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Review

A review of coccidiostats and the analysis of their residues in meat and other food

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ABSTRACT

Coccidiostats are used in the control of protozoan infections in different food producing animals. They are most widely used as feed additives in intensively reared species such as pigs and poultry to maintain animal health and in some cases enhance feed conversion. However, a number of these drugs are used in the control of infections in beef and lamb production. Coccidiostat residues have been frequently reported in meat and eggs in a number of countries since the late 1990s. This has prompted increased research and surveillance of coccidiostat residues in food. This paper reviews the various coccidiostat agents used in animal production, including their chemical properties, mode of action and activity. Legislation concerning coccidiostats, limits for residues in food, monitoring and occurrence of residues in food is discussed. Methods for residue determination in food, including screening and physicochemical methods are discussed in depth. The paper concludes with a synopsis of the current state of coccidiostat residue analysis and future perspectives.

Contents

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1. Introduction

Coccidiostat drugs are used worldwide for the treatment of coccidiosis, primarily in intensive farming. A number of these agents are also used to treat extensively reared species including cattle and sheep. Coccidiostats can be categorised as naturally occurring polyether ionophores such as monensin, narasin, lasalocid, salinomycin and maduramicin or as synthetic coccidiostats such as halofuginone, robenidine, diclazuril and nicarbazin (Dubreil-Cheneau, Bessiral, Roudaut, Verdon, & Sanders, 2009). Whilst the 'in-feed' administration of veterinary medicines and feed additives is an essential treatment for intensively farmed species, contamination of feed can and does occur (McEvoy, Smyth, & Kennedy, 2003; Yakkundi, Cannavan, Young, Elhott, & Kennedy, 2002). This can potentially cause toxic effects in non-target animals and can result in undesirable levels of residues in food. Harmful effects in animals may occur if compounds have low margins of safety in species (Nogueira, Franca, & Peixoto, 2009) or due to drug interactions as with tiamulin and ionophores. Tiamulin interferes with the metabolism and elimination of ionophores, causing cellular accumulation and acute toxicity in pigs (Roberts, Hammer, Lechtenberg, Roycroft, & King, 2011). Whilst acute toxicity in humans has never been observed, there is concern over chronic toxicity due to long-term exposure to low levels. For this reason, EU countries employ surveillance programmes to monitor and prevent unacceptable contamination of animal products intended for human consumption. The surveillance of coccidiostat residues in food has been largely limited to the ionophores and nicarbazin in eggs and poultry meat. In recent years, more extensive LC–MS/MS methods have been developed that allow the analysis of a wider range of coccidiostat residues, including drugs used in the treatment of cattle and sheep. The application of this technology will provide the potential to analyse more samples from a wider range of species and create a more comprehensive approach for coccidiostat residue monitoring.

This paper will focus on coccidiostats and their analysis in food. Review papers have been published on the analysis of ionophores but none on a broad range of coccidiostats (Elliott, Kennedy, & McCaughey, 1998; Hansen, Bjorklund, Krogh, & Halling-Sorensen, 2009). The paper reviews the chemistry of coccidiostats, their mode of action, food safety and analytical challenges.

2. Coccidiosis

Coccidiosis is an infection of the intestinal tract by parasitic protozoa of the Phylum *Apicomplexa*. Parasites belonging to the genus *Eimeria* may be prevalent in warm humid conditions affecting intensively farmed species, such as pigs (Anon, 1998; Rypula, Porowski, Kaba, Gorczykowski, & Deniz, 2012), poultry (Shirley & Lillehoj, 2012), cattle (Mitchell, Smith, & Ellis-Iversen, 2012), sheep (Chartier & Paraud, 2012) and rabbits (Akpo, Kpodekon, Djago, Licois, & Youssao, 2012). Overcrowding, poor hygiene practices and failure to isolate infected animals will encourage proliferation of the disease. Parasites are transmitted via oocysts, shed in the faeces of infected hosts and ingested by uninfected animals (Sharman, Smith, Wallach, & Katrib, 2010). Once ingested, sporozoites are released from the oocysts into epithelial cells lining the intestine. A number of asexual cycles result in the growth of merozoites which differentiate into the sexual stages. Female macrogametes are fertilised by the male microgametes to produce oocysts which are shed to potentially infect other animals once consumed. The disease can lead to intestinal lesions, diarrhoea, poor weight gain, poor feed conversion and in some cases death. With losses estimated to be in the region of $127 million annually to the United States economy (Chapman, 2009), it is considered more financially viable to administer coccidiostats as feed additives to broiler chickens for almost their entire life (28–48 days) rather than treating coccidiosis therapeutically.

3. Coccidiostats

3.1. Chemistry

The ionophore coccidiostats are produced by 53 different bacteria of the *Streptomycetaceae* family. They are characterised by multiple tetrahydrofuran rings connected together in the form of spiroketal moieties (Riddell, 2002) as shown in Fig. 1. The chemical coccidiostats (Fig. 2) can be subdivided into quinolones, pyridones, alkaloids, guanidines, thiamine analogue and triazine derivatives (Kart & Bilgili, 2008). Quinolones were discovered in 1962 and since then have undergone numerous modifications to their quinoline nucleus to improve the pharmacokinetic performance and anti-microbial spectrum (Galarini, Fioroni, Angelucci, Tovo, & Cristofani, 2009). Guanidine derivatives such as robenidine possess an imine central bond containing a carbon–nitrogen double bond, with the nitrogen atom attached to a hydrogen atom or an organic group. Triazine derivatives can be divided into two sub-groups; asymmetric (1,2,4) triazines and symmetric (1,3,5) triazines. Both of these sub-groups contain a heterocyclic ring that is analogous to the six-membered benzene ring but with three carbon atoms replaced by nitrogens atoms. Amprolium is an analogue to thiamine (vitamin B1) and is similar in structure. However, it lacks the hydroxyethyl functionality that thiamine possesses and thus is not phosphorylated to a pyrophosphate analogue (Kart & Bilgili, 2008).

4. Mode of action

Ionophores form complexes with various ions, principally sodium, potassium, and calcium, and transport these into and through membranes (Anon, 2009c; Kart & Bilgili, 2008). As result of this general mode of action, they have activity against a broad range of protozoa and have found widespread application. Voet and Voet (2004) classified ionophores into two groups based on how they transfer ions across membranes: ion carriers and channel formers. Ion carriers, such as lasalocid, form a complex with ions, shielding their charge and allowing their movement across the hydrophobic lipid bilayer. Channel forming ionophores, such as gramicidin (Bergen & Bates, 1984), facilitate the movement of ions across membranes by creating a hydrophilic channel for the ions. Disruption of the transmembrane ion concentration prevents normal function and will kill the coccidia. Ionophores affect both extra- and intracellular stages of the parasite, especially during the early, asexual stages of development. They generally depress feed intake, however body weight gain increases or is unaffected (Susin, Mendes, Pires, & Packer, 2004). The inclusion of ionophores in avian feed has been found to reduce feed consumption whilst improving feed conversion (Anon, 2009c).

Chemical coccidiostats function in a number of different ways and often act on specific developmental stages of the parasites. Amprolium inhibits uptake of thiamine by second-generation schizonts of *Eimeria tenella* and so prevents formation of thiamine coenzyme which is required for many essential metabolic reactions (James, 1980). Clopidol, buquinolate, decoquinate and nequinate inhibit mitochondrial energy production during the early stages of *Eimeria* development but act on different strains of the coccidia (Anon, 2010c; Fry & Williams, 1984). Ethopabate is a structural antagonist of folic acid or of its precursor, para-aminobenzoic acid (PABA). It is thought to inhibit the synthesis of nucleic acid, thus limiting the production of new cells (Anon, 2010c). Nicarbazin was the first coccidiostat to have broad spectrum activity and has been in use since the 1950s (Anon, 2010c). It is administered prophylactically in broiler feed and its protective action occurs on day 5 in the life-cycle of the developing coccidia so there is minimal tissue damage. Its mode of action is thought to be through the inhibition of succinate-linked nicotinamide adenine dinucleotide reduction and the energy-dependent transhydrogenase, and the accumulation of calcium in the presence of adenosine-5′-triphosphate. Research has suggested that toltrazuril primarily affects the respiratory chain and