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Review Article

MONENSIN – A REVIEW OF FACTORS INFLUENCING ITS PRESENCE IN THE ENVIRONMENT AND RECOMMENDATIONS FOR SAFE STORAGE AND USE OF MONENSIN-CONTAMINATED MANURE

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Summary: Monensin is a polyether carboxylic ionophore used in the veterinary medicine for treatment and prevention of coccidiosis in poultry. It is the most often used coccidiostat in Slovenia. It is only partially metabolised in the treated animals and gets excreted mostly in its active form. By using monensin-contaminated manure on agricultural soil, monensin enters the environment and can be potentially damaging to soil-dwelling organisms and thereby to the production potential and sustainable use of agricultural soil. There is no regulation regarding the use of manure containing monensin and much uncertainty about its dissipation and effects in the environment. We have reviewed the available literature data on the effects of monensin to soil organisms and on the expected concentrations of monensin in manure and in soil in order to ascertain what (if any) actions would be necessary to mitigate the potential harmful impacts of monensin in soil. By the most realistic scenario, if no manure storage were used, the predicted monensin concentrations in soil would exceed the no-effect levels and could therefore pose a risk to soil organisms. Manure needs to be stored not only because of monensin contamination, but also because use of manure without adequate storage poses a risk of spreading potential diseases and environmental contamination, as well as considerable loss of nutrients. Using an aging period of at least one month would sufficiently reduce the monensin levels in poultry manure to render its use safe for beneficial soil organisms. Composting is preferable to aging manure in a pile, because monensin degradation is faster.

Key words: monensin; manure; environmental risk assessment

Introduction

Monensin is a carboxyl ionophore antibiotic produced by the bacterium *Streptomyces cinnamonensis*. It is used in veterinary medicine for the prevention and treatment of coccidiosis in turkeys and chickens. Animals are treated orally via feed mixtures at a maximum dose level of 125 mg/kg dry feed. The administration to broiler chickens is recommended during the entire lifetime (up to 45 days) and to turkeys for 16 weeks, with the withdrawal period of at least one day (1). According to information gathered with a survey among the feed mixing facilities in Slovenia (unpublished data), approximately 2.4 tons of monensin were used in Slovenia in 2011.

Monensin is only partially metabolised in the treated animals and is excreted almost entirely via faeces (2-5). When the excreta are used as manure on agricultural land, monensin enters the terrestrial ecosystem. The reports on its predicted environmental concentrations (PEC) in soil vary greatly and are between 0.05 mg/kg (6) and 0.59–1.12 mg/kg (7).

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Responsible use of veterinary pharmaceuticals includes the proper management of contaminated wastes. In Slovenia, the amount of manure used on arable soil is regulated by the Decree concerning the protection of waters against pollution caused by nitrates from agricultural sources (8), which is based on the EU Nitrates Directive (9). There is, however, no legislation specifically dealing with the use of manure containing coccidiostats in general or monensin in particular. We therefore reviewed the available literature regarding the kinetics of monensin in treated broiler chickens, its fate and behaviour in the excreta, and its predicted levels and effects in the environment in order to provide a comprehensive basis for establishing the best method and the optimum time of storing monensin-contaminated chicken manure.

Pharmacokinetics of monensin in chickens

Despite the economic importance of chickens and the widespread use of monensin, there are only scarce reports on its kinetics. In 1969, Herberg and Van Duyn (2) studied the excretion and tissue distribution of ^{3}H-labelled monensin in chickens. Most of the administered radioactivity was excreted in the faeces, with less than 2% present in urine and tissues. However, most of the tissue radioactivity was associated with the tissue water, indicating that the results were not effective in defining monensin residues in tissues due to tritium exchange from the ^{3}H-monensin. Further studies therefore employed different methods. Donoho et al. (3) used ^{14}C-labelled monensin to study its pharmacokinetics in broilers. They fed the animals 120 ppm [^{14}C]monensin and measured radioactivity in edible tissues and excreta. The balance-excretion studies revealed that monensin is excreted rapidly and nearly quantitatively (94%) in the urine and faeces with the majority (60.8–83.1% of the total recovered radioactivity) excreted within the first day after treatment. Most of the residue radioactivity in the tissues was measured in the liver, but only about 7% of this radioactivity (0.06 mg/kg) was ascribed to parent monensin.

Davison (5) also used radiolabelled monensin to trace its pharmacokinetics in broilers. Chickens were fed from 2.6 to 100 mg of monensin in a single dose. Between 59.3 and 71.8% of the radioactivity was recovered in the droppings, whereas of the monensin in the tissues, most (up to 5.93 mg/kg) was recovered in the liver, followed by kidneys (up to 2.76 mg/kg), muscle (up to 1.87 mg/kg) and abdominal fat (up to 1.06 mg/kg). The elimination period of monensin from the tissues was not determined.

The development of more precise analytical methods enabled lower detection limits and measurements of trace amounts of substances. Atef et al. (10) used thin layer chromatography to detect monensin in the serum and tissues of broiler chickens after a single dose and after feeding the chickens for 2 weeks with a supplemented monensin premix. The mean elimination half-life of monensin from serum following a single intracrop administration of 40 mg/kg body weight was 2.11 ± 0.08 h. Feeding the monensin premix (120 mg/kg feed) resulted in lower tissue residues and faster elimination from the body. Most of the drug was detected in serum and liver. No monensin was detected within 48 hours after ingestion.

Similar results were obtained by Okada et al. (11) who used quantitative thin layer chromatography to determine monensin residues in broiler tissues under practical conditions of use (feed containing 120 mg/kg monensin during entire lifetime). The highest residues were determined in the fat (0.110 mg/kg body weight) and in the liver (0.039 mg/kg body weight). No monensin was detectable in tissues except fat after a 1-day withdrawal period and in any tissue after 2 days withdrawal.

Henri et al. (12) measured the bioavailability of monensin to chickens and its residues in tissues using mass spectrometry (HPLC-MS/MS). They found that the bioavailability of monensin to broilers is between 28 and 34% and concluded that the rest of the active compound is excreted in the droppings. In the body, monensin was distributed mostly in fat, followed by plasma, liver and muscles. It was still possible to quantify monensin in fat 12 h after the end of treatment (feeding the monensin premix containing 125 mg/kg of feed *ad libitum* for 33 days), whereas no residues were detectable after 6 h in plasma, liver and thigh muscle and after 2 h in breast muscle.

Presence and degradation of monensin in poultry manure

According to EFSA (13), unchanged monensin represents less than 20% of the whole metabolites in chicken excreta. However, no data could be

found on the actual amounts of monensin in broiler manure. An investigation was therefore performed on manure from a farm in Pivka, Slovenia (6), where monensin was measured in composite samples of broiler excreta every week throughout the rearing period (45 days). The results revealed that monensin levels are between 0.72 and 8.91 mg/kg wet manure. This range of antibiotic concentrations can typically be expected in the manure of treated animals (14).

The fate of monensin in manure is also not well known. Only a few studies have been reported and the results are highly variable. An overview of the reported half-lives of monensin in manure and compost is given in Table 1.

The degradation of monensin was studied in manure from chickens treated with monensin at the maximum recommended dose (7). Excreta from five consecutive twenty-four-hour periods (collected between 8-12, 13-17 and 18-22 days of treatment) were analysed for monensin. Half of the wet manure was dried at temperature 70°C, ground and analysed for monensin content. After 30 days, up to 22 and 30% of monensin was no longer detectable in wet and dry manure, respectively.

In another study, also reported by EFSA (7), monensin in manure appears to degrade much faster. After five days, approximately 50% was degraded. After 20 days the concentration in manure was below the detection limit of 0.01 mg kg^{-1}.

Experiments on monensin degradation in chicken faeces were performed on one month old chickens fed with 127 mg monensin kg^{-1} feed (13). Faeces (dry matter 26-28%) were incubated at 27 and 37°C. Levels remaining after 6 days ranged from 7 to 31% of the initial amount, but data showed a large variability.

The only comprehensive and detailed study on monensin degradation in manure was performed on turkey litter by Dolliver et al. (15). Monensin degradation was studied under different conditions of manure storage (manure pile, manure with weekly mixing and moisture adjustment, and vessel composting). The average half-life of monensin was 17 days. The fastest degradation was observed in compost.

Fate of monensin in the environment

The actual concentrations of monensin that can be expected in soil are generally unknown and no measured values can be obtained in literature regarding monensin from poultry manure on agricultural soil. All we can rely on are calculated values obtained from data on the dosage and metabolism of monensin in broilers and its manure content. The predicted environmental concentrations of monensin in agricultural soil were calculated to be between 650 µg/kg in a worst-case environmental exposure scenario obtained on the basis of doses applied in the European broiler production, and 63.4 µg/kg when taking into account data on elimination of the parent compound (16). Based on the measured monensin concentrations in manure in Slovenia (6) and the legislation on the permissible amounts of manure used on agricultural soil in the EU (9), the highest predicted environmental concentrations of monensin we might expect in Slovenia would be around 50 µg/kg soil if the manure were used without prior storage (6).

The measured soil adsorption distribution coefficients (ratio between the amount of substance absorbed to soil and the amount dissolved in water) for monensin are between 1 and 80 and are pH-dependent (7, 17). The corresponding organic carbon partition coefficients are between 125 and 5,700. This indicates low mobility of monensin between environmental compartments – the majority of the substance that enters the soil absorbs to particles and is not dissolved in water. This was confirmed in a study in Denmark (18), where no monensin was detected in waters draining agricultural soils.

When on agricultural soil, monensin undergoes both biotic and abiotic degradation, with biotic degradation being the prevalent (17). Organic matter and soil moisture appear to be the most important factors influencing monensin degradation in soil (17, 19). The soil half-lives of monensin reported in literature are highly variable (Table 2). According to Sassman and Lee (17) the half-life of monensin in soils under laboratory conditions at 23°C was approximately 2 days, while Donoho (4) obtained a half-life of 13 days in field conditions. In another laboratory experiment, Yoshida et al. (19) determined a half-life of 22.7 days in a soil with 1.9% organic carbon (OC) and 4.2 days in a soil with 4.69% OC, while no degradation was observed in air-dried soil. There was a linear relationship between soil moisture and the rate of monensin degradation. EFSA (13) reports soil half-life values of 18, 13

and 15 days for sandy loam, silty loam and clay loam soils, respectively. However, much shorter dissipation half-lives – between 2.3 and 4 days – were reported in another EFSA study (7).

Toxicity of monensin to non-target soil organisms

After knowing the potential environmental concentrations of monensin to which non-target soil organisms would be exposed, the calculation of its potential environmental risk in agricultural soil requires the data on the toxicity of monensin to soil organisms.

In spite of extensive use of monensin for more than 40 years and its potential presence in the environment, there is only little published information concerning its effects on non-target organisms. Jensen et al. (20) have found that the survival of the adult springtail *Folsomia fimetaria* was not affected by monensin at concentrations up to 800 mg/kg dry soil, whereas its EC_{50} for reproduction was 591 mg/kg. Enchytraeids (*Enchytraeus crypticus*) showed similar responses (LC_{50} >800 and EC_{50} for reproduction = 356 mg/kg dry soil). Isopods (*Porcellio scaber*) also exhibited low sensitivity to monensin in soil or food (6); LC50 was >849 mg/g. Earthworms (*Eisenia sp.*), however, are much more susceptible to monensin. Median lethal concentrations of 56 mg/kg and 49.3 mg/kg have been reported (6, 7), whereas EC_{50} for earthworm reproduction was 12.7 mg/kg (6). Some research has also been published on the effects of monensin on plants (7), which are also sensitive to its presence in soil. Wheat, mustard and red clover were tested for emergence and seedling growth. Mustard was shown to be the most sensitive of the three species and had an LC_{50} of 17 mg/kg and EC_{50} for growth of 4 mg/kg.

Recommendations for storage and use of monensin-contaminated manure

According to the EU Technical Guidance Document (21), the potential risk of a substance to the environment is calculated as

Table 1: Literature data on monensin half-lives in manure

Half-life (days)	Method	Reference
17	Wet chicken manure, no treatment	(7)
21	Chicken manure ground and dried at 70°C	(7)
5	Chicken manure, method not described	(7)
3-4	Wet chicken manure at 37°C	(13)
22	Turkey manure in a pile, no treatment	(15)
19	Turkey manure with weekly mixing and water adjustment	(15)
11	Composted turkey manure	(15)

Table 2: Literature data on monensin half-lives in soil

Half-life (days)	Method, soil properties	Reference
13	Field experiment	(4)
18	Laboratory experiment, sandy loam	(13)
13	Laboratory experiment, silty loam	(13)
15	Laboratory experiment, clay loam	(13)
2.3	Laboratory experiment, sandy loam	(7)
4.0	Laboratory experiment, clay loam	(7)
2.5	Laboratory experiment, silty clay loam	(7)
2.0	Laboratory experiment, sandy soil, 0.87% OC	(17)
1.3	Laboratory experiment, clay loam, 2.2% OC	(17)
22.7	Laboratory experiment, loam, 1.9% OC	(19)
4.2	Laboratory experiment, clay loam, 4.69% OC	(19)

a quotient between the predicted environmental concentrations of the substance and the predicted no-effect concentration (PNEC), which is derived from the available data on the toxicity of the substance to non-target organisms. If the obtained risk quotient exceeds 1, there is potential risk involved in its use and suitable measures need to be taken to mitigate the risk.

As described earlier, the predicted environmental concentration of monensin in Slovenia, calculated on the basis of actual monensin levels measured in broiler manure and the legislation regulating the amount of manure that can be used on arable land, we can expect monensin concentrations of up to 50 µg/g soil at the time of manure application.

When toxicity data are available for a producer (photosynthesizing plants), a consumer and/or a decomposer, the PNEC in soil is calculated using assessment factors (21). The lowest measured no-effect concentration (NOEC) is divided by an appropriate factor. If NOEC data are available from only one long-term toxicity test (e.g. plant emergence and growth, or earthworm reproduction), the NOEC is divided by a factor of 100.

In the case of available data on monensin toxicity, several long-term toxicity tests have been performed on consumers (6, 7, 20) and on producers (7), but no data has been provided in the EFSA report (7) on the NOEC for plant emergence and growth. Furthermore, most of the reported results (7, 20) are based on nominal and not measured values of monensin and are therefore unreliable (6). The only known NOEC from a long-term toxicity test with measured monensin concentrations is the value for earthworm reproduction (6), which was 3.5 mg/kg dry soil. This value divided by an assessment factor of 100 gives 35 µg/g soil. The PEC/PNEC ratio is thus 1.43, which indicates that using contaminated broiler manure with no prior aging could be detrimental to the environment. With the very high variability of the available data on its dissipation in soil, it is also uncertain for how long the harmful effects of monensin would persist. One half-life would be sufficient for the risk quotient to fall below 1 (a drop in the soil concentration from 50 to 25 µg/g). However, the measured half-lives vary from just over one day to more than three weeks (Table 2) and are highly dependent on soil type, organic content, moisture and temperature.

The practice of using manure with no storage should therefore be avoided, not only because of monensin contamination, but also because use of manure without adequate storage poses a risk of spreading potential diseases and environmental contamination, as well as considerable loss of nutrients. Manure should be stored long enough to reduce monensin concentrations by at least one half. With the high variability of reports on monensin half-life in manure (Table 1), there is considerable uncertainty regarding the appropriate storage time. The longest half-lives reported were 22 days in manure with no treatment (mixing, water addition or composting). We could therefore say that one month of manure storage should be sufficient to render the manure safe for use on agricultural soil. Composting (addition of plant material to increase the carbon/nitrogen ratio, aeration and water adjustments) was shown to be more efficient in reducing monensin levels, since aerobic microbial processes are the main monensin degradation pathway. If possible, composting should thus be preferred to the traditional way of aging manure with no additional treatment.

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MONENZIN – PREGLED DEJAVNIKOV, KI VPLIVAJO NA VSEBNOSTI V OKOLJU TER PRIPOROČILA ZA VARNO SHRANJEVANJE IN RABO GNOJA, KI VSEBUJE MONENZIN

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Povzetek: Monenzin je polietrni karboksilni antibiotik, ki ga v veterinarski medicini uporabljamo za preventivo in zdravljenje kokcidioze pri perutnini. Je najpogosteje uporabljan kokcidiostatik pri nas. V prebavnem traktu tretiranih živali se le delno metabolizira, večina pa se ga izloči v aktivni obliki. Z gnojenjem kmetijskih površin monenzin vnašamo v okolje, kjer lahko škoduje talnim organizmom in tako zmanjša proizvodne potenciale in trajnostno rabo kmetijskih zemljišč. O gnojenju s kontaminiranim gnojem pri nas ni pravilnikov, veliko pa je tudi negotovosti o razgradnji in učinkih monenzina v okolju. Pregledali smo dostopno literaturo o učinkih monenzina na netarčne organizme ter njegove pričakovane koncentracije v gnoju in pognojeni zemlji z namenom, da bi ugotovili, kakšni ukrepi bi bili potrebni, da bi omilili morebitne škodljive posledice prisotnosti monenzina v okolju. Po najbolj realističnem scenariju, če gnoja z monezinom zdravljenih živali ne bi starali, bi pričakovane koncentracije monenzina v okolju presegale ravni, ki nimajo učinka na talne nevretenčarje. V tem primeru bi gnoj z monenzinom predstavljal tveganje za okolje. Gnoj je potrebno shranjevati ne samo zaradi nevarnosti kontaminacije z monenzinom, temveč tudi zato, ker uporaba nestaranega gnoja predstavlja možnost širjenja bolezni ter onesnaženja in izgube hranil.
Že enomesečno obdobje staranja gnoja pomeni znižanje koncentracije monenzina na raven, ki ne predstavlja nevarnosti za talne organizme. Kompostiranje je boljše od staranja gnoja v kupu, saj je razgradnja monenzina v tem primeru hitrejša.

Ključne besede: monenzin; gnojenje; ocena tveganja za okolje