Explorative research into quality of slurry manure from dairy farms with different feeding strategies

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Abstract

To assess cattle slurry manure quality in relation to feeding strategy, a field experiment and a bio-assay were carried out with slurries from four dairy farming systems that used diets differing in protein content and digestibility. Several quality aspects were evaluated. In the field experiment the effects of slurry manure type on herbage rejection by grazing heifers and herbage yield on undisturbed plots under cages were studied for a grass monoculture and a grass/clover mixture. The bio-assay, consisting of a cress (*Lepidium sativum* L.) seed germination test, was used to study differences in phytotoxicity between the slurry types. After five weeks of undisturbed growth at equal amounts of applied inorganic nitrogen (N), the herbage yields differed statistically for the different slurries. This was probably due to immobilization of N in the case of the two slurries from farming systems in which straw was fed and used as bedding material. Herbage rejection by grazing animals was significantly shown for all slurry types and was significantly and positively correlated with the NH_3/NH_4^+-N content of the slurry. The slurries showed large differences in phytotoxicity to seeds and seedlings in the bio-assay. Ammonia and electric conductivity appeared to be the most important slurry parameters with inhibiting effects. The slurries with a high C/N ratio showed lowest phytotoxicity. Phytotoxicity in the cress seed germination test did not account for reduced herbage yields in the field experiment. On the contrary, when the slurries were ranked according to their phytotoxicity the order was the same as the ranking on the basis of undisturbed herbage yield. It was concluded that there is a need for other laboratory tests that show greater resemblance with what is observed in the field to assess slurry quality.

Additional keywords: N recovery, herbage rejection, phytotoxicity, grass, clover, bio-assay

Introduction

Nutrient efficiency of Dutch dairy farming has decreased drastically during the period 1950–1990, mainly as a result of a strong increase in the use of inorganic fertilizer on grassland (Van Keulen *et al.*, 1996). In recent years, European and national legislation have forced farmers to rapidly reduce nutrient losses (Henkens & Van Keulen, 2001). So, there is a need for knowledge on improving nutrient efficiency at farm level. From model calculations Van Bruchem *et al.* (1999a) concluded that the most effective way to reduce nitrogen (N) losses at farm level is to improve soil N efficiency by decreasing N input through inorganic fertilizer. This should be accomplished by an improved efficiency of use of internal resources like on-farm produced feed and manure (Aarts *et al.*, 2000).

Velthof *et al.* (2000) stated that the environmental problems associated with high-input livestock farming systems and inappropriate use of animal manure have given a strong impetus to re-value animal manure as a source of essential plant nutrients and as a means to improve soil quality. Animal manure has to become again the major source of nutrients for fodder crops like grass and maize. Also the farmers of the VEL and VANLA (Stuiver *et al.*, 2003) environmental co-operatives in the province of Friesland recognized this need as crucial and aim at an improvement of slurry manure through an adapted feeding strategy.

Recently, attempts have been made at the integrated mixed farm A.P. Minderhoudhoeve (APM) in Oostelijk Flevoland to modify slurry manure quality by means of adapting feeding strategy (Lantinga, 2000). To reduce ammonia emission and nitrate leaching, to stimulate microbiological activity in the soil and to increase soil organic N content, dietary crude protein content was decreased drastically and up to 3 kg straw per cow was fed daily. In the farm's grass/clover leys the amount of total N in the 0–30 cm soil layer increased with 250 kg N ha^{-1} $year^{-1}$ on average (unpublished results). This high net immobilization of soil N was attributed to the high C/N ratio of the slurry applied (cf. Whitehead, 1995). In addition, also the use of straw as animal feed or bedding material can cause immobilization (Van Faassen & Van Dijk, 1987). An increase in total soil N may lead to an increase in the inorganic soil N supply available for plant uptake (Langmeier *et al.*, 2001; Silgram & Chambers, 2002).

Herbage rejection due to fouling by cattle is a phenomenon frequently observed in pasture studies. Generally, the odour of the faeces is thought to be the main reason for herbage rejection around dung pats (Marten & Donker, 1964; Marsh & Campling, 1970; Dohi *et al.* 1999). The botanical composition of the sward may influence the degree of rejection. Marten & Donker (1964) found that faeces deposited on a monoculture of bromegrass led to greater refusal of forage than faeces deposited on a mixture of bromegrass and alfalfa. According to Mackie *et al.* (1998) there are four principal classes of odour compounds: (1) branched- and straight-chain volatile fatty acids (VFA), (2) ammonia and volatile amines, (3) indoles and phenols, and (4) volatile sulphur-containing compounds. These compounds are products from hydrolysis and fermentation of organic matter under anaerobic conditions. At the APM farm, Bosker *et al.* (2003) found considerably less herbage rejection around dung pats from cattle fed a low-protein diet that included straw than around dung pats from cattle fed diets

without straw. Besides, slurry produced on this diet and applied on a continuously grazed pasture did not lead to herbage rejection.

Phytotoxic properties of organic substances can severely damage crop yields (Mathur *et al.*, 1993). A relatively easy and quick method to test phytotoxicity of chemical substances is a bio-assay, using a germination test with cress (*Lepidium sativum* L.) seeds. This test is often used to evaluate toxicity of organic fertilizers like compost (Zucconi *et al.*, 1985). Phytotoxicity in such a seed germination bio-assay is the capability of substances to inhibit or reduce seed germination or root growth. From a bio-assay with cress seeds, using the same dung types as Bosker *et al.* (2003), Hoekstra *et al.* (2002) concluded that diets with lower protein contents and less supplementary concentrates resulted in a lower phytotoxicity of the dung to seeds or seedlings. The highest negative correlations were found between the germination index (a combined and dimensionless index for the number of germinated seeds and the root length of the germinated seeds, relative to the control) and electric conductivity (EC) or total-N concentration in the dung extracts. The authors suggested that slurry manure might be more phytotoxic than dung because of its much higher ammonia content, which is known for its phytotoxicity to seeds and seedlings (Wong *et al.*, 1983). Van Bruchem *et al.* (1999b) suggested that besides ammonia, also other nitrogenous compounds resulting from amino-acid metabolism, like biogenic amines and phenolic compounds (phenol, indol, scatol, cresol) might play a role in phytotoxicity.

In this study, slurry manures collected from four farming systems with different feeding strategies were compared in a field experiment and a bio-assay using the cress germination test. The objectives of the field experiment were to determine (1) effects of the four slurry manures on apparent N recovery and herbage yield with undisturbed growth of a grass/clover field and a grass monoculture, (2) whether these effects could be related to the amount of inorganic N applied, (3) whether these effects could be related to chemical slurry characteristics, (4) effects of these manures on herbage rejection in a grass/clover mixture and a grass monoculture, and (5) whether these rejection effects could be related to odorous compounds in the slurry.

The objectives of the bio-assay were to determine (1) whether there are differences in phytotoxicity between the slurry types, and (2) whether these differences could be related to chemical characteristics of the slurry.

The objective of the combination of the two experiments was to test whether the differences in phytotoxicity as established in the bio-assay could be related to yield differences observed in the field experiment.

Materials and methods

Slurry collection, storage and sampling

The experiments were carried out in the summer of 2001 with slurry manure from four farming systems (laid out at three different experimental farms) with different feeding strategies (Table 1). APMlac slurry was from lactating cows at experimental farm A.P. Minderhoudhoeve (APM). The diet fed to these cows had a moderate protein

Table 1. Intake, diet composition and other characteristics of the four farming systems where cattle slurry manure was collected.

	Farming system where slurry manure was collected[1]			
	APMlac	OSK	APMdry	MAR
Intake (kg DM[2] per cow per day)				
Grass silage			5.2	1.2
Maize silage				6.8
Whole-crop wheat silage	6.9		2.2	
Fresh grass	7.0	13.9		6.3
Wheat straw	1.5		1.8	
Maize straw				0.9
By-products	3.4			2.3
Concentrates	3.1	2.6	1.5	4.4
Total	21.8	16.5	10.7	22.1
Diet composition				
VEM[3] (per kg DM)	930	1050	820	1020
Crude protein (g per kg DM)	141	180	120	149
Starch (g per kg DM)	101	27	73	194
Other characteristics				
Bedding material	chopped straw	sawdust	chopped straw	sawdust
Slurry collected from:	floor	pit	floor	pit
Time between production and collection	0 weeks	1 week	0 weeks	4 weeks
Time between collection and application	3 weeks	2 weeks	3 weeks	2 weeks

[1] APMlac = A.P. Minderhoudhoeve (lactating cows); OSK = Ossekampen; APMdry = A.P. Minderhoudhoeve (dry cows); MAR = De Marke.

[2] DM = dry matter.

[3] VEM = Voeder Eenheid Melk; Dutch standard for Net Energy Lactation (1 VEM = 6.9 kJ).

content and was rather low in digestibility because of the addition of straw. OSK slurry was collected from experimental farm De Ossekampen in Wageningen, where dairy cows were fed highly digestible fresh grass with a high protein content. APMdry slurry was from dry cows at experimental farm APM, which were fed a low-protein diet including straw with low digestibility. MAR slurry was from dairy cows of experimental farm De Marke in Hengelo (Gelderland), where the cows were fed a highly digestible diet with a moderate protein content.

From each farming system 800–900 litres of slurry manure were collected and mixed in a 1000-litre polyethylene container. The APMlac and APMdry slurries were collected with a manure scraper from a concrete floor, filling the containers by hand. The OSK and MAR slurries were pumped directly from the slurry pit into the contain-

ers (Table 1). Containers were stored outdoors for 2 to 3 weeks. On 13 August 2001, after mixing the slurry in the application unit shortly before use, a sample from each slurry type was taken and analysed for dry matter (ISO 6496) and total-N (Kjeldahl method, ISO 5983) Ash was determined in a furnace at 550°C. Total C of the slurry samples was determined by elemental analysis using an EA 1110® CHN analyser (CE instruments, Milan, Italy). NH_3/NH_4^+-N was determined according to the Berthlot method (Anon., 1974). NH_3/NH_4^+-N is the sum of NH_3 (aq) and NH_4^+ present in the equilibrium: NH_3 (aq) $\Leftrightarrow NH_4^+ + OH^-$, with NH_3 (aq) being the amount of NH_3 dissolved in water.

To determine biogenic amines, phenol, indol, cresol and scatol contents, 1 gramme slurry was extracted with 1 ml methanol during 4 hours at room temperature. The extract was centrifuged at 3000 x *g* and the supernatant was used for further analysis. Biogenic amines were separated with ion-chromatography, using an amino acid analyser (Alpha Plus®, LKB, Sweden). Determination was done using spectrophotometry at 570 nm after post-column colouring with ninhydrin. For the determination of phenol the supernatant was analysed with reversed phase chromatography using a C-18 Alltime® column. The eluent consisted of 50% Na-acetate (2.5 g l^{-1}) and 50% methanol. Peak registration was done with UV-detection at 275 nm. The same methods were used for the determination of cresol, indol and scatol, using a slightly different eluent (35% Na-acetate and 65% methanol) and peak registration for cresol and indol at 215 nm and for scatol at 223 nm.

The field experiment

The field experiment was laid out on a 3.2-ha pasture of experimental farm De Ossekampen. The soil was a fine-textured river clay with 53% lutum (particles < 0.002 mm), 8.1 % organic matter (C/N ratio 7) and pH-KCl 5.0 in the 0–10 cm soil layer. The pasture had been established in June 1999, after one year of cropping with silage maize (*Zea mays* L.).

The area where the experiment was carried out consisted of an experimental part and a put-and-take part. Herbage height on the experimental part was controlled by exchanging heifers with the put-and-take part. The average herbage height aimed at was 7 cm, being the optimum height under continuous grazing in terms of both herbage intake per animal and per unit area (Lantinga, 1988). Half of the experimental part (grass/clover) was sown with a mixture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.), the other half (grass) with perennial ryegrass only. Before the start of the experiment three silage cuts were taken on the entire field. Before every cut, the field was fertilized at a rate of about 150 kg inorganic fertilizer N per ha in total. After the first cut 25 m^3 slurry manure per ha was applied, equivalent to an amount of 110 kg total-N per ha.

On the grass/clover as well as on the grass of the experimental part 20 plots of 2.4 m x 10 m were established. On 13 August 2001, 10 days after the third silage cut, the different slurry types were surface-applied on these plots in small strips, using a Schepan MMM® slurry application unit (Scheepers, 1978). Surface application was used to be able to study the rejection of fouled herbage. The intention was to apply 80

kg total N ha^{-1} for each slurry type. The actual amount applied varied between 71 and 82 kg total N ha^{-1}. On the control plots (no-slurry), 25 m^3 water ha^{-1} was applied.

To study the effect of slurry manure on undisturbed herbage growth, cages covering an area of 3.75 m x 1.05 m were placed in the centre of 10 plots in each half of the experimental part. The remaining 10 plots were used to study the effect of slurry manure on herbage rejection by grazing heifers. On both grass and grass/clover there were two replications of each treatment combination. Caged plots and grazed plots were randomized within sub-blocks.

After the application of the four slurry manures, 17 pregnant heifers were given access to the experimental part, having a free choice between grass and grass/clover. Thereafter the number of heifers was adjusted according to the average herbage height in the grazed area of the experimental plot. Herbage height was measured on each plot, using a falling plate meter with a diameter of 50 cm and a weight of 435 g. Recordings were taken at weekly intervals, starting immediately after slurry application. The faeces from the heifers grazing on the experimental plots were removed daily by hand, using a small shovel.

On 14 September 2001, because of persistently wet conditions, the field experiment was terminated. From the centre of each plot a strip of herbage was harvested using a reciprocating motor mower with a working width of 1.00 m, leaving a stubble height of about 4 cm. Patches where the fouled grass could not be cleaned satisfactorily, were not harvested. This implied that the length of the harvested strips varied. For the caged plots the length of the strips was equal to the length of the cage, i.e., 3.75 m. Harvested material was dried, weighed and sampled. Samples were analysed for (1) dry matter, after drying at 103°C, (2) ash, by combustion in a furnace at 550°C, (3) total N and total C, using a CE Instruments EA 1110® CHN analyser, (4) neutral-detergent fibre (NDF) according to Van Soest *et al.* (1991), and (5) water-soluble carbohydrates (WSC) by extraction with 80% ethanol at 80°C for 20 minutes, drying the extract, redissolving it in water, and analysing the solution with a Dionex HPAEC® system.

Because of possible contamination with soil due to the wet weather conditions, herbage organic matter yield (HOM) was used as the indicator for yield instead of dry matter yield. N use efficiency of the herbage on the caged grass plots, however, was based on dry matter yield and was expressed as apparent N recovery (ANR, kg kg^{-1}), according to Van Der Meer *et al.* (1987):

$$\mathrm{ANR} = \frac{(\text{DM yield x herbage N content})_{\text{slurry treated}} - (\text{DM yield x herbage N content})_{\text{control}}}{\text{N applied with slurry manure}}$$

where DM yield and amount of N applied with slurry manure are expressed in kg ha^{-1}.

ANR could not be calculated for the grass/clover plots due to the unknown contribution of N_2-fixation by the clover.

On the grazed plots the change in herbage height during the first week (CHH) was used as the main indicator of herbage rejection, by comparing the manured plots with the control. The difference in HOM yield between the manured plots and the control, at the end of the experiment, was used as an additional indicator for rejection.

Bio-assay

The bio-assay to study possible phytotoxicity effects of the slurries consisted of the cress seed germination test. For this test the dry matter content of the four slurry samples was standardized at 5% by adding water and shaking the mixture for 15 hours in the dark at room temperature (Paré *et al.*, 1997). The mixtures were centrifuged at 2700 x *g* for 20 minutes after which the supernatant was again centrifuged for 15 minutes. This supernatant was used in the bio-assay as the undiluted extract of the slurry. As not much was known about the phytotoxicity level of the slurries, three different dilutions were made of the undiluted extract: 1.0, 0.5 and 0.1%. Concentrations of water-extractable Cu, Zn and Cd were determined in the supernatant by Inductively Coupled Plasma-Mass Spectrometry, with the Elan 6000®, following the shaking of dried slurry with water (1:20 w/w) for 2 hours. EC and pH and were measured in all extracts.

The germination test was carried out in a non-illuminated growth cabinet at a constant temperature of 24°C and relative humidity of 90%. The experiment was of a randomized complete block design with 3 x 2 blocks divided over 3 cabinet shelves. A block comprised 17 treatments: 4 slurry manure types x 4 extract concentrations, plus a control. For the control demineralized water was used. An experimental unit consisted of 10 cress seeds placed in a 9-cm petri dish with five layers of filter paper (Schleicher & Schuell No 595, 85 mm rundfilter) onto which 5 ml of slurry extract or demineralized water were placed (Paré *et al.*, 1997). Percentage germination was recorded after 24, 48 and 72 hours of incubation. A visible root was used as the operational criterion of germination. After 72 hours the length of the roots was measured. Relative seed germination (RSG) after 24 (RSG-24), 48 (RSG-48) and 72 (RSG-72) hours and relative root growth (RRG) and germination index (GI) after 72 hours of exposure to slurry extracts were calculated as follows:

$$\text{RSG} = \frac{\text{no. of seeds germinated with slurry extract}}{\text{no. of seeds germinated with water}} \text{ x } 100$$

$$\text{RSG} = \frac{\text{mean root length with slurry extract}}{\text{mean root length with water}} \text{ x } 100$$

$$\text{GI} = \frac{\text{RSG x RRG}}{100}$$

Statistical analyses

The field experiment was analysed separately for caged and grazed plots with analysis of (co-)variance in SPSS (Anon., 2001). In the final analyses only the main effects of slurry type and field (grass/clover or grass) were included. In the analysis of HOM yields initial herbage height was used as a co-variable for both the caged and the grazed plots. Differences between slurry types within fields were tested with the Tukey

test. Pearson's method was used to calculate correlation coefficients, using mean values of the results.

In the cress germination test the effects of slurry type, concentration and shelf position in the growth cabinet on RSG, RRG and GI were analysed using Analysis of Variance in a full factorial model (Anon., 2001). Pearson correlation coefficients were calculated between RSG, RRG and GI on the one hand and slurry characteristics on the other.

Results

Slurry manure analysis

The slurry manure analysis yielded some unexpected results (Table 2). C/N ratios of the slurries were relatively high and ranged from 8.9 for APMlac to 15.5 for APMdry slurry. Total-N content of OSK slurry, the slurry that originated from cows on a diet with the highest crude protein content (180 g per kg DM) was lower than expected. A possible explanation for this low total-N content is that the ratio between faeces and urine in this slurry sample was too high compared with the original slurry due to insufficient mixing. This explanation is supported by the low content of ash, which is mainly excreted with urine, for OSK slurry compared with the other slurries (Table 2).

On the other hand, total-N and NH_3/NH_4^+-N content (57% of total N) of APMlac slurry were somewhat higher than expected (Table 2). Regular slurry analyses at APM in the summer of 2000 showed an average C/N ratio of 9.1 and a total-N content of 41 g per kg DM of which 50% was present as NH_3/NH_4^+-N. In the winter of 2000 the adapted feeding strategy at APM led to a much lower N content of the slurry, with a total-N content of 29 g per kg DM – of which only 38% was present as NH_3/NH_4^+-N – and a C/N ratio of 13.3 (unpublished data). A high protein content in the grazed grass/clover sward at the time of slurry collection can probably explain the higher N values and lower C/N ratio in our study. Because of these unexpected values, the range of total-N content and C/N ratio of the slurries was not as wide as intended.

The contents of phenolic compounds in the slurry manures were low (Table 2). Cresol could not be detected. Phenol ranged from 0.07 to 0.14 mg per kg DM and scatol from 0.04 to 0.45 mg per kg DM. Indol was hardly detectable and its content ranged from non-detectable to 0.02 mg per kg DM. Of the biogenic amines analysed in the supernatant, only putrescine could be detected in small concentrations. Its content was highest in the MAR and lowest in the OSK slurry extract. EC was lower for the OSK and APMdry than for the APMlac and MAR slurry extracts. NH_3/NH_4^+-N content was highly correlated with both phenol content and EC of all slurry extracts. The contents of putrescine, Zn and Cu were also strongly mutually correlated.

Table 2. Composition of the cattle slurry manure types used in the study.

		Farming system where slurry manure was collected[1]			
		APMlac	OSK	APMdry	MAR
Slurry (before application)					
DM[2] (g kg^{-1})		56	86	113	88
Total N (g per kg DM)		47	33	28	43
NH_3/NH^+_4-N (g per kg DM)		27	16	10	24
C/N ratio		8.9	14.4	15.5	10.2
Ash (g per kg DM)		249	191	244	284
Phenol (mg per kg DM)		0.14	0.09	0.07	0.13
Cresol (mg per kg DM)		n.d.[3]	n.d.	n.d.	n.d.
Indol (mg per kg DM)		0.02	0.02	0.01	n.d.
Scatol (mg per kg DM)		0.45	0.37	0.04	0.22
Supernatant (before germination test)					
Putrescine (mg l^{-1})		0.33	0.17	0.45	0.68
Histamine (mg l^{-1})		n.d	n.d.	n.d.	n.d.
Cadaverine (mg l^{-1})		n.d.	n.d.	n.d.	n.d.
Tyramine (mg l^{-1})		n.d.	n.d.	n.d.	n.d
Cu (mg l^{-1})		0.36	0.08	0.43	0.68
Zn (mg l^{-1})		0.73	0.19	0.93	1.43
Cd (mg l^{-1})		0.26	0.13	0.25	0.20
Extracts (before germination test)					
	Conc. (%)				
pH	5 (undiluted)	7.60	7.54	7.58	7.99
	1	7.84	7.63	7.86	8.02
	0.5	7.93	7.63	7.83	8.00
	0.1	7.93	7.61	7.86	7.99
EC[4] (mS cm^{-1})	5 (undiluted)	13.7	7.7	9.5	13.8
	1	4.4	2.2	2.1	3.4
	0.5	2.3	1.2	1.0	1.7
	0.1	0.2	0.0	0.0	0.2

[1] For acronyms used see Table 1.

[2] DM = dry matter.

[3] n.d. = not detectable.

[4] EC = electric conductivity.

Field experiment

Caged plots

The effects of the slurry types on HOM yield, total-N content of the harvested material and ANR for the grass plots after 5 weeks of undisturbed growth are summarized in Table 3. Mean response of HOM yield to MAR slurry was significantly higher than to APMlac and APMdry slurry and to no slurry (control). On the grass/clover plots, MAR and OSK slurry led to significantly higher HOM yields than APMdry slurry and no slurry. For the grass plots no statistically significant differences in (HOM) yield between slurry types were found. However, when ranking the slurry types in terms of their effects on HOM yield the order was the same as found for the grass/clover plots: MAR > OSK > APMlac > APMdry.

The amount of NH_3/NH_4^+-N applied per ha was 40.6, 40.7, 41.4 and 27.7 kg ha^{-1} for APMlac, OSK, MAR and APMdry slurry, respectively. Although similar amounts of NH_3/NH_4^+-N were applied with the first three slurries, there were statistically significant differences in HOM yield between APMlac and MAR slurry (Figure 1).

Total-N content of the harvested material varied between 21.3 and 40.6 g per kg OM. No statistically significant differences in total-N content between slurry types

Table 3. Herbage organic matter (HOM) yield, total-N content and apparent N recovery (ANR) of grass/clover and grass after 5 weeks of undisturbed growth on plots fertilized with different types of cattle slurry manure. (Means ± standard error.)

	Slurry manure type[1]				No slurry	Mean
	APMlac	OSK	APMdry	MAR		
HOM yield[2] (kg ha^{-1})						
Grass/clover[3]	2383±129 ab	2589±107 b	2188±110 a	2529±114 b	2338±107 a	2405±113
Grass	1703±114	2098±108	1673±122	2213±110	1835±113	1904±114
Mean[3]	2043±121 a	2343±108 ab	1930±116 a	2371±112b	2087±110a	2155±113
Total N (g per kg OM[4])						
Grass/clover	36.3±0.4	35.6±1.7	37.6±2.3	39.4±1.1	36.9±0.2	37.2±0.6
Grass	22.6±0.1	26.4±1.3	24.2±0.1	27.0±0.1	22.5±1.2	24.5±0.7
Mean	29.4±4.0	31.0±2.8	30.9±4.0	33.2±3.6	29.7±4.2	30.9±1.5
ANR (kg kg^{-1})						
Grass	−0.04±0.04	0.18±0.06	−0.01±0.07	0.25±0.03	n.a.[5]	0.09±0.05

[1] Acronyms refer to the farming systems where the slurry manure was collected. See Table 1.

[2] HOM yields corrected for initial herbage height.

[3] Mean yields in the same row, followed by a different letter are statistically different ($P < 0.05$).

[4] OM = organic matter.

[5] n.a. = not available.

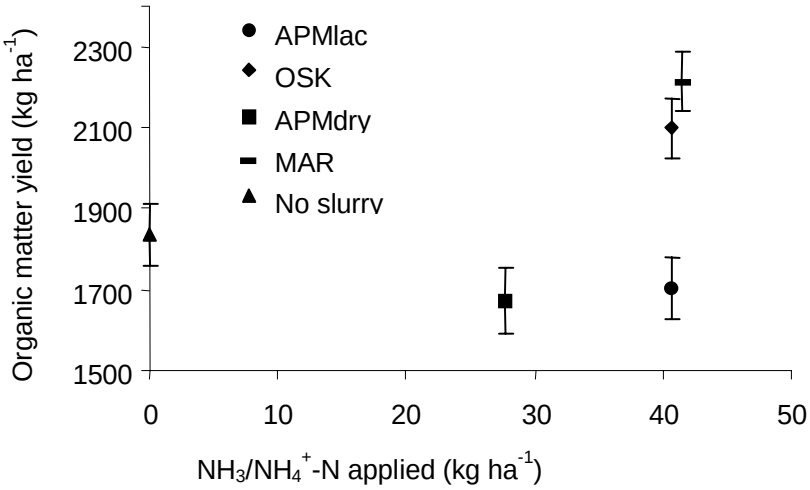

Figure 1. Relationship between amount of NH_3/NH_4^+-N applied with cattle slurry manure and corrected herbage organic matter yield for grass plots fertilized with different types of slurry manure.

were found (Table 3). WSC contents of the harvested material were very low (< 7 g per kg OM. The differences in WSC and NDF content of the harvested material between slurry types were not statistically significant (data not shown).

The two measurements of ANR (only grass plots) did not show any statistically significant difference between slurry types (Table 3). However, ANR tended to be higher ($P = 0.052$) for MAR slurry than for APMlac slurry. The ANR for both slurries from APM were even negative. No statistically significant correlation was found between ANR or HOM yield and any of the slurry characteristics.

Grazed plots

The changes in herbage height after one week (CHH) and HOM yields after 5 weeks of grazing are summarized in Table 4. Mean CHH for all slurry-treated plots was significantly higher than mean CHH for the no slurry plots. MAR slurry led to a significantly higher CHH than APMdry slurry, whereas the two other slurries had intermediate values. The ranking order of the four slurries for CHH was MAR > APMlac > OSK > APMdry. Both, the grass and the grass/clover plots showed the same ranking order.

For the grass/clover plots CHH was positively and significantly correlated with the NH_3/NH_4^+-N content of the slurry ($R^2 = 0.98$, $P < 0.05$, $n = 4$). Also for the grass plots this correlation was statistically significant ($R^2 = 0.98$, $P < 0.05$, $n = 4$).

HOM yield for the grazed plots at the end of the 5-week grazing period, the second indicator of herbage rejection, showed almost the same ranking order of the slurries as the first indicator, CHH: MAR > OSK > APMlac > APMdry. Only APMdry slurry gave no significantly higher mean HOM yield than the control plots. None of the slurry characteristics appeared significantly correlated with HOM yield after 5 weeks of grazing.

Table 4. Herbage organic matter (HOM) yield after 5 weeks and change in herbage height after 1 week (CHH) on grazed grass/clover and grass plots fertilized with different types of cattle slurry manure. (Means ± standard error.)

	Slurry manure type[1]				No slurry	Mean
	APMlac	OSK	APMdry	MAR		
HOM yield[2] (kg ha^{-1})						
Grass/clover[3]	334±0.6 ab	407±2.6 b	299±1.2 ab	434±0.6 ab	176±3.1 a	330±30.3
Grass	508±0.4	586±2.5	469±2.4	611±2.0	349	522±28.3
Mean[3]	412±50.5 bc	496±57.0 bc	384±56.4 ab	522±52.6 c	234±60.8 a	421±55.2
CHH (cm)						
Grass/clover[3]	−0.23±002 ab	−0.30±0.50 ab	−0.53±0.33 ab	0.55±0.30 b	−2.17±0.33 a	−0.53±0.32
Grass	0.03±0.13	−0.15±0.80	−0.30±0.25	0.60±0.10	−0.67±0.13	−0.10±0.19
Mean[3]	−0.10±0.09 bc	−023±0.39 bc	−0.41±0.18 b	0.58±0.13 c	−1.43±0.46 a	−0.32±0.19

[1] Acronyms refer to the farming systems where the slurry manure was collected. See Table 1.

[2] HOM yields corrected for initial herbage height.

[3] Means in the same row, followed by a different letter are statistically different ($P < 0.05$).

Bio-assay

Relative seed germination

The effects of slurry manure type and extract concentration and the slurry x extract concentration interaction on relative seed germination (RSG) were statistically highly significant ($P < 0.001$). The undiluted extracts of APMlac, OSK and MAR slurries completely inhibited germination while some germination took place with APMdry slurry (Table 5). With an extract concentration of 1% there were large differences in RSG after 24 hours (RSG-24) between the slurries. The ranking order of the slurries for their effect on RSG-24, RSG-48 and RSG-72 was APMdry > OSK > APMlac > MAR. RSG-24 was significantly higher with APMdry and OSK slurry than with the two other slurries. With a RSG-48 of 91.7 the inhibiting effect of APMlac slurry only seems to have been a delay, whereas MAR slurry had a permanent inhibiting effect, as is illustrated by a significantly lower RSG-48 and RSG-72 for this slurry than for the other ones.

No statistically significant differences between the effects of the slurries were found at an extract concentration of 0.5%. With a concentration of 0.1%, seed germination was hardly inhibited. However, RSG-48 and RSG-72 were significantly lower with OSK slurry than with the other slurries.

Relative root growth and germination index

The effects of slurry manure type and extract concentration on relative root growth (RRG) and germination index (GI) and the interaction slurry type x extract concentra-

Table 5. Results of the bio-assay (germination test). Effects of extract concentration of the different cattle slurry manures on relative seed germination (RSG) after 24, 48 or 72 hours, root length, relative root growth (RRG) and germination index (GI)[1]. (Means ± standard error.)

Extract conc./ slurry type[2]	RSG after .... hours			Root length	RRG	GI
	24	48	72	(mm)		
5 percent (undiluted)						
APMlac	0.0±0.0	0.0±0.0 a[3]	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
OSK	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
APMdry	3.5±2.2	25.0±8.5 b	36.7±6.7 b	2.7±0.9 b	5.8±1.8 b	2.6±1.0 b
MAR	0.0±0.0	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
1 percent						
APMlac	17.5±5.9 a	91.7±3.1 b	95.0±3.4 b	7.4±1.1 ab	15.7±2.3 ab	14.5±1.5 ab
OSK	91.2±3.5 b	95.0±2.2 b	96.7±2.1 b	10.2±1.8 b	21.8±3.9 b	21.2±3.9 b
APMdry	103.5±1.8 b	98.3±1.7 b	98.3±1.7 b	39.4±2.6 c	83.9±5.6 c	82.1±4.4 c
MAR	3.5±2.2 a	41.7±11.7 a	45.0±12.0 a	1.9±1.1 a	4.0±2.3 a	3.1±2.4 a
0.5 percent						
APMlac	80.7±8.0	95.0±3.4	96.7±3.3	20.2±2.9 b	42.8±6.1 b	42.1±6.6 b
OSK	94.7±3.8	95.0±3.4	98.3±1.7	38.6±2.7 c	82.0±5.8 c	80.7±6.0 c
APMdry	96.5±1.8	93.3±2.1	93.3±2.1	49.7±3.0 d	105.6±6.4 d	98.8±7.0 c
MAR	86.0±4.2	95.0±2.2	96.7±2.1	8.4±0.9 a	17.9±2.0 a	17.5±2.2 a
0,1 percent						
APMlac	105.3±0.0	100.0±0.0 b	100.0±0.0 b	41.5±3.0	88.2±6.3	88.2±6.3
OSK	100.0±3.6	95.0±2.2 a	95.0±2.2 a	44.1±2.8	93.8±5.9	89.2±6.3
APMdry	105.3±0.0	100.0±0.0 b	100.0±0.0 b	46.3±2.4	98.4±5.2	98.4±5.8
MAR	96.5±3.2	100.0±0.0 b	100.0±0.0 b	39.9±4.1	84.9±4.1	84.9±4.1
Control[4]	100.0±3.6	1000.0±0.0	100.0±0.0	47.0±3.4	100.0±7.0	100.0±7.0

[1] For an explanation of the different germination parameters see text.

[2] Acronyms refer to the farming systems where the slurry manure was collected. See Table 1.

[3] Means in a column within the same concentration, followed by a different letter are statistically different ($P < 0.05$).

[4] Demineralized water.

tion were statistically highly significant ($P < 0.001$). The undiluted extract of APMdry slurry was the only one that did not inhibit germination completely, resulting in a statistically significantly higher RRG and GI than for the other slurries (Table 5). As to the other extract concentrations, the ranking order of the slurries was the same for both RRG and GI: APMdry > OSK > APMlac > MAR (Table 5). In contrast to an extract

concentration of 0.1%, the differences in RRG and GI between slurries at concentrations of 1% and 0.5% were statistically significant (Table 5).

Correlation coefficients

With the undiluted slurry extracts, NH_3/NH_4^+-N was the only slurry parameter that was significantly and negatively correlated with RSG-24. All nitrogenous parameters, i.e., total N, NH_3/NH_4^+-N, ammonia, phenol, indol and scatol contents, were significantly and negatively correlated with RRG (Table 6). With an extract concentration of 1%, all slurry parameters except Cd were significantly and negatively correlated with RSG-24. The highest and statistically most significant correlations were found with ammonia, EC, NH_3/NH_4^+-N, total-N, phenol and indol contents (Table 6). With a concentration of 1%, RRG was significantly correlated with ammonia, EC, NH_3/NH_4^+-N, total-N, phenol, indol and scatol contents, of which the correlations with ammonia, NH_3/NH_4^+-N, total-N and indol contents were highly significant.

With a concentration of 0.5%, only EC, ammonia, NH_3/NH_4^+-N, total-N and phenol contents were significantly and negatively correlated with RSG-24. All characteristics except Cd and scatol contents were significantly and negatively correlated with RRG. Among them, the correlations with ammonia, pH, EC, NH_3/NH_4^+-N, total-N and indol contents were highest and statistically most significant (Table 6). As no statistically

Table 6. Results of bio-assay (germination test). Correlation coefficients (R^2) between relative seed germination after 24 hours (RSG-24) and relative root growth (RRG)[1] on the one hand and cattle slurry manure parameters on the other, at different slurry manure extract concentrations.

Slurry manure parameter	Extract concentration (%)					
	5		1		0.5	
	RSG-24	RRG	RSG-24	RRG	RSG-24	RRG
pH	0.03	0.06	0.41 **[2]	0.01	0.12	0.45 ***
EC	0.04	0.08	0.74 ***	0.36 **	0.24 **	0.52 ***
Ammonia	0.14	0.18 *	0.46 ***	0.90 ***	0.77 ***	0.23 *
NH_3/NH_4^+-N	0.18 *	0.41 **	0.83 ***	0.71 ***	0.23 *	0.77 ***
Total N	0.15	0.34 **	0.87 ***	0.61 ***	0.24 *	0.72 ***
Cu	0.00	0.01	0.36 **	0.00	0.04	0.29 **
Zn	0.01	0.01	0.32 **	0.00	0.03	0.27 **
Cd	0.05	0.12	0.06	0.13	0.04	0.00
Putrescine	0.00	0.01	0.28 **	0.01	0.02	0.25 *
Phenol	0.12	0.26 *	0.59 ***	0.40 **	0.23 *	0.42 **
Indol	0.05	0.45 **	0.77 ***	0.77 ***	0.08	0.76 ***
Scatol	0.06	0.30 **	0.23 *	0.36 **	0.04	0.15

[1] For an explanation of the germination parameters RSG and RRG see text.

[2] Statistically significant (n = 24). * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

significant differences in RSG-24 and RRG were found with the 0.1% slurry extracts, correlation coefficients for this concentration are not shown.

Discussion

Field experiment

Caged plots

Despite statistically significant differences in HOM yield between slurry types, no statistically significant effect of slurry type on ANR was found, which was due to the low number of replications (only two) and the large variation. Furthermore, no relation was found between HOM yield and amount of inorganic N applied with the slurries. Although this amount was the same for three of the four slurry types (APMlac, OSK and MAR), the differences in HOM yield between these slurries were statistically different. HOM yield was highest with MAR and lowest with APMlac slurry. We assume that the differences in HOM yields in this experiment were caused by a net immobilization of N with slurries APMlac and APMdry, reflected by the negative ANR values of these slurries and their lower HOM yields in comparison with the no slurry plots.

An immobilizing effect of manure can be caused by feeding poorly digestible feed components (Kyvsgaard, 2000). Furthermore, straw addition can cause net N immobilization in the short run (Van Faassen & Van Dijk, 1987), probably by its stimulating effect on the transformation of C derived from faeces, urine and soil (Sörensen, 1998). APMlac and APMdry slurry, which had the lowest ANR values and showed lowest HOM yields, were from farming systems where 1.5–1.8 kg straw cow^{-1} day^{-1} was included in the diet (Table 1). This straw has been the main cause of a lower digestibility of these diets. Moreover, these farms also added approximately 1 kg of chopped straw cow^{-1} day^{-1} to the slurry through its use as bedding material. We therefore conclude that the addition of straw, both in the form of diet and as bedding material, is the main factor determining the low HOM yields with APMdry and APMlac slurry in the short period of our experiment. However, from a long-term experiment Silgram & Chambers (2002) concluded that the addition of straw might contribute to the soil N supply in the longer run.

Herbage rejection

Under grazing, the effect of slurry manure is two-sided. Beside a stimulating effect on herbage growth, slurry can cause herbage being rejected by the animals (Garstang & Mudd, 1971). The two indicators used in our study for the rejection of herbage, i.e., change of herbage height after one week (CHH) and HOM yield after 5 weeks, are the combined result of both processes. By using indicators that are directly related to grass growth, the stimulating effect of slurry on herbage growth is intrinsically part of the evaluation of rejection. However, Prins & Van Burg (1979) concluded that in field experiments it takes at least 10 days before effects of different levels of N application can be observed, which implies that differences in change of herbage height after one

week (CHH) were primarily caused by differences in grazing behaviour.

The animals had free access to the whole pasture, but only 2% of the area was treated with slurry. Indifference of the animals to graze on the slurry-treated plots would imply a decrease in CHH similar to that of the control plots. However, all slurry-treated plots showed a significantly higher mean CHH (Table 4) than the controls, indicating that all slurries indeed caused herbage rejection. Moreover, indifference also would imply a HOM yield on the slurry-treated plots more or less equal to the yield on the control plots. However, for the APMlac, OSK and MAR slurries this yield was significantly higher (Table 4), which is another indication that these slurries did cause rejection.

Several researchers observed herbage being rejected near faeces or slurry manure (Marten & Donker, 1964; Garstang & Mudd, 1971; Bosker *et al.*, 2003). Marten & Donker (1966) showed that non-acceptability of manure-affected pasture was not associated with characteristics of the pasture but directly with the manure itself. Dohi *et al.* (1991) demonstrated that odour is the major cause of herbage rejection. Odour is most closely related to concentrations of VFA and volatile aromatic compounds (Zahn *et al.*, 2001) for which carbohydrates and proteins are biochemical precursors (Mackie *et al.*, 1998). Miller & Varel (2001) – using a starch-rich diet – concluded that starch was the most likely biochemical source of fermentation products in cattle slurry manure. According to these authors protein fermentation will become dominant after starch has become limiting. In our study, CHH as an indicator of herbage rejection was significantly and positively correlated with the NH_3/NH_4^+-N content of the slurry. This may suggest that ammonia or other nitrogenous end products of protein fermentation were responsible for the herbage rejection.

Bio-assay

Possible inhibiting factors

A number of chemical substances in the slurry extracts were significantly and negatively correlated with the results of the cress germination test (Table 6), indicating that they all may play a role in the phytotoxic effects of the slurries. However, some of the substances also showed a strong mutual correlation. Fortunately, of a number of these substances, inhibiting mechanisms and critical inhibiting values for cress seeds are known.

NH_3/NH_4^+-N in solution can be toxic to plant growth. Its toxicity is mainly caused by ammonia (NH_3), which affects plant growth and metabolism even at (low) concentrations at which NH_4^+ is not harmful (Mengel & Kirkby, 1987). The concentration of ammonia depends on the concentration of NH_4^+-N via the equilibrium $NH_4^+(aq) \Leftrightarrow NH_3\ (aq) + H^+$ and on the volatilization of NH_3 (Bennet & Adams, 1970). Several researchers have found that ammonia is playing an important inhibiting role in phytotoxicity experiments (Wong *et al.*, 1983; Tiquia & Tam, 1998). A NH_3 concentration of 0.13 mmol l^{-1} has been proven to be toxic, while a concentration of 6 $mmol^{-1}$ is lethal (Bennet & Adams, 1970). In our study the NH_3 concentrations (as calculated from pH and NH_4^+ concentration by means of the equilibrium equation) in the slurry extracts ranged from 0.02 to 4.30 mmol l^{-1} and were highly and significantly correlated with

the results of the germination test at extract concentrations where inhibition occurred. Figure 2 shows that the germination index remained above 60 when NH_3 was below 0.13 mmol l^{-1} and that it dropped rapidly with higher concentrations. It therefore is very likely that ammonia played an important role in the phytotoxicity observed, especially in the range of 0.1–1.0 mmol l^{-1}. However, above 1 mmol l^{-1} no germination took place at all, although the lethal value of 6 mmol l^{-1} was not reached. This indicates that ammonia was not the only inhibiting factor in the slurry extracts.

Also salinity can have a detrimental effect on seed germination and plant growth, especially in the seedling stage (Adriano *et al.*, 1973; Mengel & Kirkby, 1987). In general, salinity effects are mostly negligible in extracts with an EC of 2 mS cm^{-1} or less (Patrick *et al.*, 1963; Mengel & Kirkby, 1987). With extract concentrations of 0.5% and 1%, EC fluctuated around this critical value and was significantly correlated with RSG-24 and RRG. Figure 2 shows that the germination index indeed decreased rapidly around an EC of 2 mS cm^{-1}. These results indicate that apart from ammonia also

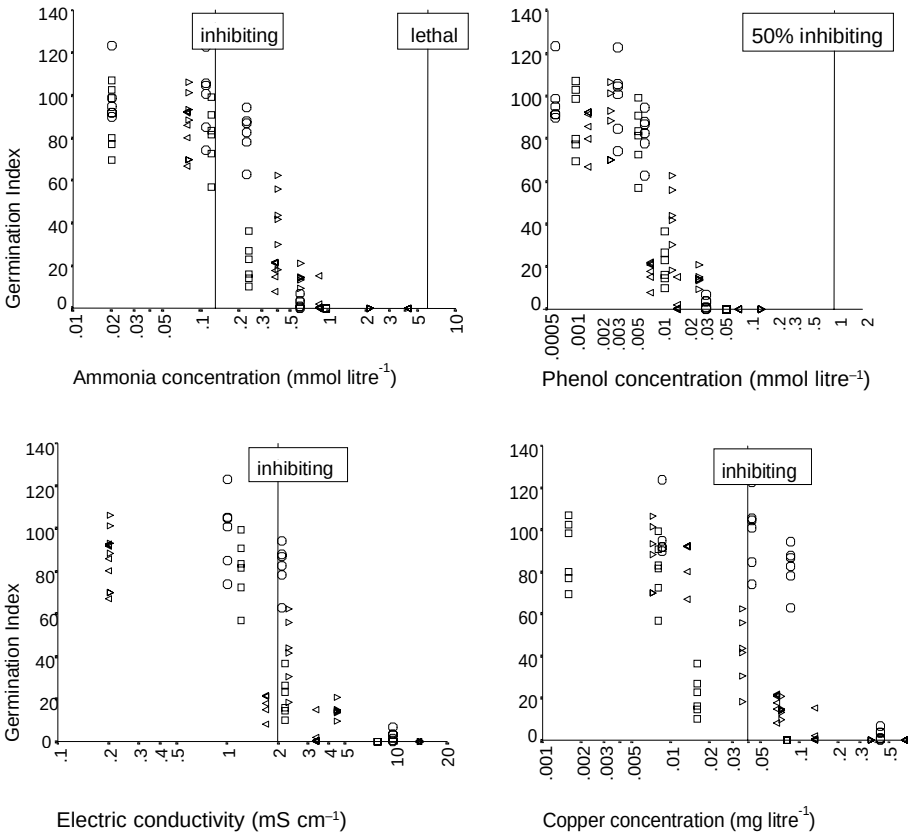

Figure 2. Effect of different levels of ammonia, phenol and copper in, and of electric conductivity of cattle slurry manure extracts on germination index of cress seeds. Vertical lines indicate inhibiting values known from literature.

salinity was an important inhibiting factor in our experiment.

Heavy metals can cause a marked delay in germination and can inhibit plant growth severely (Wollan *et al.*, 1978). However, critical concentrations at which heavy metals in slurry manure extracts become toxic are likely to be higher than the critical values found in literature, because of the relatively high amount of organic compounds that can bind heavy metals (Morel, 1983). Water-extractable Cd concentrations in this experiment ranged from 0.04 to 0.26 mg l^{-1}. The higher Cd concentrations may have had a slight inhibiting effect because they were similar to minimal inhibitory concentrations in substrates of 0.2 mg l^{-1} (Page *et al.*, 1972). However, no significant correlation of Cd concentrations with any of the results of the germination test was found. We therefore assume that Cd did not have an inhibiting effect. Water-extractable Cu concentrations in the slurry extracts ranged from 0.004 to 0.68 mg l^{-1}, whereas 0.04 mg l^{-1} has been shown to inhibit root growth of plants (Craig, 1978). With the 0.5% and 1% extracts, Cu concentrations fluctuated around the critical value. The statistically significant correlation between Cu concentration and RSG-24 with the 1% extracts and RRG and GI with the 0.5% extracts, indicate that Cu concentration may have been an inhibiting factor. However, in a number of extracts with Cu concentrations below the critical range, low GI values were found, indicating that there were more inhibiting factors involved (Figure 2). On the other hand, some extracts with Cu concentrations far above the critical values showed a high GI, indicating that the inhibiting effect of Cu was not very strong. Concentrations of Zn in this experiment ranged from 0.015 to 1.43 mg l^{-1} and were below critical values found in literature, ranging from 75 mg l^{-1} to 600 mg l^{-1} (Davies, 1977; Webber, 1977). We therefore assume that Zn was not an inhibiting factor. The statistically significant correlation between Zn concentration and RSG-24 with the 1% extracts, and RRG with the 0.5% extracts can be explained by the strong correlation between Cu and Zn.

Negative effects of slurry manure may possibly be explained by the inhibitory effect of nitrogenous compounds like biogenic amines or phenolic compounds that result from the degradation of excessive protein (Van Bruchem *et al.*, 1999b). Indeed, phenol can have a negative effect on root growth. Arambasic *et al.* (1995) found a growth inhibition with cress of 50% at 0.86 mmol l^{-1}. However, even with the 5% extract, phenol never exceeded toxic limits: the highest value for phenol in the germination test was 0.075 mmol l^{-1} (Figure 2). The statistically highly significant correlation between phenol and the germination parameters can be explained by the high correlation between phenol and ammonia concentration ($R^2 = 0.99$). Also for indol and scatol high and statistically significant correlations with the results of the germination test were found, suggesting that indol and scatol may have had an inhibiting effect. However, these compounds were also strongly correlated with the concentration of NH_3/NH_4^+-N. Unfortunately, we did not find critical inhibiting values for these compounds in literature and cannot therefore draw any conclusion as to their direct inhibiting effect. The only biogenic amine detected was putrescine, in very low concentrations. Putrescine was significantly correlated with RSG-24 for the 1% extract and with GI for the 0.5% extract, so it may have inhibited germination and root growth. On the other hand, there was also a strong positive correlation of putrescine with the amount of Cu. So, again we cannot draw firm conclusions on the direct inhibiting effect of this amine, but

considering its very low concentrations such an effect was not very likely.

Phytotoxicity and slurry quality

The bio-assay has made evident that slurry manure can be phytoxic to cress seeds and seedlings and that there were differences in this phytotoxicity between the four slurry types investigated. The ranking order of the slurries according to their phytotoxic effect (based on GI) was MAR > APMlac > OSK > APMdry for all extracts. The two slurries with a relatively high C/N ratio (OSK and APMdry; Table 2) showed highest germination indices. The undiluted slurry extracts, except APMdry, permanently damaged all cress seeds, indicating that undiluted slurry is highly phytotoxic to cress seeds. The 1% extracts showed the largest differences in germination, whereas with the 0.5% extracts the largest differences were observed in root growth. These concentrations seem to be most appropriate to test phytotoxicity of slurry with cress seeds. Ammonia and EC appeared to be the most important slurry parameters with inhibiting effects. Besides, Cu may have had a weak inhibiting effect too. These findings are in agreement with the results of Groenwold & Keuning (1988), who observed in a pot experiment with perennial ryegrass that NH_4^+ and electric conductivity were the main factors causing toxicity. It is not clear whether other nitrogenous compounds also play a specific role in the inhibiting effect of slurry manure.

However, the ranking order of slurries based on their phytotoxicty to seeds and seedling roots was similar to the ranking order when based on herbage yield after five weeks of undisturbed growth. In other words, the slurry that showed greatest phytotoxicity in the seed germination bio-assay performed best in terms of herbage yield. A number of important factors could have played a role: (1) the buffering capacity of soil, (2) the sensitivity of the type of seed used, (3) the difference between inhibition of seed germination and phytotoxicity to already existing roots, and (4) differences in time scale of effects on germination and herbage growth. Therefore, we argue that it is necessary to use or develop other laboratory tests to evaluate slurry quality that show greater resemblance with what is observed in the field. In this respect the pot experiment with barley described by Kehres (1990) for testing compost quality can be an interesting alternative.

Conclusions

The dairy farmers of the environmental co-operatives VEL and VANLA are aiming at an improvement of slurry manure through an adapted feeding strategy (Verhoeven *et al.*, 2003). In our study several characteristics of slurry manure quality that can be affected by feeding strategy were explored. The results show (1) short-term effects on nitrogen recovery and undisturbed herbage yield, (2) differences in herbage rejection by grazing heifers, and (3) differences in phytoxicity as observed in a cress seed bio-assay. However, the ranking order of slurries based on their phytotoxicty to seeds and seedling roots was similar to the ranking order when based on herbage yield after five weeks of undisturbed growth. In other words, the slurry that showed greatest phytotoxicity in the seed germination bio-assay performed best in terms of herbage yield.

Therefore, we argue that it is necessary to use or develop other laboratory tests to evaluate slurry quality that show greater resemblance with what is observed in the field.

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