Coccidiosis and coccidiostats

Coccidiosis is a very contagious infection disease in animals caused by eukaryotic single-celled parasitic organisms (protozoa) referred to as coccidia, genus Eimeria. More than 600 species of coccidia are known today but only a few of them do infect poultry. The most pathogenic among them are E. tenella, E. necatrix, E. maxima, E. acervulina, E. mitis, E. praecox and E. brunetti. In addition to poultry, turkeys and rabbits coccidia may also infect other animal species, such as pigs and sheep, but these are less sensitive. In its acute form, coccidiosis is lethal and the subacute form of the disease leads to a loss of weight due to lower food conversion and to drops in egg production.

The chemical compounds used to fight coccidiosis do not have a common denominator. They may affect the coccidia at any stage of the parasite’s life cycle. They are mostly administered as medicated feed but sometimes they are added to the drinking water or administered by spraying. Some compounds also have an antibiotic or antibacterial action, in addition to their coccidiostatic effect. Whether an antibiotic is used for therapeutic purposes or as a coccidiostat depends on the concentration.

At present, the use of 11 coccidiostats is authorised in chickens, turkeys and rabbits. In a general way, coccidiostats may be classified into two groups: ionophore coccidiostats (semduramycin[-sodium], lasalocid[-sodium], monensin[-sodium], salinomycin[-sodium], narasin and maduramicin[-ammonium]) and non-ionophore coccidiostats (halofuginon, nicarbazin, robenidine[-hydrochloride], diclazuril and decoquinate).

Legal background

Regulation (EC) No 1831/2003 provides that manufacturers who want to be authorised to market a feed additive (e.g. a coccidiostats) intended for use and processing should submit an application thereto to the European Commission. Detailed rules for the assessment of the application and for issuing authorisations as well as the requirements with respect to the application are laid down in Regulation (EC) No 429/2008. Residue studies must in any case be submitted together with the application in order to allow the European Commission to decide, upon recommendation of the European Food Safety Authority, whether maximum residue limits (MRLs) must be set for the foodstuffs obtained from animals to which the additive would have been fed.

When the assessment of the application is positive, the authorisation is granted by publishing a Regulation (EC) that lays down specific rules for the use of the additive, such as the authorised dose and the target animal species or categories for which it is meant. For five (monensin, salinomycin, narasin, robenidine, diclazuril) of the above-mentioned eleven substances MRLs have already been set for tissues of (some) target animals. References of the relevant Regulations (EC) are to be found in the List of authorised additives. (http://ec.europa.eu/food/food/animalnutrition/feedadditives/registeradditives_en.htm).

Some operators of feed businesses manufacture many different feedstuffs in one establishment whereby one production line is used for many products manufactured one after the other. In this process, it is sometimes impossible to avoid that traces of one product stay behind in the production line and end up in the beginning of the production of the next feed. This phenomenon is known as “carry-over” or “cross-contamination”. It may have as an effect that traces of coccidiostats are transferred to feedstuffs for “non target animals” that are manufactured...
next. Carry-over may occur at all stages of the manufacturing and processing of feedstuffs as well as during the storage and the transport of feedstuffs. It was therefore recommendable to set maximum limits for the carry-over of coccidiostats to non-target feed, in accordance with the ALARA principle (As Low As Reasonably Achievable). The unavoidable carry-over of active substances of authorised coccidiostats to non-target feed is considered as a case of undesirable substances in animal feed, as referred to in Directive 2002/32/EC and must therefore not involve any hazard for animal health, human health or the environment. Hence, Commission Directive 2009/08/EC amending Annex I to Directive 2002/32/EC lays down maximum levels of these substances in animal feed.

In order to allow feed manufacturers to control this unavoidable carry-over, Directive 2009/8/EC takes into account the distinct authorised carry-over ratios that depend on the final destination of the feed (fed to sensitive animal species or not) when establishing the maximum levels. So, in feed for less sensitive non-target animals a carry-over of some 3% is authorised. In other cases, a carry-over level of 1% is accepted, e.g. in feed for sensitive non-target species, in compound feed used in the period before slaughter and in feed for target animals into which no coccidiostats have been incorporated. This level must also be observed for non-target animals intended for use as permanently food producing animals such as dairy cows and laying hens when evidence has been given that a carry-over from feed to food of animal origin is possible. When feedstuffs are given directly to the animals or when additional feed is given, the use of those feedstuffs in the daily ration must not increase the exposure of the animal to a coccidiostat beyond the maximum level in force for daily rations containing only complete feedstuffs.

Another aspect of this unavoidable carry-over of coccidiostats to non-target feed is the fact that residues of such substances may end up in foodstuffs of animal origin, even if the actual carry-over is lower than the maximum levels set in accordance with Directive 2002/32/EC.

Within the framework of Regulation (EC) No 315/93 laying down rules for not intentionally added substances it was therefore necessary to establish maximum tolerances for the presence of active substances of coccidiostats in food of animal origin in order to protect public health.

For a limited number of coccidiostats that may be authorised by the national authorities for therapeutic use in some animal species and animal categories in accordance with Directive 2001/82/EC some MRLs have already been established indirectly for the relevant foodstuffs by means of Regulation (EU) No 37/2010. However, yet another legal initiative had to be taken since Regulation (EU) No 37/2010 does not include a decision on the MRLs for all coccidiostats that may lead to residues in animal tissues due to carry-over from non-target feed. At the request of the Commission the EFSA therefore issued some opinions on animal and public health risks related to the possible carry-over of coccidiostats to non-target feed. On the whole, the EFSA considers that the possible presence of coccidiostats that are authorised as additives in animal nutrition in non-target feed do not have any negative effect on animal health if certain precautionary measures are taken. In this matter, the EFSA refers to the conclusions of several scientific opinions. Moreover, the EFSA considers that the health risk for consumers related to the intake of residues of such substances in products of animals that were fed feedstuffs contaminated as a result of carry-over may be neglected.

On the basis of the opinions of the EFSA, Regulation (EC) No 124/2009, which took effect on 1 July 2009, established maximum levels in order to protect public health. These maximum levels should always be adjusted to the changes made to the MRLs for the food in question within the context of Regulation (EC) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin.
Determination of coccidiostats by the FLVVT (Federal Food Safety Laboratory in Tervuren)

Some specific methods are available at the FLVVT for checking the maximum levels of coccidiostats in feedstuffs. These methods are extremely appropriate to check the levels in terms of mg/kg but their analytical capacity is not sufficient for detecting concentrations in terms of μg/kg. That is why LC-MS methods have already been developed before in order to detect coccidiostats residues in feedstuffs in terms of μg/kg, in spite of the fact that at the time there was no legal framework for that kind of tests. The LC-MS method for ionophore coccidiostats in feedstuffs could be accredited but the LC-MS equipment of the time (ion trap type) was not appropriate to develop a satisfactory test method for non-ionophore coccidiostats. However, since late 2008 the FLVVT has a triple quadrupole type of LC-MS device which made it possible to develop one single method for the simultaneous detection of both ionophore and non-ionophore coccidiostats in feedstuffs. Besides, Directive 2009/08/EC laying down maximum limits for non-target feed, in terms of μg/kg, came into force on 1 March 2009 requiring that the LC-MS method under construction be adjusted to the new maximum limits.

On the other hand, Regulation (EC) No124/2009 laying down maximum levels of coccidiostats in food, came into force on 1 July 2009. The FLVVT already had an accredited LC-MS method for the detection of residues of ionophore coccidiostats in eggs and meat, in addition to the method for feedstuffs. But, the reporting level of that method being close to the maximum levels suggested, a decision was made to optimize this method and extend it to non-ionophore coccidiostats.

In other words, a new testing method for determining the levels of coccidiostats was required that was appropriate for the matrices eggs and feedstuffs. That method also had to be able to determine simultaneously the levels of both ionophore coccidiostats (semduramicin, lasalocid, monensin, salinomycin, narasin and maduramicin) and non-ionophore coccidiostats (halofuginon, robenidin, nicarbazin, diclazuril, decoquinate and amprolium).

For both the matrices ‘feedstuffs’ and ‘eggs’ was developed a testing method that allowed the testing of samples as referred to in Directive 2009/08/EC and in Regulation (EC) No 124/2009. As the coccidiostats to be tested belong to Group B of Annex I to Directive 96/23/EC this method must be validated in accordance with Decision 2002/657/EC. The main parameters that were determined, are selectivity/specificity, accuracy, precision, decision limit $CC_\alpha$ and detection limit $CC_\beta$. The validation files and methods were submitted for accreditation to BELAC at the audit of 29 and 30 April 2009. The BELAC audit report was received on 18 May 2009. That report does not mention any A or B non compliances for the methods and validations. The procedure for validating the extension of the method to matrix “meat” has been started.
Fig.: LC-MS equipment for determining coccidiostats

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