Bedding and Seasonal Effects on Chemical and Bacterial Properties of Feedlot Cattle Manure

Jim J. Miller,* Bruce W. Beasley, L. Jay Yanke, Francis J. Larney, Tim A. McAllister, Barry M. Olson, L. Brent Selinger, David S. Chanasyk, and Paul Hasselback

ABSTRACT

Nutrients, soluble salts, and pathogenic bacteria in feedlot-pen manure have the potential to cause pollution of the environment. A three-year study (1998–2000) was conducted at a beef cattle (*Bos taurus*) feedlot in southern Alberta, Canada to determine the effect of bedding material [barley (*Hordeum vulgare* L.) straw versus wood chips] and season on the chemical and bacterial properties of pen-floor manure. Manure was sampled for chemical content (N, P, soluble salts, electrical conductivity, and pH) and populations of four groups of bacteria (*Escherichia coli*, total coliforms, and total aerobic heterotrophs at 27 and 39°C). More chemical parameters of manure were significantly ($P \leq 0.05$) affected by season (SO_4, Na, Mg, K, Ca, sodium adsorption ratio [SAR], total C, NO_3–N, NH_4–N, total P, and available P) than by bedding (K, pH, total C, C to N ratio, NH_4–N, and available P). Bedding had no significant ($P > 0.05$) effect on the four bacterial groups whereas season affected all four groups. Numbers of *E. coli* and total coliforms (TC) were significantly higher by 1.72 to 2.02 $\log_{10}$ units in the summer than the other three seasons, which was consistent with a strong positive correlation of *E. coli* and TC with air temperature. The low ratio of bedding to manure in the pens was probably the major cause of the lack of significant bedding effects. Bedding material and seasonal timing of cleaning feedlot pens and land application of manure may be a potential tool to manage nutrients, soluble salts, and pathogens in manure.

Chemical constituents (nitrogen, phosphorus, soluble salts) and bacterial pathogens (e.g., *Escherichia coli* O157:H7) in beef feedlot manure have the potential to cause environmental pollution of air, soil, and water. Nutrients such as N and P are required for crop growth, but if they are applied in excess of crop requirements, pollution of the environment can occur. The chemical properties of feedlot manure depend on factors such as animal size, animal age and condition, animal species, housing, water consumption, climate, diet, feedlot surface, animal density, amount and type of bedding, and handling and storage of manure (Brady, 1974; Eghball and Power, 1994; Eck and Stewart, 1995; USDA, 1992). Two factors that have not received much attention are bedding and seasonal effects on the properties of feedlot manure.

Most feedlots in Alberta are bedded with barley straw, but increasing numbers of feedlots are using wood-chip bedding in their operations. Wood chips generally have a higher carbon content, lower N and P values, greater acidity, and lower amounts of soluble salts than straw (Allison and Anderson, 1951). Although the higher C to N ratios of softwoods (>400:1) compared with straw (100:1) suggest a greater potential for N immobilization and nitrate depression under wood chips (Brady, 1974; Sommerfeldt and MacKay, 1987), this effect may be less pronounced because wood has a higher lignin content and therefore decomposes more slowly (Bollen and Lu, 1957). The chemical differences in straw and wood-chip bedding suggest there is a potential for differences in feedlot manure chemistry when these two bedding materials are used.

Wood, particularly the bark, contains many different organic chemicals such as phenols, organic acids, tars, tannins, ethyl alcohol, resins, and terpentine (Goldstein, 1982). These organic chemicals in wood have the potential to be natural antibacterial inhibitors (Allison and Anderson, 1951). Research has reported evidence for (Gibbs and Werkman, 1922; Nimenya et al., 2000) and against (Gibbs and Batchelor, 1927; Allison and Anderson, 1951) inhibition of bacteria by wood in different media (soil, manure, and urine). Allison and Anderson (1951) reported that when these antibacterial compounds are present in wood at typical concentrations, they have minimal toxicity on bacteria in soil. Further, the latter authors suggested that any toxicity that may exist initially disappears within a few weeks, because the toxic substances are destroyed by soil bacteria and fungi. Kudva et al. (1998) reported that small amounts of wood-chip bedding in cattle manure may have contributed to shorter survival times for *Escherichia coli* O157:H7. Nimenya et al. (2000) found that spruce sawdust inhibited urease-producing bacteria from converting urea to NH_4 in dairy-cattle urine, and attributed the inhibitory effect to the tar contents of the wood. We are unaware of any studies that have determined *E. coli* or total coliform populations in beef-cattle manure with straw versus wood-chip bedding.

It is important to examine the effects of season on manure properties since feedlot operators may apply manure at different times of the year. Current guidelines in Alberta allow manure application at any time of the year (Province of Alberta, 2001), but most feedlot operators apply manure in the late summer or fall after

J.J. Miller, B.W. Beasley, L.J. Yanke, F.J. Larney, and T.A. McAllister, Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, AB, Canada T1J 4B1. B.M. Olson, Alberta Agriculture, Food and Rural Development, Lethbridge, AB, Canada T1J 4C7. L.B. Selinger, Department of Biological Sciences, 4401 University Drive, University of Lethbridge, Lethbridge, AB, Canada T1K 3M4. D.S. Chanasyk, Department of Renewable Resources, General Services Building, University of Alberta, Edmonton, AB, Canada T6G 2H1. P. Hasselback, Chinook Regional Health Authority, 960 19th St. S., Lethbridge, AB, Canada T1J 1W5. LRC Contribution no. 38702075. Received 17 Sept. 2002. *Corresponding author (millerjj@agr.gc.ca).

Published in J. Environ. Qual. 32:1887–1894 (2003).

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: EC, electrical conductivity; SAR, sodium adsorption ratio; TAH, total aerobic heterotrophs; TC, total coliforms.

silage harvest. Since many of the factors (diet, age, and condition of animal, water consumption, climate, etc.) that affect manure are time-dependent throughout the year, it seems reasonable to conclude that season may have a significant effect on the properties of feedlot-pen manure. We are unaware of any studies that have examined the effect of season on the chemical properties of feedlot manure; and few studies have examined the effect of season on the bacterial properties of feedlot manure (Rhodes and Hrubant, 1972). The latter authors found that microbial flora in feedlot manure were relatively constant throughout the year, although absolute numbers varied somewhat with seasonal conditions. Numbers of total coliform bacteria ranged between 10^6 and 10^8 colony forming units (CFU) g^{-1} (dry wt.) throughout the year.

The objective of this study was to determine the effect of bedding material and season on the chemical and bacterial properties of feedlot-pen manure in southern Alberta.

MATERIALS AND METHODS

Study Design and Sampling Protocol

The Lethbridge Research Centre feedlot in southern Alberta, Canada, was used for this study. The pens (14 × 20 m) held 15 beef cattle for a density of 19 m^2 per head. This density is typical of commercial feedlots in the province. The diet for the feeding period (200 d) generally consisted of about 70% barley silage and 30% barley grain for the backgrounding period (70–80 d), followed by 85 to 90% barley grain and 10 to 15% barley silage for the remainder of the feeding period (100–120 d). The experimental treatments consisted of pens bedded with either straw or wood chips (three replicates of each). The straw and wood-chip treatments were applied between April 1998 and July 2000. The exception was between 26 Sept. 1998 and 15 Apr. 1999, when the six pens all had straw bedding. The straw bedding was unchopped barley straw. The wood-chip bedding was a mixture of sawdust and bark peelings derived from 80% lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) and 20% white spruce [*Picea glauca* (Moench) Voss]. The chemical content of the two bedding materials is shown in Table 1. Generally, the pens were cleaned once a year in the spring or summer after cattle were removed. Bedding material was used mainly over the winter as needed. Dry weights of straw (18%) and wood chips (22%) added to these feedlot pens as a percentage of the total dry weight removed from the pens at cleaning were similar (F.J. Larney, unpublished data, 2002). The quantity of bedding used in this research feedlot was slightly higher than that used by commercial feedlots. Cattle were stocked in the six treatment pens from 24 Nov. 1997 to 2 July 1998; 7 Dec. 1998 to 17 June 1999; and 14 Oct. 1999 to 11 Aug. 2000.

Pen manure samples were taken for chemical analyses between 7 July 1998 and 14 June 2000, and samples for bacterial analyses were taken between 26 May 1998 and 14 June 2000. Sampling was conducted throughout the year when cattle were present or absent in the pens. Samples were collected approximately biweekly from spring to fall, and about monthly during the winter. Six to eight manure subsamples on the floor of each pen (pen-floor manure) were collected in sterile containers and composited into one sample for chemical and bacterial analyses. Samples were then transported to the laboratory within 1 to 2 h. The water content of the manure samples was determined by weighing a subsample before and after oven-drying at 60°C for seven days. Mean daily air temperature and relative humidity were obtained from a climate station at the Lethbridge Research Centre.

Chemical Analyses

All manure samples were oven-dried at 60°C for seven days, ground to pass a 2-mm sieve, and then extracted for chemical analyses. The exceptions were nitrate and ammonium, where extractions were conducted on fresh manure samples within two hours after sampling. Nitrate and ammonium in the manure were extracted using a 1:20 ratio of 10 g of manure and 200 mL of 2 *M* KCL after shaking at low speed for one hour. Ammonium N was determined using the Berthelot reaction on the autoanalyzer (Technicon Industrial Systems, 1973). Nitrate N was determined on the autoanalyzer using the copper-cadmium method (Technicon Industrial Systems, 1978). Electrical conductivity (EC), pH, and soluble salts (SO_4, Cl, Na, Mg, K, and Ca) in manure were determined on 1:5 manure and water extracts (20 g manure and 100 mL distilled water) after shaking at low speed for one hour. Soluble Ca and Mg were analyzed using atomic absorption spectroscopy, and Na and K were determined using flame emission spectroscopy

Table 1. Chemical properties of barley straw and wood-chip bedding used in experimental pens of a beef cattle feedlot in southern Alberta (1998–2000).

	Straw†		Wood‡	
Parameter	**Range**	**Mean ± SE**	**Range**	**Mean ± SE**
SO_4, g kg^{-1}	2.2–6.4	3.5 ± 0.2	0.2–0.8	0.4 ± 0.04
Cl, g kg^{-1}	0.9–10.0	4.6 ± 0.6	0.1–0.4	0.2 ± 0.02
Na, g kg^{-1}	0.2–2.9	1.2 ± 0.3	0.1–0.2	0.2 ± 0.01
Mg, g kg^{-1}	0.2–1.0	0.6 ± 0.04	0.1–0.3	0.2 ± 0.01
K, g kg^{-1}	7.9–15.2	12.1 ± 0.4	0.6–1.2	0.9 ± 0.06
Ca, g kg^{-1}	0.2–1.8	1.0 ± 0.1	0.2–0.6	0.4 ± 0.02
EC§, dS m^{-1}	4.0–8.1	5.4 ± 0.2	0.4–0.9	0.6 ± 0.04
SAR¶	0.4–4.9	2.2 ± 0.4	0.4–0.9	0.6 ± 0.03
pH	6.0–9.2	6.5 ± 0.2	3.8–5.5	4.5 ± 0.1
Total C, g kg^{-1}	439.0–462.0	452.0 ± 1.0	487.0–523.0	506.0 ± 2.0
Total N, g kg^{-1}	26.6–64.5	43.0 ± 0.2	1.0–2.8	1.7 ± 0.1
C to N ratio	68–172	111 ± 6	181–493	320 ± 21
NO_3–N, mg kg^{-1}	3.5–885.0	201.3 ± 48.2	0.0–4.8	1.0 ± 0.3
NH_4–N, mg kg^{-1}	0.0–141.5	75.3 ± 9.4	0.0–7.8	1.7 ± 0.7
Total P, g kg^{-1}	0.38–0.69	0.46 ± 0.02	0.25–0.51	0.34 ± 0.02
Available P, mg kg^{-1}	115.9–396.2	193.4 ± 15.0	12.6–62.4	31 ± 3

† Number of samples for each parameter for straw bedding ranged from 19 to 21.
‡ Number of samples for each parameter for wood bedding ranged from 18 to 19.
§ Electrical conductivity.
¶ Sodium adsorption ratio.

(Model AA5; PerkinElmer, Wellesley, MA) (Wright and Stuczynski, 1996). Soluble Cl was determined on the autoanalyzer using the mercuric thiocyanate method (Technicon Industrial Systems, 1974a). Sulfate was analyzed on the autoanalyzer using the barium chloride method (Technicon Industrial Systems, 1972). Available phosphorus (ortho-P) was extracted using a 1:25 ratio of 1 g of manure and 25 mL of Kelowna extract (Van Lierop, 1988) after shaking at low speed for one hour. Samples for total P, C, and N analyses were also finely ground to pass a 150-μm sieve. Total P was determined by a wet-oxidation procedure (Parkinson and Allen, 1975), and ortho-P was analyzed on the autoanalyzer using the ascorbic acid method (Technicon Industrial Systems, 1974b). Total C and N were determined using the Dumas automated combustion technique (McGill and Fiqueiredo, 1993) using a CNS analyzer (Carla Erba, Milan, Italy).

Barley straw and wood-chip bedding were oven-dried at 55°C for five days and then ground to pass a 2-mm sieve. Soluble anions, cations, EC, and pH of bedding were analyzed in this study, whereas C, N, and P analyses of the same bedding were taken from Larney et al. (2001). Inorganic N (NH_4–N and NO_3–N) and available P were analyzed using the same methods as described for manure. Soluble salts (Ca, Mg, Na, K, SO_4, and Cl), EC, and pH were determined on 1:10 water extracts after shaking for one hour. Subsamples were further fine-ground to pass a 1-mm sieve for analyses of total N, C, and P using methods as described for manure. Total phenol content of the bedding material was determined by manual distillation using acidified deionized water, online distillation to concentrate the phenol in solution, followed by analysis of total phenolics using the 4-aminoantipyrine (4-AAP) colorimetric method (USEPA, 1979). All chemical properties are reported on a dry weight basis.

Bacterial Analyses

Manure samples were analyzed for total aerobic heterotrophs (TAH) at 27 and 39°C, total coliforms (TC), and *Escherichia coli* (*E. coli*). Manure samples were transported to the laboratory within 1 to 2 h and then plated within four hours. Manure was weighed out in 10-g samples and added to 90 mL of sodium phosphate buffer (pH 6.5, 0.05 *M*). The samples were then blended for 2 min at a medium setting in a stomacher blender and were then serially diluted to the appropriate levels in sodium phosphate buffer. The dilutions were spread-plated (100 μL) in triplicate onto Fluorcult LMX (Merck, Darmstadt, Germany) plates for presumptive enumeration of total coliform and *E. coli* and onto tryptic soy agar plates for enumeration of TAH. The Fluorocult LMX plates were incubated aerobically at 37°C for 48 h with presumptive *E. coli* enumerated after 24 h as those colonies with the ability to hydrolyze both 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-GAL) and 4-methylumbelliferly-β-D-glucuronide (MUG). Presumptive TC were enumerated after 48 h as those colonies hydrolyzing X-GAL. The TAH were incubated at 27 and 39°C and enumerated after 48 h. Bacteria numbers on a wet weight basis were converted to a dry weight basis using the water content of the manure. The results are presented as colony forming units (CFU) per gram dry weight of manure with the standard deviation given for the replicate samples. Selected colonies of presumptive TC and *E. coli* were isolated for confirmation of identity through membrane fatty acid composition (Paisley, 1996), cellular morphology, and biochemical characteristics (Smibert and Kreig, 1994; Garthwright, 1998).

Measurement of the *E. coli* populations is the best indicator of the fecal contamination of both water (Edberg et al., 2000) and foods (Dogan et al., 2002). Since total commensal *E. coli* is also an accurate indicator of *E. coli* O157:H7 environmental persistence (Ogden et al., 2001), we measured the total *E. coli* population. In addition, since *E. coli* is the most numerous coliform in cattle manure, the TC population was determined as a check on the *E. coli* results, as well as to allow comparison with other studies. The TAH population was measured to give an indication of the magnitude of overall microbial activity in decomposing organic matter. The TAH incubated at 27°C are more indicative of environmental isolates whereas incubation at 39°C is more indicative of intestinal isolates.

Statistical Analyses

The chemical and bacteria data were analyzed using SAS-PC (SAS Institute, 1989) and a mixed model analyses (Littell et al., 1998). The main treatment effects of bedding and season were assessed for the chemical and bacterial parameters. A repeated statement with season was used when season was a factor in the model. Bacterial numbers were transformed to $\log_{10}$ values before statistical analyses. The seasons were defined based on equinoxes and solstices. The start of spring was 20 March (1998, 1999, 2000) and the start of summer was 20 (2000) or 21 June (1998, 1999). The start of fall was 22 (1998, 2000) or 23 September (1999) and the start of winter was 21 (1998, 2000) or 22 December (1999).

RESULTS AND DISCUSSION

Chemical Properties of Pen-Floor Manure

More chemical parameters of manure were significantly ($P \leq 0.05$) affected by season (SO_4, Na, Mg, K, Ca, SAR, total C, NO_3–N, NH_4–N, total P, and available P) than by bedding (K, pH, total C, C to N ratio, NH_4–N, and available P) (Tables 2 and 3). For the bedding effects, least-squares mean (LSM) values for K were 26% higher for pen manure with straw (5.9 g kg^{-1}) than with wood chips (4.7 g kg^{-1}). The mean K content of straw was 13 times that of wood chips (Table 1). The pH was 7% lower for pen-manure with wood than straw (Table 2), which was consistent with a lower pH value of wood (Table 1). Total C and the C to N ratio were 9 and 13% higher, respectively, for manure with wood chips than with straw (Table 3). This was consistent with higher total C values and C to N ratios for wood (Table 1). Even though straw bedding had 44 times more NH_4–N than wood chips (Table 1), the NH_4–N content of manure with wood chips was 133% higher than with straw (Table 3). This may be related to the lower pH of manure with wood chips than with straw (Table 1). In comparison, Larney et al. (2001) reported no significant difference in the NH_4–N content of pen-floor manure with straw or wood chips from this same feedlot. In contrast, Nimenya et al. (2000) found that spruce sawdust inhibited NH_4 production from urea in cattle urine, and this was attributed to higher tar contents in the wood. They also proposed that the NH_4 already produced could be absorbed by wheat-straw bedding. Available P in pen manure was 35% higher with wood than straw bedding (Table 3), even though available P was six times higher for straw than wood bedding (Table 1). The lower available P in pen manure with straw may be related to the higher Ca content of the straw, which could cause increased phosphate

Table 2. Bedding and seasonal effects on soluble salts, electrical conductivity (EC), sodium adsorption ratio (SAR), and pH of pen-floor manure for a beef cattle feedlot in southern Alberta (1998–2000).

Treatment	SO_4	Cl	Na	Mg	K	Ca	EC	SAR	pH
	g kg^{-1}						dS m^{-1}		
					Bedding†				
Straw	1.9 ± 0.07‡	6.9 ± 0.4	6.5 ± 0.5	1.3 ± 0.1	5.9 ± 0.2a	0.7 ± 0.1	8.9 ± 0.4	16.5 ± 1.5	7.9 ± 0.1a
Wood chips	1.9 ± 0.07	5.5 ± 0.4	6.5 ± 0.5	1.3 ± 0.1	4.7 ± 0.2b	0.9 ± 0.1	7.8 ± 0.4	17.1 ± 1.5	7.4 ± 0.1b
F value	0.21	5.82	0.00	0.00	27.51**	0.00	3.85	0.08	40.44**
					Season§				
Spring	2.1 ± 0.2a	7.0 ± 0.6	7.7 ± 0.4a	1.1 ± 0.1a	5.3 ± 0.4a	0.4 ± 0.1a	8.5 ± 0.5	22.5 ± 1.3a	7.8 ± 0.1
Summer	1.4 ± 0.2b	4.6 ± 0.7	3.0 ± 0.5b	1.2 ± 0.1a	6.2 ± 0.5a	0.7 ± 0.1b	7.5 ± 0.6	7.1 ± 1.4b	7.7 ± 0.1
Fall	2.7 ± 0.2a	6.6 ± 0.8	3.3 ± 0.5b	1.6 ± 0.1b	8.2 ± 0.5b	0.9 ± 0.1bc	9.1 ± 0.7	6.8 ± 1.6b	7.9 ± 0.1
Winter	2.2 ± 0.2a	6.3 ± 0.6	6.3 ± 0.4c	1.7 ± 0.1b	4.9 ± 0.4a	1.1 ± 0.1c	8.7 ± 0.5	13.5 ± 1.3c	7.6 ± 0.1
F value	6.47***	2.46	25.76***	8.98***	8.60***	13.19***	1.14	27.97***	2.23

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Number of samples for each bedding type ($n = 36$ for straw and 36 for wood).
‡ Least-squares mean (LSM) ± standard error. Different lowercase letters following LSM by column are significantly different at the 0.05 probability level. The LSM values followed by no or same lowercase letter are not significantly different.
§ Number of samples for each season and bacterial type ($n = 36$ in spring, 30 in summer, 24 in fall, and 36 in winter).

precipitation. Larney et al. (2001) also reported higher available P in pen manure with wood (2.1 g kg^{-1}) than with straw (1.7 g kg^{-1}). In contrast, SO_4, Cl, Na, Mg, Ca, EC, SAR, total N, NO_3–N, and total P were not significantly affected by bedding (Tables 2 and 3), even though values for these parameters were generally higher for straw than wood bedding (Table 1).

We expected more significant differences in pen manure with bedding since the straw and wood chips were chemically different (Table 1). Manure to bedding ratios of approximately 5:1 for straw and approximately 4:1 for wood chips (F.J. Larney, unpublished data, 2002) may have been too high to elicit significant bedding effects on subsequent chemical properties of manure. In addition, the high proportion of manure to bedding suggests that dilution effects caused by the bedding were probably minimal.

For the seasonal effects, sulfate in the summer was lower by 50 to 93% than in the other three seasons; and Na in the spring was higher by 22 to 157% than in the other three seasons (Table 2). Magnesium in the fall and winter was higher by 33 to 55% than in the spring and summer; and K in the fall was higher by 32 to 67% than in the other three seasons (Table 2).

Calcium in the spring was lower by 75 to 175% than in the other three seasons. The SAR in the spring was higher by 67 to 231% than in the other three seasons, indicating a higher potential risk of soil sodicity at this time. In contrast, there was no significant seasonal effect on total salt content or EC (Table 2). Increasing salinity and sodicity of soils is a serious concern with long-term application of beef cattle manure (Chang et al., 1991). Although our results indicated that bedding cannot be used to manage the EC and SAR of the pen manure, season has potential as a tool to manage the SAR values of the manure. Total C in the winter was higher by 15% than in the spring and summer (Table 3), which may be related to more bedding used in winter. Nitrate N was lower in the spring by 114 to 269% than in the other three seasons, and NH_4–N in the fall and winter were higher by 57 to 186% than in the spring and summer. Total P in the summer was higher by 20 to 71%

Table 3. Bedding and seasonal effects on carbon, nitrogen, C to N ratio, and phosphorus of pen-floor manure for a beef cattle feedlot in southern Alberta (1998–2000).

Treatment	Total C	Total N	C to N ratio	NO_3–N	NH_4–N	Total P	Available P
	g kg^{-1}			mg kg^{-1}		g kg^{-1}	
				Bedding†			
Straw	338.9 ± 4.9a‡	21.9 ± 0.7	16 ± 0.3a	18.7 ± 3.0	417.2 ± 43.3a	5.7 ± 0.2	2.3 ± 0.2a
Wood chips	368.3 ± 4.9b	20.3 ± 0.7	18 ± 0.3b	19.1 ± 2.6	973.3 ± 46.7b	5.9 ± 0.2	3.1 ± 0.2b
F value	18.24*	3.19	34.52**	2.24§	76.18**	0.55	10.55*
				Season¶			
Spring	343.0 ± 10.2a	20.9 ± 0.7	17 ± 0.5‡	10.4 ± 2.6a	615.2 ± 95.3a	5.4 ± 0.4a	1.7 ± 0.3a
Summer	343.1 ± 11.1a	20.1 ± 0.7	18 ± 0.5	22.3 ± 3.0b	407.3 ± 106.2a	6.5 ± 0.3b	4.3 ± 0.3b
Fall	372.5 ± 12.4ab	20.6 ± 0.8	18 ± 0.6	31.7 ± 3.2c	968.1 ± 116.8b	4.9 ± 0.3a	2.8 ± 0.3c
Winter	395.5 ± 10.3b	21.7 ± 0.7	18 ± 0.5	38.4 ± 2.6c	1163.2 ± 95.3b	3.8 ± 0.4c	2.5 ± 0.3ac
F value	5.86***	0.92	1.77	21.13***	11.33***	12.79***	14.26***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Number of samples for each bedding type ($n = 36$ for straw and 36 for wood).
‡ Least-squares mean (LSM) ± standard error. Different lowercase letters following LSM by column are significantly different at the 0.05 probability level. The LSM values followed by no or same lowercase letter are not significantly different.
§ Mixed-model analysis for bedding effect of NO_3–N conducted on log-transformed concentrations. The *F* value is for log-transformed concentrations and mean values and standard errors are for original untransformed data (Proc means in SAS).
¶ Number of samples for each season and bacterial type ($n = 36$ in spring, 30 in summer, 24 in fall, and 36 in winter).

than in the other three seasons, and available P in the summer was higher by 54 to 153% than in the other three seasons. In contrast, Cl, EC, pH, total N, and C to N values were not affected by season (Tables 2 and 3).

We believe that the significant seasonal differences in certain chemical properties may be related to a number of complex factors that have the potential to vary throughout the year. These may include the age, condition, and size of the cattle, whether cattle were present or absent in the pens, changes in the manure pack (depth and water content), amount of bedding used, water consumed, and climatic conditions. For example, we found a significant negative correlation ($r = -0.70$, $P = 0.0004$, $n = 21$) between mean daily air temperature and NH_4–N content of the pen-floor manure, which may be related to greater volatilization losses of NH_3 during the warmer summer season.

Bacterial Properties of Pen-Floor Manure

Throughout the study, 150 of 150 presumptive *E. coli* isolates were confirmed as *E. coli*. Of 158 presumptive coliform isolates, 136 were confirmed as coliform but not *E. coli*; the other 22 isolates were confirmed as *E. coli* when put back onto LMX plates and retested. Bedding had no significant ($P > 0.05$) effect on any of the four bacterial groups, but season had a significant effect on all groups (Table 4). The populations of *E. coli* and TC in the summer were higher by 1.72 to 3.37 $\log_{10}$ units than in the other three seasons (Table 4). In comparison, TAH at 27°C in the spring were higher by 0.36 to 0.83 $\log_{10}$ units than in the fall and winter; and TAH at 39°C in the summer were higher by 0.53 to 1.23 $\log_{10}$ units than in the other three seasons (Table 4). Overall, seasonal fluctuations in bacterial populations were much wider for *E. coli* and TC than for the TAH populations, indicating a greater seasonal effect on coliform bacteria than the overall bacterial populations. Rhodes and Hrubant (1972) found that the highest numbers of TC in cattle manure of a feedlot in Illinois, USA occurred in July and November, and the lowest counts were in January when air temperatures were coldest. Stoddard et al. (1998) also reported that fecal coliforms in soils (Kentucky, USA) declined most rapidly after fall application of manure, and attributed this to onset of freezing conditions.

Given the increased environmental persistence and increased shedding of *E. coli* O157:H7 in the summer (Hancock et al., 1997; Conedera et al., 2001), the risks of environmental and animal contamination would seem much greater during the summer season. However, increased persistence of bacteria on the pen floor does not necessarily indicate increased animal shedding, and surveys on the seasonal abundance of *E. coli* O157:H7 should be done by taking manure samples directly from the animals. The increased persistence of *E. coli* on the pen floor during the summer also raises the interesting question of whether this could lead to increased recolonization of the cattle by defecated *E. coli* O157:H7 and subsequently cause increased shedding of the organism.

We expected that the bacteria levels would be lower in pens bedded with wood chips because of the greater antimicrobial properties of wood compared with straw. This trend may not have occurred because of the low absolute concentrations of total phenols produced by the bedding material, the high ratio (5:1 straw, 4:1 wood chips) of manure to bedding, and the high quantities of organic material. The barley straw, wood sawdust, and bark peelings contained 1.30, 1.28, and 3.76 mg kg^{-1} (dry-matter basis) total phenols, respectively. Because of the high ratio of manure to bedding, the concentration of bacteria may have been so high that the low concentrations of organic acids produced by the wood may not have had an antimicrobial effect. Larney et al. (2003) also reported that bedding material had no significant effect on *E. coli* and TC during windrow composting of manure from the same feedlot used in our study.

We assessed the relationship between selected environmental variables (water content of manure, air tem-

Table 4. Bedding and seasonal effects on bacterial populations in pen-floor manure for a beef cattle feedlot in southern Alberta (1998–2000).

Season	*E. coli*	Total coliforms	Total aerobic heterotrophs (27°C)	Total aerobic heterotrophs (39°C)
	log colony forming units (CFU) g^{-1} dry wt.			
	Bedding†			
Straw	5.98 ± 0.14‡	6.27 ± 0.14	9.11 ± 0.05	8.07 ± 0.03
Wood chips	6.15 ± 0.14	6.50 ± 0.14	8.95 ± 0.05	8.06 ± 0.03
F value	0.70	1.32	5.28	0.06
	Season§			
Spring	5.89 ± 0.17bc	6.15 ± 0.17b	9.35 ± 0.11a	8.22 ± 0.10b
Summer	7.61 ± 0.20a	8.17 ± 0.20a	9.09 ± 0.13ac	8.79 ± 0.12a
Fall	5.89 ± 0.23b	6.14 ± 0.23b	8.99 ± 0.15bc	8.26 ± 0.14b
Winter	4.63 ± 0.18d	4.80 ± 0.18c	8.52 ± 0.12d	7.56 ± 0.11c
F value	40.52***	51.07***	9.27***	18.20***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Number of samples for each bedding type (n = 38 for each type, except n = 36 for *E. coli* under wood, and n = 39 for total aerobic heterotrophs at 27 and 39°C under straw).
‡ Least-squares mean (LSM) ± standard error. Different lowercase letters following LSM by column are significantly different at the 0.05 probability level. The LSM values followed by no or same lowercase letter are not significantly different.
§ Number of samples for each season and bacterial type (n = 44 in spring, except for total aerobic heterotrophs at 39°C, where n = 47; n = 30 in summer; n = 23 in fall for *E. coli* and total coliforms, n = 24 in fall for total aerobic heterotrophs at 27 and 39°C; and n = 36 in winter).

perature, and relative humidity) related to season and numbers of bacteria in each of the four groups. There was a trend for a negative relationship between the water content of manure and numbers of the bacteria in the four groups (data not shown). However, water content of the manure accounted for only 25, 23, 9, and 23% of the variation in *E. coli*, TC, and TAH at 27°C and TAH at 39°C, respectively. Therefore, our results indicated that the water content of manure had a low potential to influence bacteria numbers in pen-floor manure. Wang et al. (1996) reported that *E. coli* O157:H7 persisted in cattle manure for considerable periods of time, even at water contents of <0.10 cm cm^{-3}. In contrast, other researchers have reported lower coliform or *E. coli* O157:H7 numbers in the top layer of stockpiled cattle manure, and attributed this to drying and lower water contents (Thayer et al., 1974; Kudva et al., 1998).

There was a stronger positive relationship between mean daily air temperature (at nearby weather station) and mean numbers of the four bacterial groups (Fig. 1). Air temperature explained 47, 47, 50, and 34% of the variation in *E. coli*, TC, and TAH at 27°C and TAH at 39°C, respectively. Second-degree polynomial (quadratic) equations gave the best regression fit to the data. Populations of *E. coli* and TC increased dramatically with an increase in air temperature. In contrast, TAH at 27 and 39°C increased more gradually with temperature and then leveled off. This indicated that coliform bacteria responded to air temperature differently that the overall bacterial population. Some studies have reported that die-off of microbial pathogens in soil or water generally increased with a rise in temperature (Reddy et al., 1981; Thelin and Gifford, 1983; Flint, 1987), with microbial competition playing a major role (Jiang et al., 2002). Fukushima et al. (1999) reported decreased persistence of inoculated *E. coli* strains in cattle manure at 25°C as compared with 5 and 15°C. In contrast, Kudva et al. (1998) reported no correlation between incubation temperatures and survival of *E. coli* O157:H7 in cattle feces inoculated with this strain of bacteria. We found no relationship between the relative humidity of air and bacteria numbers (data not shown).

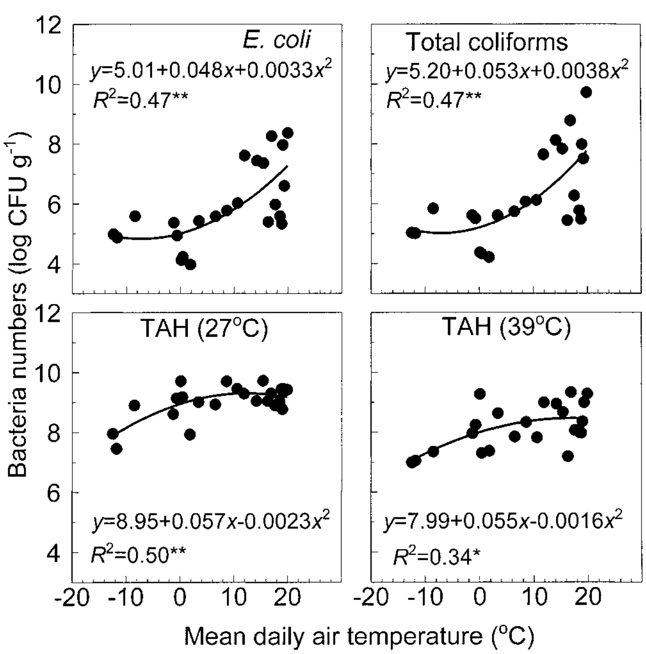

Fig. 1. Relationship between mean daily air temperature and populations of *E. coli*, total coliforms, and total aerobic heterotrophs (TAH) at 27 and 39°C (n = 23) in pen-floor manure at a beef cattle feedlot, Lethbridge, southern Alberta, Canada.

CONCLUSIONS

Higher K in pen-floor manure with straw than wood chips suggests that for situations where feedlots are currently using straw bedding and have high or excessive levels of K in their manured soils, there is a potential to reduce K loading in soils by changing from straw to wood-chip bedding. Higher total C in pen-floor manure with wood than straw indicates there is a long-term potential to increase organic C in sandy soils or reclaimed soils lacking organic matter by applying manure-containing wood chips. Lower pH values and higher NH_4 in pen-floor manure with wood than straw suggest that wood chips might be used to lower the pH and decrease ammonia volatilization, thereby increasing NH_4 in the manure. Assuming manure is applied to meet but not exceed crop nutrient uptake, increasing ammonium in manure would be desirable, as it is the dominant form of plant-available N in soils. Lower C to N ratios in pen-floor manure with straw than wood indicate a potential to use straw to lower the C to N ratio in certain seasons if N immobilization is a problem in manured soils. Sulfate concentrations were significantly higher in the spring, fall, and winter; Na and SAR in the spring; K in the fall; Mg and NH_4–N in the fall and winter; Ca and total C in the winter; NO_3–N in the summer, fall, and winter; and total P and available P in the summer.

Manure application in Alberta is currently based on the total N and NH_4–N content of manure. Our results indicated that neither bedding or season affected the total N content of pen manure. In contrast, higher NH_4–N in manure with wood than straw and higher levels in the fall and winter than the spring and summer indicate that the available N content of manure might be managed using bedding or season. Allowable manure application rates in the future may be based on phosphorus (i.e., total P) instead of N. Although total P in our study was not affected by bedding, higher concentrations in the summer than other three seasons suggests that season might be a tool to manage P application to cropland.

Season had the strongest effect on populations of *E. coli*, TC, and TAH at 27 and 39°C in the pen manure. Bedding had no effect on the bacterial populations, and may be due to the high manure to bedding ratio in our pen-floor manure. Significantly higher populations of *E. coli* and TC in the pen manure during the summer and a positive correlation of populations with mean daily air temperature indicate that this warm season is of the most concern in terms of potential environmental contamination. All bacterial populations were lower in the winter than the other three seasons. However, we believe applying manure to frozen soils is not a good practice because of potential runoff of manure over frozen soils into surface water. If manure was applied in the spring or fall, there is a potential for fecal-indicator populations to be significantly lower than in the summer, and this may minimize environmental contamination by these bacteria. Bedding material and seasonal timing of cleaning feedlot pens and land application of manure may be a potential tool to manage certain nutrients, soluble salts, and pathogens in the manure. This could increase nutrient use by crops and decrease potential environmental contamination.

ACKNOWLEDGMENTS

Field assistance was provided by Brian Handerek and Sean Robison. Laboratory assistance was provided by Jim Braglin-Marsh, Clarence Gilbertson, Wayne McKean, Bonnie Tovell, Troy Bech, and Rhett Rasmussen. Ian Walker and Wendi Smart coordinated the bedding of the cattle. Statistical advice was provided by Toby Entz and SAS programming advice by Brian Nishiyama. Enviro-Test Laboratories in Calgary, Alberta conducted the total phenol analyses of the bedding materials. Funding for this study was provided by the Canada-Alberta Beef Industry Development Fund.

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