ANTICOCCIDIAL SENSITIVITY TEST (AST) RESULTS FROM A FARM VACCINATED FOR 3 CONSECUTIVE FLOCKS WITH A COCCIDIOSIS VACCINE

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1 INTRODUCTION

In industrial poultry production, designing a preventive programme for controlling coccidiosis is one of the most important decisions to be made in order to safeguard or improve zootechnical and financial results. Usually in-feed anticoccidials are used in these programmes and, traditionally, were considered sufficient for controlling clinical coccidiosis. In spite of their use, rotating various anticoccidial products, combining chemical and ionophore treatments or shuttle programmes, resistance to anticoccidials is becoming increasingly important. Development of resistance has been described for most anticoccidials: chemicals and ionophores (Chapman, 1986, 1997). The only available method for detecting the level of drug-sensitivity of one *Eimeria* strain is the *in vivo* anticoccidial sensitivity test (AST) (Chapman, 1998).

Live coccidiosis vaccines are becoming increasingly popular, as they very often provide a solution when in-feed anticoccidials become ineffective. In fact, some live coccidiosis vaccines are able to promote the restoration of the sensitivity of *Eimeria* field strains to anticoccidials (Mathis & Broussard, 2006; Peek & Landman, 2006; Peek & Landman, 2011).

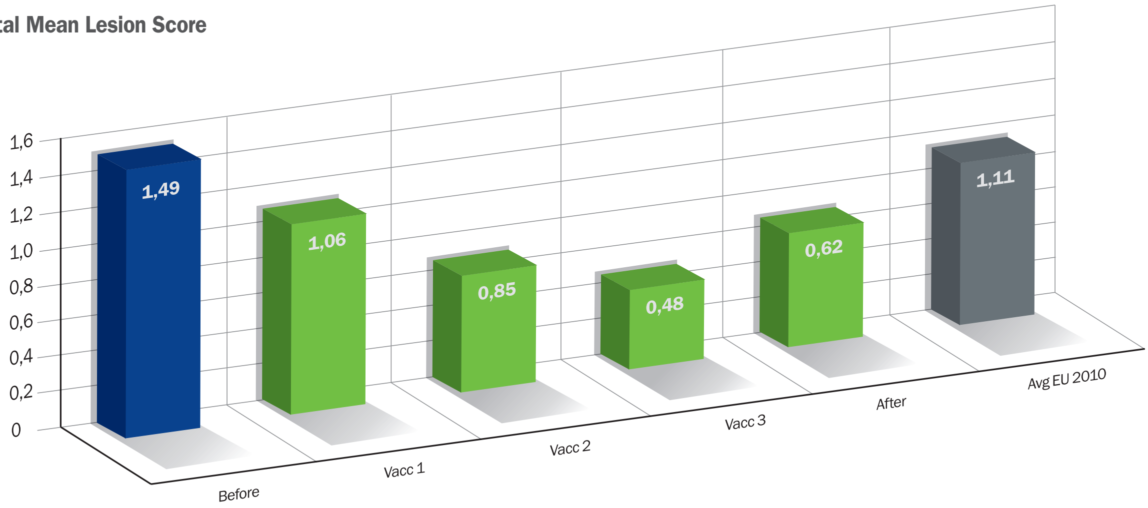
The objective of this study was to evaluate the effect of a live coccidiosis vaccine, HIPRACOX®, on the sensitivity of coccidiosis in the field. The farm used in the present study was located in The Netherlands and was composed of 5 houses, for a total capacity of approximately 160,000 birds per cycle. Birds were not sexed, with a thinning at around 32-35 days and a final slaughter age of 42 days. Before vaccination, it was considered by the veterinary supervisor as a problematic farm due to frequent occurrence of coccidiosis: frequently, signs of coccidiosis were evident via oocyst per gram (OPG) counts, lesion scores or presence of blood in the droppings. In order to make this evaluation, we performed 6 ASTs on the oocyst isolates from the farm at the end of the fattening period. We also performed Lesion Scores, OPGs and we calculated the productive results of all the cycles under study.

2 RESULTS

1. 1. TOTAL MEAN LESION SCORE (TMLS)

The TMLS is the sum of the average of the Mean Lesion Score (MLS) of *Eimeria acervulina, E. maxima* and *E. tenella* calculated at specific time points of the same cycle according to the Johnson & Reid (1970) method. For the cycle before vaccination, the TMLS was 1.49, about 34% higher than the EU average of 1.11. The average TMLS was lower than the EU average (1.06 vs. 1.11) in the first vaccination group. Over the course of the second vaccination cycle, average TMLS was further improved and lower than the EU average (0.85 vs. 1.11). In the third cycle of vaccination, the average TMLS dropped to 0.48. During the 1st cycle after vaccination, the mean TMLS increased slightly compared to the last cycle of vaccination (0.62), but it was still remarkably lower when compared to the cycle before vaccination.

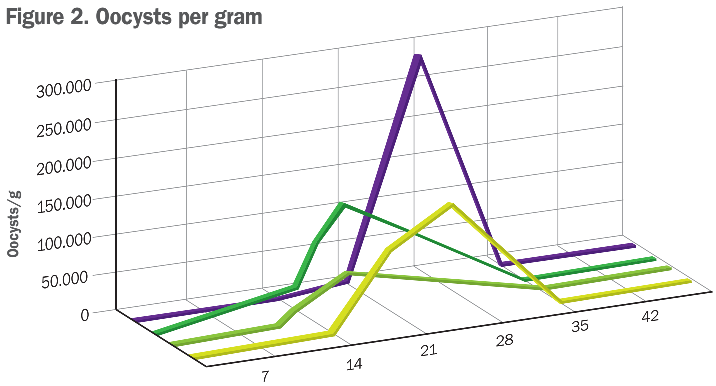
Figure 1. Total Mean Lesion Score



2. OOCYSTS PER GRAM (OPG)

Samples for OPG counts were taken on a weekly basis respectively at 7, 14, 21, 28, 35 and 42 days of age. During the 1st cycle of vaccination, the peak of infection was at 4 weeks of age, after which immunity was strong with very low counts at 35 and 42 days of age. In the 2nd cycle of vaccination, the peak of OPG counts shifted from the 4th week to the 3rd week but at much lower levels than the 1st cycle of vaccination. Over the course of the 3rd cycle of vaccination, the peak of OPG counts was again at 3 weeks of age. For the first post-vaccination group, oocyst counts started to rise and peaked at 4 weeks.

Figure 2. Oocysts per gram



3. PRODUCTIVE RESULTS

Productive performances were divided into 3 groups: before, during and after vaccination. Impact of vaccination on ADG (average daily gain) after returning to anticoccidials was 1.18 grams (58.27 grams per cycle before vaccination vs. 59.55 grams per cycle after vaccination). Body weight at slaughter age of 41 days was on average 52 grams higher after vaccination (2441 grams) compared to before vaccination (2389 grams). FCR_{2000} (feed conversion ratio) was improved by 3 points during vaccination (1.51) and 5 points after vaccination (1.49) compared to before vaccination (1.54). The average mortality before vaccination was 2.96%, whereas during vaccination this mortality dropped to 2.47%, which is a 16.6% improvement. After vaccination the mortality rose again to 2.58%, which is still a 12.8% improvement from the situation before vaccination. Finally, EPEF (European Production Efficiency Factor) was improved from 368 before vaccination to 376 during vaccination (+8 points) and 389 after vaccination (+ 22 points).

Table 1. Productive results

	Body Weight (gr)	FCR_{2000}	ADG (gr)	Mortality (%)	EPEF
Before vaccination	2389[a]	1.54[a]	58.27[a]	2.96[a]	368[a]
During vaccination	2390[a]	1.51[a]	58.31[a]	2.47[a]	376[a]
After vaccination	2441[a]	1.49[a]	59.55[a]	2.58[a]	389[a]
Difference before/after	52	- 0.05	1.28	0.38	21

4. ANTICOCCIDIAL SENSITIVITY TESTS (ASTs)

The six oocyst isolates under exam were analysed by PCR in order to identify the species of *Eimeria* present in each one. Results are shown in Table 2 and indicate that before vaccination all 5 species of *Eimeria* affecting broilers were present at the end of the fattening period on the selected farm indicating a situation of high incidence of coccidiosis. On the other hand, after vaccination, only one species appears. In general, when the anticoccidial programme controls the coccidiosis, the number of species decreases.

Table 2. PCR results

Eimeria spp.	Before	Vacc 1	Vacc 2	Vacc 3	After 1	After 2
E. acervulina	Pos.	Pos.	Pos.	Pos.	Pos.	Pos.
E. maxima	Pos.	Neg.	Pos.	Neg.	Neg.	Neg.
E. mitis	Pos.	Pos.	Pos.	Neg.	Neg.	Neg.
E. praecox	Pos.	Pos.	Pos.	Pos.	Neg.	Neg.
E. tenella	Pos.	Neg.	Pos.	Pos.	Neg.	Neg.

The ASTs were performed in accordance with the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anticoccidial drugs in chickens (Holdsworth et al., 2004). Six separated ASTs respectively coming from the cycle before vaccination, 1st vaccination, 2nd vaccination, 3rd vaccination, 1st post-vaccination and 2nd post-vaccination cycle were run in order to study drug sensitivity against the anticoccidials used in the study farm: salinomycin, monensin and narasin/nicarbazin. Table 3 shows the study design for each isolate under exam: randomised block design with 6 birds/replicate and 3 replicates/treatment group.

Table 3. Study design for each oocyst isolate

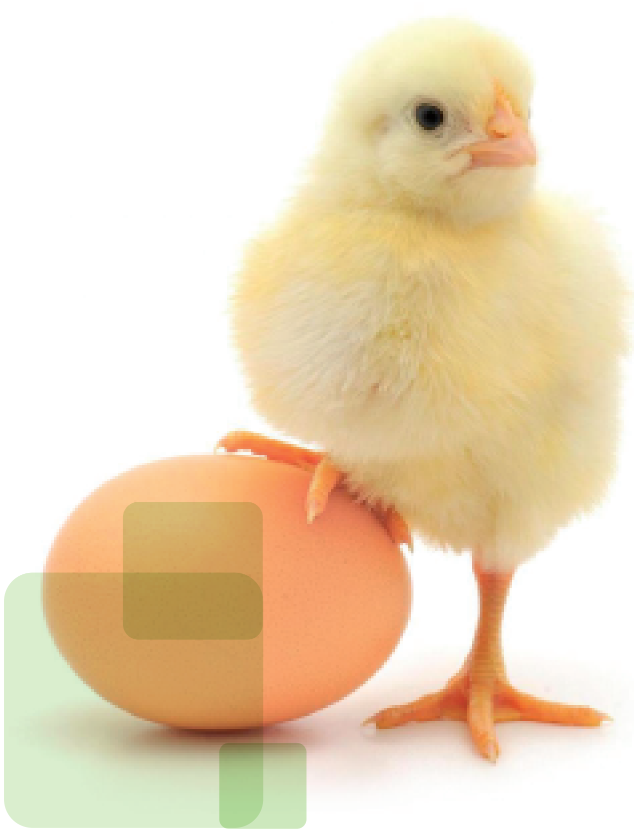
T	Treatment/Dosage (grams/ton; ppm)	Infected/ Non-infected	Cages/ Treatment	Birds/ Cage
1	Non-medicated Non-Infected (NMNI)	NI	3	6
2	Non-medicated Infected (NMI)	I	3	6
3	Salinomycin, 60 g/t or 66 ppm	I	3	6
4	Monensin, 100 g/t or 110 ppm	I	3	6
5	Narasin+Nicarbazin, 72 g/t or 79 ppm	I	3	6

Sensitivity results were studied in accordance with McDougald et al. (1986) looking at the percentage of reduction of gut lesions (Johnson & Reid, 1970) with respect to the NMI group:

- Resistance: ≤ 30 % reduction
- Partial sensitivity: 31-49% reduction
- Full Sensitivity: ≥ 50% reduction

Table 4. % Reduction of Gut Lesion Score

	% Reduction Salinomycin	% Reduction Monensin	% Reduction Narasin+Nicarbazin
Before	40.20	39.40	35.40
Vacc 1	55.40	52.30	60.90
Vacc 2	67.30	59.60	60.00
Vacc 3	54.60	57.90	62.50
After 1	50.60	41.50	49.80
After 2	47.80	41.30	46.30



3 DISCUSSION & CONCLUSION

In the present study, we observed that the TMLS before vaccination was higher than during and after vaccination and there was a drastic reduction, especially in the 3rd vaccination cycle when compared to the previous situation. Throughout the 3 cycles of vaccination, the OPG peak moved from day 28 to day 21, whereas after vaccination the peak at day 28 may indicate the onset of partial sensitivity. Regarding productive results, we saw that before vaccination final weight was similar compared to vaccination cycles, but lower than after vaccination. For the ADG, FCR_{2000}, mortality and EPEF parameters, data is always better during and after vaccination when compared to prior to it. The differences observed in the study between all the parameters monitored before vs. after vaccination clearly indicate that the vaccine helped in increasing sensitivity.

PCR and AST results indicate that oocysts present on the farm prior to vaccination were partially resistant. The profile of resistance decreased during vaccination converting the oocysts to sensitivity with the 3rd cycle of vaccination being the most sensitive. On the other hand, after vaccination and applying the same anticoccidial programme as before, the field oocysts progressively returned to a more resistant profile.

In conclusion, the data obtained indicates that three consecutive vaccinations changed the resistance profile of the study farm and controlled the coccidiosis problems at least for two flocks after vaccination. On the other hand, the 2nd cycle post-vaccination already showed the development of some resistance indicating that three consecutive vaccinations may be insufficient to change the profile to full sensitivity. This was probably due to the fact that the farm had never been vaccinated before and *Eimeria* strains had reached a marked level of resistance such that 3 consecutive cycles were not enough to fully restore their sensitivity.

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