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COCCIDIOSTATS IN POULTRY MANURE – A POTENTIAL THREAT TO BENEFICIAL SOIL INVERTEBRATES

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Abstract

Monensin and lasalocid are polyether ionophore antibiotics used in veterinary medicine for prevention and treatment of coccidiosis in poultry. They are extensively used in the poultry industry throughout Europe. Both substances are excreted with the faeces mostly in their active form. The use of manure from treated animals on agricultural soil results in contamination that could pose a threat to soil organisms and thereby reduce the production potential of farmlands. Their degradation rates in manure and soil, as well as their effects on non-target soil organisms are mostly unknown. We conducted several studies to measure the degradation of lasalocid and monensin in broiler manure and after application to soil. Ecotoxicological studies were also performed to obtain concentrations at which these coccidiostats are harmful to soil invertebrates, namely earthworms and woodlice. Degradation rates in manure and compost depend mostly on moisture levels and temperature. Half-lives in compost are significantly shorter than if manure is aged in a pile with no treatment. Avoidance of the test animals was the most sensitive endpoint in the ecotoxicity tests. On the basis of our results, we recommend that poultry manure from treated animals be stored for at least one month before application to soil.

Keywords: coccidiostats, lasalocid, monensin, manure, agricultural soil

Introduction

The growing needs of the human population drive modern agriculture into using increasing amounts of pesticides and fertilisers, thereby increasing the threat of contamination to soils and underground water. Intensive use of manure burdens the environment not only with large amounts of nitrogen and metals, but also with veterinary pharmaceuticals and feed additives. Their introduction into the environment is not controlled, a problem that has been neglected for decades (Boxall, 2004).

Coccidiosis is a protozoal infection in poultry causing diarrhoea and dysentery. It is often fatal and spreads rapidly. Coccidiostats are authorised in the European Union as feed additives for poultry. Overall in the EU, of the estimated 40.65 million tonnes of feed produced, some 18.33 million tonnes is manufactured with an in-feed coccidiostats (EC, 2008). Broilers and turkeys are treated with coccidiostats almost their entire life. The most frequently used coccidiostats, monensin and lasalocid are natural ionophore antibiotics produced by bacteria of the genus Streptomyces. In treated animals these substances are only partially metabolised

and are thus excreted predominantly in the active form. EFSA (2004) reported that 74–83% of lasalocid in broiler excreta is in the active form and more than 50% of the excreted monensin is the parent compound (Davison, 1984).
The degradation rates of monensin in manure range between 3 and 21 days (EFSA, 2004; 2005; Dolliver et al., 2008) and the rate of lasalocid degradation is mostly unknown. When in the environment, the coccidiostats undergo both biotic and abiotic degradation, with microbial degradation being the prevalent (Vertesy et al., 1987; Sassman and Lee, 2007; Hansen et al., 2009b; Hansen et al., 2012). The rate of decay depends on the organic content of the soil, soil moisture, temperature and pH (Sassman and Lee, 2007; Yoshida et al., 2010), but it has so far only been investigated under laboratory conditions. The reported monensin half-lives in soil are between 2 days (Sassman and Lee, 2007) and 22.7 days (Yoshida et al., 2010). EFSA (2004) reports lasalocid half-life values of 0.6–14.2 days. The reports on the predicted environmental concentrations (PEC) of monensin vary greatly and are in the range of 0.05–1.12 mg kg-1 soil (EFSA, 2005; Žižek et al., 2011). The PEC for lasalocid is estimated at 0.58 mg kg-1 soil (EFSA, 2004).
In spite of extensive use of monensin and lasalocid for more than 40 years and their potential presence in the environment, there is only little published information concerning their effects on non-target organisms and their fate in the environment. This lack of data was also found by (Hansen et al., 2009a), who stressed that a complete risk assessment cannot be performed without them. Several investigations into the risk of the two most common coccidiostats were therefore undertaken at the Laboratory for forensic toxicology and ecotoxicology at the Veterinary Faculty, University of Ljubljana. We studied their degradation in manure and soil and explored their effects on beneficial soil invertebrates.

Materials and Methods

Degradation experiments
Degradation of monensin in manure and compost has already been studied (Dolliver et al., 2008). In a similar way, an experiment was conducted with lasalocid. Chicken manure with no coccidiostats was obtained from a farm in Slovenia. It was divided in two piles, one of which was mixed with wood shavings to obtain a C/N ratio of approximately 30:1. Lasalocid was mixed into a sub-sample of both piles at the concentration 10.6 mg kg-1 dry weight. Lasalocid-containing manure was put into nylon mesh bags containing approximately 30g of sample. The bags were put in the middle of the manure/compost pile. The experiments were performed in 1 m3 polypropylene bioreactors with perforated bottoms and an inlet for aeration. Temperature was monitored throughout the experiment. Compost was constantly aerated and moisture was adjusted weekly to approximately 60%. Samples were taken at 2-day intervals for the first week and once a week thereafter. They were stored at -20°C until analyses. Lasalocid was measured with HPLC using the method described in Žižek and Zidar (2013).
For degradation in soil, lasalocid was mixed with the same manure to obtain a concentration of 3 mg kg-1. Manure was applied to soil at levels corresponding to 10, 20 and 30 tonnes per hectare. Samples were taken from five different locations on each plot and a cumulative sample was made at each sampling. Samplings took place at two-day intervals in the first week and in four-day intervals thereafter.
The half-life of lasalocid in manure, compost and soil was estimated using the Gustafson-Holden bi-phasic kinetic model $C_t = C_0(1+\beta t)^{-\alpha}$ where Ct and C0 are concentrations at time t and at the beginning, respectively, and α and β are the parameters of the gamma probability density function of the degradation constants. The half-life of lasalocid was calculated as $(0.5^{-(1/\alpha)} - 1)/\beta$ (Gustafson and Holden, 1990).

Toxicity tests

Ecotoxicity test were performed with both coccidiostats. The test animals used were earthworms (Eisenia andrei) and isopods (Porcellio scaber). We measured the effects of lasalocid and monensin on earthworm survival, growth and reproduction and on isopod survival, growth and food consumption. In earthworm acute and chronic toxicity testing we followed the protocol of OECD (OECD, 2004) and with isopods, the work was performed we used the procedure described in Hornung et al. (1998). The performed tests are described in more detail in Žižek et al. (2011) and Žižek and Zidar (2013).

There has been indication that standard toxicity test may not be sufficiently sensitive for assessing the threat to soil organisms. As an alternative to standard ecotoxicity tests and a rapid and cost-effective first screening tool for soil assessment, avoidance behaviour tests have been proposed (Yeardley et al., 1996; Natal da Luz et al., 2004; Loureiro et al., 2005; Amorim et al., 2008). We therefore conducted avoidance tests with lasalocid on both test species. The procedure is described in detail in Žižek and Zidar (2013).

Risk assessment

After obtaining the exposure levels with the degradation experiments and the effect concentrations with the toxicity tests, it was possible to use the newly obtained values to reassess the risk posed by monensin and lasalocid to agricultural ecosystems. This was done according to the EC Technical Guidance Document (EC, 2003). As the predicted no-effect concentrations (PNECs) we used the lowest values obtained by the toxicity tests. We formed recommendations regarding the most appropriate way and period of storing manure contaminated with coccidiostats before it is applied to agricultural soil.

Results and Discussion

The results of lasalocid degradation experiments are presented in Figures 1 and 2. Lasalocid in manure degraded with a half-life of 61.8 days and did not fall below 45% of the initial concentrations even after 84 days, whereas its half-life in compost was 17.5 days and the concentrations fell below the limit of detection (10 ng g-1) after 80 days. The average half-life of lasalocid in soil was 3.1 days. Dissipation in soil includes wash-off with rain, as well as biotic and abiotic degradation.

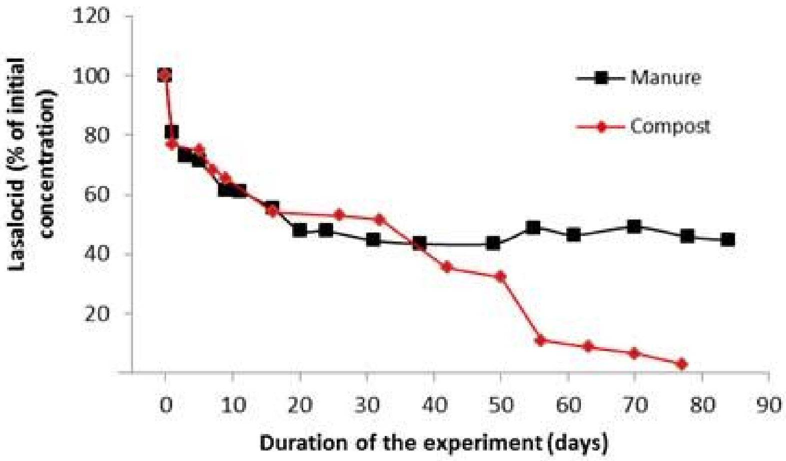

Figure 1: Degradation of lasalocid in manure and compost

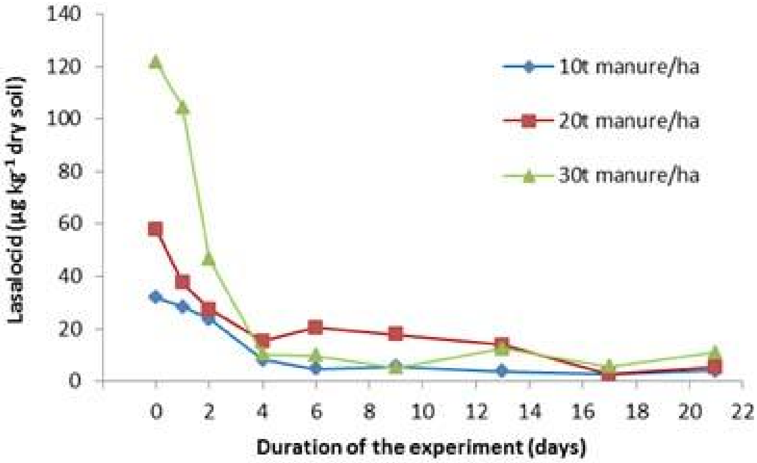

Figure 2: Degradation of lasalocid in soil

The results of the ecotoxicity tests are summarised in Table 1. In general, earthworms are more susceptible to both coccidiostats than isopods. This could be due to their thin integument and greater exposure to the environment. Avoidance test showed much higher sensitivity to ionophore antibiotics compared to the ‘classical’ sublethal toxicity tests. For earthworms, EC50 for avoidance was more than five times lower than the EC50 for reproduction. In isopods we recorded even higher sensitivity. While the highest lasalocid concentration had no significant effects on isopod growth or survival, already the lowest used concentration in the behavioural assay (4.51 mg kg-1) caused significant impact on isopod behaviour. Avoidance behaviour could indicate a potential adverse effect of lasalocid on the habitat function of soils where lasalocid-contaminated manure is applied.

Table 1: Results of the toxicity tests (LC50 – 50% lethal concentration, EC50 – 50% effect concentration, NOEC – no observed effect concentration)

Test species	Test compound	Effect
Eisenia andrei	Monensin	LC_{50} = 49.3 mg kg^{-1} $EC_{50\ reproduction}$ = 12.7 mg kg^{-1} $NOEC_{reproduction}$ = 3.5 mg kg^{-1}
	Lasalocid	LC_{50} = 156 mg kg^{-1} $EC_{50\ reproduction}$ = 69.6 mg kg^{-1} $NOEC_{reproduction}$ = 4.77 mg kg^{-1} $EC_{50\ avoidance}$ = 12.3 mg kg^{-1}
Porcellio scaber	Monensin	LC_{50} > 849 mg kg^{-1} $EC_{50\ growth}$ > 849 mg kg^{-1} $NOEC_{growth}$ > 849 mg kg^{-1}
	Lasalocid	LC_{50} > 277 mg kg^{-1} $EC_{50\ growth}$ > 277 mg kg^{-1} $NOEC_{growth}$ > 277 mg kg^{-1} $EC_{50\ avoidance}$ = 4.9 mg kg^{-1}

By applying the obtained toxicity data in the risk assessment, we saw that using fresh manure contaminated with coccidiostats could pose a threat to soil invertebrates and thereby harm the production potential of agricultural soil. It is strongly recommended that manure should be aged for at least one month before application.

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