Research on the persistence of tetracyclines contained in pig slurry

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Abstract

Tetracyclines are among the most common antibiotics administered *via* medicated feed to weaning piglets, in concentrations of several hundreds of mg kg^{-1} feed, to prevent the outbreaks of enteric diseases. A significant part is excreted still in active form, remaining in the slurry, where it can persist particularly when bound to the organic matter.

A study has been carried out to assess the risk of the transfer of oxytetracycline, chlortetracycline and their metabolites to grain maize fertilised with pig slurry, at two experimental sites, one in Northern Italy (Reggio Emilia) and the other in Central Italy (Rome). Slurries were either injected between rows or distributed by sprinkling, splashing onto the plants. The tetracycline concentrations in the slurries, in the soil and in the different parts of the maize plant (stalks, cobs, grains and roots) were measured by means of liquid chromatography with UV/DAD detection (LoD = 20 ng g^{-1}).

The average concentration in the weaning piglets slurry was measured at 24 mg kg^{-1} of oxytetracycline and 6 mg kg^{-1} of chlortetracycline. In soil, oxytetracycline was detected sporadically both at the beginning and the end of the Reggio Emilia experiment while chlortetracycline was found at the end of the same experiment with concentrations of up to 300 ng g^{-1}. It was not possible to identify the presence of tetracycline above the detection limit of the analytical method in any of the samples of the different parts of the maize plants taken from the two field trials.

However, in an experiment conducted on maize seedlings cultivated in jars over a short time-scale it was possible to detect oxytetracycline concentrations in the roots of the plants which were in direct relation to that in the soil while in the aerial part of the plants only some traces were detected. This could indicate a general possibility of uptake for tetracycline from the soil but results of the field trials and the calculated carry-over rate of 5% for roots/soil and of <1% for aerial part/soil in jars plants excluded accumulation in the edible parts of the crop in real growing conditions.

Keywords: antibiotics, liquid chromatography, maize, soil, solid fraction, uptake

Introduction

It is common practice during the weaning of piglets to use feeds medicated with a wide range of antimicrobials to prevent possible diseases outbreaks. Tetracyclines are among the most in use antibiotics administered with the feed, in concentrations of several hundreds of mg kg^{-1} feed. They are partially adsorbed and metabolised; a significant part is then excreted still in active form, remaining in the slurry, to be found particularly in the solid fraction (del Castillo *et al*., 2003).

One of the risk posed by the presence of antibiotics in slurry involves their possible transfer to crop products through slurry spreading on soil or directly onto the plants (De Liguoro *et al*., 2003). Research has been carried out to assess the risk of the transfer of tetracyclines to maize fertilised with the slurry.

Materials and methods

Grain maize was cultivated for two years at two experimental sites:
- Reggio Emilia site (Northern Italy). The trials were carried out in a field entirely devoted to maize cultivation, on a sub-alkaline not calcareous silty loam soil already fertilised with pig slurry over preceding years;
- Rome site (Central Italy). The neutral not calcareous sandy loam soil had never been treated with livestock manure and was therefore free of tetracycline residues.
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Using a split-plot design, maize was fertilised with both slurries from weaning piglets and slurries from finishing pigs (main plots). Rates were sufficient to meet the crop nitrogen requirements. Slurries were either injected between rows or distributed by sprinkling, splashing onto the plants (subplots).

Concentrations of oxytetracycline and chlortetracycline in the slurries, in the soil and in the different parts of the maize plant (stalks, cobs, grains and roots) were measured by means of liquid chromatography with UV/DAD detection (Brambilla *et al*., 2007).

A further experiment was conducted on maize seedlings cultivated in jars over a shor time scale (45 days) using increasing concentrations of oxytetracycline in the soil (up to 1,000 ng g^{-1} in dry soil) obtained by adding a prepared mixture for medicated pig feed, serially diluted in distilled water.

Results and discussion

Slurry from weaning piglets (fed with feed medicated with antibiotics) had tetracycline concentrations significantly higher than those found in slurry from finishing pigs where the use of antibiotics is sporadic and in any case affected by suspension times (Table 1).

It is interesting to note that appreciable concentrations were also found in slurry from finishing pigs, probably due to the delay in the excretion of the antibiotic with respect to its ingestion over the weeks immediately following the transfer of the animals to this sector.

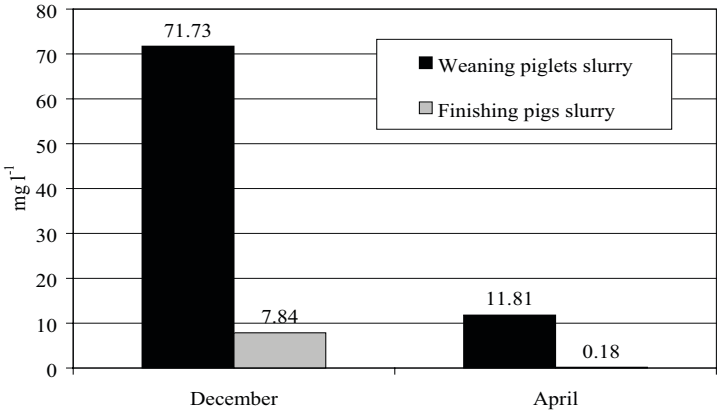
Table 1. Average characteristics of the slurry used in the trials

Parameters	Unit of measurement	Weaning piglets slurry	Finishing pigs slurry
pH	-	7.6	7.2
Total solids	%	4.1	4.6
Volatile solids	%	2.7	3.3
Total organic carbon	% total solids	37.6	43.2
Total Kjeldahl nitrogen	g kg^{-1}	3.24	3.35
Oxytetracycline(1)	mg kg^{-1}	24.5	3.2
Chlortetracycline(2)	mg kg^{-1}	5.9	1.0

(1) Concentrations measured over the first year of the trial when the feed contained oxytetracycline
(2) Concentrations measured during the second year of the trial when the feed contained chlortetracycline

There was a strong reduction in antibiotic concentrations over the storage period of the slurry but in spite of this there were still significant differences between the materials originating from the two sectors (Figure 1). Analysis of reduction trends leads to the conclusion that, with the extended storage times envisaged by the Action Programmes drawn up under the Nitrate Directive 676/91, this may result in lowering of concentrations, even if not enough to eliminate antibiotics altogether from weaning piglets slurry.

Figure 1. Reduction of concentrations of oxytetracycline in slurry over 4 months of storage.



Concentrations over the limit of detection (20 ng g^{-1} of dry weight) were found in the soil only in the Reggio Emilia site, which had already been fertilized with pig slurry for several years before the research activities were initiated. Oxytetracycline was detected sporadically both at the beginning and the end of the experiment while chlortetracycline was found at the end of the research with concentrations of up to 300 ng g^{-1}, where slurry from weaning piglets had been used. Since chlortetracycline degrades faster than oxytetracycline in liquid solutions like slurry, once it is in the soil it bonds effectively with the organic matter and thus tends to persist.

It was not possible to identify the presence of tetracycline above the detection limit of the analytical method in any of the samples of the different parts of the maize plants taken from the two field trials.

However, in the experiment conducted on maize seedlings cultivated in jars over a short time-scale it was possible to detect oxytetracycline concentrations in the roots of the plants which were in direct relation to that in the soil, while in the aerial part of the plant only some traces were detected (Table 2). This could indicate a general possibility of uptake for tetracycline from the soil but results of the field trials and the calculated carry-over rate of 5% for roots/soil and of <1% for aerial part/soil in jars plants excluded accumulation in the edible parts of the crop in real growing conditions.

Table 2. Oxytetracycline concentrations in the experiment on maize seedlings cultivated in jars

Treatment (ng g^{-1} soil)	Maize aerial parts (ng g^{-1})	Maize roots (ng g^{-1})
Control	-	-
62.5	1.84	7.50
125	1.69	9.70
250	1.01	10.29
500	1.88	18.23
1000	2.81	54.46

Furthermore, in the experiment conducted in Rome, the height of the plants and the number of leaves and cobs were found to be significantly higher in some treatments, in particular for the maize which had been fertilised with the weaning piglets slurry and using the sprinkling method. In the trials using laboratory jars too, it was found that tetracyclines stimulate maize seedlings growth at low concentrations and inhibited it at high concentrations.

For the moment it is only possible to hypothesise that these differences were due to a hormetic response. In conceptual terms, the hormesis theory states that there are certain substances which have a stimulating effect when administered at low concentrations causing an inhibiting or toxic effect at high levels, when interacting with a living organism (Stebbing, 1997; Calabrese and Baldwin, 2002; Migliore *et al.*, 2007).

Conclusions

An appreciable level of tetracycline residue was found in fresh slurries from weaning piglets while the concentrations of the antibiotics tended to reduce significantly in finishing pig slurry and following a lengthy period of storage.

The accumulation of chlortetracycline in soils treated with slurry from weaning piglets was significant, probably due to the formation of a complex with the organic matter.

In real cultivation conditions however, no detectable transfer of the antibiotics or their metabolites was measured inside those parts of the maize plant tested. This was true both with the soil injection of the slurry containing tetracycline and with the spraying of the slurry on the plants during the phenological stages prior to flowering.
These findings reassured about the possible transfer risk for tetracyclines trough the food chain.

Questions raised which go beyond the purposes of this research but merit further study regard both possible hormetic effects on crop growth due to tetracycline and the effects that the emission of antibiotics and their metabolites have on the microbiology of agricultural soils.

References

Brambilla G., Patrizii M., De Filippis S. P., Bonazzi G., Mantovi P., Barchi D., Migliore L. (2007) - Oxytetracycline as environmental contaminant in arable lands. Analytica Chimica Acta, 586: 326–329.

Calabrese E.J., Baldwin L.A. (2002) - Defining hormesis, Human and Experimental Toxicology, 21: 91-97.

del Castillo J.R.E., Beauchamp G., Martineau G.P., Besner J.G. (2003) - Short-term effects of in-feed supplementation of tetracyclines for disease control on feed intake pattern and growth in weaned pigs. Livestock Production Science, 76:115-124.

De Liguoro M., Cibin V., Capolongo F., Halling-Sørensen B., Montesissa C. (2003) - Use of oxytetracycline and tylosin in intensive calf farming: evaluation of transfer to manure and soil. Chemosphere, 52: 203-206.

Migliore L., Amendola A., Cerioli N.L., Fiori M., Cozzolino S. (2007) - Hormesis in plant (Lythrum salicaria L.): a case study. Environmental Management Engineering, Planning and Economics (A. Kungolos et al. eds.), Vol. I, pp. 245-250, Grafima Publ., Thessaloniki, Greece.

Stebbing A.R.D. (1997) - A theory for growth hormesis. Mutation Research, 403: 249-258.