Nutritive Value of Timothy Fertilized with Chloride or Chloride-Containing Liquid Swine Manure

S. Pelletier,*¹ G. F. Tremblay,* G. Bélanger,* M. H. Chantigny,* P. Seguin,† R. Drapeau,‡ and G. Allard§

*Agriculture and Agri-Food Canada, Soils and Crops Research and Development Centre, Québec, Canada G1V 2J3

†Department of Plant Science, Macdonald Campus, McGill University, Sainte-Anne-de-Bellevue, Canada H9X 3V9

‡Agriculture and Agri-Food Canada, Soils and Crops Research and Development Centre, Normandin, Canada G1M 4K3

§Département de phytologie, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec, Canada G1K 7P4

ABSTRACT

Chloride fertilization of timothy (*Phleum pratense* L.) decreases forage dietary cation-anion difference to an acceptable value [<250 mmol_c/kg of dry matter (DM)] for dry dairy cows (*Bos taurus*). However, high Cl concentrations in forages as a result of fertilization might affect nutritive value. Two experiments were used to evaluate the effects of chloride fertilization on timothy spring growth and summer regrowth by determining concentrations of crude protein and neutral detergent fiber (NDF), in vitro true digestibility of DM (IVTD), and in vitro digestibility of NDF (dNDF). In an inorganic fertilization experiment, forages grown at 4 locations were fertilized with CaCl₂ (0, 80, 160, and 240 kg of Cl/ha per yr) or NH₄Cl (160 kg of Cl/ha per yr) in combination with 2 N application rates (70 and 140 kg of N/ha per yr). The increase in Cl fertilization rate affected forage NDF concentration (+1.4%), IVTD (−0.8%), and dNDF (−1.2%) only at the highest rate of N fertilization, but this effect was not of biological importance. Crude protein concentration was not affected by Cl fertilization. Both Cl fertilizer types had a similar impact on forage nutritive value. In an organic fertilization experiment, forages grown at 2 locations received 1 of 7 experimental treatments [unfertilized control, inorganic fertilizer, raw liquid swine manure (LSM), and liquid fractions of 4 pretreated LSM types (decanted, filtered, anaerobically digested, and flocculated)] that provided, respectively, 0, 60, 41, 44, 44, 36, and 101 kg of Cl/ha per yr. The last 6 fertilizer treatments also provided 140 kg of N/ha per yr. The IVTD, dNDF, and concentration of NDF in timothy forage were not affected by the Cl content of the different LSM types. Nitrogen fertilization increased concentration of forage NDF and decreased IVTD and dNDF, but this effect was not biologically important. In both

experiments, soil types and harvests had a negligible effect on forage nutritive value. Organic or inorganic Cl fertilizers applied to decrease timothy dietary cation-anion difference have little or no effect on forage nutritive value.

Key words: digestibility, *Phleum pratense*, chloride fertilization, liquid swine manure

INTRODUCTION

Chloride is an important determinant of the DCAD, a concept used to determine the susceptibility of a ration to cause postcalving hypocalcaemia in dairy cows (Pelletier et al., 2007a,b, 2008). The DCAD can be calculated with equations that include the cations K⁺ and Na⁺ and the anions Cl[−] and SO₄^{2−} (Ender et al., 1971; Goff et al., 2004). Pelletier et al. (2007b, 2008) report that applications of inorganic Cl fertilizers to forage grasses reduce their DCAD by as much as 349 mmol_c (millimoles of charge)/kg of DM depending on the soil type, growth period, forage species, and the maturity of the sward. Forages can therefore be produced with DCAD levels that are acceptable for dry dairy cows. Liquid swine manure (LSM) with a high Cl content also proved to be effective in reducing forage DCAD (Pelletier et al., 2008).

Decreases in forage DCAD following Cl or LSM fertilization are the result of an increase in forage Cl concentration (Pelletier et al., 2007a,b, 2008). With an increased application from 0 to 240 kg of Cl/ha, Cl concentration of timothy forage increased by 8.5 and 15.1 mg/g of DM for forages grown on Canadian and Australian soils, respectively. In both cases, the greatest forage Cl concentration observed was close to 20 mg/g of DM. Nonwoody species, such as timothy, can generally tolerate plant Cl concentrations as high as 15 to 50 mg/g of DM (Xu et al., 2000). The NRC (2001) reports that the average Cl concentration of forage is 6.6 mg/g of DM. The effect of high forage Cl concentrations, as observed by Pelletier et al. (2007a,b, 2008), on the nutritive value of timothy has never been investigated.

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¹Corresponding author: pelletiers@agr.gc.ca

Although the effects of Cl fertilization are site-specific and vary during the growing season, Cl fertilization may have an impact on grass development (Fixen, 1993). Chloride plays a role in several processes associated with DM production, including enzyme activation, phloem loading and unloading of sugars, interactions with N metabolism, and the increases in cell hydration and turgor pressure that support cell expansion and elongation (Fixen, 1993; Taiz and Zeiger, 1998; Xu et al., 2000; Britto et al., 2004).

Although Cl fertilization up to 240 kg of Cl/ha did not affect timothy DM yield (Pelletier et al., 2007a), mechanisms affected by Cl (e.g., N metabolism, cell expansion and extension) may have, in turn, affected forage digestibility and concentrations of CP and NDF. To our knowledge, there are no reports that document the effects of Cl fertilization on forage digestibility and concentrations of CP and NDF. The objective of our study was to document the effects of applying organic and inorganic Cl fertilizers on forage CP and NDF concentrations, in vitro true digestibility of DM (IVTD), and in vitro digestibility of NDF (dNDF) of timothy grown on different soils and harvested twice during the growing season.

MATERIALS AND METHODS

Study Sites and Experimental Setup

Inorganic Fertilization Experiment. This experiment is described in detail by Pelletier et al. (2007a). Briefly, timothy (*Phleum pratense* L., cv. Champ) was sown in 1998 at Sainte-Perpétue (46°05' N, 72°28' W), and in 2002 at Sainte-Anne-de-Bellevue (45°24' N, 73°57' W), Normandin (48°51' N, 72°32' W), and Saint-Augustin-de-Desmaures (46°44' N, 71°27' W), Quebec, Canada. In 2003 and 2004, 10 fertilizer treatments were applied (0, 80, 160, and 240 kg of Cl/ha as CaCl_2 ; 160 kg of Cl/ha as NH_4Cl ; all combined with N fertilization to provide 70 or 140 kg of N/ha as NH_4NO_3 for CaCl_2 treatments, or a mix of NH_4NO_3 and NH_4Cl for NH_4Cl treatment) in a split application: 60% before the start of spring growth and 40% after the first harvest. Plots were harvested twice during the growing season when timothy reached the late-heading stage of development. The second harvest was taken approximately 7 wk after the first one. Plots were harvested to a 5-cm height with a self-propelled flail forage harvester (Carter MGF Co., Inc., Brookston, IN) at Normandin and Sainte-Perpétue and with a REM flail forage harvester (Swift Machine & Welding, Swift Current, Saskatchewan, Canada) at Sainte-Anne-de-Bellevue and Saint-Augustin-de-Desmaures.

Average DM yields for spring growth and summer regrowth were, respectively, 3.70 and 1.45 Mg/ha at

Sainte-Anne-de-Bellevue (hereafter called Sainte-Anne), 3.76 and 2.58 Mg/ha at Normandin, 4.22 and 3.34 Mg/ha at Saint-Augustin-de-Desmaures (hereafter called Saint-Augustin), and 1.96 and 1.49 Mg/ha at Sainte-Perpétue. Details of DM yields are provided in Pelletier et al. (2007a).

LSM Fertilization Experiment. The study sites and experimental setup were part of a larger experiment described in detail by Chantigny et al. (2007). Timothy was sown in 2000 at Saint-David-de-Lévis (46°48' N, 71°23' W) and at Saint-Lambert-de-Lévis, Quebec, Canada (46°05' N, 71°02' W). In 2001 and 2002, 7 fertilization treatments were applied: 4 pretreated LSM (decanted, filtered, digested, and flocculated), 1 raw LSM, 1 inorganic fertilizer, and 1 unfertilized control.

Raw LSM was obtained during the winters of 2001 and 2002 from a commercial swine finishing operation. Part of the collected LSM was transferred into a batch anaerobic digester. After standing for 1 mo in the batch digester, anaerobically digested LSM was transferred into a 1-m³ plastic container and labeled "digested LSM". The rest of the collected LSM was stored for 6 wk in four 1-m³ plastic containers. After this period, the upper half of the raw LSM was pumped out of 2 plastic containers and transferred to an empty 1-m³ container. This liquid material was labeled "decanted LSM" and represented the clarified fraction of raw LSM after the natural settling of solids. Manure from a third plastic container was strained through a bed of wood shavings and sawdust. The filtrate was collected in a plastic container and labeled "filtered LSM". A fifth LSM type was obtained from another commercial swine finishing operation. This manure was chemically treated with a CaCl_2 -based coagulant to remove solids, and 1 m³ of the liquid fraction was collected in a plastic container and labeled "flocculated LSM". Selected characteristics of the LSM are presented in Table 1.

Fertilizer treatments were split in 2 applications to provide 80 kg of total N/ha in early spring (between May 8 and 22) and 60 kg of total N/ha 3 to 6 d after the first harvest (between June 18 and 25). Therefore, the average Cl provided by the fertilization treatments each growing season was 0 kg/ha for the unfertilized control, 60 kg/ha for the inorganic fertilizer, 41 kg/ha for the raw LSM, 44 kg/ha for the decanted and the filtered LSM, 36 kg/ha for the digested LSM, and 101 kg/ha for the flocculated LSM. The various LSM types were continuously stirred in the containers during field applications. At both locations, plots of the inorganic fertilizer treatment also received 50 kg of P_2O_5 /ha applied as triple superphosphate and 80 kg of K_2O /ha applied as KCl, following regional recommendations.

Table 1. Characteristics of the different liquid swine manures (LSM) in 2001 and 2002

Characteristic	LSM type				
	Raw	Decanted	Filtered	Digested	Flocculated
2001					
pH	7.5	7.8	7.9	8.2	7.7
	kg/m ³				
DM	41.6	24.0	28.4	15.2	17.2
Total C	22.4	17.3	17.3	9.1	10.6
Total N	5.54	5.35	5.24	4.85	4.42
K	2.78	2.74	2.52	2.33	4.34
Cl	1.60	1.52	1.51	0.41	4.03
2002					
pH	7.2	7.4	7.6	8.4	7.7
	kg/m ³				
DM	45.5	29.5	32.5	14.7	14.2
Total C	24.8	19.6	21.0	7.2	7.9
Total N	5.35	4.99	4.95	4.47	2.79
K	2.42	2.49	2.34	2.34	2.11
Cl	1.46	1.60	1.42	1.05	2.54

Plots were harvested twice to a 5-cm height when timothy reached the early-heading stage of development with an REM flail forage harvester (Swift Machine & Welding) at Saint-Lambert-de-Lévis and with a self-propelled flail forage harvester (Carter MGF Co. Inc.) at Saint-David-de-Lévis. The second harvest was taken approximately 6 wk after the first one. Average DM yields for spring growth and summer regrowth were, respectively, 3.66 and 2.77 Mg/ha at Saint-Lambert-de-Lévis (hereafter called Saint-Lambert), and 5.66 and 2.05 Mg/ha at Saint-David-de-Lévis (hereafter called Saint-David).

Chemical Analyses

Manure. For each field application of manure, a 2-L composite sample was collected from each LSM type for analysis. Samples were homogenized with a Polytron homogenizer (model PT 3100, Kinematica AG, Littau-Lucerne, Switzerland); pH was then measured directly by reading with a glass electrode. Dry matter content was determined as the weight of materials remaining after drying 100 mL of homogenized LSM at 55°C for 96 h. Total C concentrations were measured in the homogenized LSM samples by direct injection in an automated combustion C analyzer (model Formacs, Skalar Analytical, De Breda, the Netherlands).

Nitrogen and K concentrations of the various LSM types were determined by acid digestion modified from the method of Isaac and Johnson (1976). Nitrogen was measured with an automated continuous-flow injection analyzer (model QuikChem 8000 FIA+, Zellweger Analytics Inc., Lachat Instruments, Milwaukee, WI) as described in Chantigny et al. (2007), and K was deter-

mined by flame emission with a Perkin Elmer 3300 atomic absorption spectrometer (Perkin Elmer, Überlingen, Germany). Finally, Cl was extracted by mixing 5 mL of the LSM sample with 20 mL of distilled water for 30 min; extracts were centrifuged at $32,570 \times g$ for 10 min and filtered, and Cl concentration was determined in the supernatant by chromatography as previously described by Pelletier et al. (2007a).

Plants. For both experiments, sample preparation and N analyses are described in Pelletier et al. (2007a). Briefly, a fresh forage sample of approximately 500 g was taken from each plot, weighed, and dried at 55°C in a forced-draft oven for 2 d (LSM fertilization experiment) or 3 d (inorganic fertilization experiment) to determine the DM concentration. Samples were then ground using a Wiley mill (standard model 3, Arthur H. Thomas Co., Philadelphia, PA) to pass through a 1-mm screen. Nitrogen was extracted using a method adapted from Isaac and Johnson (1976) and measured as described for manure extracts. Crude protein was determined by multiplying the forage N concentration by 6.25.

For the LSM fertilization experiment, all forage samples were analyzed chemically. The NDF concentration was determined using the Ankom Fiber Analyzer (Ankom Technology Corp., Fairport, NY). The IVTD was measured using the method of Goering and Van Soest (1970) based on a 48-h incubation with buffered rumen fluid followed by an NDF determination of the postdigestion residues. The rumen fluid incubation was performed with Ankom F57 filter bags and an Ankom Daisy II incubator, using the batch incubation procedures outlined by Ankom Technology Corp. Rumen fluid was obtained from a lactating, ruminally fistulated dairy

cow that was offered a diet of good quality silages [40% timothy, 60% alfalfa (*Medicago sativa* L.)], corn (*Zea mays* L.) grain, and a concentrate mix. The diet was formulated to meet the nutritional requirements of a 636-kg lactating cow producing 9,580 kg of milk per year.

The IVTD (g/kg of DM) and dNDF (g/kg of NDF) were calculated as follows:

$$\text{IVTD} = [1 - (\text{postdigestion dry weight following NDF wash/predigestion dry weight})] \times 1,000,$$

$$\text{dNDF} = [1 - (\text{postdigestion dry weight following NDF wash/predigestion dry weight of NDF})] \times 1,000.$$

For the inorganic fertilization experiment, milled samples were scanned using a near-infrared spectrophotometer (Foss NIRsystems 6500, Foss, Silver Spring, MD). Reflectance from 400 to 2,500 nm at 2-nm intervals was collected from all samples. The near infrared spectroscopy calibration equations were developed using a modified least squares regression method of the WinISI III software (Infrasoft International LLC, State College, PA). The 160 spectra with the greatest relative significance were selected from all samples of the 2 production years and the 4 locations. Of these samples, 140 were selected for the calibration set, and the other 20 samples were selected for the validation set. The 160 selected samples were analyzed in duplicate for NDF concentration and IVTD as previously described. The calibration equations were selected based on standard errors of prediction bias corrected, and R^2 for the validation set; these statistics were, respectively, 0.949, 0.103, and 0.97 for the NDF concentration, and 2.029, -1.02, and 0.92 for the IVTD. The NDF concentration and IVTD were predicted in all samples using the selected calibration equations. The dNDF (g/kg of NDF) was then calculated as follows:

$$\text{dNDF} = \{1,000 - [(1,000 - \text{IVTD}) / \text{predigestion NDF concentration}]\}.$$

Statistical Analyses

Data of the inorganic fertilization experiment were analyzed by ANOVA as a split-split-plot design with 4 replicates. Locations were assigned as main plots, fertilizer treatments as subplots, and harvests as sub-subplots; sources of variation are presented in Table 2. The experimental design at each location of the LSM experiment was a randomized complete block with 4 replicates; sources of variation are presented in Table 3.

For both experiments, production years and replicates within locations were considered to be random effects, and harvests within years were considered to be repeated measurements. Data were analyzed using the MIXED procedure (Littell et al., 1996) with the Repeated option of SAS (SAS Institute Inc., 1999). In both experiments, plots were not rerandomized each year. Years were not treated as repeated measurements because there was no residual effect of Cl and N fertilizations in the spring of the second year. Indeed, soil Cl, NO_3 , and NH_4 contents from the inorganic fertilization experiment and soil NO_3 content from the LSM fertilization experiment at the beginning of the second year were not significantly different among fertilization treatments. Statistical significance was postulated at $P \leq 0.05$. Least squares means and standard error of the means (SEM) were calculated. Averaged SEM allowing comparison of values within fertilization treatments (Tables 2 and 3) and within the interaction location $\times$ harvest (Figure 1) were calculated from SEM given for these sources of variations in the difference of least squares means output of SAS. Contrasts defined a priori were performed on treatments of the inorganic fertilization experiment. Comparisons of least squares means from the LSM fertilization experiment were carried out using the predicted difference (PDIFF) option of SAS.

RESULTS

Inorganic Fertilization

Chloride fertilization treatments significantly affected NDF concentration, IVTD, and dNDF of timothy (Table 2). Chloride fertilization significantly increased NDF concentration ($P = 0.040$) and decreased IVTD ($P = 0.026$) and dNDF ($P = 0.028$) but only at the greatest application of N fertilization (Table 2). These variations, as a proportion of values when no Cl was applied, remained small (from 1.2 to 1.9%) for all variables. Moreover, when analyzed for each location, the linear effect of Cl fertilization on NDF concentration was not significant at any of the locations, and the quadratic effect of Cl fertilization on IVTD and dNDF was significant only at Sainte-Perpétue (IVTD, $P = 0.035$; dNDF, $P = 0.026$; data not shown). The CP concentration was not affected by Cl fertilization. Both types of Cl fertilizer (CaCl_2 vs. NH_4Cl) had the same effect on the nutritive value of timothy (Table 2).

Increasing N fertilization generally increased CP and NDF concentrations ($P < 0.001$) and decreased IVTD and dNDF ($P < 0.001$) of timothy forage (Table 2). The significant interaction between fertilization treatments and locations for NDF concentration and IVTD (Table 2) was due to N fertilization. Variations in forage NDF concentration and IVTD with increasing N fertilization

Table 2. Crude protein and NDF concentrations, in vitro true digestibility of DM (IVTD), and in vitro digestibility of NDF (dNDF) of forage from predominantly timothy swards subjected to 10 inorganic fertilizer treatments (seasonal applications) and harvested at late heading, in Québec, Canada (mean values over 4 locations, 2 harvests, and 2 production years)

	Cl application	N application	CP	NDF	IVTD	dNDF
	kg of Cl/ha	kg of N/ha	g/kg of DM		g/kg of NDF	
Fertilizer treatment						
CaCl ₂ + NH ₄ NO ₃	0	70	124	573	830	705
	80	70	124	575	835	715
	160	70	125	573	830	705
	240	70	125	576	829	705
	0	140	142	578	825	700
	80	140	140	585	821	696
	160	140	137	588	815	687
	240	140	140	584	820	693
NH ₄ Cl + NH ₄ NO ₃	160	70	125	579	830	708
	160	140	140	586	819	693
SEM ¹			1.8	3.4	3.6	5.0
<hr/>						
<i>F</i> -values						
Sources of variation	df					
Locations (L)	3		12.84***	42.72***	52.87***	74.53***
Fertilization (F)	9		38.97***	4.98***	6.19***	5.75***
L × F	27		1.49	1.55*	1.56*	1.5
Harvests (H)	1		4.25*	798.65***	17.18***	7.47**
L × H	3		85.73***	85.75***	18.04***	5.74***
F × H	9		1.91	2.86**	1.17	0.91
L × F × H	27		0.4	0.33	0.49	0.45
Contrasts						
N1 vs. N2 ²			***	***	***	***
CaCl ₂ linear at N1						
CaCl ₂ linear at N2				*		
CaCl ₂ quadratic at N1						
CaCl ₂ quadratic at N2					*	*
CaCl ₂ vs. NH ₄ Cl at N1						
CaCl ₂ vs. NH ₄ Cl at N2						

¹Standard error of the mean for comparing values within a column (n = 64, df = 280).

²N1 = 70 kg of N/ha; N2 = 140 kg of N/ha; N applied as NH₄NO₃.

P* < 0.05; *P* < 0.01; ****P* < 0.001.

were greater at Sainte-Anne (+14.5 g/kg of DM for NDF; −12.2 g/kg of DM for IVTD) and Saint-Augustin (+15.4 g/kg of DM for NDF; −24.5 g/kg of DM for IVTD) than at Normandin (−0.5 g/kg of DM for NDF; −2.6 g/kg of DM for IVTD) and Sainte-Perpétue (+6.0 g/kg of DM for NDF; −3.1 g/kg of DM for IVTD). The effect of N fertilization on forage NDF concentration also varied with harvests (Table 2); the increase in NDF concentration with increasing N application rates was generally more important in summer regrowth than in spring growth (data not shown).

Forage CP and NDF concentrations, IVTD, and dNDF were affected by an interaction between locations and harvests (Table 2; Figure 1). The CP concentration was greatest at Normandin in spring growth and at Sainte-Perpétue in summer regrowth (Figure 1A). Neutral detergent fiber concentration was greatest at Saint-Augustin in spring growth and at Normandin and Saint-Augustin in summer regrowth (Figure 1B). The

IVTD (Figure 1C) and dNDF (Figure 1D) were greatest at Saint-Anne and Normandin in spring growth and greatest at Sainte-Anne only in summer regrowth. Variations with harvests and locations were, on average, 45 g/kg of DM for CP, 73 g/kg of DM for NDF concentration, 75 g/kg of DM for IVTD, and 109 g/kg of NDF for dNDF (Figure 1). Proportionally, these variations remained small for NDF concentration, IVTD, and dNDF (9 to 14%), but were larger for CP (28%).

LSM Fertilization

The flocculated LSM, which had the greatest Cl content of all LSM types (Table 1), did not affect forage NDF concentration, IVTD, and dNDF compared with the other LSM types (Table 3). Timothy fertilized with LSM had greater CP and NDF concentrations and generally lower IVTD and dNDF than the unfertilized control forage. On the other hand, timothy fertilized with

Table 3. Crude protein and NDF concentrations, in vitro true digestibility of DM (IVTD), and in vitro digestibility of NDF (dNDF) of timothy unfertilized (control), or fertilized with inorganic fertilizers, a raw liquid swine manure (LSM), or liquid fractions of 4 pretreated LSM (decanted, filtered, digested, and flocculated) and harvested at early heading, in Québec, Canada (mean values over 2 harvests, 2 locations, and 2 production years)

Treatment		CP	NDF	IVTD	dNDF
		g/kg of DM		g/kg of NDF	
Unfertilized control		120 ^a	521 ^a	858 ^c	731 ^c
Inorganic fertilizer		146 ^d	548 ^b	840 ^a	709 ^a
Raw LSM		131 ^b	541 ^b	849 ^b	723 ^{bc}
Decanted LSM		137 ^c	540 ^b	848 ^b	719 ^b
Filtered LSM		135 ^{bc}	546 ^b	847 ^{ab}	719 ^b
Digested LSM		135 ^{bc}	543 ^b	846 ^{ab}	717 ^{ab}
Flocculated LSM		138 ^c	544 ^b	847 ^{ab}	718 ^{ab}
SEM ¹		2.8	5.5	3.6	5.1
<i>F</i> -values					
Source of variation	df				
Locations (L)	1	85.28***	48.58***	17.63***	0.38
Fertilization (F)	6	16.17***	5.27***	4.62***	3.57**
L × F	6	0.29	0.56	1.11	2.06
Harvests (H)	1	7.77**	213.18***	32.38***	7.71**
L × H	1	75.94***	91.38***	45.71***	23.44***
F × H	6	9.06***	5.57***	1.56	0.95
L × F × H	6	1.06	0.99	0.51	0.36

^{a-d}Means within a column with different letters differ ($P < 0.05$).

¹Standard error of the mean allows comparison of values within a column ($n = 32$, $df = 98$).

** $P < 0.01$; *** $P < 0.001$.

LSM had lower CP concentration, especially with the raw LSM, and generally greater IVTD and dNDF than forage fertilized with the inorganic fertilizer (Table 3).

The effect of fertilization treatments on forage CP and NDF concentrations varied with harvests (Table 3). The CP concentration was generally similar or lower in summer regrowth than in spring growth, except for the unfertilized control timothy, which had greater CP concentration in summer regrowth (data not shown). On the other hand, NDF concentration was smaller in summer regrowth than in spring growth for all treatments, but the difference between harvests was greater for the unfertilized control timothy.

Concentrations of CP and NDF, and IVTD and dNDF of timothy, were affected by an interaction between locations and harvests (Table 3; Figure 1). From spring growth to summer regrowth, CP concentration decreased in timothy grown at Saint-Lambert and increased in forage grown at Saint-David; on average for both harvests, CP concentration remained greater in forage grown at Saint-Lambert (Figure 1A). The NDF concentration was greater in forage grown at Saint-David, especially in spring growth (Figure 1B). Finally, forages grown in spring at Saint-David had lower IVTD (Figure 1C) and dNDF (Figure 1D) than those grown at Saint-Lambert. In summer regrowth, IVTD and dNDF of forages grown at Saint-David were greater

than those of forages grown at Saint-Lambert. These variations over harvests and locations were of 47 g/kg of DM for NDF concentration, 16 g/kg of DM for IVTD, 2.5 g/kg of NDF for dNDF, and 31 g/kg of DM for CP. Similar to the inorganic fertilization experiment, these variations remained small for NDF, IVTD, and dNDF (3 to 8%) but were greater for CP concentration (20%).

DISCUSSION

Timothy fertilized with an inorganic fertilizer or LSM, whether high or low in Cl, and harvested in spring growth had NDF concentration, IVTD, and dNDF similar to those observed in other studies conducted in eastern Canada (Bélanger and McQueen, 1996, 1998; Claessens et al., 2004).

Chloride Fertilization

The effect of Cl fertilization on forage NDF concentration and digestibility of DM and NDF (Figure 2), although small, was surprising. Plants contain several compounds with covalently bound Cl (White and Broadley, 2001), but this element is biochemically inert (Fixen, 1993). Forage digestibility (Claessens et al., 2004) and Cl concentration (Xu et al., 2000) are both related to plant growth. Plant Cl concentration may improve plant growth because its accumulation in-

Figure 1. Forage CP concentration (A), NDF concentration (B), in vitro true digestibility of DM (IVTD; C), and in vitro digestibility of NDF (dNDF; D) for spring growth (black bars) and summer regrowth (gray bars) at each location of the inorganic fertilization experiment [Sainte-Anne (Ste-A), Normandin (Norm), Saint-Augustin (St-Aug), Sainte-Perpétue (Ste-P)] and the liquid swine manure (LSM) fertilization experiment [Saint-Lambert (St-Lamb), Saint-David (St-Dav)]. Values are averages for 10 fertilization treatments for the inorganic fertilization experiment (seasonal applications of 0, 80, 160, and 240 kg of Cl/ha as CaCl_2 , 160 kg of Cl/ha as NH_4Cl ; all combined with 70 or 140 kg of N/ha as NH_4NO_3 or a mix of NH_4NO_3 and NH_4Cl), 7 fertilization treatments for the LSM fertilization experiment (unfertilized control, inorganic fertilizer, raw LSM, and decanted, filtered, digested, and flocculated LSM), and 2 production years for the inorganic fertilization experiment (2003 and 2004) and the LSM fertilization experiment (2001 and 2002). Vertical dashed lines delineate the results of the 2 experiments.

creases cell hydration and turgor pressure needed for cell expansion and elongation (Xu et al., 2000), whereas the production of cellulose, hemicellulose, and lignin, which are NDF components, is needed to maintain constant wall thickness during cell expansion (Carpita and McCann, 2000). Thus, cell expansion and continued deposition of NDF polymers into the cell wall must be tightly integrated events. Therefore, high Cl concentration could be associated with increased cell expansion and plant growth which could, in turn, be associated with increased deposition of NDF components.

Forages in the inorganic Cl fertilization experiment had greater Cl concentration than the minimal require-

ment for crop growth (1 g/kg of DM; White and Broadley, 2001). However, in some treatments, especially those with the lowest application rate of Cl, forage Cl concentrations were close to this limit (1.3 g/kg of DM; Pelletier et al., 2007a). These low Cl concentrations may have affected plant growth and NDF deposition, explaining the lower NDF concentration and generally greater IVTD and dNDF of forages not fertilized with Cl.

The effect of Cl in the inorganic fertilization experiment at the greatest N application rate could be due to interactions between Cl and N in their biochemical functions. Indeed, it is known that Cl plays a role in N

Figure 2. Relationship between Cl fertilization [organic (open symbols) or inorganic sources (solid symbols)] and forage CP (○, ●) and NDF concentrations (□, ■), in vitro true digestibility of DM (IVTD; ▽, ▼), and in vitro digestibility of NDF (dNDF; ◇, ◆). Values are averages of 17 fertilization treatments (inorganic fertilization experiment; seasonal applications of 0, 80, 160, and 240 kg of Cl/ha as CaCl_2 , 160 kg of Cl/ha as NH_4Cl ; all combined with 70 or 140 kg of N/ha as NH_4NO_3 or a mix of NH_4NO_3 and NH_4Cl ; liquid swine manure (LSM) fertilization experiment: unfertilized control, inorganic fertilizer, raw LSM, and decanted, filtered, digested, and flocculated LSM), 6 locations (Sainte-Anne, Normandin, Saint-Augustin, Sainte-Perpétue, Saint-Lambert, and Saint-David), 2 harvests (spring growth and summer regrowth), and 2 yr (inorganic fertilization experiment: 2003 and 2004; LSM fertilization experiment: 2001 and 2002).

metabolism and that a number of proteins are involved in modulating the requirement for Cl in the evolution of O_2 (Britto et al., 2004).

Nitrogen Fertilization

Conflicting results have been reported for the effect of N fertilization on the DM digestibility of timothy (Bélanger et al., 2001). In studies conducted in the Netherlands (Deinum et al., 1968), Sweden (Thorvaldsson and Andersson, 1986), and Canada (Kunelius et al., 1976), increasing application rates of N fertilization decreased timothy DM digestibility. However, no effect of applied N on forage DM digestibility was reported by Calder and MacLeod (1968) and St-Pierre and Pelletier (1977) in eastern Canada. In a more recent study with inorganic N fertilization, Bélanger and McQueen (1998) report that N fertilization decreased DM digestibility of timothy, an effect that was attributed mainly to a decreased proportion of leaves. Bélanger and McQueen (1998) conclude that one possible explanation for the conflicting reports concerning the effect of N fertilization on the digestibility of DM is the varying degree of crop N deficiency in the studies. A relative yield (DM yield obtained for a given treatment divided by the

maximal observed DM yield) of less than 60% usually resulted in increased DM digestibility.

In the LSM experiment, the DM yield with no N applied was 66% of the maximal DM yield obtained with the inorganic fertilizer (Chantigny et al., 2007). The intermediate values for DM yield and IVTD of forages fertilized with LSM are probably due to lower availability and greater losses of N from LSM compared with the inorganic fertilizer (Chantigny et al., 2007). Our results confirm that increasing N fertilization with LSM or inorganic fertilizer can have a negative effect on the digestibility of DM, at least for situations in which the relative yield is 66% or less in the absence of N fertilization. In the inorganic fertilization experiment, the average DM yield over Cl fertilization treatments, years, and locations of forages fertilized with 70 kg of N/ha was 90% of the maximum DM yield obtained with forage fertilized with 140 kg of N/ha (Pelletier et al., 2007a), suggesting that timothy growth was only slightly limited by N. The IVTD of forage fertilized with 140 kg of N/ha was only 10 g/kg of DM lower than that of forage fertilized with 70 kg of N/ha. This small difference supports the hypothesis that when N limitation is negligible, the digestibility of DM and NDF is not affected by N fertilization.

In the LSM experiment, N fertilization increased NDF concentration and decreased dNDF. Similar results were reported by Bélanger and McQueen (1998). Increasing N fertilization with LSM or inorganic fertilizer also resulted in an increased forage CP concentration. This was reported by MacLeod and MacLeod (1974) and Guertin et al. (1979). Although statistically significant, the effect of N fertilization on the 4 attributes of nutritive value was relatively small (Table 3). When no N was applied, CP concentration was 14% lower, NDF concentration was 4% lower, and both IVTD and dNDF were 2% greater than when N was applied. Our results confirm that when timothy is grown under nonlimiting N conditions, the increase in DM yield caused by N fertilization does not result in a biologically significant decrease in nutritive value.

CONCLUSIONS

The NDF concentration, IVTD, and dNDF of timothy were slightly affected by the inorganic Cl fertilization applied to decrease forage DCAD only at the greatest N fertilization application (140 kg/ha). However, variations in forage NDF concentration, IVTD, and dNDF with the application of Cl fertilizer at a seasonal application of up to 240 kg/ha remained small (10 to 12 g/kg) and were not of biological importance. In the LSM fertilization experiment, the flocculated LSM, which was the treatment with the greatest Cl content, did

not affect forage NDF concentration, IVTD, and dNDF compared with the other LSM types and the inorganic fertilization treatment. Nitrogen fertilization decreased forage nutritive value, but the magnitude of this effect was not of biological importance. These results show that the practice of applying Cl fertilizer, whether from an inorganic (CaCl_2 or NH_4Cl) or an organic (LSM) source, to produce low-DCAD timothy does not affect, or only minimally affects, forage nutritive value.

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