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Original Research Article

Effects of supplementation of *Brachiaria brizantha* cv. Piatá and Napier grass with *Desmodium distortum* on feed intake, digesta kinetics and milk production in crossbred dairy cows



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ABSTRACT

This study was planned to analyse the basis that make *Brachiaria* species with greater feeding value than Napier grass (*Pennisetum purpureum*) for lactating dairy cows. Forty lactating crossbred cows were stallfed on *Brachiaria brizantha* cv. Piatá or Napier grass cv. French Cameroon supplemented (mixed on fresh matter basis) or unsupplemented with *Desmodium distortum*, a forage legume. All cows were fed on fresh matter basis under small-holder farming conditions. Results showed that *B. brizantha* cv. Piatá had higher contents of dry matter (DM), crude protein (CP) and organic matter (OM), but lower contents of neutral detergent fibre (NDF) and acid detergent fibre (ADF) than Napier grass (P < 0.001). The legume supplementation increased intakes of CP and metabolizable energy (ME; P < 0.001), with higher effect on cows fed *B. brizantha* cv. Piatá than on cows fed Napier grass. Average daily milk yield was lower on diets based on Napier grass than those based on *B. brizantha* cv. Piatá (P < 0.001). The retention time of the particle phase of digesta in the digestive tract was longer on Napier grass (83.1 h) than on *B. brizantha* cv. Piatá increases nutrient intake, hence resulting in higher milk yields than supplementation of Napier grass.

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1. Introduction

Napier grass (*Pennisetum purpureum*) has been recognised as a major fodder grass that has supported sustainable climate-smart

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intensified smallholder dairy farming in East Africa. It is a multipurpose grass for feed with land sparing because it produces high biomass on small size of the land. It is also a key rotation component for control of stem-borer of maize (Pretty et al., 2011). Brachiaria grasses could provide similar benefits (Pickett et al., 2014) with the additional advantage of reducing nitrous oxide emission from the soil through biological nitrification reduction (Subbarao et al., 2009). Increasing ruminant livestock populations in many areas of the sub-Saharan Africa (SSA) have increased the demand for land for fodder production. Traditionally, mixed crop-livestock agriculture is feasible where the management facilitates reciprocal nutrient and energy flows between crop and livestock components with minimum demand for external nutrient supply (Andrieu et al., 2015). The mechanisms for reciprocation include the use of crop residues as animal feed, animal waste in integrated soil fertility management,

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animal draught in farm mechanization, fodder grasses and legumes for erosion control, legumes for biological nitrogen fixation and reduction of greenhouse gas emissions, and promotion of reforestation using multipurpose fodder trees. However, this synergy is compromised when farmers are confronted with competing interests between land use for food or fodder crops; and between crop wastes for mulching and livestock feed (Homann-Kee Tui et al., 2015). These accentuate the need for cultivated fodder for sustainable intensification.

Chemical analyses of Brachiaria grass and Napier grass have consistently ranked the two forages to be similar in their nutritional qualities (Mutimura et al., 2015). However, a few feeding trials and farmers' perceptions have indicated that animals and farmers preferred Brachiaria to Napier grass. There is indication that palatability and response of animal improves when fed on Brachiaria grass (Rao et al., 2015). These perceptions suggest that voluntary dry matter intake (DMI) in ruminants fed on Brachiaria grass is less constrained by gut fill than in other grasses. Gut-fill is predominantly determined by rates of physical degradation of feed particles and the outflow rates of undegraded material from the reticulo-rumen of the animal (Zebeli et al., 2012). On tropical, high-fibre grasses, the most adversely affected animals are those that had been selected (under feedlot conditions) for high milk yields (Niu et al., 2014). On systems based on tropical forages, DMI is the major limiting factor for livestock productivity, especially in dairy cattle (Hills et al., 2015). Here, we tested this phenomenon using stall-fed crossbred lactating dairy cows, to compare the nutritional superiority of Brachiaria brizantha cy. Piatá over Napier grass when fed with and without supplementation by Desmodium distortum, a tropical forage legume adapted to cut and carry system.

2. Materials and methods

2.1. Study site, animals and trial management

This study was carried out in smallholder farms in semi-arid area of Rwanda. The choice of farms was based on easy access and proximity to Karama Research Station of the Rwanda Agriculture Board (RAB) to guarantee the supply of fresh-cut forage legume.

The experimental animals were Ankole Longhorn \times Holstein Friesian crossbred cows in second parity with 319 \pm 14 kg of body weight and in early lactation (10 to 15 days in milk). The animals belonged to the farmers who stall-fed them in individual pens in the cowsheds at each farm.

2.2. Digesta flow markers and marker preparation

Fluid and particulate phase markers used were cobalt ethylene diamine tetraacetic acid (Co-EDTA) and ytterbium oxide (Yb₂O₃), respectively. The Co-EDTA was prepared according to Uden et al. (1980) and modified by Nsahlai (1991). It involved dissolving and gently heating (while stirring) Na-EDTA (297.2 g), CoCl₂·6H₂O (190.4 g) and NaOH (32.0 g) in distilled water (1,600 mL). Additional NaOH pellets (6.8 to 7 g) were added to ensure complete solubilisation. The solution was allowed to cool to room temperature; 160 mL hydrogen peroxide was added and allowed to stand at room temperature for 4 h before adding 95% ethanol (vol/vol; 2,400 mL). The solution was stored under refrigeration overnight for crystal formation. Crystals were filtered, repeatedly washed with 80% ethanol (vol/vol) and dried overnight at 100 °C.

2.3. Feed, experimental design and data collection

Basal diets were fresh *B. brizantha* cv. Piatá and Napier grass (*P. purpureum*) harvested at farmers' field where they were established without fertiliser application. These grasses were harvested after 90 days of establishment at a height of 15 cm from the ground. Either one of these grasses was fed with or without forage legume (*D. distortum*) used as supplement. This legume was established without fertiliser application and harvested at 90 days after regrowth from the Karama Research station of RAB. Fresh forage, water, and mineral block ([per kg forage] vitamin A: 100,000 IU; vitamin D₃: 20,000 IU; vitamin E: 40,000 IU; calcium: 40,000 mg; phosphorus: 50,000 mg; magnesium: 5,000 mg; iron: 2,000 mg; cobalt: 50 mg; iodine: 50 mg; manganese: 2,000 mg; zinc: 1,000 mg; selenium: 10 mg) were provided *ad libitum*.

Four diets (Table 1) were compared in this experiment. Ten cows corresponding to 10 farms were randomly assigned to each dietary treatment in a completely randomised block design. Fourteen days for feed adaptation were allotted to individual cows. Before feeding, fresh feed and refusals were also weighed. Feed sampling in each farm was done twice a week for a period of 17 weeks. Milk yield data were recorded daily and summarised weekly. Milking was done twice daily, in the morning between 07:00 and 08:00, and in the evening between 16:00 and 18:00 for 17 weeks. Forage grasses and legume were chopped manually at 10 cm length (using machete), before feeding. Daily feed DM, crude protein (CP) and metabolizable energy (ME) intakes were calculated as the difference between feed offered and refusal corrected for their respective contents. Initial data on body weight were recorded and used as covariates during statistical analysis of feed intake and milk yield data.

Farmers recorded data of forage on offer and refusals (fresh weight basis) twice a day (morning and evening). Data collected by farmers were validated during weekly test-day visits, when samples of forage on offer and refusals were collected for chemical analysis. Farmers also recorded daily milk yields, which were validated during the test day visits.

2.4. Markers administration, sampling and laboratory analysis

Four dairy cows in each dietary group of 10 lactating cows were selected for the administration of external markers. Since animals used were not fistulated, markers were administrated orally (pulse dose). Ytterbium oxide (600 mg) was weighed and mixed with small amount of feed and ensured total ingestion of the marker. According to Pinares-Patiño et al. (2007), ytterbium oxide can be used as a solid marker to estimate faecal output. The Co-EDTA (20 g) was dissolved in water (1 L) for the same reason (Huhtanen and Kukkonen, 1995). Faecal samples were taken from the rectum during the following times: 0, 2, 4, 8, 10, 12, 24, 27, 30, 33, 36, 48, 54, 60, 72, 96, 120 and 144 h post marker administration. Faecal samples were kept in cool box (4 °C) and delivered to the

Table 1

Experimental details on diet composition (ratio, as-fed) and number of animals and farms used in the study.

Treatments	Diet composition ¹	Animals (farms)
1	100NG (NG)	10 cows (10 farms)
2	70NG:30DD (NGD)	10 cows (10 farms)
3	100PG (PG)	10 cows (10 farms)
4	70PG:30DD (PGD)	10 cows (10 farms)

¹ NG = Napier grass; DD = *Desmodium distortum*; NGD = Napier grass + DD; PG = *Brachiaria brizantha* cv. Piatá grass; PGD = *B. brizantha* + DD.

laboratory. Frozen samples of faeces were dried in forced-air oven (105 °C) for 24 h. Dried samples were ground to pass through 1 mm mesh and 1 g of each sample was ignited at 550 °C in a muffle furnace for 8 h to collect ash. Ash samples were analysed for Yb and Co concentrations using Inductively Coupled Plasma (ICP) Optical Emission Spectrometer (Varian 720-ES Series) at the University of KwaZulu-Natal, South Africa.

2.5. Chemical composition of feeds used

Dry matter (DM, g/kg) contents were determined according to (AOAC (1990); method ID 942.05) while organic matter (OM) and ash were determined according to (AOAC (2006a); method ID 984.13). Crude protein (CP) content (g/kg DM) was calculated as $6.25 \times N$ (Kjeldahl nitrogen) content in the feed. The N content was determined according to (AOAC (2006b); method ID 942.05); method ID 984.13). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest et al. (1991).

2.6. Metabolizable energy estimation

Metabolizable energy (ME) contents of forages were estimated from *in vitro* gas production over a 24-h incubation period (Menke et al., 1979, Eq. (1)).

$$ME (MJ/kg DM) = 2.2 + 0.136V_{24} + 0.057CP + 0.0029CP^2$$
(1)

where V_{24} is gas volume (mL) at 24 h; CP is crude protein (%).

2.7. Passage rate calculations

The passage outflows (k_1 and k_2) and transit time (*TT*) were calculated based on the model (Eq. (2)) developed by Blaxter et al. (1956) and cited by Nsahlai (1991).

$$Z = A \left[\left(e^{-k_1(t-TT)} - e^{-k_2(t-TT)} \right) \right] e^{-k_1(t-TT)} - e^{-k_2(t-TT)};$$

$$t \ge TT; \ Z = 0 \text{ for } t < TT;$$
(2)

where *Z* and *A* are the marker concentrations in the faecal DM; k_1 and k_2 are passage rate constants; *TT* is the estimated time for the first appearance of marker in faeces while *t* is the time of sampling after a single marker had been administered.

For each marker, the natural logarithm (ln) of marker concentration in the dried faeces was plotted against time with the regression analysis produced on the linear portion of the descending slope. The regression coefficient and Z-intercept correspond to the slowest rate constant (k_1) and A_1 , respectively. Fitted values were estimated for all collection times that corresponded to the ascending phase and the peak portions of the curve. Then, the anti-logarithm of the fitted values minus the actual concentrations measured at these times gave residuals. Regression analysis involving the natural logarithm of the residual concentrations and the collection time would give the Z-intercept A_2 and the second slowest rate constant (k_2). The two lines intersect at the point (*TT*, *A*) helped to calculate *TT* (Eq. (3)).

$$TT = \frac{(A_2 - A_1)}{(k_2 - k_1)} \tag{3}$$

 A_1 and A_2 in this equation are the derivatives of natural logarithm. Then, total mean retention time (MRT; h) that represents the mean retention time of particles in the whole digestive tract was calculated as the reciprocal of the natural logarithmic of slopes of descending and ascending phase of the curve $(1/k_1 + 1/k_2)$ plus the *TT* (Eq. (4)).

$$TMRT(h) = \left(\frac{1}{k_1} + \frac{1}{k_2}\right) + TT$$
(4)

2.8. Statistical analysis

Data on chemical compositions of diet were analysed using General Linear Model (GLM) procedures of the Statistical Analysis System (SAS, 2010). The model used is given as follows (Eq. (5)):

$$Y_{ijk} = \mu + G_i + L_j + P_k + (G \times L)_{ij} + (G \times L \times P)_{ijk} + \varepsilon_{ijk}$$
(5)

where Y_{ijk} : variable dependent; μ : overall mean; G_i : effect of grass; L_j : effect of forage legume; P_k : effect of period of the experiment (weeks); $(G \times L)_{ij}$: interaction between grass and forage legume; $(G \times L \times P)_{ijk}$: interaction effect of grass-legume-experimental period; ε_{ijk} : random residual error.

In addition, DMI, ME and CP intakes, and milk yield were subjected to a two-way analysis of variance (ANOVA) in a randomised complete block design using GLM procedures of SAS and differences between diet means were detected using pairwise *t*-test (PDIFF option of SAS). The model for the ANOVA is given as follows (Eq. (6)):

$$Y_{ijk} = \mu + B_0 + G_i + L_j + P_k + (G \times L)_{ij} + (G \times L \times P)_{ijk} + \varepsilon_{ijk}$$
(6)

where Y_{ijk} : dependent variable; μ : overall mean; B_0 is initial body weight of the cows, used as covariate; G_i : effect of grass; L_j : effect of forage legume; P_k : effect of lactation period; $(G \times L)_{ij}$: interaction between grass and legume; $(G \times L \times P)_{ijk}$: interaction of grass, legume and lactation period; ε_{iik} : random residual error.

Kinetics passage rate (k_1 and k_2), *TT* and MRT data were analysed using the GLM procedures of SAS (2010). The model of ANOVA is given (Eq. (7)) and pairwise *t*-test (PDIFF option of SAS) was used to separate the means.

$$Z_{ij} = \mu + G_i + L_j + (G \times L)_{ij} + \varepsilon_{ij}$$
⁽⁷⁾

where Z_{ij} : dependent variable; μ : overall mean; G_i : effect of grass; L_j : effect of forage legume; $(G \times L)_{ij}$: interaction between grass and legume; e_{ij} : random residual error.

3. Results

3.1. Chemical composition of diets

Chemical compositions of *Desmodium* during the experimental period were 245.1, 182.5, 871.5, 507.3 and 259.7 g/kg for DM, CP, OM, NDF and ADF, respectively. Chemical compositions of experimental diets differed significantly (Table 2). Dry matter content was higher for *B. brizantha* cv. Piatá than for Napier grass based diets. Napier grass–*Desmodium* (NGD) mixture had higher DM content than Napier grass alone (NG). On the other hand, *B. brizantha* cv. Piatá sole fed (PG) had similar DM content to *B. brizantha* cv. Piatá–*Desmodium* mixture (PGD). Organic matter (OM) and CP contents were significantly higher in PG than in NG forage. Supplementation of grasses with *Desmodium* improved the CP content

 Table 2

 Dry matter content (DM, g/kg) and chemical composition (g/kg DM) of the diets.

-										
	Parameters	NG	NGD	PG	PGD	RMSE	Significance ¹		Significance ¹	
	(n = 1,360)						G	L	$G\timesL$	
	DM	156.6	196.1	238.5	241.4	37.4	***	***	***	
	СР	124.5	153.1	157.9	169.1	11.7	***	***	***	
	OM	861.7	862.4	882.3	872.8	12.3	***	***	***	
	NDF	386.3	431.0	329.3	426.6	39.8	***	***	***	
	ADF	372.6	300.0	323.8	300.7	32.6	***	***	***	

n = the number of observations; NG = Napier grass; NGD = Napier grass + *Desmodium distortum*; PG = *Brachiaria brizantha* cv. Piatá grass; PGD = *B. brizantha* + *Desmodium distortum*; RMSE = root mean standard error; CP = crude protein; OM = organic matter; NDF = neutral detergent fibre; ADF = acid detergent fibre.

***: *P* < 0.001.

 $^1\,$ Effects of grass (G), legume (L) and G \times L.

(P < 0.001) of diets. However, supplementation with legume did not improve OM content of NG and it reduced OM content of PG based diet. The NDF and ADF contents were lower in PG than in NG. Supplementation of grasses with *Desmodium* increased their NDF contents, but decreased their ADF contents (Table 2). This pattern was consistent across the experimental period, except that CP contents varied with progress in the experiment. Changes in CP contents with progress in experiment were grass species and supplement dependent.

3.2. Nutrient intake and milk yield

Relative to Napier grass, *B. brizantha* cv. Piatá increased DMI (P < 0.05), CPI (P < 0.001), and metabolizable energy (MEI) (P < 0.001) and as such improved milk yield (Table 3; P < 0.001). *Desmodium* had no effect on DMI (P > 0.05) but increased CPI (P < 0.001) and MEI (P < 0.001).

3.3. Kinetics of fluid and particle phases of digesta

Kinetics of both fluid and particles did not differ between the grass-only diets, except that NG had longer (P < 0.05) total MRT of particles than PG (Table 4). *Desmodium* increased the rate of passage of liquid (P < 0.05) and solids (P < 0.01) resulting in shorter MRT of fluid (P < 0.05) and particles (P < 0.001) than grass-only diets. *Desmodium* increased the rate of passage of fluid in Napier grass diets than in *B. brizantha* cv. Piatá diets with an effect on the interaction (P < 0.05). The interaction of grass-*Desmodium* on total MRT of solids was significant, showing pronounced reductions in Napier grass diets than in *B. brizantha* cv. Piatá diets. No other effect was significant.

Table 3

Daily intakes of dry matter (DMI), crude protein (CPI) and metabolizable energy (MEI) and milk yield of dairy cows fed the experimental diets on-farm.

Parameters	NG	NGD	PG	PGD	RMSE	Significance ¹		
(n = 4,760)						G	L	$G\timesL$
DMI, kg/day	8.3	8.2	9.1	9.2	2.1	**	NS	NS
CPI, kg/day	1.0	1.2	1.4	1.5	0.3	***	***	NS
MEI, MJ/day	59.6	71.7	81.9	88.7	18.0	***	***	NS
Milk, L/day	5.4	7.1	8.1	9.0	1.5	***	*	NS

n = the number of observations; NG = Napier grass; NGD = Napier grass + *Desmodium distortum*; PG = *Brachiaria brizantha* cv. Piatá grass; PGD = *B. brizantha* cv. Piatá + *Desmodium distortum*; RMSE = root mean standard error.

¹ Effects of grass (G), legume (L) and G × L.

Table 4

Fractional rate of passage from the rumen (k_1) and hind gut (k_2) , transit time (TT) and total mean retention time (TMRT) of liquid and particle phases of digesta in lactating dairy cows fed on Napier grass (NG) or *Brachiaria brizantha* cv. Piatá (PG), unsupplemented or supplemented with *Desmodium distortum* (NGD and PGD, respectively).

Parameters ¹	NG	NGD	PG	PGD	RMSE	Significance ²		
(<i>n</i> = 32)						Grass DD Grass \times		$\text{Grass} \times \text{DD}$
Fluid phase								
k1, /h	0.04	0.04	0.04	0.05	0.006	NS	*	NS
k ₂ , /h	0.06	0.11	0.15	0.1	0.046	NS	NS	*
TT, h	2.5	0.7	1.1	2.0	1.59	NS	NS	NS
TMRT, h	46.5	35.4	37.6	34.7	5.26	NS	*	NS
Particle phase								
k1, /h	0.02	0.03	0.03	0.03	0.005	NS	**	NS
k ₂ , /h	0.03	0.06	0.06	0.06	0.017	NS	NS	NS
TT, h	2.4	2.1	1.8	2.3	2.19	NS	NS	NS
TMRT, h	83.1	49.7	62.8	50.7	7.77	*	***	*

n = the number of observations; NG = Napier grass; NGD = Napier grass + *Desmodium distortum*; PG = *B. brizantha* cv. Piatá grass; PGD = *B. brizantha* cv. Piatá + *Desmodium distortum*; RMSE = root mean standard error; DD = *Desmodium distortum*.

*: *P* < 0.05; **: *P* < 0.01; ***: *P* < 0.001; NS: *P* > 0.05.

 1 k₁ is proportion per hour at which particles pass out of the rumen; k₂ is proportion per hour at which large particles are reduced to small particles within the rumen.

² Grass: effect of NG and PG; Grass \times DD: interaction effect of grass and DD.

4. Discussion

This study compared *B. brizantha* cv. Piatá with locally existing Napier grass, either supplemented or unsupplemented with *D. distortum*, a forage legume, on digesta kinetics and milk production by dairy cows under smallholder farming conditions. The finding provided insights supporting the use of *B. brizantha* cv. Piatá for improved feed intake and thereby improved milk production. The study also found evidence for the affordable use of forage legume as a supplement of grass-based diets. Furthermore, the study contributed to improved understanding of the relationship between digesta kinetics, nutrient intake and animal production response.

In the present study, CP and OM contents of Napier grass were higher than those found by other studies (Lounglawan et al., 2014; Mutimura et al., 2015). Nonetheless, Salgado et al. (2013) reported similar CP content, but higher NDF and ADF concentrations than those found in the present study. In the present study, chemical composition of Napier grass and *B. brizantha* cv. Piatá based grass diets differed with *Desmodium* supplementation, which in agreement with reports by Avilés-Nieto et al. (2013), who found an increase or decrease depending on the forage legume. The differences in nutritional compositions between the diets were expected given that the grasses differed in chemical composition and hence nutritive value. Changes in chemical composition with progress in experiment (due to maturity progress) were also expected (Kozloski et al., 2005).

Feed and animal factors that influence intake of roughages are the chemical composition and gut fill, respectively. Extensive reviews have validated that, fibre components of roughages impose physical constraints on intake but improved NDF digestibility increases intake and milk yield in dairy cattle (Oba and Allen, 1999). Contrary to expectations, DMI in cows fed mixed grass-legume was not significantly higher than the DMI in cows fed sole grass. However, the differences in DMI of Napier grass and *B. brizantha* cv. Piatá as sole diets and of their mixes with the legume seemed to be related to the effect of grass. The likelihood of biological significance of higher DMI in cows fed with *B. brizantha* cv. Piatá is illustrated in the significantly and consistently higher milk yield. Cows fed with sole *B. brizantha* cv. Piatá had 33% more milk yield than cows fed with sole Napier diets. Cows fed with *B. brizantha* cv. Piatá-legume diets produced approximately 21% more milk than cows fed with Napier-legume diet. This finding is consistent with reports that forage grass supplemented with legume increase milk yield in lactating dairy cows (Halmemies-Beauchet-Filleau et al., 2014). The milk production recorded in this study is typical of *Bos taurus* and *Bos indicus* crossbreds in East and Central African region.

Although Co-EDTA was used as a proxy indicator for small particle dynamics in the rumen and hind gut, it effectively determined the fluid phase dynamics. As expected (Clauss and Lechner-Doll, 2001), the fluid phase had higher rumen outflow rate (k_{1C0}) than larger particles (k_{1Yb}) . Our values for k_{1C0} are similar to values reported for lactating Jersey cows (Aikman et al., 2008). The high values of k_{1Co} observed with B. brizantha cv. Piatá-Desmodium and Napier-Desmodium diets might be attributed to supplementation effect of forage legumes, which are known to have faster passage (Kammes and Allen, 2012) and fermentation rates in the rumen (Hebel et al., 2011). Conversely, k_{2Co} and k_{2Yb} values for small and large particles did not differ either in cows fed B. brizantha cv. Piatá or in the cows fed with Napier grass. However, shorter MRT of both fluid and large particles and higher DMI of B. brizantha cv. Piatá-legume or sole feed were observed. This agrees with Gorniak et al. (2014) who demonstrated the associations of particle size reduction through physical and digestive functions. This relationship is based on the fundamental property of physical effectiveness of forage NDF, which varies with sources and not contents of NDF (Zebeli et al., 2012). We therefore postulate that differences in nutritional attributes of B. brizantha cv. Piatá and Napier grass are associated with the biophysical attributes of these two grasses.

5. Conclusion

Findings from this study indicate that the higher feeding values (higher DMI and milk yield) of diets based on *B. brizantha* cv. Piatá (Piatá grass) than those based on Napier grass (*P. purpureum*) were most likely due to the better chemical composition of Piatá grass compared with that of Napier grass. Accordingly, faster outflow rates from the rumen were observed with *B. brizantha* cv. Piatá than with Napier grass diets. However, the legume (*D. distortum*) supplementation improved the outflow rates of both grasses.

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