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TOXICITY OF SALINOMYCIN AND NARASIN IN TURKEYS[1]

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Abstract

Coccidiosis continues to be one of the most serious diseases in poultry breeding, causing major economic losses in this industry. Many trials are conducted to prevent and control this disease. However, only a few medications are approved for use in the prevention and treatment of coccidiosis. Ionophore coccidiostats (e.g. salinomycin and narasin) act by altering the transmembrane movement of monovalent or divalent ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Rb^+, Cs^+), resulting in altered ionic gradients and disturbed physiological processes in coccidia. In broiler and turkey breeding, these agents are used for nearly the whole fattening period. Ionophore coccidiostats have a narrow safety margin. Their toxicity is probably due to a disturbed ion balance or to oxidative damage. Although ionophore coccidiostats are considered to be relatively safe for target animals, there are numerous reports of poisoning cases caused by these medications in birds. This paper summarizes the current state of knowledge on the toxicity of salinomycin and narasin in turkeys. It reviews the data concerning the symptoms, mortality rate and possible causes of poisoning with these agents. Moreover, the paper discusses the legal regulations regarding the use of these drugs in poultry.

Key words: ionophore coccidiostats, salinomycin, narasin, toxicity, turkeys.

TOKSYCZNOŚĆ SALINOMYCYNY I NARAZYNY U INDYKÓW

Abstrakt

Kokcydioza jest nadal uznawana za jedną z najważniejszych chorób w hodowli drobiu, stanowiąc główną przyczynę strat ekonomicznych w przemyśle drobiarskim. Podejmuje się wiele działań na rzecz zapobiegania i kontroli tej choroby. Do profilaktyki i terapii kokcydiozy zatwierdzono tylko kilka leków. Mechanizm działania kokcydiostatyków jonoforowych (np. salinomycyny i narazyny) jest związany z zakłóceniem transbłonowego transportu monowalentnych

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i dwuwalentnych jonów (Na^+, K^+, Ca^{2+}, Mg^{2+}, Rb^+, Cs^+), prowadzącym do zmiany gradientu jonowego i wtórnego zaburzenia procesów fizjologicznych u kokcydiów. U brojlerów i indyków leki te podawane są przez prawie cały okres tuczu. Kokcydiostatyki jonoforowe mają wąski margines bezpieczeństwa. Ich toksyczność jest związana z zaburzeniem równowagi jonowej lub uszkodzeniami oksydacyjnymi. Chociaż kokcydiostatyki jonoforowe są uznawane za stosunkowo bezpieczne dla zwierząt docelowych, w literaturze można znaleźć liczne doniesienia na temat zatruć tymi lekami u ptaków. W artykule przedstawiano bieżący stan wiedzy na temat toksyczności salinomycyny i narazyny u indyków, a także dane dotyczące objawów, upadków i możliwych przyczyn zatruć wywołanych tymi lekami. Omawiano również regulacje prawne w zakresie ich stosowania.

Słowa kluczowe: kokcydiostatyki jonoforowe, salinomycyna, narazyna, toksyczność, indyki.

INTRODUCTION

Coccidiostats were discovered at the early 1950s and described as a new class of chemotherapeutics in 1964 (Moore, Pressman 1964). New regulations introduced in the UE member states, which allow contamination of turkey fodder with ionophore coccidiostats like salinomycin and narasin, can be justified although the acceptable amounts set for these compounds as undesirable substances do not seem to be on an optimum level. This creates the risk of poisoning among older turkeys due to contamination of non-target feed stuffs even though the permissible threshold of an undesirable substance has not been exceeded. Moreover, the use of all antimicrobial growth promoters was prohibited in the European Union as of 2006. The consequences of this step on the incidence of *Eimeria* related diseases or the prevalence of *salmonella* and *campylobacter* are essentially unknown (Johansen et al. 2007).

COCIDIOSTATIC IN GENERAL

Currently, there are several substances possessing coccidiostatic properties, which can be divided into two groups. The first one consists of chemical compounds of natural origin, which are products of actinobacterial fermentation from *Sterptomyces* spp. and *Actinomadura* spp. They include narasin, monensin, lasalosid, maduramycin, salinomycin and *semduramicin* (Mehlorn 2008). The second group consists of synthetic coccidiostats, which vary in their mode of action, biological activity and chemical structure, e.g. robenidine (from the guanidine group), decoquinate (from quinolones), diclazuril (from benzeneacetonitriles), halofuginone (from quinolines) and nicarbazin.

Coccidiostats from the first group (of natural origin) are referred to as ionophore antibiotics. As such, they have found application in veterinary medicine, mainly as fodder additives used for the prevention and treatment of coccidiosis, a disease caused by protozoas *Eimeria (Report from the*

Commission (WE)... 2008, *Community Register*... 2012). There are seven main pathogenic species identified: *Eimeria* in poultry (*E. necatrix, E. mitis, E. brunetti, E. acervulina, E. tenella, E. maxima and E. praecox*) and other types peculiar to turkeys (*E. meleagrimitis, E. adenoides, E. gallopavonis and E. dispersa*). As well as poultry, coccidiosis may affect cattle, sheep, pigs and rabbits (Olejnik, Szprenger-Juszkiewicz 2007, Koutoulis et al. 2013).

A wide spectrum of effects against all pathogenic types of *Eimeria* spp. which appear in poultry ensures adequate control of these protozoa (i.e. sporozoites *Eimeria tenella* die after 12-24 hour incubation in a liquid with salinomycine and monensin). However, their widespread use induces cases of immunity to these medications, first observed in the USA and Europe during the early 1980s (Mehlorn 2008). Currently, there are strains resistant to all ionophore coccidiostats. Noteworthy is the cross resistance in this group of medicines, i.e. when microorganisms gain resistance to one antibiotic, simultaneously they become resistant to other antibiotics from the same group (EFSA... 2008).

THE MODE OF ACTION

Ionophores modify the permeability of biological membranes by forming lipid soluble, dynamically reversible cation complexes, which transport cations across membranes. Each ionophore group has its own, typical, inorganic ion selectivity pattern. The mechanism of action of ionophore coccidiostats is related to their binding in lipophilic substances with monovalent or divalent ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Rb^+, Cs^+), causing perturbation that depends on the grade of transport concentration through cell membranes, which in consequence leads to quick depletion of intracellular energy stores and cell death (EFSA... 2008).

Salinomycin selectively facilitates the transmembrane exchange of sodium and potassium. As a result of this uncontrolled movement, ion gradient and concentration across the cell membrane are altered and the physiological processes in coccidia are deranged (Rizvi et al. 2008).

COCCIDIOSTATICS IN PRACTICE OF FEED FORMULATION

In the European Union, conditions for approval of coccidiostats and their application are regulated by the European Union legislature and laws of particular countries (*Council Regulation (EEC)*... 1990, *Regulation (EC)*... 2003, *Act on Animal* ... 2006, *Regulation on the European Parlament (WE)*... 2009, *Community Register*... 2012). Coccidiostats are used as fodder additives in

poultry and rabbit breeding (ESFA... 2004b, *Report from Commission (WE)...* 2008, *Community Register...* 2012). Currently, 27 pharmaceutical products are authorized in the EU member states for the prevention of coccidiosis in one or more animal species, but only 9 are allowed to be used in turkey breeding: Elancoban (monensin sodium), Elancogran (monensin sodium), Coxidin (monensin sodium), Cycostat (robenidine hydrochloride), Cygro (maduramicin ammonium), Avatec (lasalocid A sodium), Halofuginone (halofuginone hydrobromide), Clinacox 0.2 (diclazuril), Clinacox 0.5 (diclazuril) (*Community Register...* 2012). An approval document sets the minimum and maximum level in which coccidiostats can be used as feed additives in the animal's diet. It may specify the animal species as well as the species categories (for example chickens for fattening and chickens reared for egg laying) and in some cases withdrawal periods (*Council Regulation (EEC)...* 1990, EFSA... 2008).

In broiler chicken, turkey and rabbit breeding, coccidostats are used for almost the entire fattening period, with a 0-, 1-, 3- or 5-day (depending on the substance) withdrawal period before slaughter (*Report from the Commission (WE)...* 2008). Medicated substances are added to 86% of starter/grower fodders for broilers, 97% of starter/grower for turkeys, 45% of fodders for rabbits and 15% of fodders for young hens which start the egg yield period (Olejnik et al. 2009). The application of ionophore coccidiostats in laying hens' fodder of finisher type is forbidden.

In accordance with the European Commission's Regulation and European Food Safety Authority (*Regulation (WE)...* 2007, ESFA... 2004a, EFSA... 2008), salinomycin is used as sodium salt in the product called Sacox 120 microGranulate. The description of the use of fodder with sodium salinomycin should contain the warning: 'Salinomycin is dangerous to horses and turkeys', as well as some additional information like 'This additive in the fodder contains ionophore; when used with certain medicinal substances (like tiamulin) it can be contraindicated.' Therefore, such fodders can be used, as recommended, only for broilers provided the salinomycin content does not exceed a dose of 60-70 mg kg^{-1}, chickens reared for laying until the age of 12 weeks with the salinomycin content no more than 50 mg kg^{-1} and rabbits reared for fattening with the salinomycin content which does not exceed a dose of 20-25 mg kg^{-1} of the fodder. Despite the prohibition of the use of salinomycin in fodder additives for turkeys, cases of intoxication with this and other coccidiostat are still reported.

Salinomycin in the form of sodium salt is an authorized feed additive in non-target feed following unavoidable carry-over. For equine species, turkeys, laying birds and chickens reared for laying (over > 12 weeks), its acceptable amount is 0.7 mg kg^{-1} of feed regarding feeds with 12% water content (*Commission Regulation (EU)...* 2011).

The above assumption has been made because a certain level of contamination of fodders is impossible to avoid due to economical and technological

reasons. It has been established that the content of coccidiostats including salinomycin in fodders for animals other than target species should not exceed the amount of 1-3% of the highest concentration for target animals. The level of 1% is accepted in the fodders for laying chickens and dairy animals as well as for the species which are considered to be sensitive, namely equines and turkeys. The accepted limit of the content of salinomycin in fodders for animals different than the target animals arises from an arbitrary assessment of a possible level of contamination of fodders. As the acceptable concentration (mg/kg) of this coccidiostatic in the case of broilers is the dose of 70 mg kg^{-1} of the fodder, 1% is a dose of 0.7 mg kg^{-1} of salinomycin in the fodder. This quantity is the amount of undesirable substance that is accepted by the legislator. However, it does not mean that the acceptable amount of salinomycin which appears in the fodder cannot cause intoxication in turkeys. Researchers and clinical veterinarians know of the falls of older turkeys (20 weeks) after feeding with the fodder in which salinomycin appeared in the amount of 0.26 mg kg^{-1}. This supports the suggestion that the acceptable amount of salinomycin as an undesirable fodder additive should be lower, especially in the case of older turkeys (over 12 weeks old), which are more sensitive to this coccidiostatic.

SIGNS OF INTOXICATION

Signs of intoxication, including cardiovascular effects (raised blood pressure and myocardial degeneration), anorexia, weakness, ataxia and paralysis, are reported in various non-target animal species (EFSA... 2008). These signs correlate with the mode of action of ionophores and occur also in target animal species at dose levels exceeding the maximum authorized level. Animals particularly sensitive to salinomycin are turkeys, pre-ruminant calves and cattle, horses, cats and dogs, less pigs. In dogs, signs of neurotoxicity was observed experimentally. Toxicity is described in some non-target animal species at salinomycin concentrations in feed below the maximum level authorized for chickens for fattening, hence reflecting the significant species difference and a small margin of safety of sodium salinomycin used as a coccidiostat. The Panel on Contaminants in the Food Chain (CONTAM) concluded that ingestion of the maximum authorized level of salinomycin in poultry feed of 70 mg kg^{-1} feed may cause intoxications and constitute a health risk for several non-target animal species (EFSA... 2008).

ANALYSIS OF SALINOMYCIN IN PREMIXES AND ANIMAL FEEDS AND TOXICOSIS

All substances from the ionophore coccidiostat group have a narrow safety margin, i.e. there is a small gap between a therapeutic and a toxic dose. Some of them, such as salinomycin, narasin, maduramycin and – to a lesser degree – monensin, may be toxic to animals. Ionophore coccidiostats can be ordered in terms of increasing toxicity as follows: salinomycin < lasalosid < narasin < monensin < maduramycin (Oehme, Pickrell 1999). Because equines and turkeys are very sensitive to salinomycin, its application in these species is forbidden. Salinomycin causes bad food intoxication in turkeys. Its symptoms are decreased fodder and water intake, growth inhibition, diarrhoea, emaciation, sleepiness, lack of motor coordination, muscle impairment, sultriness and lying on the sternum with legs extended forward. Intoxication caused by these coccidiostats often ends up in falls (Reece 1988). Moreover, severe intoxication with ionophores appears when these substances are given with other medicines administered to poultry. Such poisoning cases are noted among birds after a combined application of salinomycin with tiamulin (Fowler 1995, Mehlorn 2008). Other researchers proved that the application of salinomycin in the amount of 44 or 66 mg kg^{-1} in fodder causes a higher mortality rate among 32-week turkey cocks (13 out of 20 of the examined birds were dead) than among 7-week turkey cocks (one out of 84 examined birds was dead) (Potter et al. 1986). Thus, older turkeys are evidently more sensitive to salinomycin than younger ones. Higher toxicity of salinomycin among older turkeys was confirmed by data included in the Anticoccidial Compendium – Table 1 (Fowler 1995). In another examination, a fall of 400 30-week birds in a flock consisting of 700 turkeys occurred within seven days from the beginning of feed application with salinomycin at a dose of 50 mg kg^{-1} of fodder (Griffiths et al. 1989). Among the dead birds, no anatomopathological features were noted and histopathological examinations disclosed segmental muscle necrosis. The toxic effect of salinomycin is also described in 10- and 13-week turkeys fed fodder for chickens, which included

Table 1

Toxicity of salinomycin in turkeys

Age	Dose	Effect
Turkeys 0-12 week	40 ppm	no effect level
12 + week	20 ppm	no effect level
Adult females	10-15 ppm	no effect level for adult breeding females
Growing	< 50 ppm	no negative effects
Adult	< 50 ppm	dyspnoea, ataxia, death
Breeders	15-30 ppm	< 16% mortality
Fatteners	60 ppm	12-14% mortality
14 week old	16 ppm	dyspnoea, some mortality/hot
Young ages	22 ppm	depressed growth
Older ages	22 ppm	growth prevented or decreased

salinomycin at a dose of 60 mg kg^{-1} of fodder (HARRIES 1991). First symptoms of food poisoning such as loss of appetite, muscle impairment and falls appeared on the second day of feeding with this feed. In a flock of 10-week turkeys consisting of 3000 birds, 1200 birds died, and in a flock of 13-week turkeys consisting of 300 birds, 75 birds were dead. Similar observations were made by other researchers (ANDREASEN, SCHLEIFER 1995), who found that due to feed contamination with salinomycin at a dose of 13 mg kg^{-1} of fodder (an assembly line on which cock fodder had been prepared was not cleaned properly) 180 birds died in a flock consisting of six-hundred 48-week turkeys. Degeneration and necrosis of skeletal muscles were asserted in histopathological examination.

SALINOMYCIN - ANTICOCCIDIAL ACTIVITY

Salinomycin, like other polyether ionophores, is effective against sporozoites as well as early and late asexual stages of coccidia in the intestines of chickens. The biological activity is based on the ability of ionophores to form lipid soluble and dynamically reversible complexes with ions (preferably the alkaline ions K^+ and Na^+). Salinomycin encloses the cation in a hollow ball, in the centre of which the cation is fixed and immobilised. It functions as a carrier of ions, mediating an electrically-neutral exchange of cations across the membranes. The resultant changes in transmembrane ion gradients and electrical potentials often produce profound effects on cellular function and metabolism that can lead to the death of coccidia (EFSA... 2008).

SALINOMYCIN - ANTIBACTERIAL ACTIVITY

Salinomycin shows selective antibacterial activity when applied in a concentration range of 0.5 to 16 mg L^{-1}. It is effective against many Gram -positive bacteria species, but *Enterobacteriaceae* are resistant. The minimum inhibitory concentration of salinomycin for common intestinal bacterial species such as *Enterococcus faecalis*, *E. faecium*, *Staphylococcus* spp. and *Clostridium perfringens* is between 0.5 and 16 mg L^{-1}. Inhibitory concentrations of salinomycin to susceptible bacterial strains are thus lower than the concentration in supplemented feed (EFSA... 2008).

DRUG-DRUG INTERACTION

Drug-drug interaction with salinomycin (used as a feed additive) and tiamulin (used therapeutically against infections with *Mycoplasma* spp.)

is frequently reported and results in up to 60% mortality in some poultry herds (Lin 1995). Moreover, serious poisoning with ionophore antibiotics may appear in cases of their delivery with other medicines administered to poultry. Such intoxication was noted among birds after combined application of salinomycin with tiamulin (Fowler 1995, Mehlorn 2008). The interaction between these medicines hinders the process of biotransformation of salinomycin and its accumulation in the birds' system, particularly in livers (Mézes et al. 1992, Fowler 1995, Mehlorn 2008). It is know that tiamulin (and also valnemulin, a related pleuromutilin) is a potent inhibitor of the activity of hepatic cytochrome P450 enzymes. Subsequently, the latter accumulate following daily ingestions, and signs of toxicity occur due to a relative overdose (Witkamp et al. 1995, Szucs et al. 2004, EFSA... 2008).

Earlier studies on the fattening of male chickens (28 day-old) fed with a diet containing 60 mg kg^{-1} salinomycin with and without experimental treatment with tiamulin given intraoesophageally (50 mg kg^{-1} b.w.) showed that the hepatic malondialdehyde concentration rose in the salinomycin-treated group, indicating lipid peroxidation. At the same time, glutathione concentrations and glutathione peroxidase activity decreased rapidly and these effects preceded clinical signs of toxicity in the animals and indicated that salinomycin and tiamulin exerted a synergistic effect in affecting the antioxidant (glutathione) system (Mézes et al. 1992, EFSA... 2008).

EGGS

Several studies show that salinomycin was detected in eggs from laying hens that were treated with salinomycin sodium in their feed (Kan, Petz 2000, Rizvi et al. 2008). When hens were fed with the doses of 30, 60, 90 or 150 mg kg^{-1} of salinomycin sodium for 14 days, their eggs contained salinomycin in amounts of <10, 80, 110 and 200 µg kg^{-1} in the egg white, and 1400, 2000, 2800 and 3700 µg kg^{-1} in the yolk, respectively. However, in hens treated with a dose of 60 mg kg^{-1} in their feed for 7 days, the excretion of salinomycin with eggs resulted in concentrations of 50 µg kg^{-1} in the egg whites, and 1500 µg kg^{-1} in the yolks. In another study, hens given 60 mg kg^{-1} for 5 days had salinomycin concentrations of <10 µg kg^{-1} in the egg white and 220 mg kg^{-1} in the yolk (EFSA... 2008). Another feeding trial showed that the accumulation of salinomycin in eggs was 3.3 µg kg^{-1} egg per mg kg^{-1} feed (Kennedy et al. 1998). Mean egg salinomycin concentrations did not exceed 60 µg kg^{-1} at any time (EFSA... 2008).

EXCRETA

Salinomycin is extensively metabolised by chicken and unchanged salinomycin represents only a very small fraction of total radioactive residues in the excreta. More than twenty metabolites have been separated and identified from the excreta, each representing less than 10% of the total salinomycin-derived compounds (EFSA... 2008).

NARASIN

A similar problem appears in the case of narasin, which according to the community legislator (*Regulation (WE)...* 2006) can be used as a fodder additive in a product called Monteban. The document informs (analogously to salinomycin) that the same information should be included for this narasin-enriched fodder as for Sacox. As indicated, the fodder is intended for broilers of any age. The dose of narsin in Monteban is 60-70 mg of active ingredient/kg of complete compound feed stuff.

Although narasin, as well as other ionophore coccidiostats, is considered to be effective and safe for target animals, the literature describes numerous examples of birds poisoned with narasin (Braunius 1985). The main reason for intoxication is wrong application, e.g. overdosing or incorrect fodder mixing (Novilla 1992, Fowler 1995, Oehme, Pickrell 1999). Symptoms of intoxication and a high death rate (up to 32%) were observed in a flock of 3000 turkeys aged 11 weeks, which had been fed with fodder containing 42.8 mg kg^{-1} of narasin (Salyi et al. 1988). The falls of birds took place during the first week after the first symptoms of poisoning such as incoordination, dyspnoae and diarrhoea. The post mortem revealed gastritis, degeneration of kidneys and pulmonary congestion. The symptoms of poisoning and falls were also noted among turkey cocks aged over 12 and 18 weeks which had been fed with fodder containing 70 mg kg^{-1} dose of narasin (Davis 1983). Falls of turkeys were also observed after the intake of a dose of 25-40 mg of narasin kg^{-1} in fodder given for 3 days. A much higher death rate was observed among turkeys at the age of 18 and 22 weeks when compared to 8-week-old birds – Table 2 (Fowler 1995).

In turkeys, narasin is an authorized feed additive in non-target feed following unavoidable carry-over. The admissible amount of narasin is 0.7 mg kg^{-1} of feed and its content in feed was calculated same as for salinomycin (*Commission Regulation (EU)...* 2011).

In conclusion, salinomycin and narasin intoxication may create serious health and production problems in poultry keeping. This issue should be discussed and veterinarians have to educate breeders about the current problem caused by contaminated or toxic feeds.

Table 2

Toxicity of narasin in turkeys

Age	Dose	Effect
Turkeys 11 week old	43 ppm	- 30% mortality < 7 days
	28 ppm	- 30% mortality
		in both the above there was also slight monensin contamination.
Adult males	20 ppm/10 day	- no negative effects
8 week		- 7.2% mortality
18 week	25/40 ppm /3+ day	- 84% mortality
22 week		- 69% mortality
		anorexia, ataxia, paralysis, drooping, wings, dyspnoae, diarrhea.
18 week old males	70 ppm	- 30% mortality: reduced feed consuption
Adult females		weight gain, incoordination

REFERENCES

Andreasen J.R., Schleifer J.H. 1995. *Salinomycin toxicosis in male breeder turkeys*. Avian Dis., 39(3): 638-642.

Braunius W.W. 1985. *Ionophorous anticoccidial drugs in coccidiosis control*. World`s Poult. Sci. J., 41(3): 198-209.

Commission Regulation (EU) No 574/2011 of 16 June 2011 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for nitrite, melamine, Ambrosia spp. and carry-over of certain coccidiostats and histomonostats and consolidating Annexes I and II thereto. O. J. EC Nr L 159, 7-24.

Community Register of Feed Additives: Animal Nutrition - Feed Additives Revision 134 released 8 February 2012.

Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. O. J. EC Nr L 224, 1-8, with subsequent amendments.

Davis C. 1983. *Narasin toxicity in turkeys*. Vet. Rec., 113, 627.

EFSA (European Food Safety Authority) 2004*a*. *Opinion of the Scientific Panel on Additives and Products or Substances Used in Animal Feed on a request from the Commission on the re-evaluation of coccidiostat Sacox 120 microGranulate as feed additive in accordance with article 9G of Council Directive 70/524/EEC*. EFSA J., 76: 1-49.

EFSA (European Food Safety Authority) 2004*b*. *Opinion of the Scientific Panel on Additives and Products or Substances Used in Animal Feed on a request from the Commission on the evaluation of coccidiostat Kokcisan 120G*. EFSA J., 63: 1-41.

EFSA (European Food Safety Authority) 2008. *Opinion of European Commission on Cross-contamination of non-target feedingstuffs by salinomycin authorised for use as a feed additive*. EFSA J., 591: 1-38.

Fowler N. G. 1995. *Anticoccidial compendium*. Beerse, Belgium, Janssen Animal Health., 57: 72-73.

Griffiths G.L., Hillier P., Sutherland R. J. 1989. *Salinomycin poisoning in point-of-lay turkeys*. Aust. Vet. J., 66(10): 326-329.

Harries N. 1991. *Alberta. Salinomycin toxicity in turkeys*. Can. Vet. J., 32(2): 117.

Johansen C.H., Bjerrum L., Pedersen K. 2007. *Impact of salinomycin on the intestinal microflora of broiler chickens*. Acta Vet. Scand., 49:30. DOI:10.1186/1751-0147-49-30

Kan C.A., Petz M. 2000. *Residues of veterinary drugs in eggs and their distribution between yolk and white.* J Agric Food Chem., 48(12): 6397-6403.

Kennedy D.G., Hughes P.J., Blanchflower W.J. 1998. *Ionophore residues in eggs in Northen Ireland: incidence and cause.* Food Addit Contam, 15(5): 535-541.

Koutoulis K.C., Kefalas G., Minos E. 2013. *Salinomycin toxicosis in broiler breeders and turkeys: report of the first case.* Am. J. Anim. Vet. Sci., 8(4): 190-196.

Lin J.A. 1995. *Mass salinomycin toxicity death to concomitant use of tiamulin in a breeder flock.* Taiwan. J. Vet. Med. Anim. Husb., 65: 339-346.

Mehlorn H. 2008. *Encyclopedia of parasitology*. Springer-Verlag Berlin Heilderberg, 3rd edition, 274-276.

Mézes M., Sályi G., Bánhidi G., Szeberényi S. 1992. *Effect of acute salinomycin-tiamulin toxicity on the lipid peroxide and antioxidant status of broiler chicken.* Acta Vet. Hung., 40(4): 251-257.

Moore C., Pressman B.C. 1964. *Mechanism of action of valinomycin on mitochondria.* Biochem. Biophys. Res. Commun., 15: 562-567.

Novilla M. N. 1992. *The veterinary importance of the toxic syndrome induced by ionophores.* Vet. Hum. Toxicol., 34(1): 66-70.

Oehme F.W., Pickrell J.A. 1999. *An analysis of the chronic oral toxicity of polyether ionophore antibiotics in animals.* Vet. Hum. Toxicol., 41(4): 251-257.

Olejnik M., Szprenger-Juszkiewicz T. 2007. *Residues of coccidiostats in poultry tissues and eggs.* Med. Wet., 63(12): 1539-1545. (in Polish)

Olejnik M., Szprenger-Juszkiewicz T., Żmudzki J. 2009. *Coccidiostats in animal food and fodder.* Med. Wet., 65(12): 807-811. (in Polish)

Potter L.M., Blake J.P., Blair M.E., Bliss B.A., Denbov D.M. 1986. *Salinomycin toxicity in turkeys.* Poult. Sci., 65(10): 1955-1959.

Reece R.L. 1988. *Review of adverse effects of chemotherapeutic agents in poultry.* World`s Poultry Sci. J., 44(3): 193-216.

Rizvi F., Anjum A.D., Khan A., Mohsan M., Shazad M. 2008. *Pathological and serum biochemical effects of salinomycin on layer chicks.* Pakistan Vet. J., 28(2): 71-75.

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. O. J. EC Nr L 268, 29-43.

Regulation (EC) No 545/2006 of 31 March 2006 amending Regulation (EC) No 1464/2004 as regards the conditions of authorisation of the feed additive Monteban belonging to the group of coccidiostats and other medicinal substances. O.J.E.C., No 94, 26-27.

Regulation (EC) No 500/2007 of 7 May 2007, amending Regulation (EC) No 1463/2004 as regards the introduction of a maximum residue limit for the feed additive Sacox 120 micro-Granulate, belonging to the group of coccidiostats and other medicinal substances. O.J.E.U. No L 118, 3-4.

Regulation of the European Parliament and the Council (EC) No 470/2009 of 6 May 2009, laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No. 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No. 726/2004 of the European Parliament and of the Council.

Salyi G., Szabo E., Bago G., Banhidi G., Szilagyi M. 1988. *Narasin poisoning in turkeys.* Acta Vet. Hung., 36(1-2): 107-114.

Szucs G., Tamasi V., Laczay P., Monostory K. 2004. *Biochemical background of toxic interaction between tiamulin and monensin.* Chem. Biol. Interact., 147(2): 151-161.

Report from the Commission to the Council and European Parliament on the use of coccidiostats and histomonostats as feed additives, of 5 May 2008 pursuant to Art. 11 of Regulation (EC)

No 1831/2003 of the European Parliament and Council of 22 September 2003 on the use of feed additives in animal nutrition.

Act on Animal Feeds of 22 July 2006. Journal of Law No 144 item 1045, 7499-7520.

Witkamp R.F., Nijmeijer, S.M., Monshouwer, M., Van Miert, A.S. 1995. *The antibiotic tiamulin is a potent inducer and inhibitor of cytochrome P4503A via the formation of a stable metabolic intermediate complex. Studies in primary hepatocyte cultures and liver microsomes of the pig.* Drug Metab. Dispos., 23(5): 542-547.