DEGRADATION OF DORAMECTIN DURING THERMOPHILE PHASE OF COMPOSTING AND MANURE STORAGE

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OBJECTIVES

Avermectins derived from soil microorganism *Streptomyces avermitilis* (Fisher 1990). Doramectin is an avermectin derivate, is an endectocide. In veterinary medicine, doramectin is used as an antiparasitic drug for extermination of numerous parasites in all species of domestic animals. Their mode of action based on strong binding on chloride channels in nerve cells of parasite, disruption of nerve impulses and its transmission (Martin, Robertson, & Wolstenholme 2002). Following animal treatment, it is excreted mostly (ab to 80%) in an active, non metabolized form irrespective on formulation, dosage and route of administration (Shoop, Mrozik, & Fisher 1995). Excretion period is usual long (several weeks), but main amount is excited at sheep in 7 days (Hennessy & Alvinerie 2002; Stenersen 2004). The maximum concentration of 2186 ±145 ng/g dry faeces after single subcutaneous administration of 0.2 mg/kg body weight of doramectin in dry faeces was detected on day 2 (Kolar et al. 2006). Doramectin can persist in environment for longer period of time, dependent on chemical, physical and biological conditions (Eržen Kožuh Nevenka et al. 2005). Doramectin is among avermectins medicine with the greatest harmful effect in the environment, because of their specific metabolism and action on non-target organisms. Presence of avermectins in animal faeces and pasture causes killing of some adult insects, of young, barely hatched insects; it increases destruction of their larval forms and can lead to reduction in biotic diversity (Barth et al. 1993; Halley, VandenHeuvel, & Wislocki 1993; Iwasa et al. 2005; Sommer & Bibby 2002; Suarez et al. 2003; Svendsen et al. 2003). Avermectins are extremely toxic as well to water organisms in spite of poor water solubility (Tišler & Kožuh Eržen 2006). In good animal husbandry are some possibilities like composting or anaerobic digestion to influence on containing of some pollutants. Chemical properties of pollutants have strong influence during degradability process. Substances with low water solubility, a large soil/sediment adsorption coefficient and cyclic substances degraded slowly then chain and water soluble substances (Lavrance P.Wackett & Dougls Hershberger 2001). Composting as a biotechnological process is used in organic waste management and bio-remediation of contaminated organic materials and soil (Wolfgang Fritsche & Martin Hofrichter 2005). Therefore we investigated possible degradation of doramectin during composting after a single addition into compost mixture of sheep manure to minimised spreading in to the environment. As a comparison, influence of sterile compost and manure storage on doramectin degradation was assessed.

MATERIALS AND METHODS

Degradation of doramectin followed 21 days during thermopile phase of composting and manure storage. Degradation of doramectin addition during composting of sheep faeces was studied in pilot scale ($1m^3$) insulated vessels in the dark controlled conditions. Four different suspensions of doramectin were used for addition into compost mixtures.

Table 1: Composition of suspension used as addition in our study

Components	Suspension 0	Suspension 1	Suspension 2	Suspension 3
Dectomax®	0 ml	0.5 ml	1.0 ml	2.0 ml
Ethyoleate 98% (Acros Organics, USA)	11.8 g	11.8 g	11.7 g	11.6 g
Purified sesame oil (for pharnaceutical use) (KEFO, Slovenia)	up to 100 ml (86.4 ml)	up to 100 ml (85.9 ml)	up to 100 ml (85.6 ml)	up to 100 ml (84.7 ml)
Achieved concentration of doramectin	0	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml

The equal quantities of each suspension were added into the compost mixtures in the single test. Target concentrations in compost were:

a) Concentration 0 (C0) didn't contain any doramectin.
b) Concentration 1 (C1) contend the half of concentration C2
c) Concentration (C2) contend the maximum concentration detected in dry faeces after single subcutaneous administration of 0.2 mg/kg body weight of doramectin at sheep – 2186 ng/g of dry sample (Kolar, Cerkvenik Flajs, Kužner, Marc, Pogačnik, Andrej, Cornelis van Gestel, & Kožuh Eržen2006)
d) Concentration 3 (C3) contend double value of concentration C2.

Each concentration of doramectin was tested in during composting in three batches (B1, B2, B3) and six samples.

Table 2. Sampling plan

Sampling number	Sampling day
1	0
2	7
3	14
4	21

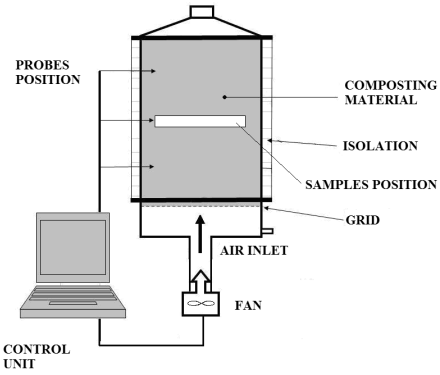

Figure 1. Scheme of composting vessel.

Homogenous mixed material of sheep faeces from deep litter with addition of water and pine bark has been used so that content of moisture reached approximately 60%. Temperature was limited upward to 68ºC, was controlled in vessels by PT 100 probes using computer program "Visi DaQ" ® (Advantech, USA) and maintained by fans which were used for aeration of material as well. Humidity was determinate by drying of samples at 105ºC for 24 hours and weighing. The pH value was determinate in air dried samples after addition of five amount of $CaCl_2$, 0.01 mol/l and standing of two hours, by calibrated pH meter (Hanna HI 221, Germany). Total carbon (C), total nitrogen (N) and C: N ratio where determinate by elementary analyzer Vario MAX CNS (Elementar, Hanau, Germany) by thermal conductivity detector after combustion at 900ºC.

Concentrations of doramectin was analyzed using validated analytical procedure employed HPLC with fluorescent detection. Homogenized, moist samples (2.0 g) were extracted with 25 ml of acetonitrile (Merck, Darmstadt, Germany) by shaking on a mechanical shaker (Vibromix 313 EVT, Tehtnica Zelezniki, Slovenia) at room temperature for 15 minutes at 400 rpm. After centrifugation for 10 min at 3000 rpm (20ºC), using a centrifuge (ROTIXA/RP, Hettich, Germany), a 15 ml portion of extract was taken and mixed with 50 µl TAE and doubly distilled water to 50 ml. Bakerbond SPE Octyl (C_8) cartridges 500 mg, 6 ml (J.T. Baker, Philipsburg, New Jersy) were introduced into the clean-up procedure and to pre-concentrate doramectin extracted from samples. Doramectin was eluted with 5.0 ml of acetonitrile. After that fallowed evaporation to dryness under nitrogen at 60ºC and derivatisation. To the samples was then added 100 µl N-methylimidazole – acetonitrile (1:1, v/v) and 150 µ trifluoroacetic anhydride-acetonitrile (1:2, v/v), all supplied by Merck (Germany) (De Montigny et al., 1990), and analyzed by HPLC. The Thermo Separation Products (USA) HPLC system consisted of a Spectra Systems P2000 pump, an AS300 auto injector and a Shimadzu (Japan) RF-535 fluorescence (excitation wavelength 365 nm; emission wavelength 470 nm) detector. The separation was carried out on a Phenomenex (Phenomenex USA) Luna C18 (2) column (150 x 4.6 mm ID; 3 µm particle size) with a Phenomenex pre-column C18 (4.0 x 4.6 mm ID; 5 µm particle size). The column temperature was maintained at 28ºC. Mobile phase consisting of acetonitrile, methanol (Merck, Germany) and water (47.5:47.5:6.0, v/v/v), was pumped at 1.1 ml/min and 50 µl of sample was injected into the HPLC system. Results were evaluated according to the external standard method and corrected for recovery (Kolar et al., 2004). The stock solution of doramectin in a concentration of 100 mg/ml and working standard solutions were prepared in acetonitrile. The recovery of the method was tested daily within the set of sample determinations by addition of

doramectin to blank moist samples at two concentrations expected in the measured samples. The blank samples (added suspension 0) served as a negative control. All samples were analyzed in four parallel determinations. Low detection limit (1.0 µg/kg of dry sample), good repeatability (RSD < 15%), recovery of the method in above 80.0%, enabled the determination of doramectin in our samples.

RESULTS AND DISCUSSION

Average 21 days temperatures were in compost mixtures between 48.9 and 56.3°C and in manure storage 39.5°C (Figure 2). Differences in average temperatures between manure storage and all batches of composting were statistically significant ($P<0.05$). Average moisture contain was in compost samples at the beginning of the study between 57.2% and 63.5% (Figure 3). Losses of the moisture were in composting mixtures between 12 and 40% and in manure storage only 5.5% (Figure 3). Losses of moisture in composting mixtures are most likely consequence of high temperatures and aeration of material (Zhu et al. 2004) in the mean time both parameters in manure storage were absent.

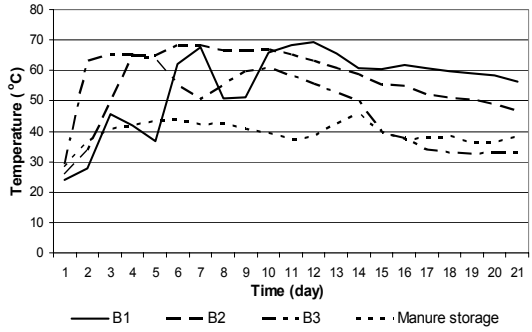

Figure 2. Temperature trends during composting (batches B1, B2, B3) and manure storage

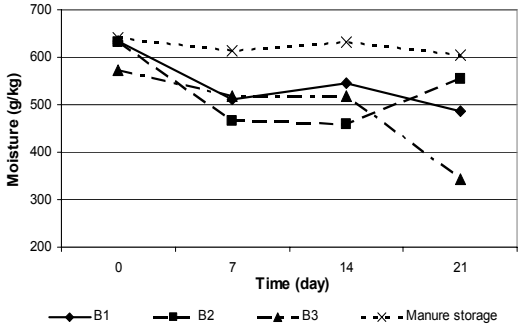

Figure 3. Average moisture contents during composting (batches B1, B2, B3) and manure storage.

Values of pH ranged in all samples between 6.9 and 7.66 (Figure 4). High pH values at the beginning of the study we assigned to raw material used in study (deep litter), where degradation

processes already started. Degradation products (NH_3, NH_4+, urea, uric acid, proteins) that originated from anaerobic degradation and can influence on pH, (Peigne & Girardin 2004); (Veeken, de Wilde, & Hamelers 2004). Values of pH decrease during composting, but in manure storage raised a little. Decrease in C: N ratio from 26:1 at the beginning of the study to 20:1 after 21 days in composting mixtures was statistically significant (P<0,05) (Figure 5). The tendency in C: N ratio in manure storage was opposite to composting and was grown during 21 days of storage in range 16.6: 1 at the beginning of storage up to 23.4 in day 21. Increase in inorganic matter – ash contain, decrease in C:N ratio and changes of temperatures in composting mixtures were in our study indicators of good composting process (Nakasaki et al. 1992).

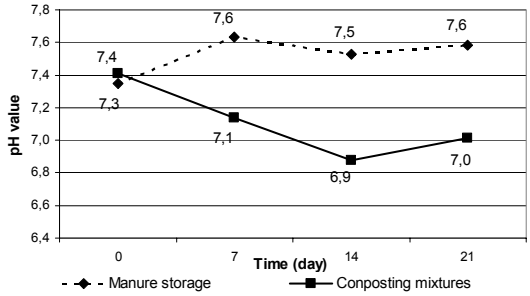

Figure 4. Average pH values during composting and manure storage

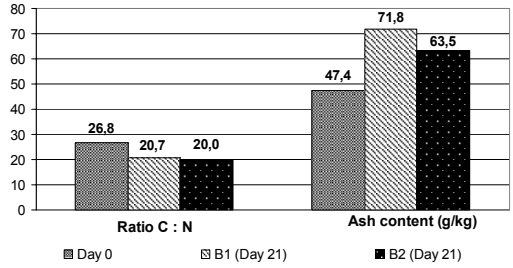

Figure 5. Average ratio C: N and average ash contain on day 0 and day 21 in composting mixtures studied; B3 was not analyzed.

Degradation of doramectin during composting was also found and was in average 36. 6% (Figure 6). Differences in content of doramectin in samples before composting and day 7, 14 and 21 were statistically significant (P<0.05). Differences in degradation rate between varied concentrations were insignificant. Degradation of doramectin in manure storage was in average 12.2% and difference in doramectin contains in samples before storage and day 14 and 21 were statistically significant (P<0.05) (Figure 7).

Statistically evaluation of composting parameters showed on joining doramectin degradation and loss of moisture in samples especial in B3 (r=0.969) and correlation was statistical significant (P<0.05). About influence of moisture was published in other studies (Kolar & Kožuh Eržen 2007). We believe that decries in doramectin concentration could not be significant dependent with aeration of material noir evaporation account of strong tendency to bind to particles and low water solubility (Bloom & Matheson 1993). During composting process formed different substances and as well humic substances (humic acids, fulvic acids, humins) (Miikki et al. 1994;

Mondini et al. 199; Huang et al. 2006). Known are sorption properties organic and inorganic molecules and also pesticides and herbicides (Bollag, Myers, & Minard 1992; Fliedner 1997; Jones & Bryan 1998).

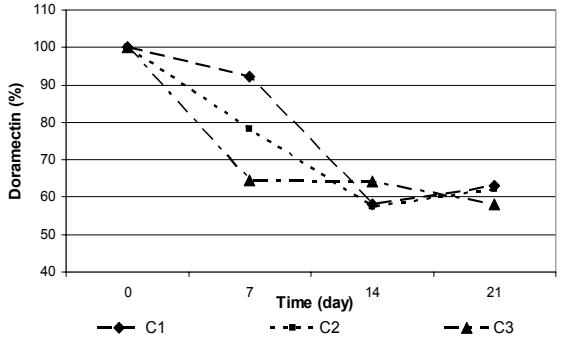

Figure 6. Average doramectin contain during composting.

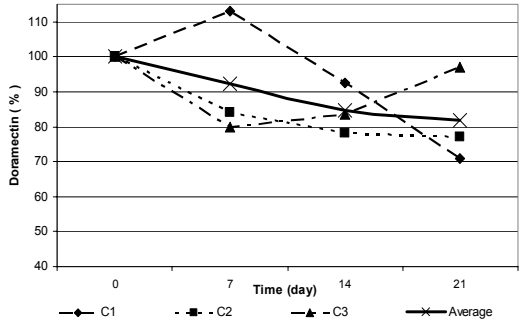

Figure 7. Average doramectin contain during manure storage.

CONCLUSIONS

Rapid rising of temperatures in composting mixtures was proved by biodegradation of composting material. Loss of the humidity in compost mixtures result from aeration of composting mixtures. We observed gradual degradation of doramectin under composting and storage conditions. Degradation rate in 21 days was greater during composting then manure storage. This difference is due to more intensively biological degradation and loss of humidity which can influence on sorption behaviour of doramectin against organic carbon in dry matter. For final estimation, these influences should be assessed further. Faster degradation during composting could be turned into account to reduce enter of medicine in to environment.

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