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Comparative nitrogen use efficiency of urea and pig slurry for regrowth yield and nutritive value in perennial ryegrass sward

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Objective: The study aimed to assess the N use efficiency (NUE) of pig slurry (in comparison with chemical fertilizer) for each regrowth yield and annual herbage production and their nutritive value.
Methods: Consecutive field experiments were separately performed using a single application with a full dose of N (200 kg N/ha) in 2014 and by four split applications in 2015 in different sites. The experiment consisted of three treatments: i) control plots that received no additional N, ii) chemical fertilizer-N as urea, and iii) pig-slurry-N with five replicates.
Results: The effect of N fertilization on herbage yield, N recovery in herbage, residual inorganic N in soil, and crude protein were significantly positive. When comparing the NUE between the two N sources (urea and pig slurry), pig slurry was significantly less effective for the earlier two regrowth periods, as shown by lower regrowth dry matter (DM) yield, N amount recovered in herbage, and inorganic N availability in soil at the 1st and 2nd cut compared to those of urea-applied plots. However, the effect of split application of the two N sources was significantly positive at the last two regrowth periods (at the 3rd and 4th cut). The two N sources and/or split application had little or no influence on neutral detergent fiber (NDF) content, acid detergent fiber (ADF) content, and *in vitro* DM digestibility, whereas cutting date was a large source of variation for these variables, resulting in a significant increase in *in vitro* DM digestibility for the last two regrowth periods when an increase in NDF and ADF content occurred. Split application of N reduced the N loss via nitrate leaching by 36% on average for the two N sources compared to a single application.
Conclusion: The pig slurry-N was utilized as efficiently as urea-N for annual herbage yield, with a significant increase in NUE especially for the latter regrowth periods.

Keywords: *Lolium perenne*; Nitrate Leaching; N Use Efficiency; Nutritive Value; Pig Slurry; Regrowth

INTRODUCTION

Manure emission from pig production accounts for 38.2% of the total quantity of manure issued from livestock production (46 million tons per year) in Korea [1]. Pig slurry is the most important organic manure resource in Korea, estimated to be more than 80% of recycled animal manure [2]. The use of pig slurry as an alternative organic fertilizer is the most viable recycling option because pig farms usually have little or no arable surface for forage production in Korea.

When manure is applied to land, the N in manure, which is bound in organic compounds, must be mineralized by the soil microbes to be in a form available to plants (e.g., ammonium or nitrate). N losses after pig slurry application to soil are attributed to ammonia volatilization, nitrate leaching, denitrification, and/or microbial immobilization. At the time of application, pig slurry generally contains a large portion of total N as NH_4^+, which can be lost through volatilization as the largest pathway of pig slurry N loss [3,4]. Pig slurry NH_4^+ is rapidly nitrified in soil after

Table 1. Soil properties of experimental sites in 2014 and 2015

Site (year)	pHwater (1:5)	EC (Ds/m)	OM (%)	Total N (%)	P_2O_5 (mg/kg)	Exchangeable cation (cmol^+/kg)		
						K	Ca	Mg
Site A (2014)	5.6	0.76	2.45	0.15	256.2	0.23	3.06	1.98
Site B (2015)	5.9	0.61	1.98	0.12	229.8	0.19	2.25	1.49

EC, electrical conductivity; OM, organic matter.

application [5-7]. This process leads to N loss through denitrification and leaching [8] when crop uptake is limited. In soils amended with pig slurry, N losses via denitrification were found to vary from 1% to 30% depending on soil texture and moisture condition [9,10]. The season of slurry application into the grassland sward is also an important determinant for improving N use efficiency (NUE). Large quantities of manure are commonly applied to grasslands in the autumn and early winter when agricultural machinery access is possible. However, there is a risk of nitrate leaching during the subsequent winter period [11,12]. In contrast, summer application results in high N losses through ammonia volatilization, as a result of warmer, drier air and soil [8,13]. As an N management strategy, split applications of N are often considered more efficient and environmentally sound [12,14]. However, the positive effects of N splitting are not always evident depending on various factors, such as the rate and timing of application, growth stage and crop species, soil type, and climatic condition.

Perennial grasses in grassland systems regrow successively after harvests by cutting or grazing. The regrowth yield and nutritive value at each harvest are crucial determinants for the productivity of sward. During vegetative regrowth, soil mineral N and N reserves meet the N requirements for shoot regrowth. Thus, proper N management during the whole period of regrowth is essential to improve annual herbage yield and forage quality. A number of studies have investigated the NUE of different types of organic manures in cropping systems [15] and for forage yield [14,16]. However, the effects of different N sources and split application on regrowth yield and nutritive value of perennial grass swards have not been fully elucidated for successive regrowth periods. The present study aimed to assess the NUE of pig slurry (in comparison with chemical fertilizer) for each regrowth yield and annual herbage production and their nutritive value. The effect of split application of N was also investigated by interpreting the yield response, N residues in soil, CO_2 efflux, and NO_3^--N leaching throughout four successive regrowth cycles.

MATERIALS AND METHODS

Sites and weather condition

The present study is based on a field experiment conducted at two sites located in the west-southern upland area of South Korea (site A, E126°90′, S35°18′; site B, E126°76′, S34°97′) from February 2014 to November 2015. The experiment was carried out on a permanent grass sward, consisting mainly of perennial ryegrass (*Lolium perenne*), which was used for grass silage in the year preceding the treatment application. The two sites are located on a sandy loamy soil with the chemical properties presented in Table 1. The local climate is semi-continental with mean temperatures of 14.3°C and 14.8°C in 2014 and 2015, respectively, ranging from a low of 1.9°C in January to a high of 26.1°C in August (Figure 1). Annual rainfall was 1,288 and 1,076 mm in 2014 and 2015, respectively, with intensive precipitation during summer (June to August) (53% and 46% of annual precipitation

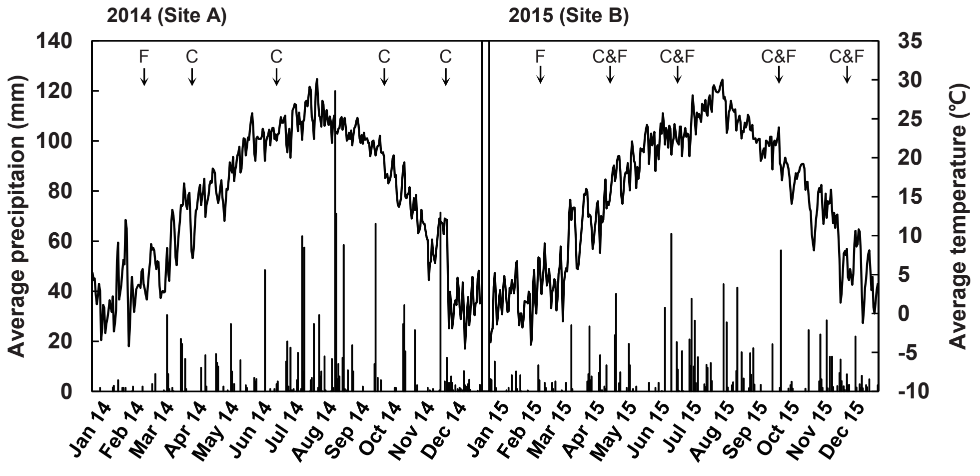

Figure 1. Mean daily temperature (solid line) and daily precipitation (vertical bars) of the experiment sites in 2014 and 2015. Arrows indicate the time of N fertilization with pig slurry or urea (F) and fertilization after cutting (C&F).

in 2014 and 2015, respectively).

Experimental design and treatments

The experiment consisted of three treatments: i) control plots that received no additional N, ii) chemical fertilizer-N as urea, and iii) pig slurry-N with five replicates. Experiments with two application methods of the N sources were carried out separately at the two sites in subsequent years with a single application in 2014 at site A and for four split applications in 2015 at site B. Each treatment plot measured 2.5 m×10 m. Adjacent plots were separated by a 2 m margin and bordered with 45 cm metal retainers pressed 30 cm deep into the soil to prevent surface runoff and contamination between plots. Pig slurry was incubated at 25°C for 2 weeks under anaerobic condition in a polyvinyl chloride container. At the time of application, subsamples of the applied slurry were analyzed. The pig slurry contained on average (kg/m^3): 1.57±0.11 total N, 0.206±0.012 NH_4^+-N, 0.218±0.017 NO_3^--N, 0.79±0.03 P, and 1.06±0.01 K with pH_{water} (1:5) of 7.8±0.02.

In 2014, a single application of N was done at site A (E126° 90′, S35°18′) with a target rate of 200 kg N/ha. For the application of pig slurry, 318 L of pig slurry, which contained 101 kg P/ha and 135 kg K/ha, was applied to the 25 m^2 plots in the early spring (February 12). For the plots of chemical fertilizer-N, an equivalent amount of N was applied as urea and P and K fertilizers were supplemented to match the amount applied by the pig slurry treatment (e.g., 1,072 g urea, 1,107 g KH_2PO_4, and 96 g K_2SO_4 in a plot). In control plots, no additional nutrients were supplied. In 2015, for the split application of N, the amount of urea and pig slurry corresponding to one fourth of the target rate of N (50 kg N/ha), including P and K, was applied in the early spring (February 15) and after the 1st, 2nd, and 3rd cuttings at site B (E126°76′, S34°97′).

The perennial ryegrass sward was cut four times in each year according to the regrowth status. The regrowth period varied from 50 to 110 days depending on the local climate (Figure 1). Overall, the most rapid regrowth occurred from May to early June. After the 2nd cut, regrowth was very slow owing to the heat and high humidity of the summer. A second peak of regrowth occurred from the end of September when temperatures began to decrease.

Herbage, soil, and leachate sampling

Soil and herbage samples, along with successive regrowth, were collected four times with five replicates in each year of the experiment. For the sampling at each regrowth period, a motor scythe was used to cut five randomly placed bands 2 m long and 0.5 m wide, leaving stubble of approximately 5 cm. The herbage mass harvested by a motor scythe was designated as the regrowth yield of each cutting time. After herbage sampling, all areas of experimental plot were cut with a motor scythe and allow to regrowth. About 500 g of the harvested herbage tissues was sliced into 2 cm long segments, lyophilized, ground, and stored in a vacuum desiccator for further analysis. Soil sampling was conducted in the same bands where herbage sampling was done. Soil cores (0- to 30-cm depth) were taken randomly with a 3-cm diameter tube auger. The collected soil samples were air-dried and fine ground to <0.15 mm. The soil properties of experimental sites were shown in Table 1. A minimum of 50 cm at the periphery of the plots was left unsampled to minimize edge effects. Three suction cups (P80, eco Tech, Bonn, Germany) in each plot were installed at a depth of 50 cm to obtain leachate samples for NO_3^--N analysis. Soil water samples were obtained by applying a tension of -240 hPa. Sampling was done twice per month, and combined for use as a representative sample of the monthly cumulated and stored at –20°C.

Measurements and chemical analysis

At each cutting time, the biomass harvested by a motor scythe from a 0.5 m×2.0 m area was converted to kg/ha. This estimate was multiplied by the N concentration determined in the subsamples in order to calculate the N recovery in herbage (kg N/ha) at a given cutting time. The values measured in unfertilized plots were used as the control to compare apparent efficiency of urea and pig slurry application because soil N was not significantly different among the three experimental plot types before application of urea or pig slurry. Thus, apparent NUE of urea-N or pig slurry-N for regrowth yield was compared using the difference method, i.e., NUE = (DM yield in urea- or in pig slurry-applied plot – dry matter [DM] yield in unfertilized control)× 100/DM yield in unfertilized control). Similarly, apparent N recovery in herbage was calculated by dividing the amount of N applied by the difference in the amount of N of the herbage between urea- or pig slurry-applied plots and unfertilized control plots, as previously described [13].

The nutritive value of the herbage harvested at each cutting time was determined with the subsamples collected for DM yield. Crude protein (CP) content was obtained by multiplying N content by 6.25. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of [17] using a Dosi-Fiber extractor (SELCTA, Barcelona, Spain). *In vitro* DM digestibility (IVDMD) was determined according to Tilley and Terry [18] using a Daisy incubator (ANKOM tech. Co, Fairport, NY, USA). Around 500 mg of herbage sample were incubated for 48 h at pH 6.8 with ruminal fluid. The substrate was fermented for a further 24 h in an acidic solution with pepsin. After fermentation, the bags were withdrawn and dried at 105°C for 8 h. The percentage difference between the initial and final DM was designated as the digestibility of the herbage sample.

Total N of soil or pig slurry samples was determined by Kjeldahl digestion. For ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) determination, the samples were extracted with 250 mL of 2 M KCl. The extracts were put in a distillation flask and steam-distilled with MgO for the NH_4^+ fraction. The samples in the flask then were distilled again after addition of

Devarda's alloy for NO_3^- determination. The liberated NH_3 was collected into H_3BO_3-indicator solution [19]. The concentration of NH_4^+-N and NO_3^--N was determined by titration with standard H_2SO_4 and converted to kg N/ha using soil bulk density concurrently determined from the soil core. The concentration of NO_3^--N in leachate was determined by ion chromatography (Dionex, DX-120, Sunnyval, CA, USA) as previously described [20].

Statistical analysis

An analysis of variance (ANOVA) was performed on data of regrowth yield, herbage N uptake, and inorganic N at each cutting time to test statistical significance of the main factors (N source and application method and of their interaction. For the variables of nutritive value (CP, NDF, ADF, and IVDMD), seasonal effect (i.e., cutting time) nested within each year was included in the model as a time-repeated factor with the polynomial option of the general linear model procedure in SAS. All analyses were performed using SAS 9.1.3 software (SAS Institute, Cary, NC, USA).

RESULTS

Regrowth yield response to N source and split application

The effects of urea and pig slurry application as the N source and split application on herbage DM yield throughout four successive regrowth cycles are presented in Table 2. N fertilization with urea and pig slurry significantly ($p<0.01$) enhanced annual herbage DM yield (sum of four cutting times) by 141% and 133%, respectively, compared to the non-fertilized control. When comparing the two N sources during regrowth cycle, DM yield in urea-applied plots was significantly ($p<0.05$) higher than that of pig slurry-applied plots for the first two regrowth periods (the 1st and 2nd cuts), while the average of apparent NUE for the 1st and 2nd cuts was 33.0% and 12.5% in urea- and pig slurry-applied plots, respectively. However, no significant ($p>0.05$) difference was observed for the latter two regrowth periods (the 3rd and 4th cuts). The DM yield in the split application was significantly lower at the 1st regrowth period, whereas it was higher at the last two regrowth periods compared to the single application (Table 2). Total annual DM yield by split application in 2015 was significantly ($p<0.05$) higher than that by single application in 2014, resulting in a significant ($p<0.05$) N source×year interaction.

Table 2. Effects of N source and split application on herbage yield (kg dry matter/ha) throughout four cycles of regrowth[1],[2]

Treatments	Regrowth period				Total
	1st	2nd	3rd	4th	
N sources					
Control	2,488^{c}	2,776^{c}	1,609^{b}	1,712^{c}	8,585^{c}
Urea	3,300^{a} (32^{a})	3,717^{a} (34^{a})	2,497^{a} (55^{a})	2,553^{b} (49^{a})	12,066^{a} (41^{a})
Pig slurry	2,915^{b} (14^{b})	3,145^{b} (13^{b})	2,495^{a} (55^{a})	2,843^{a} (40^{b})	11,397^{b} (24^{b})
LSD	176	227	172	173	438
Application method					
2014 (Non-split)	2,987^{a}	3,280^{a}	2,024^{b}	2,218^{b}	10,511^{b}
2015 (Splitting)	2,814^{b}	3,145^{a}	2,376^{a}	2,519^{a}	10,854^{a}
LSD	144	186	140	141	308
N source (N)	***	***	ns	*	**
Splitting (S)	*	ns	***	***	*
S × N	*	ns	**	**	*

LSD, least significant difference; ns, not significant.

[1] The number in parentheses is the apparent use efficiency (%) of the two N sources.

[2] Results of N source effect and splitting effect are means of two years (n = 10) and three N sources (n = 15), respectively, at each regrowth period.

Means designated by the same letter in a column do not differ significantly ($p > 0.05$) by Tukey's test.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

N recovery in herbage as affected by N source and split application

The effect of N fertilization on the N content in harvested herbage was significant ($p<0.01$) at all regrowth periods (Table 3). Total amount of N recovered in herbage during four regrowth periods increased by 168% in urea- and 158% in pig slurry-applied plots compared to the unfertilized control. When comparing the two N sources over the course of regrowth, the amount of N recovered in herbage was significantly ($p<0.05$) higher in urea-applied plots for the first two regrowth periods, with apparent N recovery of 10.8% and 6.1% on average in urea- and pig slurry-applied plots, respectively. Split application tended to increase N recovery in

Table 3. Effects of N source and split application on the amount of N recovered in herbage (kg N/ha) throughout four cycles of regrowth[1],[2]

Treatments	Regrowth period				Total
	1st	2nd	3rd	4th	
N sources					
Control	51.7^{c}	55.7^{c}	29.8^{b}	33.6^{b}	170.7^{b} (68.0^{a})
Urea	71.2^{a} (9.8^{a})	79.3^{a} (11.8^{a})	64.3^{a} (17.2^{a})	72.0^{a} (19.2^{a})	286.7^{a} (68.0^{a})
Pig slurry	63.3^{b} (5.8^{b})	68.3^{b} (6.3^{b})	64.1^{a} (17.2^{a})	74.6^{a} (20.5^{a})	270.2^{a} (49.7^{a})
LSD	6.2	6.4	5.0	6.0	13.2
Application method					
2014 (Non-split)	63.8^{a}	67.2^{a}	49.7^{b}	55.8^{b}	236.5^{b}
2015 (Splitting)	60.3^{a}	68.3^{a}	55.8^{a}	64.3^{a}	248.6^{a}
LSD	5.0	5.3	4.1	4.9	10.8
N source (N)	**	***	***	***	***
Splitting (S)	ns	ns	**	**	ns
S × N	ns	ns	ns	**	*

LSD, least significant difference; ns, not significant.

[1] The number in parentheses is the apparent N recovery (%) of the two N sources.

[2] Results of N source effect and splitting effect are means of two years (n = 10) and three N sources (n = 15), respectively, at each regrowth period.

Means designated by the same letter in a column do not differ significantly ($p > 0.05$) by Tukey's test.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

herbage, with significantly ($p<0.05$) higher N content in herbage harvested at the last two regrowth periods (Table 3). Split application of N significantly enhanced the total amount of N recovered in herbage by 5.1% compared to the single application.

Residual mineral N in soil

The effect of N fertilization on residual amounts of NH_4^+-N and NO_3^--N in soil was also significant ($p<0.01$) at all regrowth periods (Table 4). At the end of the 4th regrowth period, the total amount of residual inorganic N (sum of NH_4^+-N and NO_3^--N) in soil was 54.0 and 63.6 kg N/ha in urea- and pig slurry-applied plots, respectively, while only 21.2 kg N/ha was found in non-fertilized plots. When comparing the effect of the two N sources over the course of regrowth, residual NH_4^+-N and NO_3^--N in the urea-amended soil were significantly higher than those of pig slurry-amended soil only for the 1st regrowth period. After the 2nd regrowth period, there was no significant difference in NO_3^--N. Split application tended to enhance the availability of inorganic N in the soil (except for NH_4^+-N after the 1st regrowth period), as shown by the significantly higher NH_4^+-N in the split application from the 2nd to 4th regrowth periods and the higher NO_3^--N after the 4th regrowth period with a significant ($p<0.01$) N source×year interaction (Table 4).

Nutritive value of herbage

ANOVA results for nutritive value (i.e., CP, NDF, ADF, and IVDMD) are presented in Table 5. The effects of N source and split application on the variables were statistically poor or not significant. The significance of the N source on CP content resulted from the variation between N fertilized and non-fertilized plots rather than between urea-N and pig slurry-N. However, cutting time effects, reflecting seasonal variation, on all variables of nutritive value were significant ($p<0.001$) having the largest source of variance given this finding, changes in nutritive value of herbage harvested at each cutting time are presented separately in Figure 2.

Table 5. Analysis of variance for the descriptive parameters of nutrient values

Source	DF	CP	NDF	ADF	IVDMD
N source (N)	2	**	ns	ns	ns
Splitting (S)	1	ns	ns	ns	ns
Cutting time (C)	3	***	***	**	**
N×S	2	ns	ns	ns	ns
N×C	6	*	ns	ns	ns
S×C	3	ns	ns	ns	*
N×S×C	6	ns	ns	ns	ns

DF, degrees of freedom; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; IVDMD, in vitro dry matter digestibility; ns, not significant.
* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

The CP content in non-fertilized control plots was relatively constant within a range of 120 to 132 g/kg DM) during the four regrowth periods, while that in urea- and pig slurry-applied plots continuously increased up to 175 and 164 g/kg DM at the 4th cut, respectively (Figures 2A and 2B). In both urea- and pig slurry-applied plots, the increase in CP content was latent for the first two regrowth periods, whereas it was relatively higher for the last two regrowth periods.

The NDF content in non-fertilized control plots slightly decreased from 573 at the 1st cut to 500 g/kg DM at the 4th cut (Figures 2C and 2D). However, N fertilization tended to increase NDF, especially for the first two regrowth periods, resulting in a significant ($p<0.05$) N source effect. However, no significant difference in NDF responses to urea and pig slurry was observed (except for the 1st cut in 2014). The effect of the split application on NDF was not significant (Table 5).

Table 4. Effects of N source and split application on the residual N amount (kg N/ha) of the ammonium and nitrate fraction in the soil throughout four cycles of regrowth[1)]

Treatments	Regrowth period							
	1st		2nd		3rd		4th	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
N source								
Control	14.6^{c}	27.9^{b}	10.6^{b}	23.1^{b}	7.7^{b}	16.1^{b}	6.5^{c}	14.7^{b}
Urea	41.8^{a}	46.8^{a}	28.5^{a}	45.3^{a}	21.1^{a}	42.9^{a}	15.8^{b}	38.2^{a}
Pig slurry	29.7^{b}	34.5^{b}	30.9^{a}	42.8^{a}	26.2^{a}	44.0^{a}	23.5^{a}	38.1^{a}
LSD	5.5	5.2	3.1	4.2	2.3	3.7	2.2	4.1
Application method								
2014 (Non-split)	32.2^{a}	36.1^{a}	19.5^{b}	36.3^{a}	15.0^{b}	33.2^{a}	13.3^{b}	28.0^{b}
2015 (Splitting)	25.2^{b}	36.7^{a}	27.2^{a}	38.8^{a}	21.7^{a}	35.5^{a}	17.1^{a}	32.7^{a}
LSD	4.5	4.2	2.6	3.4	1.8	3.0	1.8	3.3
N source (N)	***	**	**	**	***	***	***	***
Splitting (S)	**	ns	***	ns	***	ns	***	**
S×N	ns	**	***	ns	***	***	***	**

LSD, least significant difference; ns, not significant.
[1)] Results of N source effect and splitting effect are means of two years (n = 10) and three N sources (n = 15), respectively, at each regrowth period.
Means designated by the same letter in the column do not differ significantly ($p>0.05$) by Tukey's test.
* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

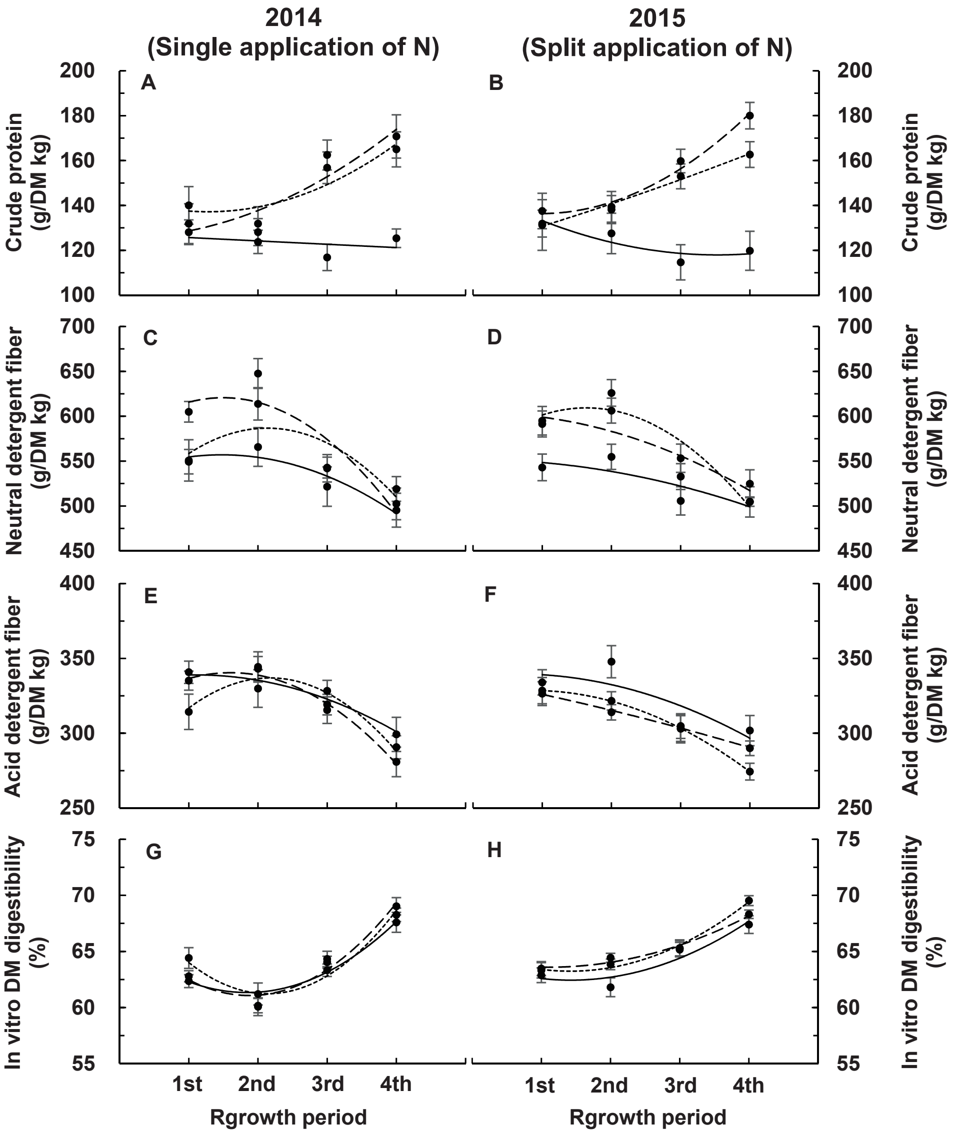

Figure 2. Changes in crude protein (A, B), neutral detergent fiber (C, D), acid detergent fiber (E, F), and *in vitro* dry matter (DM) digestibility (G, H) of herbage harvested at non-fertilized control (solid line), urea-applied (dashed line), and pig slurry-applied (dotted line) plots throughout four cycles of regrowth under single application in 2014 (A, C, E, F) and split application of N in 2015 (B, D, F, H). Each value is the mean±standard deviation for n = 5.

The ADF content was not significantly affected by N source. The ADF decreased from 345 at the 1st cut to 300 g/kg DM at the 4th cut in non-fertilized control plots, and similarly from 331 to 283 g/kg DM on average in N fertilized plots (Figures 2E and 2F). The effect of split application on NDF was also not significant.

Neither N source nor split application had significant influence on IVDMD (Table 5). Mean values of IVDMD over two years were 64.3%, 65.7%, and 65.0% in control, urea-, and pig slurry-applied plots, respectively, and over four regrowth periods were 64.5% and 65.3% in 2014 (single application) and 2015 (split application), respectively (Figures 2G and 2H). IVDMD slightly decreased or remained similar for the first two regrowth periods (62% on average of three N sources over two years), then significantly increased up to the 4th regrowth period (69.4%).

Nitrate leaching

In non-fertilized control plots, the monthly cumulative nitrate concentration in leachate was relatively constant within a range from 3.2 to 6.1 NO_3^--N mg/L over the two years of measurement, resulting in an average of 53.8 mg/L over the two years for the cumulative concentration over the whole regrowth period (Figure 3). N application increased the cumulative concentration by 222.9% and 253.9% in urea- and pig slurry-applied plots, respectively, when compared to the non-fertilized control. In 2014 (single application), nitrate concentration in the leachate sharply increased after N fertilization, maintained a plateau through spring to early summer, then peaked in July in both urea- and

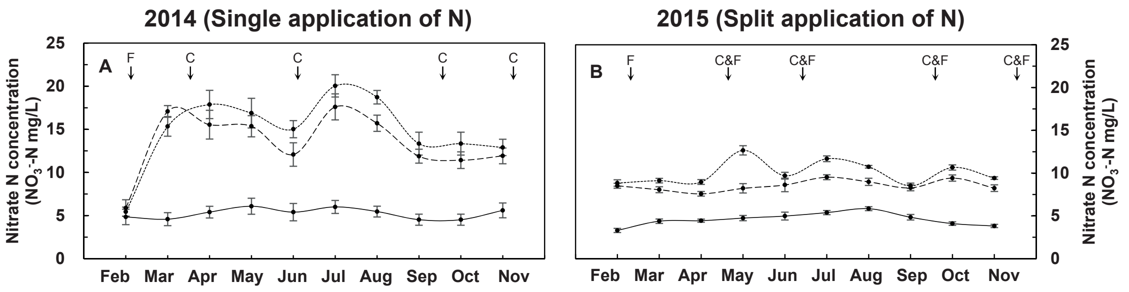

Figure 3. Changes in the monthly accumulated nitrate concentration in leachate (mg NO_3^--N/L) at non-fertilized control (solid line), urea-applied (dashed line) and pig slurry-applied (dotted line) plots under single application in 2014 (A) and split application of N in 2015 (B). Each value is the mean±standard deviation for n = 3. Arrows indicate the time of N fertilization with pig slurry or urea (F) and fertilization after cutting (C&F).

pig slurry-applied plots (Figure 3A). Split application of N in 2015 reduced the cumulative nitrate concentration by 37.5% and 33.7% in urea- and pig slurry-applied plots, respectively, compared to a single application (Figure 3B).

DISCUSSION

Regrowth yield, N recovery in herbage and residual N in soil during four successive regrowth

The N input into grassland swards increases the regrowth rate leading to higher herbage yield for a given harvest time by cutting or grazing [13,21] or more rapid attainment of a given regrowth yield [22]. In this study, annual yield response to N fertilization was 17.4 and 13.9 kg DM/kg N applied, respectively for urea- and pig slurry-applied sward. The annual yield response to pig slurry N with a four-cut regime was very similar to that (13.4 kg DM/kg N applied) of cattle slurry in a three-cut regime [13], or less than that (15 kg DM/kg N applied) of the average of different cutting frequency [23]. The present study showed that pig slurry was a poorer N source for the earlier two regrowth periods, but it was equal or better than urea for the latter regrowth periods, as shown by the significantly lower regrowth yield and apparent N recovery in herbage for the 1st and 2nd regrowth periods in pig slurry-applied plots than those of urea-applied plots (Tables 2 and 3). Similar results were observed for grass in a pot experiment [24] and grassland sward [13]. These may reflect a common characteristic of organic manure which releases plant available N more slowly than chemical fertilizers. Indeed, the residual ammonium-N and nitrate-N amounts in soil after the 1st cut in slurry-applied plots was significantly less than those of urea-applied plots (Table 4). The N uptake gradually increased with the progression of the cutting in perennial ryegrass swards applied with cattle slurry throughout three cycles of regrowth [13]. Similarly, it was estimated by ^{15}N tracing that more N was released from applied organic amendments and beneficial effects on N uptake and growth in annual crops (e.g., Chinese cabbage) were more distinct in the latter growth period than the earlier 30 days [23,25]. The relatively lower N recovery in herbage for the earlier regrowth period in pig slurry-applied plots was possibly due to insufficient mineralization of organic N and partially to the immobilization of ammonium. It has been reported that soils receiving organic N input released inorganic nitrogen in a gradual manner depending on the mineralization process [15].

Synchronization of the supply of plant available N and crop N demand is essential to optimize N utilization and it can be improved by split application of N, as demonstrated in various crops [12,14]. The present study confirmed the positive effect of split application of N on annual herbage DM yield (+6.9% compared to single application with full N dose) and N uptake in herbage (+8.7%), resulting from a significant ($p = 0.005$ for herbage yield and $p = 0.013$ for N uptake) N source×year interaction (Tables 2 and 3). These benefits of N splitting were significant for the two latter regrowth periods (i.e., the 3rd and 4th cut). This result may be attributed mainly to the increased availability of ammonium-N from the 2nd cut by split application of N (Table 4). This result suggested that the need for additional N fertilization was apparently less for the earlier two regrowth periods, as the plant available N is sufficient through mineralization of organic N in pig slurry or mineral N itself.

Ruminant nutrition of herbage during four successive regrowth

The response of CP content of grasses is nearly linear up to the levels of N applied [26]. In the present study, it was estimated that N fertilization with urea and pig slurry resulted in an increase in annual CP yield of 15.2 and 12.4 kg/ha/kg N applied, respectively, when compared to non-fertilized plots. However, the N source as urea or pig slurry and split application had less influence on the CP content in most regrowth periods (Figures 1A and 1B).

Cell wall fraction of the forage refers to the insoluble portion (NDF) which contains cellulose, hemicellulose, lignin, and silica [27]. The most commonly isolated fraction of the forage cell wall is the ADF (cellulose and lignin), because ADF has been shown to be negatively correlated with the digestibility of forage. In the present study, a significant effect of N fertilization on NDF content was found only for the earlier two regrowth periods as shown by lower NDF in non-fertilized plots. In addition, N sources of either urea or pig slurry and split N application effects on NDF and ADF were not significant through four successive regrowth cycles (Table 5). Several studies have shown that there is a significant relationship between accumulated forage mass and nutritive value [28,29]. However, in this study, a poor relationship between herbage mass and NDF or ADF was found (data not shown). Indeed, NDF and ADF remained relatively constant compared to the DM yield response to N source and split application throughout four regrowth periods, consistent with previous findings [30,31].

The present study, however, indicated that nutritive value of herbage harvested at each cut was more affected by seasonal impacts rather than N source or split N application. Overall, lower CP content in spring to early summer harvest (the 1st and 2nd cut in this study) than those of autumn harvest (the 3rd and 4th cut) were found and a reversed pattern in NDF and ADF were observed (Figure 2). Pontes [21] reported a seasonal pattern in the nutritive value over thirteen native grasses. It has been clearly established that cell wall formation is a stronger sink for assimilates at high rather than at low temperatures [29,32]. Therefore, it was suggested that cell wall content increased along with the increase in DM accumulation (Table 2 and Figures 2C, 2D, 2E, and 2F), resulting in a lower IVDMD (Figures 2G and 2H) during the 1st and 2nd regrowth periods because more assimilates are available for cell wall formation than for cellular compound synthesis [27] with the gradual increase in daylight and temperature.

The seasonal pattern of IVDMD determined in this study was consistent with that of previous work [21,29]. The decrease in DM digestibility with the progress of the spring season could be associated with high temperature, which tends to increase lignin content and decrease in cell wall digestibility [27,33]. The progressive increase in DM digestibility in the autumn harvest, the 3rd and 4th cut in this study, might be owing to less deposition of lignified material [33], which in turn leads to a compensatory accumulation of non-structural carbohydrates [21] and cellular compounds [32,29] as temperature decreased.

Nitrate leaching

The NH_4^+-N in mineral N fertilizer or organic manure is rapidly nitrified in soil after application [5-7]. The present study showed that pig slurry-N tended to cause slightly higher nitrate leaching compared to urea-N (Figure 3) which might be caused by the asynchrony of crop N demand and the release of the organically bound N [11,13]. In the single N application, nitrate concentration in the leachate peaked in July in both urea- and pig slurry-applied plots (Figure 3A), reflecting the increased percolation of water by higher precipitation (Figure 1) and higher availability of inorganic N (Table 4). In addition, the monthly accumulated nitrate concentration in leachate at a given sampling time did not exceed the threshold value of 50 mg/L of Korean or European Union ground water directives [34]. In this study, it was estimated that split application of N resulted in a reduction of 35%, suggesting that N splitting can improve the synchronization of the supply of plant available N and crop N demand in accordance with results previously reported for various crops [12,14]. In this context, although the present data do not support the positive effects of N splitting for the earlier regrowth periods, the split application of pig slurry-N or urea-N should be considered to reduce the risk of nitrate leaching during subsequent seasons, especially from early summer when rainfall is intensive and plant NUE is severely depressed by high temperatures in Korea.

IMPLICATIONS

Pig slurry-N supplied by a single application with 200 kg N/ha at early spring or by the split application at each regrowth can be efficiently used for four successive regrowth periods of perennial ryegrass sward without hazardous ground water pollution by nitrate leaching. When compared to urea, the positive effect of pig slurry on regrowth yield and N recovery in herbage was equal or better than urea only for the last regrowth periods (the 3rd and 4th cut). N source and/or split application have less or no direct influences on ruminant nutritive value, whereas the seasonal pattern of nutritive value was distinct, as shown by the significantly higher quality in the autumn harvest. Split application of urea-N or pig slurry-N effectively alleviated the loss of N via nitrate leaching. However, this study failed to explain a significant corresponding increase in apparent NUE, especially for later regrowth. Thus, for further study, 15N tracing would be powerful method for investigating the long-term fate of N derived from organic manure to determine the mineralization dynamics, NUE for DM accumulation, N loss of ammonia volatilization, denitrification, and microbial immobilization.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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