Multiplex flow cytometric immunoassay of coccidiostats in eggs and their cross-contamination in non-targeted feed

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OBJECTIVES WP2a

Coccidiostats are widely used to control Coccidiosis but, a potential cross-contamination from targeted to non-targeted feeds (laying hens feed) exists and to protect the consumers, MRL's in eggs have been set by the European Union (regulation 124/2009).

The main goal of this work concerns the simultaneous detection of residues of selected coccidiostats lasalocid, monensin, nicarbazin, salinomycin, narasin and diclazuril in eggs and in non-targeted feed. To this end a flow cytometry-based immunoassay is under development using the Luminex™ platform.

Moreover, a carry-over study of lasalocid from laying hens feed to eggs is forecast aiming at contribution to a predictive hazard behaviour model.

RESULTS

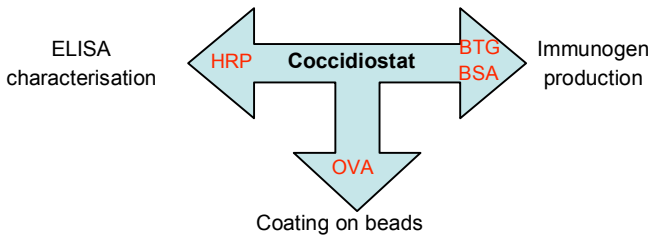
Test materials

Eggs incurred with selected coccidiostats were produced and used by the assay developers. Salinomycin, lasalocid, diclazuril and nicarbazin incurred egg samples at the target concentration corresponding to 75µg kg^{-1} were obtained whereas test materials containing monensin and narasin showed maximal concentration at 37 and 30µg kg^{-1}, respectively. It means that transfer of these two molecules to eggs is quite poor; this observation was confirmed for monensin (1-2).

All produced batches showed a good homogeneity according to criteria described by Fearn and Thompson (3). Stability tests are also in progress.

Synthesis of conjugates

Each of the six coccidiostats (or derivatives there of) were chemically conjugated :



Need to synthesize a specific immunogen for narasin because anti-salinomycin showed very low affinity towards free narasin although they present similar chemical structures.

Antibodies characteristics

Inhibition concentration 50 values and determination of specificity in buffer obtained by ELISA and / or Luminex™:

pABs	IC_{50} (ng ml^{-1})	specificity	method used
anti-lasalocid	1	to be determined	Luminex
anti-monensin	ND (max response too low)	-	Luminex
anti-nicarbazin	7.8 / 21	DNC specific	Luminex / ELISA
anti-salinomycin	0.3	2% narasin	Luminex
anti-narasin	to be determined	to be determined	Luminex
anti-diclazuril	0.6 / 5.2	specific	Luminex / ELISA

ND = not determined, DNC = dinitrocarbanilide

CONCLUSIONS and PERSPECTIVES

The multiplex assay for salinomycin, diclazuril, lasalocid and nicarbazin are ready to be performed in real matrixes (egg and feed). The monensin and narasin assays need further optimisation in buffer. The next challenge is to provide simple and reliable sample preparation.

References

1) Kennedy *et al.*, Food Additives and Contaminants, 1998, **15** (5), 535-451
2) EFSA Journal, 2008, **592**, 29-40
3) Fearn, T. and Thompson M., Analyst, 2001, **126**, 1414-1417

The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013*) under *grant agreement* n° 211326: CONffIDENCE project (www.conffidence.eu).