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Influence of cattle manure on the bacteriological quality of organic Iceberg lettuce

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Introduction

Manure may harbour human pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes*. Pathogenic bacteria can be present in the intestinal tract of animals without causing disease, and are therefore not easily detectable. Results from an experiment by Kudva *et al.* (1998) indicated that *E. coli* O157:H7 survived for more than a year in a manure pile that was stored outside under fluctuating environmental conditions. In organic agriculture manure is more frequently used as fertilizer for vegetables than in conventional farming. The use of manure as fertilizer in organic food crop production has led to concern about the potential for contamination of the products with human pathogenic bacteria. It is especially products that are eaten raw that may pose a risk for food borne diseases. This risk of transferring pathogenic bacteria from manure to vegetables during production has to a lesser degree been investigated.

Composting is suggested as a possible method for sanitizing fresh manure, but there are no standards and requirements for such a treatment before the manure is used in organic or conventional food crop production. The defined composting method called Controlled Microbial Composting® (CMC) is said to give fully decomposed sanitized compost in 6-8 weeks (Lindström 1995). The process has to be aerobic, and the temperature must quickly rise to 55-65ºC and stay there for a week. In organic vegetable production in Norway use of composted manure is rather common, but controlling the process with a compost turner according to the temperature and CO_2 during decomposition is not common.

The aim of this project was to investigate the bacteriological quality and the occurrence of selected pathogenic bacteria from organically grown Iceberg lettuce fertilized with slurry, solid manure and manure-based compost.

Materials and methods

The field trials were carried out in 2001 (Trial I) and 2002 (Trial II) in Tingvoll at the west coast of Norway. Four fertilizing treatments were randomized with four replications on 16 experimental plots, each of 4 × 3.25 m. The fertilizing treatments were manure-based compost (30 t/ha), firm manure (40 t/ha), slurry (35 t/ha) and inorganic fertilizer as control (0.4 t/ha Fullgjødsel 25-2-6, Norsk Hydro, Norway). All manure was from bovine origin and from the same livestock.

Before each field trial a modified form of the CMC-method (Lindström 1995) was applied on a mixture composed of 86 % firm manure, 10 % straw and 4 % soil, on weight basis. The method recommends addition of a compost microbial starter culture and fresh plant material. The compost microbial starter culture is not permitted for use in Norway and was thus not added to the compost. Fresh plant material was not available when the composts were started, in March (I) and November (II).

Seedlings of lettuce (*Lactuca sativa* var. Crispum, 'Iglo') were transplanted in the plots one week after fertilizing, 160 plants per plot. A 1.5 m broad buffer zone with unfertilized lawn was established between the plots. A fence surrounded the field trial in order to keep out larger animals.

Samples of soil, fertilizer, fertilized soil, seedlings and lettuce at harvest were analysed for the presence of bacteria used for indication of contamination with intestinal material (thermotolerant coliform bacteria (TCB) and *E. coli*), *E. coli* O157, *Salmonella* spp. and *L. monocytogenes* by the use of standard microbiological methods.

Results and discussion

<u>Bacterial analyses of soil, fertilizer and fertilized soil</u>

The results from analysing soil, fertilizer and fertilized soil for faecal indicator bacteria (TCB and *E. coli*) are listed in Table 1. As expected there were large differences in the numbers of indicator organisms between inorganic fertilizer and the different organic fertilizers. Difference between number of TCB and *E. coli* in composted manure and untreated manure was expected, but not found. The reason for this was probably that temperature did not exceed the expected 55-60°C for the required time.

The impact of the different fertilizers on the numbers of TCB in soil was not obvious. There was a significant difference in the numbers of TCB in soil fertilized with inorganic fertilizer and slurry. By comparing untreated manure (slurry and solid manure), treated manure (compost) and inorganic fertilizer, the only significant difference was detected between untreated manure and inorganic fertilizer (results not shown). In trial II, the numbers of TCB and *E. coli* steadily declined from application of manure to a barely detectable level 41 weeks after fertilization. The numbers of the different indicator organisms isolated from the samples differed significantly between the years which suggest that the climate influence persistence of faecal bacteria in the environment along with other factors such as other microorganisms present in soils etc.

Table 1. Numbers of faecal indicator bacteria (TCB and *E. coli*) in soil, fertilizer and fertilized soil (n = 10) one week after fertilizing in trial I and II. Results and are given as mean $\log_{10}$ CFU (colony forming units) g^{-1} wet weight (S.D).

	Type of bacteria	Soil bef. fertili zing	Inorganic fertilizer		Composted manure		Firm maure		Slurry	
			Alone	And soil	Alone	And soil	Alone	And soil	Alone	And soil
I	TKB	<1	<1	<1	3,11 (1,36)	1-1,6**	3,78*	1*	4,23 (0,48)	1,17 (1,05)
	E. coli	<1	<1	<1	2,81 (1,36)	1-1,6**	3,78*	1*	4,23 (0,48)	1,17 (1,05)
II	TKB	2,44 (0,56)	<1	1,90 (0,73)	3,00 (1,35)	2,33 (0,33)	2,49 (1,36)	2,46 (0,31)	4,49 (0,28)	2,84 (0,42)
	E. coli	2,14 (0,92)	<1	1,89 (0,74)	0,77 (1,25)	2,07 (0,83)	2,49 (1,36)	2,44 (0,32)	4,49 (0,28)	2,84 (0,42)

** Four positive samples, * One positive sample

In Trial II *E. coli* O157:H7 was isolated from solid manure (8 of 10 samples) and slurry (10 of 10 samples); and in soils fertilized with these types of manure one week after fertilizing, respectively 1 of 10 samples and 9 of 10 samples. Samples of fertilized soil 7, 9, 13, 17 and 41 weeks after fertilizing were negative for *E. coli* O157:H7. The finding of *E. coli* O157:H7 was very surprising since the monitoring programme shows that the occurrence of *E. coli* O157:H7 in Norwegian cattle is low compared with other countries in Europe (Hofshagen *et al.*, 2004).
Salmonella spp. and *Listeria monocytogenes* were not found in any of the samples.

Bacterial analyses of lettuce at harvest

The results from bacterial analyses of lettuce at harvest are given in Table 2. Although significant differences were found for TCB counts from soils fertilized with the different types of fertilizer, this was not reflected on the lettuce. The levels of faecal indicator bacteria in the lettuce at harvest were low and there was no significant difference in the bacteriological quality of the lettuce among the different fertilizer treatments. The results from the bacterial analyses agree with results found in an investigation of Norwegian organic lettuce (Loncarevic *et al.* 2005).

Table 2. Numbers of faecal indicator bacteria (TCB and *E. coli*) on seedlings and lettuce (n = 10) at harvest from trial 1 and 2. Results and are given as mean $\log_{10}$ CFU g^{-1} (S.D).

Trial	Type of Bacteria	Seedlings	Lettuce fertilized with inorganic fertilizer	Lettuce fertilized with compost	Lettuce fertilized with firm manure	Lettuce fertilized with slurry
I	TCB	2,58 (1,03)	<1	1,48*	1*	<1
	E.coli	<1	<1	1,48*	1*	<1
II	TCB	4,1*	1*	<1	<1	<1
	E.coli	4,1*	1*	<1	<1	<1

* One positive sample.

E. coli O157:H7, *Salmonella* spp. and *Listeria monocytogenes* were not isolated from any of the samples. In a greenhouse experiment with lettuce fertilized with manure inoculated with *E. coli* O157:H7 the same trend was seen; the pathogen persisted in soil eight weeks after fertilizing when the lettuce was harvested, but was not recovered from the lettuce (Johannessen *et al*. 2005). Other Norwegian investigations of bacteriological quality of lettuce and other vegetables for fresh consume, both conventional and organic, shows that such products may contain pathogens and derby pose a risk of food-borne diseases (Johannessen *et al*. 2002, Loncarevic *et al*. 2005).

Contamination of fresh produce from farm level to consumers may take place during all stages of production, processing and storage. Besides direct contamination with faeces or fertilized soil possible sources are irrigation water, wash water, equipment and handling of the product, insects, birds and rodents.

Conclusion

In conclusion, no difference in bacteriological quality could be detected in lettuce at harvest after application of various types of cattle manure.

The results are published in: Johannessen, G.S., R.B. Frøseth, L. Solemdal, J. Jarp, Y. Wasteson & L. M. Rørvik. 2004. Influence of bovine manure as fertilizer on the bacteriological quality of organic Iceberg lettuce. Journal of Applied Microbiology 96:787-794

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