

Indication of cross-resistance between different monovalent ionophores as determined by an anticoccidial sensitivity test (AST)

M. MARIEN^{1*}, M. DE GUSSEM¹, D. VANCRAEYNEST¹, G. FORT² and M. NACIRI²

¹Alpharma Animal Health, Laarstraat 16, B-2610 Wilrijk, Belgium, ²INRA, INRA – UR 1282 Infectiologie Animale et Santé Publique, 37380 Nouzilly – France.

*Corresponding author: maja.marien@alpharma.com

Anticoccidial sensitivity tests (AST's) are used to determine the anticoccidial sensitivity profile of field *Eimeria* strains with the goal to decide rationally on future anticoccidial programs in order to avoid resistance.

In 2006 an AST was performed for an integration without clinical coccidiosis problems but with an increased incidence of bacterial enteritis. The farms of the integration were using monensin (full program) for 8 years.

When comparing parasitological results and zootechnical performances it was shown that the field isolate proved totally resistant to diclazuril. Robenidine, maduramicin and lasalocid significantly reduced *E. acervulina* gross lesions, whereas salinomycin and monensin (both monovalent ionophores) reduced them only numerically. Against *E. tenella*, three anticoccidials proved very significantly effective: robenidine, lasalocid and maduramicin. Salinomycin also significantly reduced *E. tenella* gross lesions, but monensin showed no effect. Except diclazuril, all other tested anticoccidials were effective in improving zootechnical performance, although, compared to the monovalent ionophores, lasalocid (divalent) and maduramicin (monovalent glycoside) showed the tendency to be more efficacious (numerically higher daily weight gain, higher weight gain improvement), indicating cross-resistance between different ionophore classes did not arise. All results were comparable for monensin and salinomycin, although salinomycin had not been used previously in the farms.

In this AST, although not all the data proved to be significant in this set-up, an indication for the existence of cross-resistance between ionophores of the same class was seen.

Keywords: anticoccidials; cross-resistance; rotation; monensin; lasalocid

Introduction

Anticoccidial sensitivity tests (ASTs) enable to identify *Eimeria* species present in field samples, quantify them, evaluate their pathogenicity, evaluate and compare the efficacy of various anticoccidials with a view to select the most effective ones and eventually predict their efficacy in the field by recommending them in a strategy against coccidiosis (full or shuttle program) in poultry farms under investigation, taking into account the farm commemoratives (including anticoccidials previously used) (Naciri *et al.*, 2004). The use of anticoccidials in the end inevitably leads to the development of resistance which results in reduced efficacy of the used molecule (Chapman, 1986; 1998). By rotating to another molecule, the efficacy may be restored. A complicating factor however is that one particular class of anticoccidials (the ionophores) contains different molecules which share a common mode of action. Three classes of ionophores exist: monovalent ionophores (monensin, salinomycin, narasin), monovalent glycoside ionophores (maduramicin, semduramicin), and divalent ionophore (lasalocid). If resistance develops to one ionophore then it will also be apparent to the others, mainly between the ionophores of the same class (cross-resistance) (Chapman, 2007). Rotating between ionophores of different classes would decrease very much the risk for cross-resistance.

Materials and Methods

Field samples were collected from different broiler farms of one integration (meaning that all the farms are on the same anticoccidial program). Both intestinal and caecal droppings were collected and treated with potassium dichromate (2.5%). First identification was done under the optical microscope by morphometry, afterwards quantification was done of the number of oocysts of the different species by using a McMaster counting chamber and finally calculation of the percentage of each species, was performed. On top of this, the *Eimeria* species were identified by PCR technique (Naciri *et al.*, 2004). After this, the field isolates were reproduced by inoculating them in coccidia-free chickens and harvesting the oocysts that were produced. Viable coccidia were identified as *E. acervulina* (42%), *E. mitis* (16%), and *E. tenella* (42%) by microscopic examination. PCR technique did not confirm the presence of *E. mitis* which were then considered to be probably *E. acervulina*. For the actual AST, day-old male broiler chickens (Ross) were used, housed in wire-floored battery-cages in a climate controlled room. On day 9, they were wing-banded, weighed and randomly assigned to one of the groups (6 birds per cage, 3 replications *i.e.* 18 birds per group) so that there was no difference in average weights among the groups. The following experimental groups were included: an uninfected, untreated control group (UUC), an infected, untreated control group (IUC), and then six infected, treated (with different anticoccidials) groups, *i.e.* maduramicin 5 ppm (Cygro® 1%), monensin 100 ppm (Elancoban® 200), salinomycin 60 ppm (Salinomax® 120G), robenidine 33 ppm (Cycostat® 66G), lasalocid 125 ppm (Avatec® 15%cc), and diclazuril 1 ppm (Clinacox® 0.5%). Starting from day 9, the birds received the anticoccidials in the feed until the end of the experiment (day 24).

On day 16, the birds were orally inoculated with a dosage of 270,000 sporulated oocysts (243,125 *E. acervulina* + 26,875 *E. tenella*) from the field isolate propagated in sensitive birds. The inoculum was administered in a volume of 1 ml with a calibrated syringe. On day 9, day 16 and day 23, the chickens were weighed individually, and the feed consumption of the intermediate periods was determined per cage (3 cages per group). Half of the chickens were necropsied and scored for gross lesions in the gut (according to Johnson and Reid, 1970) on day 23. Faeces were collected from day 21 to day 24 (the time period corresponding to the peak oocyst excretion) and excreted oocysts were counted using a McMaster counting chamber. Data (weight and weight gain) were analysed by one-way analysis of variance using the General Linear Models (GLM) procedure of SIMSTAT pour windows version 2.04, June 18, 2002 (Provalis Research). The differences between groups were assessed using the Newman – Keuls mean comparison test and considered significant at $p < 0.05$. For lesion scores a Kruskal-Wallis 1-way ANOVA was carried out and when its significance was comparable to GLM-analysis of variance, the differences between groups were assessed using Newman-Keuls test at $p < 0.05$.

Results and discussion

Taking into account an analytic error of $\pm 20\%$, assay results were in accordance with the target values for the different anticoccidials.

At day 16 (before inoculation), no significant differences ($p < 0.05$) in live body weight, daily weight gain, feed consumption and feed conversion ratio were noted between groups. Over the period from day 16 to 23, the inoculation with the isolated oocysts, induced moderate coccidiosis in the IUC group characterized by mortality (3 out 18 chicks), coccidial lesions scored 3.0, 2.4 and 3.3 in the duodenum, jejunum-ileum and caeca, respectively (Table 1), and a significant drop in the daily weight gain by 30.0% compared to UUC (Table 2). In the medicated groups, mortality due to coccidiosis was noted in three groups: 3 birds in the monensin group at 5 and 6 days post-inoculation, 2 birds in the salinomycin group at 4 and 5 dpi and 9 birds in the diclazuril group at 5 and 6 dpi. In the duodenum, gross lesions were mainly due to *E. acervulina*. Robenidine, maduramicin and lasalocid significantly reduced *E. acervulina* gross lesions ($p < 0.05$) (Table 1), whereas salinomycin and monensin reduced them only numerically, and diclazuril showed no effect. Against *E. tenella*, three anticoccidials proved very significantly effective: robenidine followed by lasalocid and maduramicin. Salinomycin also

significantly reduced *E. tenella* gross lesions, but monensin and diclazuril showed no effect. Total oocyst excretion was decreased by 72 %, 51% and 30% in the robenidine, salinomycin and monensin groups, respectively, but in the other medicated groups, total oocyst shedding was not decreased (Table 1).

Table 1: Lesion scores¹ on day 23 and daily oocyst shedding of broilers (over period day 21-24) medicated with various anticoccidial products and inoculated on day 16.

Groups	Dose (ppm)	Mortality due to coccidiosis	Lesion scores ¹		Daily total oocysts / bird x 10 ⁶
			<i>Duodenum</i>	<i>Caeca</i>	
UUC	-	0	0	0	0
IUC	-	3	3.0 ^{a2}	3.3 ^a	485
Maduramicin	5	0	1.8 ^{bc}	0.9 ^c	447
Monensin	100	3	2.1 ^{abc}	3.2 ^a	340
Salinomycin	60	2	2.4 ^{ab}	2.4 ^b	240
Robenidine	33	0	1.1 ^c	0.1 ^c	137
Lasalocid	125	0	2.0 ^{bc}	0.4 ^c	541
Diclazuril	1	9	3.1 ^a	3.9 ^a	729

¹ Lesion scores (scale from 0 to 4 according to Johnson and Reid, 1970)

² In a column, means not sharing a common letter differ significantly ($p \leq 0.05$).

Table 2: Zootechnical performances (from day 16 to 23) of broiler chickens medicated with various anticoccidial products and inoculated on day 16, and calculation of daily weight loss in percentage (UUC = 100%) and anticoccidial weight gain improvement in percentage (UUC-IUC = 100%).

Products	Dose ppm	*Weight day 16	*Weight (g) day 23	*Weight gain (g) day 16-23	**Daily weight gain (g) day 16-23	Daily weight reduction in % (UUC=100%)	Anticoccidial improvement (%) (UUC-IUC = 100%)	**Daily feed consumption (g) day 16-23	**FCR day 16-23
UUC	-	586.1 ^{a1}	997.9 ^a	412.5 ^a	59.0 ^a	0	100	95.8 ^{ab}	1.62 ^c
IUC	-	588.0 ^a	890.9 ^b	296.9 ^b	41.3 ^b	30	0	92.9 ^{ab}	2.28 ^b
Maduramicin	5	582.9 ^a	982.1 ^a	399.2 ^a	56.8 ^a	3.7	87.6	92.6 ^{ab}	1.64 ^c
Monensin	100	591.6 ^a	973.6 ^a	379.2 ^a	52.4 ^a	11.2	62.7	89.7 ^{ab}	1.71 ^c
Salinomycin	60	610.5 ^a	976.9 ^a	367.3 ^a	51.3 ^a	13.1	56.5	93.9 ^{ab}	1.83 ^c
Robenidine	33	582.5 ^a	992.3 ^a	409.8 ^a	58.1 ^a	1.5	94.9	101.9 ^a	1.77 ^c
Lasalocid	125	583.0 ^a	990.1 ^a	407.1 ^a	58.4 ^a	1.0	96.6	100.5 ^a	1.72 ^c
Diclazuril	1	576.1 ^a	856.7 ^b	250.9 ^b	31.6 ^c	46.4	-54.8	82.2 ^b	2.66 ^a

¹ In a column, means not sharing a common letter differ significantly ($p \leq 0.05$).

* Mean weight and weight gain of surviving animals

** Parameters (Daily weight gain, feed consumption and FCR) calculated per cage, including dead birds' weight

Regarding zootechnical performance, the mean live body weight was significantly higher in all medicated groups compared to the IUC group and not different from UUC except in the diclazuril group. The mean weight gain was not significantly improved in the diclazuril group compared to the IUC group; all other medicated groups did not differ from the UUC group. Regarding the mean daily weight gain, all anticoccidials (except diclazuril) improved it significantly compared to IUC, and no difference was noted compared to UUC. Numerically, the best performance was seen by lasalocid, followed by robenidine, maduramicin, monensin and salinomycin, which significantly reduced the drop in the daily weight gain due to coccidial infection by 96.6%, 94.9%, 87.6%, 62.7% and 56.5 %,

respectively (*Table 2*). Feed intake was only significantly reduced in the diclazuril group (*Table 3*). The feed conversion ratio (FCR) was significantly increased in the IUC group compared to the UUC group ($p < 0.05$) (*Table 2*). It was significantly improved in all medicated groups and was not different from the UUC control group, except in the diclazuril group where it was significantly increased compared to IUC (*Table 2*).

To conclude, this field isolate, containing the two species *E. acervulina* and *E. tenella*, proved totally resistant to diclazuril 1 ppm in the feed. All other tested anticoccidials were effective in improving zootechnical performance. However, among these effective anticoccidials, mortality (due to statistically significant lesser control of *E. tenella*) was only observed in monensin 100 ppm and salinomycin 60 ppm groups (3 and 2 out of 18 chicks, respectively) and they were less effective to reduce gross lesions than maduramicin 5 ppm, robenidine 33 ppm and lasalocid 125 ppm. Moreover, for both monovalent ionophores tested in this AST, monensin (Elancoban®) and salinomycin (Salinomax®), differences, although not significant, were observed in the improvements in the weight gain drop (compared with the weight gain difference between the uninfected, untreated and the infected, untreated groups): only 62.7% and 56.5% respectively compared to lasalocid (96.6% improvement), robenidine (94.9% improvement) and maduramicin (87.6% improvement). Results were very comparable for monensin and salinomycin, although, as far as could be remembered, salinomycin had not been used previously in the concerned farms. The numerically lesser results for monensin on the other hand were not unexpected since it had been used for 8 years on the field. Evaluated products from two other ionophore classes, i.e. lasalocid (divalent ionophore, Avatec®) and maduramicin (monovalent glycoside ionophore, Cygro®) showed the tendency to be more efficacious (numerically higher daily weight gain, higher weight gain improvement, although not statistically significant) compared to the two monovalent ionophores, indicating that cross-resistance between different classes of ionophores did not arise. In this AST, although not all the (especially zootechnical) data proved to be significant in this set-up, a strong indication for the existence of cross-resistance between ionophores of the same class was seen. These findings consolidate the importance of sound anticoccidial rotations in order to preserve efficacy.

References

- CHAPMAN, H.D. (1986). Isolates of *Eimeria tenella*: studies on resistance to ionophorous anticoccidial drugs. *Research in Veterinary Science* **41**(2):281-282.
- CHAPMAN, H.D. (1998). Evaluation of the efficacy of anticoccidial drugs against *Eimeria* species in the fowl. *International Journal of Parasitology* **28**(7):1141-1144. Review.
- CHAPMAN, H.D. (2007). Rotation programmes for coccidiosis control. *International Poultry Production*, **15**: 7-9.
- NACIRI, M., CHAUSSÉ, A.M., FORT, G., BERNARDET, N., NÉRAT, F., and DE GUSSEM, K. (2004). Value of anticoccidial sensitivity tests (ASTs) in the prevention of chicken coccidiosis. XXII World's Poultry Congress, Istanbul June, 8-13, 2004.