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Quality Herbage Production of Dwarf Napiergrass with Italian Ryegrass Cropping under Digested Effluent Application in Southern Kyushu, Japan

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Abstract: Digested effluent produced by a biogas-plant contains essential plant nutrients to solve the treated and disposal problems of livestock manure. Dwarf variety of late-heading type (dwarf) napiergrass as a perennial summer crop and intercropping of over-sown Italian ryegrass as an annual winter crop were applied to 3 levels of digested effluent in southern Kyushu from 2007 to 2009. Digested effluent revealed so effective fertilizer as chemical one to increase dry matter yield, wintering ability and forage quality in crude protein concentration and *in vitro* dry matter digestibility (digestibility) with increasing application rate. Digestibility in leaf blade of napiergrass and that in whole Italian ryegrass were positively correlated with acid detergent lignin and neutral detergent fiber concentrations, respectively, which might be mediated with reducing tiller size with defoliation proceeded. The present cropping systems have a potential under sufficient application of the effluent to achieve sustainable quality herbage production in southern Kyushu, Japan.

Keywords: Digested Effluent, Dwarf Napiergrass, Italian Ryegrass, Herbage Quality, Wintering Ability

Introduction

Animal manure contains valuable nutrients and organic matter that can be used as a nutritional source for forages to produce high yield with high nutritive value and be suitable to build adequate environments of grassland farming (Idota *et al*., 2005). Livestock wastes can be processed to Digested Effluent of Manure (DEM) by biogas-plant (Thy and Buntha, 2005), which requires arable crops to utilize these nutrients efficiently. Intensive management of grassland systems requires considerable amount of N fertilizer to sustain high production and nutritive value of herbages (Sunusi *et al*., 1999; Wadi *et al*., 2003). Defoliation is an important management factor because of the multiple influences on the growth attributes as well as forage qualities, either by cutting operation or by animal grazing (Hodgson, 1979; Thomas, 1980).

Napiergrass (*Pennisetum purpureum* Schumach) is a C_4 tropical grass which produces abundantly nutritious green forage and is considered to be excellent feeds for livestock under both green-chopping and grazing systems in the tropics (Vicente-Chandler *et al*., 1959; Woodard and Prine, 1993) as well as in temperate Kyushu, Japan (Sunusi *et al*., 1999; Wadi *et al*., 2004; Idota *et al*., 2005; Hasyim *et al*., 2010), affected by the various conditions such as growth stages, climatic factors and defoliation intensity (Zewdu *et al*., 2002). Several genotypes of napiergrass were investigated for the potential biofuel production in the tropics (Williams and Hanna, 1995; Rengsirikul *et al*., 2011) and in temperate Kyushu (Khairani *et al*., 2013). Napiergrass required heavy fertilization and careful management to maintain a rapid growth with quality forage under the subtropical climate in Australia (Jones, 1985) as well as in southern Kyushu, Japan (Sunusi *et al*., 1999; Wadi *et al*., 2003). Dwarf variety of late-heading type (dwarf) napiergrass, bred in Florida, USA (Sollenberger *et al*., 1988) and introduced from Thailand (Ishii *et al*., 1998), was evaluated for the adaptability in southern Kyushu areas (Utamy *et al*., 2011; Ishii *et al*., 2013) and can be used for grazing of beef cattle (Ishii *et al*., 2005). In the temperate Kyushu, lower temperature with shorter optimal growing period requires napiergrass to have a rapid growth with vigorous tillering ability in an optimal growing season and maintain higher wintering ability (Ishii *et al*., 1995), which might be achievable by the high rate of DEM application.

Since napiergrass, C_4 tropical species, is suffered from low temperature to retard the growth in the wintering season, it is necessary to over-sow temperate species into the interrow space of napiergrass during the winter-spring period, so as to achieve maximum annual herbage yield. Recently, Italian ryegrass (*Lolium multiflorum* Lam.) has been recognized as the rotation crop for the plowing-down management (Dart and Fulkerson, 2014), since it is a cool-season annual, leafy and palatable grass to livestock and can produce significant amount of herbages under favorable growing conditions. Therefore, this study was examined for the cut-and-carry cropping of dwarf napiergrass with over-sown Italian ryegrass under different levels of DEM application compared with chemical fertilization to examine the potential quality herbage production for 2 years after establishment.

Materials and Methods

Plant Culture and Design of Experiment

The experiment was conducted on andosols at 31 m above sea level in Kibana Field, University of Miyazaki in southern Kyushu, Japan (131.41°E, 31.83°N, 31 m a.s.l.) in the two cropping years from 2007 to 2009. Dwarf napiergrass was grown by transplanting a rooted tiller with 2 plants m^{-2} on 10 May, 2007 and Italian ryegrass (cv. Ace, Snow Brand Seed Co. Ltd. Sapporo, Japan) was sown into the inter-row space as an intercrop after harvesting dwarf napiergrass in autumn on 30 October, 2007 and 27 October, 2008. The plots (13.5 m^2/plot) were set into a randomized blocked design by 3 replications and were applied into 4 treatments, which have 3 levels of DEM application and chemical compound fertilizer (C) application at the same rate as high DEM level with additional split application.

Fertilizer Application

Application of DEM solution was 3 levels at 5.04, 2.52 and 1.26 g N m^{-2} $time^{-1}$ for high (H), medium (M) and low (L) levels, respectively and chemical compound fertilizer was supplied as a check (C: 5.04 g N m^{-2} $time^{-1}$). Application to napiergrass was conducted 4 times at a monthly interval from June to September in both 2007 and 2008. The same levels of fertilizing treatments were imposed to Italian ryegrass 3 times of application each cropping season on 29 October, 7 November, 2007 and 3 May, 2008 and on 26 November, 2008, 23 February and 29 April, 2009, for the first and second season, respectively.

Plant Sampling and Measurement

Dwarf napiergrass plants were sampled 3 times and 4 times almost in a monthly interval in 2007 and 2008 cropping season, respectively and divided into plant fractions; Leaf Blade (LB), stem inclusive of leaf sheath (ST) and dead part (D). Wintering ability of this species was determined in the following spring on 20 May, 2008 and 9 June, 2009, by the Percentage of Overwintered Plants (POP) which had one or more regrown tillers from stubbles and Regrown Tiller Number (RTN) per m^{-2}. Italian ryegrass plants were harvested 2 times in a monthly interval by quadrat (0.25 m^2) at 5 cm above the ground level both in 2008 and 2009. Sampled plants were dried at 70°C for 72 h. by ventilating oven to determine percentage of dry matter. Dried samples were milled to analyze herbage quality attributes, such as concentrations of total Nitrogen (N) and Crude Protein (CP), *in vitro* Dry Matter Digestibility (IVDMD), concentrations of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL). The N concentrations of Leaf Blade (LB) and stem inclusive of leaf Sheath (ST) of dwarf napiergrass and whole plants of Italian ryegrass at each cutting were determined by CN corder (Model: NC-220F; Sumigraph). The IVDMD was determined in ANKOM filter bag by pepsin-cellulase digestion assay method with *in vitro* incubator (Model: DAISY II-200/220, ANKOM Technology Co. Ltd.) at 39.0°C. The NDF, ADF and ADL concentrations of forages were determined by detergent assay method using fiber analyzer (Model: ANKOM 200/220, ANKOM Technology Co. Ltd.).

Climatic Conditions

Changes in the mean and minimum air temperatures and precipitation were monitored from the data of Miyazaki Meteorological Observatory (JMA, 2013) in the summer (May to November) and winter periods (December to April) for napiergrass and Italian ryegrass, respectively. Climatic conditions of both periods were not significantly different from those in the normal year, obtaining on the 30-year average for 1980-2010 (JMA, 2013), except for the variation of summer precipitation, which was extremely higher in July 2007, contrary to the extremely drought condition in July 2008. The minimum monthly temperatures occurred at 1.1°C in February 2008 in the first season, compared with higher wintering temperature at 3.2°C in January 2009 in the second season.

Statistical Analysis

Analysis of variance (ANOVA) was carried out using SPSS software (version 15.0) by one-way analysis procedures for yield and quality attributes of napiergrass and Italian ryegrass in a randomized complete design. Mean separation was tested using the Least Significance Difference (LSD) method at the 5% level.

Results

Wintering Ability of Dwarf Napiergrass in 2008 and 2009

Wintering ability, as assessed by POP and RTN per m^{-2} in the spring of both 2008 and 2009, is shown in Fig. 1. In the first post-wintered season, POPs were quite stable and almost perfect averaged at 99.2%, while they increased with the increase in DEM application rate from 86.7% (L level) to 93.3% (H level) in the second season. The RTN showed the positive response to the increase in DEM application rate both years, except for the erratic lowest value in M plot in early June 2009. Chemical fertilization caused almost similar effect on POP and RTN with H level of DEM application in mid-May 2008, contrary to the significantly highest POP and RTN across treatments in early June 2009 (Fig. 1).

Yield and Crude Protein of Herbages in Dwarf Napiergrass and Italian Ryegrass

Both of dry matter yield and CP concentration in dwarf napiergrass and Italian ryegrass increased significantly with the increase in DEM application rate across all cuttings both years (Table 1). With cutting, dry matter yield tended to decrease, while CP concentration tended to increase in both dwarf napiergrass and Italian ryegrass both years. Annual total yields increased from the first year to the second year across all treatments and grass species, while CP concentration tended to increase slightly from the first to the second year in dwarf napiergrass, while it decreased from the first to the second year in the DEM-applied Italian ryegrass. Dry matter yields between DEM and chemical fertilization were non-significantly different in any cutting for both species, except for the highest yield of dwarf napiergrass in C plot at the third cutting under the lowest temperature period in 2007.

Annual total yield had a positive and linear regression with annual total N input both for dwarf napiergrass ($r = 0.929$, $p<0.10$ and $r = 0.965$, $p<0.05$ in the first and second year, respectively) and for Italian ryegrass ($r = 0.991$, $p<0.01$ and $r = 0.978$, $p<0.05$ in the first and second year, respectively). Correlation coefficient of dry matter yield with CP concentration was significantly negative ($r = -0.671$, $p<0.01$) for dwarf napiergrass, while it was non-significantly negative ($r = -0.341$, $p>0.10$) for Italian ryegrass (Table 2).

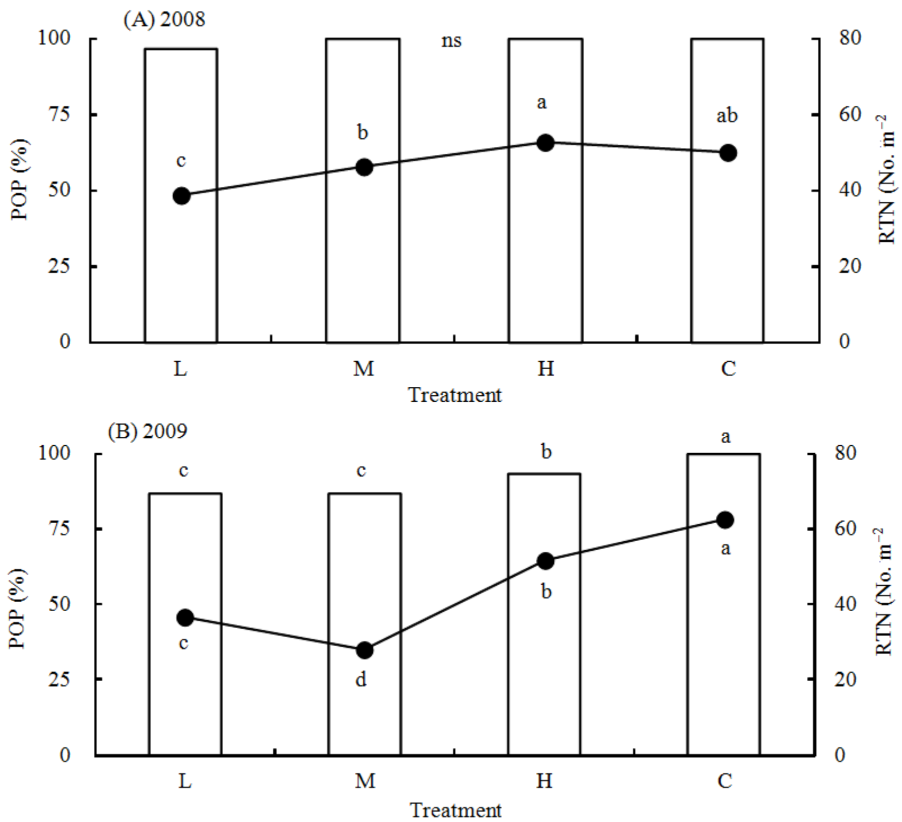

Fig. 1. Percentage of overwintered plants (POP, Bar□) and regrown tiller number (RTN, ●) in the overwintered plants in dwarf napiergrass pasture on May 20, 2008 (A) and June 9, 2009 (B). Symbols with different letters denote significant difference among treatments on the same date at the 5% level. ns: $p>0.05$. Treatment: DEM application of L, M, H level (1.26, 2.52, 5.04 g N m^{-2} $time^{-1}$, respectively), chemical (5.04 g N m^{-2} $time^{-1}$)

Digestibility of Herbages in Dwarf Napiergrass and Italian Ryegrass

Changes in IVDMD of LB, ST and LB + ST in dwarf napiergrass and that of whole plants in Italian ryegrass are shown for each cutting both years in Fig. 2. In dwarf napiergrass, IVDMD of LB maintained above 60 and 65% in 2007 and 2008, respectively and that of ST almost above 70% both years. The IVDMD in Italian ryegrass maintained above 70% both years. The IVDMD in dwarf napiergrass did not respond clearly to the increase in DEM application rate, except for LB at the first cutting in 2007, while positive responses were obtained among treatments at the first cutting both years in Italian ryegrass. Differences in IVDMD between H and C plots were small at all cuttings for both species both years (Fig. 2).

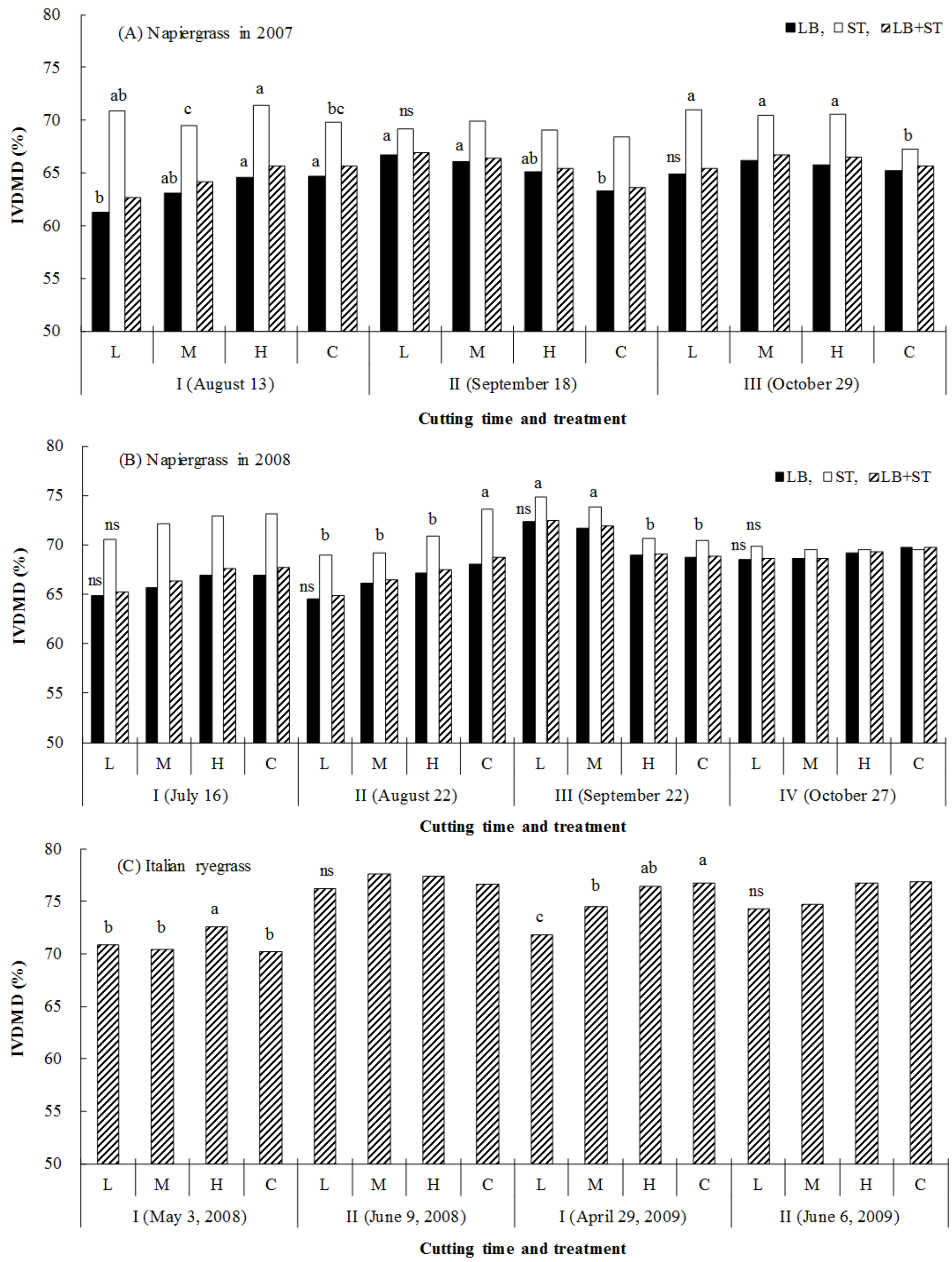

Fig. 2. Changes in *in vitro* Dry Matter Digestibility (IVDMD) of herbage with cutting under different treatments in dwarf napiergrass in 2007 (A) and 2008 (B) and in Italian ryegrass (C). For the treatments (L, M, H, C), refer to Fig. 1. LB: Leaf blade, ST: Stem inclusive of leaf sheath, LB+ST: Leaf blade and stem inclusive of leaf sheath. Symbols with different letters denote significant difference in each organ among treatments on the same date at 5% level. ns: p>0.05

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Table 1. Changes in dry matter yield and crude protein in dwarf Napiergrass pasture in 2007-2008 and Italian ryegrass pasture in 2008- 2009 under DEM applications of different levels

Cutting time			Dry matter yield (gm^{-2}) Treatment†				Crude protein (%) Treatment			
Species	Year	and date	L	M	H	C	L	M	H	C
Napiergrass	2007	I(August13)	188.9b††	273.9ab	356.8a	344.3a	7.4c	7.9b	8.5a	8.1ab
		II(September18)	109.4b	155.7b	177.9ab	196.4a	9.7b	9.6b	11.0a	11.0a
		III(October29)	112.0b	147.5b	162.1b	222.3a	10.8b	11.1a	11.1a	10.7b
		Annual total/mean	410.3	577.1	696.8	763.0	9.3	9.5	10.2	9.9
	2008	I(July16)	245.4c	307.3b	371.1a	333.4ab	8.2c	8.9b	9.6a	8.7bc
		II(August22)	193.3b	251.8ab	293.5a	277.1ab	9.5c	10.3b	10.9a	10.7ab
		III(September22)	109.4c	137.9b	156.6ab	175.1a	11.8b	12.3b	13.5a	12.7ab
		IV(October27)	76.6c	96.6b	117.4a	113.6ab	13.2c	13.7b	14.7a	14.4a
		Annual total/mean	624.7	793.6	938.6	899.2	10.7	11.3	12.2	11.6
Italian ryegrass	2008	I(May3)	36.5ns	102.8ns	164.1ns	193.9ns	7.7ns	7.6ns	8.1ns	7.2ns
		II(June9)	39.4b	50.4ab	76.0a	58.7a	10.6b	11.9a	11.2ab	10.6b
		Annual total/mean	75.9	153.2	240.1	252.6	9.2	9.7	9.7	8.9
	2009	I(April29)	102.5c	247.2bc	463.2ab	568.9a	4.8b	4.9b	6.0a	6.5a
		II(June6)	68.4b	139.0ab	210.4a	249.8a	8.8c	9.2c	11.5b	13.7a
		Annual total/mean	170.9	386.2	673.6	818.7	6.8	7.1	8.8	10.1

†DEM application of L level (1.26 g N m^{-2} $time^{-1}$), M level (2.52 g N m^{-2} $time^{-1}$), H level (5.04 g N m^{-2} $time^{-1}$); chemical (5.04 g N m^{-2} $time^{-1}$).

††Symbols with different letters in a row denote significant difference for yield or crude protein among treatments on the same date at the 5% level. ns: non-significant (p>0.05).

Table 2. Correlation coefficients of Dry Matter Yield (DMY) with Crude Protein (CP) content and *in vitro* dry matter digestibility (IVDMD) in dwarf Napier grass and Italian ryegrass

Species		DMY	CP	IVDMD
Napiergrass	DMY	-		
(n = 28)	CP	-0.671**†	-	
	IVDMD	-0.343+	0.684**	-
Italian ryegrass	DMY	-		
(n = 16)	CP	-0.346	-	
	IVDMD	0.246	0.572*	-

†+, *, **: Significant at the 10, 5 and 1% level, respectively.

Table 3. Changes in Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) contents across cuttings in dwarf Napier grass pasture in 2007 and 2008 and in Italian ryegrass in 2008 and 2009

				NDF (%)				ADF (%)				ADL (%)			
Species	Year	Plant part	Cutting time	L†	M†	H†	C†	L	M	H	C	L	M	H	C
Napier grass	2007	Leaf blade	I (August 13)	64.1c††	65.6b	67.0a	67.2a	37.5b	38.3a	38.2a	37.6b	8.4a	8.5a	8.1b	7.8b
			II (September 18)	57.4c	58.5bc	58.8b	63.9a	38.5ns	39.2	39.2	39.4	9.6ns	9.3	9.2	8.8
			III (October 29)	63.0a	60.9b	59.9b	60.1b	36.6ns	35.6	37.2	38.3	11.1a	10.5ab	10.1b	9.7b
			Annual mean	61.5	59.7	59.4	62.0	37.5	37.7	38.2	38.5	9.7	9.4	9.1	8.8
		Stem with leaf sheath	I (August 13)	61.9ns	61.1	61.2	60.2	37.4ns	37.3	37.2	37.9	6.8ns	7.1	6.9	7.1
			II (September 18)	63.3c	64.2b	64.7ab	65.6a	38.4ns	39.2	39.3	37.6	6.9ns	6.7	7.6	7.7
			III (October 29)	61.0c	61.1c	62.5b	64.0a	37.1c	37.1c	38.3b	39.3a	6.9ns	6.9	6.6	6.9
			Annual mean	62.1	62.2	62.8	63.3	37.6	37.8	38.3	38.3	6.9	6.9	7.0	7.2
	2008	Leaf blade	I (July 16)	64.1ns	62.9	63.6	64.0	39.3ns	40.1	40.7	40.2	10.5ns	10.1	9.5	10.5
			II (August 22)	66.1ns	66.0	66.5	64.6	41.1b	42.6ab	42.7ab	43.8a	10.9ns	11.7	10.2	10.4
			III (September 22)	61.9ns	62.2	61.8	61.7	39.9ns	41.4	40.9	40.7	8.9ns	11.9	10.2	9.8
			IV (October 27)	62.1a	61.5ab	58.3c	59.5bc	40.1ab	40.9a	38.7b	39.7ab	11.9a	11.4b	10.9b	10.6b
			Annual mean	63.6	63.2	62.6	62.5	40.1	41.3	40.8	41.1	10.6	11.3	10.2	10.3
		Stem with leaf sheath	I (July 16)	65.1ns	63.4	62.7	64	38.2ns	37.9	38.2	38.7	10.7ns	11.8	10.2	8.3
			II (August 22)	65.5a	62.5b	63.3b	62.7b	40.5ns	40.1	41.1	41.8	7.6ns	8.4	8.8	7.7
			III (September 22)	63.8ns	63.7	62.9	64.4	41.2bc	40.5c	42.8a	42.1ab	7.9ns	8.7	9.3	8.5
			IV (October 27)	62.1b	62.8ab	62.6ab	63.5a	39.4bc	40.5a	38.9c	39.9ab	9.2a	8.5b	7.8b	7.8b
			Annual mean	64.1	63.1	62.9	63.7	39.8	39.8	40.3	40.6	8.9	9.4	9.0	8.1
Italian ryegrass	2008	Whole plant	I (May 3)	58.4ns	59.1	59.9	60.5	37.6ns	38.2	38.6	38.4	10.8a	10.6a	10.3ab	9.4b
			II (June 9)	60.7b	61.0b	62.1b	64.1a	37.1b	37.2b	38.1b	39.6a	6.5ns	5.7	5.6	5.5
			Annual mean	59.5	60.0	61.0	62.3	37.4	37.7	38.4	39.0	8.6	8.1	8.0	7.5
	2009	Whole plant	I (April 29)	60.8ns	61.2	63.0	61.3	39.2ns	39.9	40.1	39.6	11.4ns	11.1	10.8	11.4
			II (June 6)	62.1b	63.9a	64.3a	63.9a	39.4ns	40.3	40.5	40.3	9.2ns	7.9	7.4	7.8
			Annual mean	61.4	62.6	63.7	62.6	39.3	40.1	40.3	39.9	10.3	9.5	9.1	9.6

† As for the treatment, refer to the footnote of Table 1.

†† Symbols with different letters in a row denote significant difference for NDF, ADF and ADL among treatments on the same date at the 5% level. ns: Non-significant (p>0.05).

Table 4. Correlation coefficients between *In vitro* Dry Matter Digestibility (IVDMD) and fiber content in dwarf Napier grass and Italian ryegrass

Species	Plant part		IVDMD	NDF†	ADF†	ADL†
Napiergrass	Leaf blade	IVDMD	-			
	(n = 28)	NDF	-0.360^{+}	-		
		ADF	0.437*††	0.268	-	
		ADL	0.486**	-0.186	0.386*	-
	Stem with	IVDMD	-			
	leaf sheath	NDF	-0.094	-		
	(n = 28)	ADF	0.192	0.376*	-	
		ADL	0.321^{+}	0.279	0.221	-
Italian ryegrass	Whole plant	IVDMD	-			
	(n = 16)	NDF	0.686*	-		
		ADF	0.247	0.738**	-	
		ADL	-0.615*	-0.486^{+}	0.199	-

†Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL)
†† $^{+}$, *, **: Significant at the 10, 5 and 1% level, respectively

The positive correlations of IVDMD with CP concentration were significant in both dwarf napiergrass (r = 0.684, p<0.01) and Italian ryegrass (r = 0.572, p<0.05), while there was a negative tendency of IVDMD with dry matter yield (r = -0.343, p<0.10) in dwarf napiergrass, contrary to the non-significant correlation in Italian ryegrass (Table 2).

The NDF concentration maintained above 60% for both dwarf napiergrass and Italian ryegrass and ADF concentration maintained below 41 and 40% for dwarf napiergrass and Italian ryegrass, respectively (Table 3). The increase in DEM application rate lead to no consistent effect on NDF or ADF concentration for either species, while ADL concentration tended to decrease with the increase in DEM application rate for both species (Table 3).

The IVDMD was related with fiber concentrations in LB and ST of dwarf napiergrass and in whole plants of Italian ryegrass (Table 4). In LB of dwarf napiergrass, there were negative and positive correlations of IVDMD with NDF and ADL concentration, respectively, contrary to the positive and negative correlations of IVDMD with NDF and ADL concentration, respectively, in whole plants of Italian ryegrass. Among fiber concentrations, positive correlations or tendencies of ADF concentration with NDF and ADL concentrations were obtained in both species (Table 4).

Discussion

Napiergrass exhibits the best performance in deep and fertile soils with moderate to fairly heavy texture based on its origin (James, 1983) and is tolerant to the wide climatic range of annual precipitation in 2,000-4,000 mm and annual temperature in 13.6-23.7°C (Duke, 1978), which are just fit to the climatic conditions in southern Kyushu, Japan. Cultivation of napiergrass at the border between subtropical and temperate zones such as southern Kyushu, is not so popular among the world, while the grass is available for its cultivation only limited to slightly frosted areas because aboveground herbage is easily suffered to be dead from light frost and underground stem parts remain alive if the soil is not frozen (Burton, 1989). The present study revealed that the wintering ability in dwarf napiergrass was almost perfect under the favorably fertilized H and C plots in Miyazaki both years, as well as in our previous research (Ishii *et al*., 2013). The increase in DEM application rate was beneficial for improving wintering ability in dwarf napiergrass, probably due to the increase in the density of stubble tillers, which had underground tiller buds, being potential for spring regrowth (Ishii *et al*., 1995). Thus, dwarf napiergrass can be grown as perennial cut-and-carry herbages under 3 or 4 cuttings per year fertilized at around 20 g N m^{-2} $year^{-1}$.

Napiergrass can be intercropped with herbaceous and tree leguminous crops to increase total yield and nutritive value (Mureithi *et al*., 1995). In the present study, Italian ryegrass as temperate winter crop has an opposite growing season to tropical species of napiergrass. The combination of dwarf napiergrass with Italian ryegrass cropping proved to be satisfactory in the region with succeeding cropping years that annual total yield increased from 486.2 (L level) -936.9 (H level) g DM m^{-2} in the 2007-8 season to 795.6 (L level) -1612.2 (H level) g DM m^{-2} in the 2008-9 season by 64-72% as shown in Table 1. The increase in annual total yields from the year established to the following growing season in this cropping system was brought about by the increasing yielding ability due to the faster spring regrowth of dwarf napiergrass and more stable establishment of Italian ryegrass in the following year. It is a common feature that annual total yields of napiergrass increased from the year of establishment to the following year when the spring regrowth started from the overwintered stubbles (Ishii *et al*., 1995; Sunusi *et al*., 1999; Wadi *et al*., 2004) as well as other perennial tropical grasses, if pasture management was suitable for the grass species. As for the establishment of Italian ryegrass, the way of DEM application was changed from the first to the second year; in the first year, DEM was applied twice just at pre-and post-sowing of Italian ryegrass, which may hinder the emergence of over-sown Italian ryegrass to produce poorer establishment, resulting in lower dry matter yield. However, in the second year, the first application of DEM was carried out one month after the sowing of Italian ryegrass to improve the establishment of the species without disturbing the early growth by DEM solution.

In accordance with the increase in dry matter yield from the first to the second year in dwarf napiergrass, CP concentration increased in a range of 9.3-10.2% in the first year to 10.7-12.2% in the second year as shown in Table 1. The IVDMD in both dwarf napiergrass and Italian ryegrass increased from the first to the second growing season especially in the first-cut plants (Fig. 2), which were composed of principal yielding harvest in the annual yields for both grasses (Table 1). Even though negative correlations were obtained of dry matter yield with CP concentration in both grasses and that with IVDMD in dwarf napiergrass across whole cuttings and treatments (Table 2), dry matter yield was positively correlated with both CP concentration and IVDMD across DEM-applied plots at the same cutting date. Thy and Buntha (2005) examined in Chinese cabbage that both fresh biomass yield and CP concentration increased with the increase in application rate of bio-digester effluent up to 15 g N m^{-2}. Both crop nutrient requirements and soil properties are crucial factors to judge the proper agronomic practice of DEM as a fertilizer. Application of DEM to soil surface was proved to improve soil chemical properties without major contamination to the environments of arable lands (Hasyim *et al*., 2014). Several studies in Thailand (Rengsirikul *et al*., 2011) and Florida, USA (Woodard and Prine, 1993) noted that the recommended application rate at 20-30 g N m^{-2} are crucial to sustain high yield and quality in napiergrass.

Chemical compositions, particularly IVDMD and CP concentration, become good indices for feeding herbage to dairy and beef cattle. In the present study, IVDMD maintained above 60 and 70% in dwarf napiergrass and Italian ryegrass, respectively and stem inclusive of leaf sheath had always higher IVDMD than the leaf blade in dwarf napiergrass. As the minimum CP requirement for livestock production is 6-8% for maintenance (Minson, 1990), annual average of CP concentration in dwarf napiergrass, ranging in 9.3-10.2 and 10.7-12.2% across DEM-supplied plots in 2007 and 2008, respectively, maintained satisfactory level for feeding to cattle under the examined DEM levels. The situation was the similar to CP concentration in DEM-supplied Italian ryegrass ranging in 9.2-9.7 and 6.8-8.8% in 2008 and 2009, respectively. Thus, based on IVDMD and CP concentration, sustainable herbage-cropping system of dwarf napiergrass with Italian ryegrass can produce satisfactorily quality herbage to feed cattle.

In general, the level of CP concentration of herbages is affected by direct and indirect factors with the application of fertilizer including DEM. The former direct factor appears rapidly in forages after the supply of inorganic fertilizer (Moss, 2009), as well as liquid DEM which contained mainly inorganic elements, in accordance with the change of leaf color to become dense green. The latter indirect factor is mainly attributable to dilution of CP concentration by the rapid accumulation of cell wall carbohydrates at the latter stages of growth (Van Soest, 1994), which appeared typically in the first-cut Italian ryegrass in 2009 due to the heavy accumulation of biomass (Table 1). One of the other indirect factors is the change of leaf percentage with advance in growth stage. It is a frequent phenomenon in napiergrass that CP concentration is higher in the early growth stage than in the late and senescent growth stage, as reported in Africa (Seyoum *et al*., 1998; Tesema *et al*., 2002). In the temperate southern Kyushu, regrowth rate in dwarf napiergrass was suppressed by the decline in temperature and solar radiation as well as in Australia (Russel and Webb, 1976) after the first and second defoliation in 2007 and 2008, respectively. The optimal temperature for napiergrass growth is above 25°C up to 40°C and its growth ceases when temperature falls below 10°C (Bogdan, 1977). Under the sub-optimal temperature below 25°C, stem elongation of napiergrass is suppressed severely, causing the higher leaf percentage to lead to the increase in CP concentration, which happened in dwarf napiergrass after August both years. The similar situation of sub-optimal temperature and termination of spring vigorous growth in the second-cut Italian ryegrass proved to increase CP concentration to the large extent, compared with the first-cut plants (Table 1).

The compositions of fiber fraction can be classified into NDF, which is a measure of the amount of structural carbohydrates, including digestible (hemicellulose), less digestible (cellulose) and indigestible (lignin) components and ADF, which is a measure of less digestible and indigestible components. In napiergrass, ADF and NDF concentrations are reported at 36.6 and 63.4%, respectively (NARO, 2001), which are almost corresponded with the current average of ADF concentration at 38 and 40% and NDF concentration at 62 and 63% in 2007 and 2008, respectively (Table 3). Fiber fractions, such as NDF, ADF and lignin concentrations, are negatively correlated with dry matter digestibility of forages in general (Minson, 1990). However, in the present study, positive correlations were obtained of IVDMD with ADF and ADL concentrations in leaf blade of napiergrass and of IVDMD with NDF concentration in whole plants of Italian ryegrass (Table

4). In leaf blade of napiergrass, IVDMD tended to increase seasonally from the first to the last defoliation both years (Fig. 2), which were concurrent with the increase in ADL and ADF concentrations and the decrease in NDF concentration across all treatments both years (Table 3). The seasonal diverse trend of NDF and ADL concentrations in leaf blade of dwarf napiergrass may be derived from the reduction of tiller size with time. The first-cut plant was mainly composed of primary tillers, which was emerged from the underground stem and had large size of leaf, while the third- or fourth-cut plants were composed of large number of daughter tillers with smaller leaf size. The difference between NDF and ADF concentration can be estimated as hemicellulose concentration, which tended to decrease seasonally from the first to the last defoliation, suggesting poor cell wall components accumulated in the third- or fourth-cut plants. In Italian ryegrass, IVDMD increased from the first to the second defoliation across all treatments both years (Fig. 2), which was associated with the slight increase in NDF and ADF concentrations and the great decrease in ADL concentration with time (Table 3). The seasonal diverse trend of NDF and ADL concentration in Italian ryegrass may be caused by the reduction of heading tiller size with time. The first-cut plants were composed of large heading tillers, possessing of high lignin concentration to support stem-hardened heading tiller, while the second-cut plants had a smaller size of heading tiller possessing poor accumulation of non-structural carbohydrates, resulting in the slight increase of NDF and ADF concentrations with time.

Rate of DEM application will be determined by several agronomic and economic factors. In the present study, DEM solution was produced by a biogas-plant, built in Faculty of Agriculture, University of Miyazaki and was transported to the experiment field by the plastic tank. Therefore, authors cannot estimate costs of processing DEM by biogas-plant and transporting DEM to the field, but can do the fertilizing effect on dwarf napiergrass and Italian ryegrass in the present cropping system. This standpoint has some sort of reasonable aspect that biogas-plant is used to build at the region where the density of livestock is relatively higher than the area of fields and suitable manure treatment is highly required without influencing on the environments neighboring to the field. Thus, it is worthwhile to omit the cost of purchasing DEM. From the present study on herbage yield and quality in dwarf napiergrass with Italian ryegrass, combining with the former study in the effect of DEM on soil and soil water environments and plant production (Hasyim *et al*., 2014), herbage yield and quality of dwarf napiergrass and Italian ryegrass increased in accordance with the increase in plant persistence of dwarf napiergrass with increasing DEM application rate up to 35 g N m^{-2} yr^{-1}, without influencing on the major changes in the environments.

Conclusion

The application of DEM to dwarf napiergrass with Italian ryegrass cropping system had a comparable effect with chemical fertilization on herbage yield and quality in both grasses and DEM had a positively linear effect on enhancing dry matter production within the examined level of 20 and 15 g N m^{-2} for summer and winter crop, respectively. Plant regrowth after defoliation was almost perfect both in the growing and post-wintering seasons for 2 years. Thus, the present cropping system can be maintained for several years only by the over-sowing of Italian ryegrass just after the final defoliation of dwarf napiergrass under the sufficient use of DEM to achieve sustainable quality herbage production system in southern Kyushu, Japan.

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Author's Contributions

We certify that all persons who have made substantial contributions to the work reported in this manuscript.

Hadijah Hasyim and Ahmad Wadi: Both of them conducted field researches, made the literature review, analyzed and interpreted the results and drew conclusions.

Yasuyuki Ishii: He conducted field researches, revised the manuscript and conducted the correspondence of the submitted paper.

Sachiko Idota: She carried out the advice for the chemical analysis and put the comment on the manuscript, especially on the discussion section.

Ethics

The manuscript presents an original and valid work and does not infringe or violate any copy rights and neither this manuscript nor one with substantially similar content has been published or being considered for publication elsewhere.

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