas (Boonman 1993).

The work reported here evaluated new perennial ryegrass varieties and novel festulolium hybrids with the aim of quantifying their production potential in the dairy-dominated Nyandarua County (Mwendia et al. 2015) of Central Kenya. The grass varieties tested include new and novel products from the breeding program at the Institute of Biological, Environmental and Rural Sciences (IBERS) at Aberystwyth University in Wales, UK, including diploid and tetraploid perennial ryegrass as well as festulolium hybrids generated by combining

*L. multiflorum* or *L. perenne* with either *F. arundinacea* var. *glaucescens* or *F. mairei*.

## Materials and Methods

### *Site description*

Two on-farm trials were conducted at Ol-joro-Orok in Nyandarua County in Central Kenya (00°09' S, 36°17' E; 2,808 masl; Farm 1); and (00°09' S, 36°18' E; 2,667 masl; Farm 2). Soils in the area are lithosols with humic topsoil, dark reddish brown, well-drained, highly fertile clay and clay loam soils (Jaetzold and Schmidt 1983). The soils on the 2 farms were classified as slightly acidic clay loams with similar characteristics (Table 1).

The Ol-joro-Orok climate is described as temperate. Average annual temperature is 13.7 °C, with a mean minimum of 6.5 °C and about 950 mm annual precipitation (Climate-Data.org 2016), with rainfall occurring throughout the year, although most falls in August and the least in January-February. Weather conditions during the study are summarized in Table 3a and for calendar years 2015 and 2016 in Table 3b.

### *Approach and experimental design*

We conducted the study in a participatory approach with Nyamarura farmers' group. The individual farmers keep 2–5 dairy cows on their farms, and a group discussion was held to determine which 2 farms (described above) were to host the trials.

Seed of 2 varieties of perennial ryegrass and 5 hybrids of festulolium (Table 2) was sourced from IBERS. The experiments were conducted in a randomized complete block design with 3 replicates for each of the entries on each farm.

### *Trial establishment and maintenance*

Farmers prepared the land using hoes to break soil lumps and obtain a fine seedbed and, on 12 May 2015, seed was sown in plots of 2 $m^2$ for perennial ryegrass (20 kg/ha) and 1 $m^2$ for festulolium (16 kg/ha) in furrows about 6 mm deep with 10 cm between rows. A mixed NP fertilizer (23:23) was applied at a rate of 90 kg N/ha to all plots. A thin layer of soil was placed over the seed. Plots were hand-weeded according to need.

### *Plant height and dry matter yield measurement*

Plant height (PH) was measured from the base of a bunch of tillers to the tips of the leaves at 4 randomly selected positions within each plot, just before each harvest. Unlike the conventional monthly harvests for ryegrass and festulolium, a 3-month growth period was nominated to accumulate substantial biomass that would be preferable under a manual cut-and-carry scenario. However, to allow sufficient time for establishment, the growth period for the first cycle was longer than this target, while leaf senescence was observed in the third cycle, leading to earlier harvesting than the desired time. To assess dry matter yield (DMY), forage in quadrats of

**Table 1**. Soil properties at trial farms in Ol-joro-Orok, Central Kenya.

| Site | Total N (%) | Total C (%) | Bray P (mg/kg) | pH | Clay (%) | Sand (%) | Silt (%) | Soil texture |
|---|---|---|---|---|---|---|---|---|
| Farm 1 (N = 5) | 0.27 | 2.9 | 12.9 | 5.6 | 37.5 | 34.5 | 28.0 | Clay loam |
| Farm 2 (N = 5) | 0.30 | 3.5 | 12.6 | 5.0 | 36.1 | 31.6 | 32.3 | Clay loam |

Source: International Center for Tropical Agriculture (CIAT) Laboratory, Duduville, Nairobi.

**Table 2.** Details of forage grasses assessed in Ol-joro-Orok, Central Kenya.

| Variety or hybrid | Ploidy level, hybrid[1] |
|---|---|
| Perennial ryegrass | |
| AberBite (AB) | *Lolium perenne* (4x) |
| AberWolf (AW) | *Lolium perenne* (2x) |
| Festulolium | |
| AberNiche (AN)[2] | *Lolium multiflorum* × *Festuca pratensis* |
| Hybrid 1 | *L. perenne* (2x) × *F. arundinacea* (6x) |
| Hybrid 2 | *L. perenne* (2x) × *F. arundinacea* var. *glaucescens* (4x) |
| Hybrid 3 | *L. perenne* (4x) × *F. arundinacea* var. *glaucescens* (4x) |
| Hybrid 4 | *L. perenne* (4x) × *F. mairei* (4x) |

[1]Information from Humphreys et al. (2014b), Wilkins and Lovat (2011) and M.W. Humphreys (pers. comm.). [2]First festulolium registered in the UK.

0.5 × 0.5 m located at the center of each plot was hand-harvested at a stubble height of 4 cm with a sickle on 2 September and 11 December 2015 and 13 January 2016, i.e. 1st, 2nd and 3rd growth cycle, respectively.

Total fresh herbage was weighed in the field with a digital weighing balance and subsamples of about 250 g were selected at random. These samples were then dried to constant weight in an oven at 65 °C for 48 h for DM determination. Samples were then ground in a mill (Retich RM 100, Germany) to pass a 1 mm sieve and stored in sample bottles for subsequent laboratory analyses. Dry matter concentration was used to calculate DMY for each growth cycle as well as the herbage growth rate (HGR) during the growth period. Rainfall use efficiency (RUE, kg DM/ha/mm) was calculated by dividing daily herbage growth rate by total daily rainfall. After each harvest, the remaining forage within each plot was cut back to the uniform stubble height in preparation for subsequent growth cycles.

*Chemical analyses*

Cost limitations restricted laboratory analyses to samples from the initial harvest only. For precision and accuracy, nitrogen concentration was determined by the combustion method at 900 °C with Max Cube Elementar, Hanau, Germany (AOAC 1990) and the results multiplied by 6.25 to obtain crude protein (CP) concentration. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were estimated by Ankom bag technique analyzer (Ankom 143 Technology Fairport, NY, USA) following the AOAC procedure (AOAC 1975) at CIAT laboratory, Duduville, Nairobi.

*Digestible dry matter, dry matter intake and relative feed value*

Acid detergent fiber and NDF values were used to calculate/estimate digestible dry matter (DDM), dry matter intake (DMI) relative to body weight and, subsequently, relative feed value (RFV) using the formulae according to Jeranyama and Garcia (2004) as follows: DDM = 88.9 − (0.779 × %ADF); DMI = 120/(%NDF); and RFV = (DDM × DMI)/1.29.

*Participatory evaluation*

At 3 months after planting towards the end of the first growth cycle, 61 dairy farmers from Nyamarura group were invited to undertake a participatory evaluation of the varieties/hybrids. In a focus group discussion, the farmers generated a list of criteria they considered important in forages, before scoring these criteria on a scale of 1 to 10, where 1 represents the least important and 10 the most important. All plots were tagged with labels bearing the name or hybrid number. Farmers were requested to stand around the perimeter of the entire experimental block and were guided in identifying the varieties or hybrids. A variety or hybrid was pointed out in each of the 3 replicates, and farmers scored it on a particular criterion, while interacting amongst themselves, and only an agreed consensus score was recorded. This was repeated in sequence for all varieties/hybrids on that criterion before repeating the procedure for the second criterion. The process continued until scores had been allocated to all varieties/hybrids for all criteria.

*Statistical analyses*

Data were managed in Microsoft Excel software and analyzed in GenStat statistical software 14 (GenStat 2011). All data except for the scores and correlation coefficients were subjected to repeated measures analysis of variance and means separated by least significant difference at P≤0.05. The participatory scores were weighted according to Abeyasekera (2001) by the formula:

$$ws = \frac{\sum(c_1 V_1 + \cdots + c_n V_1)}{c_1 + \cdots + c_n}$$

where: ws = weighted score; c = score of criterion; and v = score of variety/hybrid. This was repeated for each variety/hybrid. Significance of correlation coefficients (r) was checked by the Pearson's correlation coefficient critical values table at 16 degrees of freedom (df) for perennial ryegrass and 22 df for festulolium.

## Results

*Climatic conditions*

Key weather variables, including average maximum and minimum temperatures and rainfall during the growth cycles, are summarized in Table 3. True minimum temperature (Table 3a) is the lowest temperature recorded during the experimental period. The 2015 annual precipitation was 899 mm, only 9% less than the long-term mean precipitation stipulated for Ol-joro-Orok (Climate-Data.org 2016).

*Plant height and herbage yields*

Generally, the ryegrasses and festuloliums produced similar (P>0.05) plant heights, dry matter yields, growth rates and rainfall use efficiencies within each of the 3 growth cycles (Table 4). The only exception was that festulolium hybrid AberNiche grew taller than all other varieties and hybrids

(Table 5). Estimated plant height, dry matter accumulated, daily herbage growth rate and rainfall use efficiency were lowest in the third growth cycle for all varieties and hybrids. Total DMY for the 8-month period ranged from 14.3 t/ha (Hybrid 4) to 20.9 t/ha (AberNiche).

*Forage quality*

There were no differences between any of the hybrids and/or varieties for the quality attributes assessed, including CP, ADF, NDF, DDM, DMI or RFV (Table 6). Acid detergent fiber range was 264–272 g/kg DM in perennial ryegrass and 273–281 g/kg DM in festulolium, while the corresponding NDF values were 449–467 g/kg DM and 448–493 g/kg DM, respectively. Crude protein concentrations were in the ranges 169–190 g/kg DM and 163–180 g/kg DM for perennial ryegrass varieties and festuloliums, respectively. In perennial ryegrass, RFV range was 139.8–142.5% and 126.6–140.2% in festulolium.

Herbage DMY was positively correlated with plant height ($P<0.001$), ADF concentration ($P<0.001$), NDF concentration ($P<0.01$) and surprisingly with CP concentration ($P<0.01$) (Table 7). CP concentration was also unexpectedly positively correlated with plant height.

*Participatory evaluation*

Farmers developed the following criteria as most important to them in identifying a forage grass of choice, and in order of importance: biomass production; growth rate; plant height; leafiness (leaf:stem ratio); frost tolerance; and disease tolerance (Table 8). Weighted scores were similar for ryegrass varieties AberBite and AberWolf (6.8 and 6.7), while those for all festulolium hybrids were higher with AberNiche rated most highly (9.6) and Hybrid 2 the lowest (7.9). No evidence of disease or frostbite was observed by farmers, leading them to assign maximum scores on these attributes for all varieties/hybrids. Although participatory evaluation was not conducted in Cycles 2 and 3, no visible symptoms of disease or frostbite were observed in either of these cycles.

**Table 3a**. Summary of growth conditions during 3 growth cycles at Ol-joro-Orok, Central Kenya.

| Growth cycle | Period | Duration (days) | Temperature (°C) | | | Rainfall (mm) | Mean rainfall/ day (mm) |
|---|---|---|---|---|---|---|---|
| | | | Mean max. | Mean min. | True min. | | |
| 1 | 12/05/15–02/09/15 | 113 | 22.5 | 8.6 | 1.9 | 263 | 2.3 |
| 2 | 03/09/15–11/12/15 | 99 | 22.1 | 9.9 | 2.6 | 289 | 2.9 |
| 3 | 12/12/15–13/01/16 | 32 | 21.0 | 9.9 | 6.6 | 127 | 4.0 |

**Table 3b**. Monthly temperatures and rainfall over 2015 and 2016 calendar years at Ol-joro-Orok, Central Kenya.

| Year | Parameter | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2015 | Mean max. temp. (°C) | 24.0 | 25.0 | 25.0 | 23.0 | 22.2 | 21.7 | 22.0 | 22.5 | 24.0 | 23.5 | 21.0 | 21.1 |
| | Mean min. temp. (°C) | 5.8 | 7.0 | 6.7 | 9.6 | 9.0 | 9.3 | 6.1 | 6.2 | 6.3 | 8.8 | 11 | 10 |
| | Total rainfall (mm) | 2 | 28 | 1 | 115 | 99 | 102 | 71 | 36 | 68 | 52 | 167 | 158 |
| | Number of wet days | 1 | 2 | 4 | 14 | 15 | 14 | 11 | 8 | 9 | 9 | 21 | 13 |
| 2016 | Mean max. temp. (°C) | 21.8 | 23.3 | 25.6 | 24.1 | 22.0 | 21.8 | 20.0 | 20.1 | 22.6 | 23.1 | 21.3 | 21.8 |
| | Mean min. temp. (°C) | 10.1 | 8.1 | 8.9 | 10.2 | 9.4 | 7.1 | 8.4 | 7.3 | 6.1 | 7.2 | 9.0 | 8.4 |
| | Total rainfall (mm) | 61 | 30 | 41 | 166 | 164 | 160 | 157 | 172 | 61 | 53 | 61 | 15 |
| | Number of wet days | 11 | 2 | 3 | 11 | 12 | 11 | 18 | 16 | 9 | 8 | 19 | 6 |

Source: Weather data obtained from KALRO Ol-joro-Orok.

**Table 4**. Growth attributes of perennial ryegrass varieties and festulolium hybrids assessed over 3 growth cycles in Ol-joro-Orok, Central Kenya.

| Attribute | Grass | Growth cycle | | | LSD |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | |
| Plant height (m) | Festulolium | 0.47a[1] | 0.40a | 0.27bc | |
| | Ryegrass | 0.40a | 0.29b | 0.20c | 0.07 |
| DMY (t/ha) | Festulolium | 8.3a | 8.8a | 1.0b | |
| | Ryegrass | 8.0a | 7.6a | 0.7b | 2.7 |
| HGR (kg DM/ha/d) | Festulolium | 73.3a | 88.7a | 29.8b | |
| | Ryegrass | 70.6a | 76.9a | 21.6b | 24.7 |
| RUE (kg DM/ha/mm) | Festulolium | 35.1a | 30.4a | 7.5b | |
| | Ryegrass | 33.8a | 26.3a | 5.4b | 11.3 |

[1]Within attributes, means followed by different letters are significantly different (P<0.05).
DMY = dry matter yield; HGR = herbage growth rate; RUE = rainfall use efficiency.

**Table 5.** Growth attributes of ryegrass varieties and festulolium hybrids averaged over 3 growth cycles (May–Jan), and total yields in Ol-joro-Orok, Central Kenya.

| Grass | Variety/hybrid | Plant height (m) | Total DMY (t/ha) | HGR (kg DM/ha/d) | RUE (kg DM/ha/mm) |
|---|---|---|---|---|---|
| Ryegrass | AberBite | 0.33bc[1] | 14.6b | 53.8a | 21.8a |
| | AberWolf | 0.31c | 18.0ab | 64.4a | 26.5a |
| Festulolium | Hybrid 1 | 0.37bc | 18.6ab | 68.3a | 27.0a |
| | Hybrid 2 | 0.36bc | 20.2ab | 70.5a | 28.4a |
| | Hybrid 3 | 0.38bc | 16.0ab | 58.7a | 23.6a |
| | Hybrid 4 | 0.39b | 14.3b | 55.2a | 22.2a |
| | AberNiche | 0.50a | 20.9a | 76.0a | 30.8a |
| LSD | | 0.07 | 4.7 | 23.0 | 10.5 |

[1]Within attributes, means followed by the same letters do not differ (P>0.05).
DMY = dry matter yield; HGR = herbage growth rate; RUE = rainfall use efficiency.

**Table 6**. Crude protein (CP), acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations and calculated values for digestible dry matter (DDM), dry matter intake (DMI) and relative feed value (RFV) for perennial ryegrass varieties and festulolium hybrids at 113 days after planting at Ol-joro-Orok, Central Kenya.

| Grass | Variety | CP (g/kg DM) | ADF (g/kg DM) | NDF (g/kg DM) | DDM (g/kg DM) | DMI (g/kg LW) | RFV (%) |
|---|---|---|---|---|---|---|---|
| Ryegrass | AberBite | 190a | 264a | 449a | 684a | 26.9a | 142.5a |
| | AberWolf | 169a | 272a | 467a | 677a | 26.7a | 139.8a |
| Festulolium | Hybrid 1 | 180a | 280a | 465a | 671a | 25.8a | 134.6a |
| | Hybrid 2 | 165a | 281a | 493a | 670a | 24.3a | 126.6a |
| | Hybrid 3 | 177a | 273a | 452a | 676a | 26.7a | 139.8a |
| | Hybrid 4 | 163a | 278a | 448a | 672a | 26.9a | 140.2a |
| | AberNiche | 178a | 277a | 476a | 673a | 25.4a | 132.4a |
| LSD (P=0.05) | | 34.4 | 20.6 | 51.4 | 16.0 | 3.4 | 18.7 |

[1]Within columns, means followed by the same letter do not differ (P>0.05) for either ryegrass or festulolium.

**Table 7**. Correlation coefficients (r) amongst measured attributes during the first growth cycle pooled for ryegrass and festulolium in Ol-joro-Orok, Central Kenya.

| Attribute | DMY | PH | CP | ADF | NDF |
|---|---|---|---|---|---|
| DMY (t/ha) | 1.000 | | | | |
| PH (m) | 0.791*** | 1.000 | | | |
| CP (g/kg DM) | 0.382** | 0.360** | 1.000 | | |
| ADF (g/kg DM) | 0.500*** | 0.439** | 0.176 | 1.000 | |
| NDF (g/kg DM) | 0.421** | 0.146 | 0.027 | 0.294* | 1.000 |

DMY = dry matter yield; PH = plant height; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber.

**Table 8.** Weighted scores (1 = low; 10 = high) for ryegrass and festulolium varieties/hybrids according to criteria generated by Nyamarura dairy farmers in Ol-joro-Orok, Central Kenya, during the first growth cycle.

| Grass | Variety | Criterion score | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Biomass | Growth rate | Height | Leafiness | Frost tolerance | Disease tolerance | Weighted score |
| | | 10 | 9 | 6 | 6 | 4 | 2 | |
| | | Variety score | | | | | | |
| Ryegrass | AberBite | 6 | 6 | 6 | 7 | 10 | 10 | 6.8 |
| | AberWolf | 6 | 7 | 6 | 5 | 10 | 10 | 6.7 |
| Festulolium | Hybrid 1 | 8 | 9 | 7 | 9 | 10 | 10 | 8.7 |
| | Hybrid 2 | 7 | 8 | 7 | 8 | 10 | 10 | 7.9 |
| | Hybrid 3 | 8 | 8 | 8 | 7 | 10 | 10 | 8.2 |
| | Hybrid 4 | 9 | 9 | 8 | 8 | 10 | 10 | 8.8 |
| | AberNiche | 9 | 10 | 9 | 10 | 10 | 10 | 9.6 |

## Discussion

### *Growth and herbage yield*

The new perennial ryegrass varieties and novel festulolium hybrids performed very well, especially during the first 2 growth cycles. During these cycles, daily herbage rate exceeded 70 kg DM/ha, which was much greater than the 10 and 17 kg DM/ha/d reported by Boonman (1993) for kikuyu grass (*Cenchrus clandestinus* syn. *Pennisetum clandestinum*) and star grass (*Cynodon* spp.), respectively, in the area. However, growth rate in Cycle 3 of 21.6 (ryegrass) and 29.8 (festulolium) kg DM/ha/d was much lower than the initial yields. As mean maximum temperature was only 1–1.5 ºC lower than in cycles 1 and 2 and mean minimum temperatures, true minimums and rainfall per day were at least equal to those in cycles 1 and 2, some other factor must have been responsible for the drop in growth. Since no N fertilizer was applied after the 90 kg N/ha at planting, it would seem that low amounts of available N may have been largely responsible for the poor growth in cycle 3. Further studies with N application after each cut would clarify this hypothesis.

Plant heights and biomass yields realized in this experiment (Tables 4 and 5) were comparable with those reported elsewhere. For example, Humphreys et al. (2014b) quoted plant heights of 0.22–0.62 m in festulolium under field conditions following a month of growth, which were similar to the 0.27–0.47 m recorded in the various growth cycles in the current study. Yield studies on ryegrass and festulolium in a subtropical environment in Australia reported annual yields of 12.5–20.7 t DM/ha in various ryegrasses and 20.1 t DM/ha in a festulolium hybrid Felopa (Lowe and Bowdler 1995). These performances in monthly estimates translate to about 1.08–1.78 t DM/ha/month for ryegrass and 1.57 t DM/ha/month for festulolium. While mean monthly yields of 2.2–2.6 t DM/ha in cycles 1 and 2 were markedly greater, over a complete year mean monthly yields would probably be lower. This is supported by the 0.85 t DM/ha in cycle 3, which is synonymous with reports that show perennial ryegrasses are most productive early in the season (Givens et al. 2000). The good yields when soil moisture and available N were not limiting in cycles 1 and 2 were possibly a reflection of the favorable soils in the study farms (Table 1). Clay-loam soils have better water holding capacity than sandy soils, coupled with P and N values that were at medium levels (Table 1) according to ratings by Hazelton and Murphy (2007). Further, available N and P were boosted by fertilizer application at planting with no top dressing subsequently, possibly leading to the low yields in cycle 3.

### *Herbage nutritive value*

Forage nutritive value of both perennial ryegrass and festulolium was much superior to the commonly used Napier grass, although the values were from the first cut

only. The RFVs we calculated (126.6–142.5%) were nearly double those reported by Mwendia et al (2016) for Napier grass. These values for perennial ryegrass and festulolium exceed those for lucerne/alfalfa (*Medicago sativa*), which is used as the standard of 100, i.e. the reference fodder crop in the estimations (Jeranyama and Garcia 2004). This would make either of them preferable to lucerne when quality is the main consideration. The relatively low NDF values (448–493 g/kg DM) compared with 675 g/kg DM observed in Napier grass (Mwendia et al. 2016) are also beneficial, as they imply better digestibility. The estimated DMIs (24.3–26.9 g/kg LW) (Table 6) were largely close to 24.3–24.7 g/kg LW reported by Kidane et al. (2018), which is a key driver of animal performance. Although there is a dearth of information on quality of ryegrass or festuloliums grown in tropical environments, the range of crude protein concentrations (163–190 g/kg DM) was greater than the 94–156 g/kg DM observed by Humphreys et al. (2014b) in festulolium, but within the range of 112–239 g/kg DM reported by Weller and Cooper (2001) in perennial ryegrass planted without N in the UK. However, observed values in this study were much greater than the 80–130 g/kg DM recorded in Napier grass under tropical conditions (Wijitphan et al. 2009), indicating the superior quality of forage produced by perennial ryegrass and festulolium varieties under the study conditions. Despite quality attributes not being measured in the 2nd and 3rd cuts, it is doubtful they could have deteriorated to less than those for Napier grass given the herbage had a high leaf:stem ratio.

*Potential farmers' acceptance and fit into production systems*

Studies on fodder adoption in Kenya have shown that farmers' perceptions of different attributes like drought tolerance govern adoption along with other factors like labor availability and economy on land (Sinja et al. 2004). The 6 important attributes, identified by the farmers (Table 8) and duly scored, rated biomass production highly as in a previous study in the area (Mwendia et al. 2015). As such, the likelihood of farmers adopting the studied forages in the area would be promising. We consider that involving farmers in the observations from an early stage of the evaluation should enhance the likelihood of adoption of these pastures. Further, in the central Kenyan highlands it is only in the study area, where individual farms are large enough for some farmers to practice some grazing (Muia et al. 2011), that these pasture grasses would be well suited. However, further observations and especially during dry seasons are essential. Perennial ryegrass may look much better after its usually slow establishment, and scrutiny over more growth cycles would be important, especially in this regard. Although the farmers involved in this participatory evaluation did not have a chance to compare these grasses with other pasture grasses, the evidence generated on yield, quality and farmers' ratings is likely to boost potential adoption in an area where only about 41% of the farmers use improved fodders (Muia et al. 2011). However, seed availability could be a major impediment, and sustainable supply possibly via the private sector would be most appropriate to account for situations when persistence is low and re-seeding is required.

## Conclusion

The preliminary forage evaluation work reported here has shown potential for using the tested new perennial ryegrass and novel festulolium varieties bred for temperate Europe to improve forage resources and, therefore, cattle nutrition in Nyandarua, and other similar tropical highland areas. From the study, we deduce the following:

i) Herbage production of these varieties and hybrids is acceptable, coupled with farmers' positive perceptions, but further assessment including nitrogen application and management is needed as well as in a range of environments;
ii) Persistence needs to be evaluated for a longer period including under grazing conditions, as well as animal production; and
iii) Reliable seed supply would be essential to allow adoption, and preferably through the private sector. Essentially, it would entail registration and licensing the varieties first, then encouraging seed companies to list them in their portfolios.

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***Research Paper***

# Enhanced germination performance of dormant seeds of *Eragrostis tef* in the presence of light

## *Germinación mejorada de semillas de* Eragrostis tef *con latencia en presencia de luz*

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**Abstract**

Lack of germination or low germination due to seed dormancy prevents successful crop establishment in several important plants. To determine the presence of innate seed dormancy and effects of stress-related plant hormones on germination performance of Teff (*Eragrostis tef*) seeds, we primed seeds in 1% $KNO_3$ for 24 hours in dark conditions at 21 ± 0.5 ºC along with varying concentrations of chemicals known to influence seed germination, including: acetyl salicylic acid (ASA); methyl jasmonate (JA-Me); giberellic acid ($GA_3$); and indole acetic acid (IAA). Primed seeds were incubated either in constant light (210 µM/$m^2$/s) or in darkness at 21 ± 0.5 ºC. The results indicated that priming significantly improved final germination percentage (FGP) in both light (92.5%) and dark (89.4%) conditions compared with untreated seeds. The inclusion of plant hormones in the priming media generally had limited effects, except for 10 µM ASA (94.5%) and 100 µM $GA_3$ (92.5%). ASA generally provided faster seed germination than seeds primed in 1% $KNO_3$ only, while the other plant hormones had no effect on the time required for 50% of FGP in the dark. Priming had no significant effect on time span of germination in either light or dark incubation conditions. The results demonstrate that *E. tef* has light-inducible seed germination and about half of freshly harvested seeds can be dormant, which can be eliminated to some extent by priming seeds in 1% $KNO_3$.

**Keywords**: Dormancy, hormone, photoblastism, priming, Teff.

**Resumen**

La ausencia de germinación o la germinación baja debido a la latencia (dormancia) de las semillas puede impedir el establecimiento exitoso de cultivos importantes. Para determinar la presencia de latencia endógena y los efectos de hormonas vegetales en la germinación de semillas de Teff (*Eragrostis tef*), muestras de estas fueron remojadas en $KNO_3$ al 1% durante 24 horas en condiciones de oscuridad a 21 ± 0.5 ºC aplicando concentraciones variables de cuatro hormonas vegetales que influyen en la germinación de las semillas: ácido acetilsalicílico (ASA); jasmonato de metilo (JA-Me); ácido giberélico ($GA_3$); y ácido indolacético (IAA). Las semillas tratadas fueron incubadas a luz constante (210 µM/$m^2$/s) o en la oscuridad a 21 ± 0.5 ºC. Los resultados indicaron que el acondicionamiento mejoró significativamente el porcentaje de germinación final (FGP), tanto en condiciones de luz (92.5%) como de oscuridad (89.4%), en comparación con las semillas no tratadas. La inclusión de hormonas vegetales generalmente tuvo solo efectos limitados, a excepción de 10 µM de ASA (94.5%) y 100 µM de $GA_3$ (92.5%). El ASA generalmente proporcionó una germinación de semillas más rápida que cuando se trataron solo con $KNO_3$ al 1%, mientras que las otras hormonas no tuvieron efecto en el tiempo requerido para el 50% de germinación final en condiciones de oscuridad. El acondicionamiento no tuvo un efecto significativo sobre el período de tiempo para la germinación en condiciones de incubación a luz ni en la oscuridad. Los resultados demuestran que (1) la germinación de semillas de *E. tef* es inducida por la luz y (2) que aproximadamente la mitad de semillas recién cosechadas

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pueden estar en estado de latencia la cual podrá eliminarse, hasta cierto punto, mediante acondicionamiento de las semillas en $KNO_3$ al 1%.

**Palabras clave**: Fotoblastismo, hormonas, latencia, osmoacondicionamiento, Teff.

## Introduction

Teff (*Eragrostis tef*), also known as lovegrass or annual bunch grass, is an ancient gluten-free cereal crop mostly adapted to environments with warm seasons (Spaenij-Dekking et al. 2005; Assefa et al. 2015). Teff originated in Ethiopia (Ketema 1993; Girma et al. 2014), and has been introduced to regions and countries with a range of climates, including the USA, India, Africa, Western Europe and Australia (Ketema 1997; Girma et al. 2014) owing to its importance for supplying good quality proteins for human use and for animal feeding as a forage or silage crop (Hagos and Melaku 2009; Zhu 2018).

Teff seeds are the world's smallest cereal grain, with an average l,000-seed weight of 0.3–0.4 g (Kreitschitz et al. 2009). The market value of Teff seed is determined mainly by seed color, which varies from white to dark brown (Ketema 1993). An often temporary state of physiological seed dormancy means many cereal seeds either do not germinate or have low germination percentage (Khan et al. 2017), which prevents a successful start to the season and uniform stand establishment in several important plant species including *Lolium rigidum* (Steadman et al. 2003), *Elymus elymoides* (Meyer et al. 2000) and *Bromus tectorum* (Bauer et al. 1998). Some research into Teff seed has been conducted including hydrothermal time modelling (van Delden 2011), plus grain structure and properties of caryopses of Teff seeds (Kreitschitz et al. 2009). However, there is still a lack of information about the severity of innate seed dormancy, and the effects of stress-related plant hormones and light on germination performance of Teff seed.

Seed priming, or osmoconditioning, which is influenced by several factors such as temperature, water potential and duration, is a relatively common method for treating seed to initiate seed germination. Priming is done prior to the emergence of the primary root during later seed imbibition (Pill 1995), thereby enhancing germination under conditions marginal for establishment. Osmotica used in priming include polyethylene glycol (PEG), sodium or potassium salts (e.g. $K_3PO_4$, $KH_2PO_4$, $MgSO_4$, NaCl), which control water uptake of seeds during seed imbibition and can provide a higher germination percentage and rate than unprimed vegetable and grass seeds (Frett and Pill 1995). Previous reports indicated that dormancy in freshly harvested seeds of *Amaranthus cruentus* (Tiryaki et al. 2005), *Poa pratensis* (Tiryaki et al. 2006) and *Phacelia tanacetifolia* (Tiryaki and Keles 2012) could be eliminated, to some extent, by priming in the presence of stress-related plant hormones like methyl jasmonate (JA-Me), 1-amino-cyclopropane-1-carboxylic acid (ACC) and 6-benzyl-amino-purine (BAP) (Tiryaki and Buyukcingil 2009).

The objectives of this study were to determine the presence and severity of innate seed dormancy of *Eragrostis tef* seeds, and to reveal the effects of stress-related plant hormones on germination performance of the seeds when incubated with and without light.

## Materials and Methods

### *Material*

*Eragrostis tef* (accession PI 197210) seeds used in the experiments were harvested by hand from plants grown under field conditions on 12 October 2017, in Canakkale, Turkey. Harvested seeds came from a single lot and were immediately used for germination experiments at Canakkale Onsekiz Mart University, Canakkale.

### *Methods*

Pre-experimental trials indicated the best priming medium and duration of priming were 1% $KNO_3$ for 24 hours (Tiryaki unpublished data). A single layer of seeds (0.3 g) was placed on double layers of filter paper and 3 mL of 1% $KNO_3$ was applied plus various concentrations of the following primers: acetyl salicylic acid (ASA – 1, 5, 10 and 15 µM), methyl jasmonate (JA-Me – 0.3, 0.6, 0.9 and 1.2 µM), gibberellic acid ($GA_3$ – 50, 100, 150 and 200 µM) and indole-3-acetic acid (IAA – 0.5, 1.0, 1.5 and 2.0 µM) for 24 h at 21 ± 0.5 ºC in darkness. Prior to the germination test, the priming solution was removed from the primed seeds by washing with tap water for 1 minute and then placing seeds on paper towels for 2 h under room conditions. Surface-dried and primed seeds were then used for the germination experiments. Seeds treated with 1% $KNO_3$ only, seeds treated with distilled water ($dH_2O$) only (hydropriming) and non-primed seeds were used as controls.

Germination experiments were conducted in a growth chamber held at 21 ± 0.5 °C either in constant light (210 µmol/$m^2$/s for 24 h/d) or darkness. A completely randomized block design with 4 replications of 50 seeds was arranged as a factorial. Replicates of each treatment

were placed either in different shelves of the same growth chamber or were arranged in different positions in light-sealed boxes in light and dark experiments, respectively, and were randomized after each count. Seeds, which showed a radicle exceeding 2 mm in length emerged from the testa, were counted as germinated and were removed daily from the petri dishes for 7 days in light and 12 days in dark germination experiments. The final germination percentage (FGP) was determined and its angular transformation (arcsine√FGP) was used to provide a normal distribution for data analysis. Germination rate (estimated as days to 50% of FGP) and the span of germination (time from 10% to 90% of FGP) were calculated from the total number of seeds germinated using a method used successfully on sugarbeet seeds (Murray et al. 1993).

SAS statistical software was used to analyze the data (SAS 1997) and Fisher's least significant difference (LSD) test was applied, if the F test was significant at $P<0.05$.

## Results

*Effects of priming media and plant hormones in darkness*

Approximately half of the Teff seeds were considered dormant shortly after harvesting when incubated in the absence of light. Priming seeds in 1% $KNO_3$ for 24 h at 21 ± 0.5 ºC in the dark significantly improved germination percentage over that of untreated control seeds (89.4 vs. 56.5%, respectively; Figure 1). The results also revealed that hydropriming significantly reduced FGP and gave the lowest FGP (40.5%) in comparison with untreated control seeds.

The inclusion of plant hormones in the priming medium generally had little effect on FGP with values ranging from 79.5 to 94.5% (Figure 1).

Priming in the presence of hormones generally had little effect on the speed of germination of seeds, while seeds primed in $dH_2O$ had the slowest ($G_{50}$ = 3.76 days; $P<0.05$) seed germination (Table 1).

*Effects of priming media and plant hormones in light*

In general, seeds germinated in light had higher FGP than seeds germinated in the dark (Figure 1). In light, priming seeds in 1% $KNO_3$ significantly improved FGP compared with untreated control seeds (92.5 vs. 72.5%; Figure 1). Adding 150 µM $GA_3$ and 1 µM ASA to the priming medium (Figure 1) improved FGP to 99 and 98.5%, respectively, while adding the other plant hormones provided intermediate results. Hydroprimed seeds had significantly higher FGP (82.5%) than untreated control seeds (72.5%), which was in complete contrast to the results obtained in darkness (Figure 1).

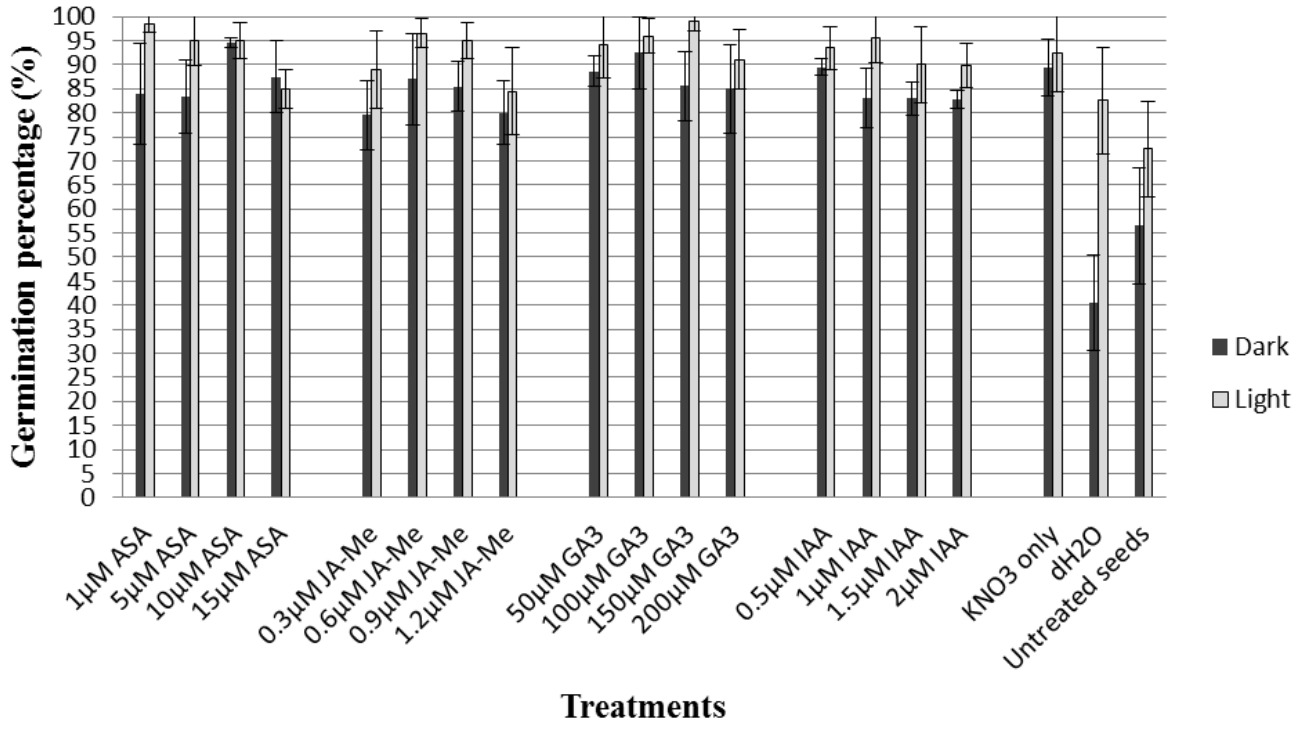


**Figure 1.** The final germination percentage (FGP) of Teff seeds primed in 1% $KNO_3$ supplemented with various concentrations of different plant hormones for 24 h at 21 °C and germinated under light or dark conditions at 21 °C. Bar indicates SD (n = 4).

**Table 1**. The angular transformation [FGP] of final germination percentage (FGP), speed ($G_{50}$) and span ($G_{10-90}$) of Teff seed germination incubated in dark or light at 21 °C following priming for 24 hours at 21 °C in 1% $KNO_3$ combined with different plant hormones at various concentrations.

| Treatment[1] | FGP [FGP] | $G_{50}$ (days) | $G_{10-90}$ (days) | FGP [FGP] | $G_{50}$ (days) | $G_{10-90}$ (days) |
|---|---|---|---|---|---|---|
| | Dark | | | Light | | |
| Acetyl salicylic acid | | | | | | |
| 1 | [66.9]ab[2] | 2.13c | 4.91 | [85.0]ab | 1.69b | 2.38 |
| 5 | [66.5]ab | 2.70bc | 6.29 | [79.3]a-c | 1.84b | 2.53 |
| 10 | [76.4]a | 2.18c | 6.78 | [77.7]a-c | 1.68b | 2.46 |
| 15 | [69.9]ab | 2.08c | 5.96 | [67.3]cd | 1.79b | 3.00 |
| Methyl jasmonate | | | | | | |
| 0.3 | [63.3]b | 2.91bc | 6.29 | [73.8]a-c | 1.88b | 3.03 |
| 0.6 | [71.6]ab | 2.44bc | 6.26 | [79.7]a-c | 1.52b | 2.36 |
| 0.9 | [67.8]ab | 2.47bc | 5.19 | [77.7]a-c | 1.53b | 2.32 |
| 1.2 | [63.7]b | 2.76bc | 5.11 | [67.4]cd | 1.78b | 2.62 |
| Giberellic acid | | | | | | |
| 50 | [70.4]ab | 2.27bc | 5.65 | [77.1]a-c | 1.75b | 2.55 |
| 100 | [76.6]a | 3.10ab | 4.95 | [80.3]a-c | 1.56b | 2.44 |
| 150 | [68.3]ab | 2.44bc | 5.06 | [87.1]a | 1.71b | 3.11 |
| 200 | [68.0]ab | 2.88bc | 5.27 | [73.4]a-c | 1.79b | 2.74 |
| Indole acetic acid | | | | | | |
| 0.5 | [71.2]ab | 2.55bc | 7.46 | [77.1]a-c | 1.55b | 2.26 |
| 1.0 | [65.9]ab | 2.80bc | 5.47 | [80.0]a-c | 1.86b | 2.54 |
| 1.5 | [65.7]b | 2.80bc | 7.39 | [75.2]a-c | 1.86b | 2.88 |
| 2.0 | [65.5]b | 2.35bc | 5.32 | [71.8]b-d | 1.54b | 2.15 |
| Controls | | | | | | |
| 1% $KNO_3$ only | [71.6]ab | 2.54bc | 5.68 | [76.5]a-c | 1.89b | 2.40 |
| $dH_2O$ | [39.4]d | 3.76a | 7.36 | [69.6]cd | 2.65a | 3.37 |
| Untreated seed | [48.8]c | 2.50bc | 6.80 | [58.6]d | 2.43a | 2.47 |

[1]$KNO_3$ + related plant hormone at given concentrations (µM).
[2]Values within columns followed by different letters differ ($P<0.05$).

Seeds primed in 1% $KNO_3$ had significantly enhanced germination rate ($G_{50}$ = 1.89 days) compared with untreated control seeds ($G_{50}$ = 2.43 days) (Table 1), while there was no further improvement from inclusion of plant hormones (Table 1). Hydroprimed ($G_{50}$ = 2.65 days) and untreated ($G_{50}$ = 2.43 days) control seeds had the slowest germination speeds (Table 1). There were no significant differences between primed and control seeds for span of germination ($G_{10-90}$) in either light or dark germination conditions (Table 1).

## Discussion

One of the most important prerequisites for a successful plant stand is even, fast and successful seed germination. This can depend on external factors such as light, temperature and water supply to the seed as well as endogenous seed factors including ripeness (seed maturity), hormone levels and other forms of dormancy (van Delden 2011). Of those, seed dormancy can delay and spread seed germination over time, and is a characteristic of several plant species (Schonbeck and Egley 1981; Gu et al. 2003). Several methods are used to reduce or overcome seed dormancy, and these can vary among species (Steadman et al. 2003; Nee et al. 2017). Priming seeds in an appropriate osmoticum improves germination and seedling emergence of several plant species (Tiryaki and Keles 2012), and a positive photoblastism, light-inducible seed germination, was recently reported for *Eragrostis ciliaris* (Khan et al. 2017). Although existence of slight embryo dormancy was previously mentioned in fresh seeds of *Eragrostis abyssinica* [synonym of *E. tef* (Zuccagni) Trotter] (Katayama and Nakagama 1972; van Delden 2011), this study has demonstrated that *E. tef* has light-inducible seed germination and half of freshly harvested seed can be dormant. In addition, the study revealed that untreated control seeds had higher FGP (72.5%) in the presence of light than untreated control seeds germinated in darkness (56.5%). Priming of dormant seeds in 1% $KNO_3$ for 24 h at 21 ± 0.5 ºC significantly improved FGP and provided faster seed germination in the presence of light (Figure 1), while the same priming treatment had no effect on the span of

germination in both light and dark conditions (Table 1). In addition, hydropriming appeared to decrease FGP (40.5%) in darkness in comparison with untreated control seeds (56.5%), whereas hydroprimed seeds had a higher FGP (82.5%) than untreated control seeds (72.5%) in the light (Figure 1). These results suggest that hydropriming of Teff seed could be considered if planting alone or in a mixture with other grasses as a seeding material for temporary nurse grass or erosion control.

Light is one of the most important regulatory factors of seed germination for many plant species including reed canary grass (Lindig-Cisneros and Zedler 2001), weedy rice (Lee et al. 2010), *Arabidopsis* (Auge et al. 2018), lettuce (Sawada et al. 2008), tomato (Auge et al. 2009) and phacelia (Tiryaki and Keles 2012). Positively photoblastic species often have small and dormant seeds (Floresa et al. 2011). Previous reports indicated that seed dormancy was influenced by the nitrate concentrations in both soil and seed (Matakiadis et al. 2009; Huang et al. 2018) and phytochrome plays a very significant role in this action (Footitt et al. 2013). However, this may not be the case in Teff seeds since seeds primed in $KNO_3$ gave about the same FGPs with (92.5%) and without (89.4%) light during incubation (Figure 1). It may be that use of $KNO_3$ as a priming agent with Teff seeds enhances seed germination by balancing endogenous nitrate content or by accelerating the decrease in abscisic acid (ABA) level prior to the completion of germination triggered by the production of N-related signals (Footitt et al. 2013).

It is well known that the level of physiological seed dormancy is influenced by ABA and GA (giberellic acid) homeostasis during seed maturation (Seo et al. 2009). Although the exact dormancy-release mechanisms of the hormones are still unknown for many plant species (Nakabayashi et al. 2012), recent new molecular approaches partially shed light on these mechanisms (Shen et al. 2018; Auge et al. 2018). In general, inclusion of plant hormones with priming solution used in this study did not provide any further improvement on FGP under both light and no light conditions except for ASA and $GA_3$ at certain concentrations. A high concentration of ASA (10 μM), which had FGP of 94.5%, appeared to be more effective under no light conditions, while the lower concentration of ASA (1 μM) provided a better improvement in FGP under light conditions (98.5%) (Figure 1). In contrast, a higher $GA_3$ concentration (150 μM) gave a higher FGP (99.0%) in the light than the seeds primed in the presence of 100 μM $GA_3$ (92.5%) under no light conditions. The differences are relatively small, but indicate germination and dormancy-release processes of Teff seeds may be influenced by ASA and GA homeostasis with light playing an important role in this regulation. A similar regulation of ASA and GA was previously shown for lettuce (Sawada et al. 2008) and pepper seeds (Korkmaz 2005).

Recent advances in genetic and biochemical studies have revealed that GA metabolism of seeds is controlled by light-response (Varbanova et al. 2007; Footitt et al. 2017). Involvement of light and GA in seed germination was also previously shown in *Arabidopsis* at the molecular level (Ogawa et al. 2003). Those authors suggested that increased levels of GA in germinated seeds were controlled mainly by both transcriptional activation and repression of GA biosynthetic as well as GA catabolic genes (Ogawa et al. 2003). However, this is not the case for Teff seeds, since exogenous application of ASA and $GA_3$ produced similar additional increments in FGP under both light and no light conditions (Table 1).

Jasmonic acid (JA) or methylated jasmonic acid (JA-Me) affects several biological activities in plants, including plant growth and development, as well as responses to biotic and abiotic stresses (Tiryaki and Staswick 2002). Previous reports indicated that exogenous applications of JA or JA-Me stimulate germination of dormant seeds in various plant species (Berestetzky et al. 1991; Ranjan and Lewak 1992), while non-dormant seed germination was inhibited in some other plant species (Corbineau et al. 1988; Daletskaya and Sembdner 1989).

In this study we found that JA-Me neither stimulated nor inhibited the germination percentage of dormant *E. tef* seeds when primed with $KNO_3$. This may indicate that JA-Me has no, or only a limited, function in the germination processes of Teff seed. It is well known that JA-Me and IAA have signaling cross talk to control or to regulate several important plant growth parameters, including response to light (Westfall et al. 2016; Kućko et al. 2017; Sherp et al. 2018). Regulatory involvement of these two plant hormones in seed germination was previously shown in wheat (Xu et al. 2016), pepper (Korkmaz 2005), Kentucky bluegrass (Tiryaki et al. 2006) and *Arabidopsis* (Shu et al. 2016). Altogether the results of this study suggested that JA-Me and IAA neither regulate Teff seed germination nor are involved in N-related signals with ABA (Footitt et al. 2013; Jacobsen et al. 2013), further physiological or molecular studies are needed to confirm these results.

In conclusion, this study indicates that Teff has light-inducible seed germination and a substantial proportion of freshly harvested *E. tef* seeds can be dormant. This can be overcome to some extent by priming seeds in 1% $KNO_3$. The results also revealed that including ASA and $GA_3$ in priming media may play a role in enhancing the Teff germination process. These findings have possible implications for treatment of seed following harvesting and prior to sowing.

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*(Note of the editors: All hyperlinks were verified 11 April 2019.)*


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***Research Paper***

# Effectiveness of inoculation with rumen fluid containing *Synergistes jonesii* to control DHP toxicity in ruminants in eastern Indonesia

## *Efectividad de la inoculación con fluido ruminal conteniendo* Synergistes jonesii *para controlar la toxicidad de DHP en rumiantes en el este de Indonesia*

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**Abstract**

The feasibility and efficacy of inoculating with rumen fluid as a method to control hydroxypyridone (DHP) toxicity in ruminants on high leucaena diets in eastern Indonesia were investigated. Rumen fluid collected from 2 buffalo identified as 'protected', due to low levels of DHP excretion in urine, was orally administered to animals identified as 'unprotected' and concentrations of urinary DHP monitored. Control animals were dosed with water only. Treatments were randomly allocated to 10 recipient animals: 3 goats and 7 cattle. All animals were fed a diet containing freshly cut 100% leucaena during the 18 day study period. Measurement of urinary DHP via colorimetric analysis commenced 8 days prior to animals being drenched with rumen fluid or water and continued for 10 days afterwards. Urinary DHP levels in animals that received the inoculum did not differ from those in the control group 10 days post-inoculation (mean 425 mg DHP/L; P = 0.50). Unexpectedly, DHP levels in all animals (rumen fluid and water) declined with time, although the difference did not reach statistical significance (P = 0.12), and remained above considered safe threshold levels. These results suggest that transfer of rumen fluid to overcome leucaena toxicity in animals in eastern Indonesia may not be effective despite great care having been taken to ensure the viability of the anaerobic organisms during the inoculation process; this methodology is also not a practical solution to replicate on a commercial scale. The findings suggest that inoculation may not be necessary if animals previously naïve to leucaena are able to adapt to DHP toxicity by other means.

**Keywords**: Dihydroxypyridine, hydroxypyridone, *Leucaena leucocephala*, mimosine, urine.

**Resumen**

Se investigó la viabilidad y la eficacia de la inoculación con líquido ruminal que contenía *Synergistes jonesii* como método para controlar la toxicidad de hidroxipiridona (DHP) en rumiantes que reciben dietas con altos porcentajes de leucaena (*Leucaena leucocephala*). Para el estudio, a animales (cabras y bovinos) identificados como 'no protegidos' se les administró por vía oral líquido ruminal recolectado en dos búfalos identificados como 'protegidos' de la toxicidad, debido a los bajos niveles de excreción de DHP en la orina. Además fueron incluidos animales testigo que se dosificaron solo con agua. Los tratamientos fueron aplicados en forma aleatoria a tres cabras y siete bovinos como receptores. Todos los animales recibieron una dieta de 100% de forraje de leucaena fresca. La medición de la DHP urinaria se hizo por colorimetría y comenzó 8 días antes de la administración del líquido ruminal o agua (testigo) a los animales y fue continuada durante 10 días posteriores. Diez días después de la inoculación, los niveles en orina de DHP (425 mg DHP/L)

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en animales que recibieron el inóculo no difirieron de los del grupo control (P = 0.50). En todos los animales se observó una tendencia a la reducción de DHP en orina a través del tiempo, aunque la diferencia respecto a los niveles antes de la inoculación no fue significativa (P = 0.12). Los niveles se mantuvieron por encima del nivel límite que se considera seguro. Estos resultados muestran que en el este de Indonesia la transferencia de fluido ruminal puede ser no eficaz si los animales, que nunca habían consumido leucaena, son capaces de adaptarse por otros medios a la toxicidad de DHP.

**Palabras clave**: Dihidroxipiridina, hidroxipiridona, *Leucaena leucocephala*, mimosina, orina.

## Introduction

*Leucaena leucocephala* (leucaena) is a productive forage tree legume which contains the toxic non-protein amino acid mimosine. While this secondary plant compound can seriously affect animal health, in the case of ruminants, rumen microbes and endogenous plant hydrolase enzymes rapidly convert mimosine to hydroxypyridone (DHP), which is chronically rather than acutely toxic (Hegarty et al. 1964; Lowry et al. 1983). The widely published method of protection against DHP toxicity is via microbial degradation by a specialized rumen bacterium, *Synergistes jonesii*, strains of which, including the type strain (78-1), can degrade the mimosine-metabolite 3-hydroxy-4(1*H*)-pyridone (3,4-DHP) to 3-hydroxy-2(1*H*)-pyridone (2,3-DHP) and then to non-toxic by-products (Allison et al. 1992). Despite originally being considered non-ubiquitous (Jones 1994), *S. jonesii* is now considered a rumen microbe indigenous to the rumen (Halliday et al. 2013), albeit with discrete mutations or variants which have been detected in DNA gene sequences associated with different geographical locations. Variants of *S. jonesii* have been identified in ruminants in Indonesia (Padmanabha et al. 2014), which may account for the varying capacity of *S. jonesii* in certain animals to degrade DHP, and hence the historically perceived non-ubiquity of the microbe.

Seminal work on research into leucaena toxicity has demonstrated that functional *S. jonesii* can be rapidly and readily transferred from 'protected' animals to susceptible animals using rumen fluid inoculum (Jones and Lowry 1984). This methodology is effective in Australia with a cultured frozen inoculum available commercially, but may not be practical in eastern Indonesia, where vast distances separate 'protected' animals from those potentially susceptible to toxicity, including between islands. For effective microbial protection against leucaena toxicity to occur in susceptible animals, a practical method is required to successfully transfer rumen fluid from 'protected' to 'unprotected' ruminants, which may be in remote locations and often of a different species (e.g. goats, buffalo plus *Bos indicus* and *Bos javanicus* cattle).

Accordingly, this trial was conducted to investigate the feasibility and efficacy of inoculation of cattle and goats consuming high levels of leucaena in West Timor Island and excreting high levels of urinary DHP, i.e. not fully degrading all DHP. The inoculation consisted of rumen fluid from buffalo on nearby Sumba Island, which were consuming high leucaena diets but were excreting low levels of urinary DHP and therefore considered 'protected'. As no commercial inoculum is currently available in Indonesia, it was also important to evaluate the practical feasibility of collecting, storing and transporting the rumen fluid anaerobically, with subsequent inoculation of smallholder animals, as a possible long-term solution for controlling DHP toxicity in eastern Indonesia.

## Materials and Methods

The experiment was conducted on the islands of Sumba and West Timor in eastern Indonesia (Figure 1), over an 18 day period beginning in December 2012.

Donor animals were 2 female buffalo (*Bubalus bubalis*) from Melolo, Sumba. These buffalo were selected from a herd where *S. jonesii* had been previously detected (Halliday et al. 2014; Padmanabha et al. 2014) and were excreting low urinary concentrations of DHP (<10 mg DHP/L), while consuming diets containing >70% leucaena leaf and fine stems. Recipient animals were selected from areas known to be prone to DHP toxicity (Halliday et al. 2014), including: 3 kacang goats (*Capra hircus*) from Sumlili, Timor; and 4 Bali bulls (*Bos javanicus*) from Bone, Timor. All were consuming diets containing 50–100% leucaena and excreting high urinary concentrations of DHP (>900 mg DHP/L). Three Bali cattle (*Bos javanicus*, 2 bulls and 1 cow) from Tarus, Timor, previously consuming low levels of leucaena (<30% leucaena) in their diets, were also included. All animals were maintained on diets of 100% freshly cut leucaena leaf and fine stems throughout the monitoring period of 18 days. Recipient animals were randomly allocated to receive either the Sumba rumen fluid inoculum or a control treatment consisting of a water drench (Table 1).

Urinary DHP concentration was estimated visually following color development using the iron(III) chloride method (Figure 2) (Graham et al. 2014). Buffalo urine

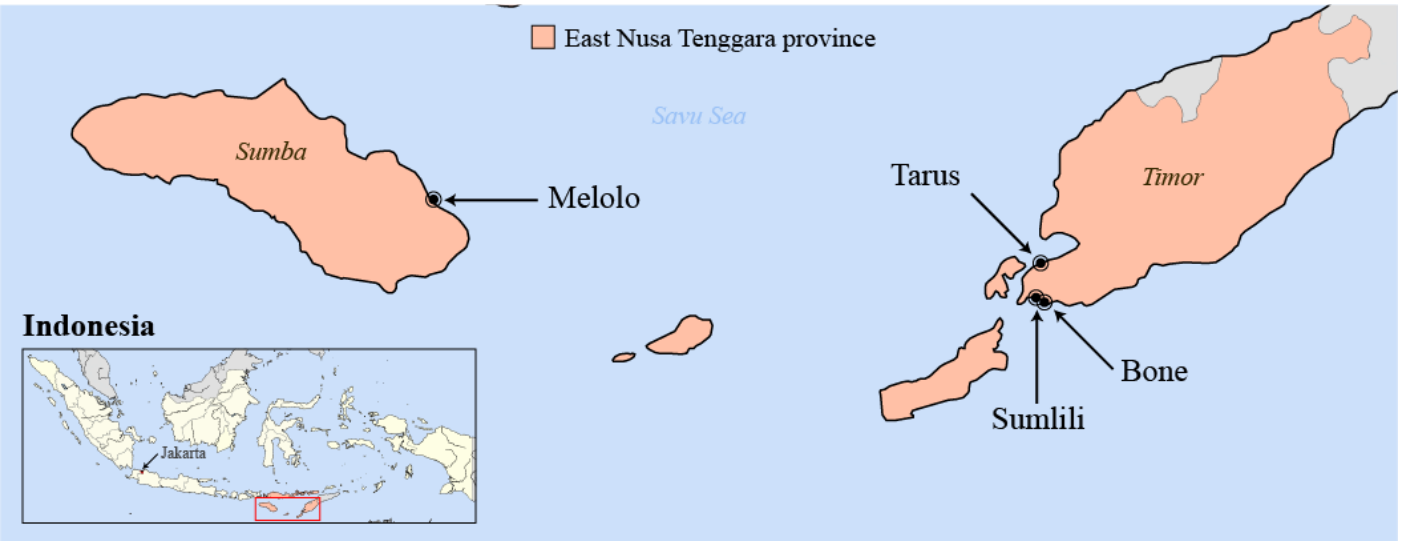


**Figure 1.** Map of locations involved in this experiment. Source: naturalearthdata.com

**Table 1.** Number of animals allocated to each treatment.

| Treatment | Melolo, Sumba buffalo (n) | Sumlili, Timor goats (n) | Bone, Timor cattle (n) | Tarus, Timor cattle (n) |
|---|---|---|---|---|
| Sumba donors | 2 | | | |
| Sumba inoculum | | 2 | 1 | 1 |
| Control | | 1 | 3 | 2 |

from donors was spot-sampled daily for 3 days prior to collection of rumen fluid, while recipient animals were monitored 2–4 times during the 8 days pre-inoculation, with further urine collections at 5 and 10 days post-inoculation. Methodology for hydrolysis of DHP to the free form was adjusted by omitting boiling for 1 hour (Graham et al. 2014), and instead allowing samples to hydrolyze over several days at ambient temperature (up to 37 ºC). During this period the color intensified, indicating hydrolysis of conjugated DHP consistent with the findings of Graham et al. (2014). Approximate concentrations of DHP, representative of free DHP after acid hydrolysis, were estimated based on color hue and intensity (Figure 2); the overwhelming majority of samples were dominated by the isomer 2,3-DHP, indicating at least partial degradation (from the isomer 3,4-DHP to 2,3-DHP) was occurring.

Rumen fluid for inoculation was collected via an orogastric tube (Graham et al. 2013), strained through muslin cloth, pooled, mixed and stored in a glass thermos and glass Schott® bottles. Animals were inoculated on the same day as collection, via an orogastric tube. All containers were initially flushed with rumen fluid, and young fresh ground leucaena leaves were added as a mimosine substrate. The bottles were kept at ~37 ºC and an anaerobic environment was maintained via the active fermentation of leucaena. Doses were: goats from Sumlili – 100 mL; bulls from Bone – 240 mL; and cattle from Tarus – 150 mL. The trial was sanctioned under animal ethics approval # SAFS/144/11/ACIAR.

Repeated observations over time were analyzed using Minitab® statistical software 16 (©2010, Minitab Inc., State College, PA, USA) with a General Linear Model ANOVA using a split-plot design (with treatment as the main plot, time as the subplot and individual animals as a random factor, nested with treatment and location).

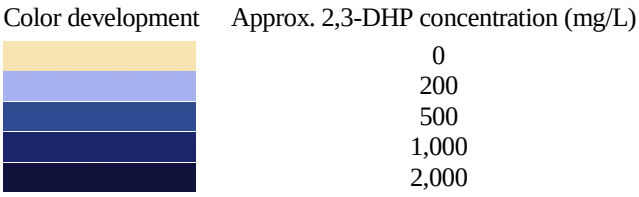


**Figure 2.** Examples of color hues from the colorimetric test with estimated 2,3-DHP concentration.

## Results

Urinary DHP levels in recipient animals (in both the Sumba inoculum and control group) were high prior to inoculation (Table 2), at levels exceeding the considered safe threshold of 100 mg DHP/L (Dalzell et al. 2012).

**Table 2.** Mean (± s.e.) urinary DHP excretions from animals receiving either the inoculum of rumen fluid from Sumba buffalo or a control water drench as estimated by iron(III) chloride test.

| Treatment | Period | Mean DHP (mg DHP/L) | n (samples) | n (animals) |
|---|---|---|---|---|
| Sumba inoculum | Pre-inoculation | 1,030 ± 140 | 12 | 4 |
| | 5 days post-inoculation | 1,280 ± 230 | 5 | 4 |
| | 10 days post-inoculation | 350 ± 220 | 5 | 4 |
| Control | Pre-inoculation | 730 ± 170 | 12 | 6 |
| | 5 days post-inoculation | 730 ± 340 | 5 | 6 |
| | 10 days post-inoculation | 500 ± 220 | 5 | 6 |

There were no significant (P = 0.50) differences in mean urinary DHP levels between animals dosed with the Sumba inoculum and those on the control treatment, either pre- or post-inoculation. Despite the lack of a treatment effect at 10 days post-inoculation, urinary DHP levels in all animals, while still high, had declined (most notably in the dosed animals), although they were not significantly different (P = 0.12) from pre-inoculation levels. The overwhelming majority of DHP in urine was excreted as the secondary isomer, 2,3-DHP. Mean urinary DHP levels for the 2 donor buffalo from Sumba (mean of 5 samples) were low (<10 mg DHP/L).

Treatment means are presented as an average of all animals receiving each treatment; animal species and location had no significant (P = 0.84) effect on DHP level. There was also no significant (P = 0.70) interaction effect between treatment and location.

## Discussion

The results of this study indicate direct rumen fluid transfer was not effective in reducing urinary DHP levels in animals newly introduced to leucaena. This was evident as both a lack of difference between treated and control animals, and a lack of significant difference between pre- and post-inoculation DHP levels in the treated animals. Although there was an apparent decline in DHP levels in treated animals 10 days post-inoculation, this did not reach statistical significance, likely due to the small sample size and the high variability between animals. The main isomer excreted in animals was 2,3-DHP, indicative of partial degradation occurring; despite having originally been thought to be a transitory isomer, the presence of 2,3-DHP is now found to be the common isomer excreted.

This result contrasted with earlier reports where urinary DHP levels declined rapidly to safe levels following inoculation with rumen fluid, within 5 days in Indonesia (Jones and Lowry 1984) and within 3 days in Thailand (Palmer et al. 2010). However, while Jones and Lowry (1984) reported a "dramatic" decline in DHP excretion after inoculation, the 2 dosed goats in their experiment were already degrading 45–62% of ingested DHP prior to inoculation. This natural decline in DHP excretion and apparent inherent capacity to partially degrade ingested DHP was common in both these past, and the present, studies.

Although the scale of this study did not permit molecular analysis of rumen fluid samples, the donor buffalo belonged to the same herd which previously tested positive for *S. jonesii*. As the animals were consuming up to 100% leucaena diets, it was assumed that functional *S. jonesii* was present in the rumens of these donor animals owing to the negligible DHP levels in urine. In contrast, since mean urinary DHP levels in recipient animals, tested up to 4 times each pre-inoculation, were high (Table 2), exhibiting normal variation (most likely a function of the differential adaptation of individual animals to high leucaena diets within their first month on the diet) (Giles et al. 2013; Graham et al. 2013), we concluded that these animals were not fully 'protected' by rumen bacteria.

It is known that *S. jonesii* exists in the rumen in low populations, often below the limits of detection by nested PCR ($<10^4$–$10^5$ cells/mL) (Graham et al. 2013), and it is possible that the dosed inoculum did not increase 'functional' *S. jonesii* populations sufficiently to achieve protection. Although performed in vitro, the study of McSweeney et al. (1993) required 2 weeks for *S. jonesii* to establish a competitive population capable of degrading 3,4-DHP, quantified as abundance of *S. jonesii* RNA in a mixed-culture chemostat. Therefore, it seems our study should have continued for a longer duration. Doses in this study were limited by the volume of rumen fluid collected from the buffalo, and although dosage volumes were smaller than used in the work of Jones and Lowry (1984) (350 mL), they were greater than used by Palmer et al. (2010) (30 mL) (concentrations of organisms not measured). As *S. jonesii* was neither confirmed nor quantified in this study, it was also possible that the selected donor animals did not have effective *S. jonesii* populations. Possible alternative explanations for the low urinary DHP in donor buffalo include: degradation by microbes other than *S. jonesii*; the diurnal variation of urine volume affecting toxin concentration; and the possibility of a period of low leucaena levels in the diet.

## Conclusions

Despite a natural decline in urinary DHP excretion in inoculated animals over time, differences failed to reach significance within the 10 day monitoring period (P>0.10). There was also no significant (P = 0.50) effect of inoculation, relative to the control animals. While limited by the length of monitoring, outcomes following inoculation were different from those originally reported over 30 years ago, using a similar methodology.

This study also highlighted the technical and logistical difficulties involved in collecting and transferring rumen fluid containing *S. jonesii* anaerobically as a method of inoculation against DHP toxicity, especially in multi-island countries, where supply-chain systems are limited. The methodology evaluated was not practically suitable for eastern Indonesia. The equipment and skills required to complete these actions are not currently available in the Indonesian Government livestock and extension services, and would require specialist training, the uptake of which would be problematical. If inoculation were to be viable, further work to develop suitable techniques is required, including exploring the possibility of transferring animals to allow the natural spread of rumen bacteria in a herd, if there was a need.

The apparent decline in urinary DHP levels in control animals indicated an inherent ability to adapt to DHP. Possible adaptation mechanisms include: the presence of an indigenous variant of *S. jonesii* or other DHP-degrading microbes; and metabolic detoxification of DHP by conjugation. Although the method of acid hydrolysis of DHP had been improved to optimize color development in iron(III) chloride reagent (Graham et al. 2014), it is acknowledged that there is a possibility that the apparent decline in measured DHP levels could be attributed to inconsistent hydrolysis among samples. Any remaining conjugated DHP would be unable to bind with Fe in solution, thus preventing the development of strength of color representative of the total DHP in urine. However, in the absence of chromatographic quantification of total DHP (be it free or conjugated), consistent hydrolysis is assumed.

Further work should aim to study: (a) the presence, functional capacity and relative contribution to degradation of DHP-substrate consumed of indigenous microbes within eastern Indonesia to better understand their role in initial degradation; and (b) the role of conjugation in detoxification of remaining undegraded DHP-substrate. At a practical level, finding an alternative control mechanism to eliminate the practical need for inoculation would greatly benefit these systems.

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***Retracted Article***

# RETRACTED: Agro-morphological characterization of *Urochloa* grass accessions in Kenya

## *Caracterización agro-morfológica de accesiones de* Urochloa *en Kenia*

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The Editors and Publisher have retracted this article because the text had already been published in Njauri et al 2016. The content conveyed by this article is therefore not original and redundant. The corresponding author, Donald Njarui, recognizes this involuntary duplication publication and agrees to the retraction of this article. The online version contains the full text of the retracted article as electronic supplementary material.

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*(Note of the editors: All hyperlinks were verified 28 June 2019.)*

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