THE ANTICOCCIDIAL EFFICACY OF ROBENIDINE HYDROCHLORIDE IN *EIMERIA* CHALLENGED RABBITS

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ABSTRACT

Coccidiosis remains one of the most important infectious causes of digestive disorders in fattening rabbits. The importance of the disease even increased with the onset of Epizootic Rabbit Enteropathy. It is thus of utmost importance to adequately prevent this disease in rational rabbit production. One of the most applied methods of prevention is the incorporation of an anticoccidial product in rabbit feed. In the present trial, the efficacy of robenidine hydrochloride, registered under the tradename Cycostat® 66G for breeding and fattening rabbits, was compared with a diclazuril treated and a non treated control group in an *Eimeria* challenge model. The trial confirmed the beneficial effects of robenidine hydrochloride incorporation in the feed, leading to significantly better zootechnical results in animals inoculated with *Eimeria media* and *Eimeria magna*.

Key words: Coccidiosis, Challenge, Robenidine hydrochloride, Zootechnical performance, OPG.

INTRODUCTION

Rabbit coccidiosis is caused by parasites of the genus *Eimeria*, which are true pathogens that are always present on rabbit farms as they are virtually impossible to eradicate. Coccidiosis has a direct impact on performance, but also acts in synergy with Epizootic Rabbit Enteropathy (ERE) (Coudert *et al.*, 2000). Therefore, prevention of coccidiosis remains of utmost importance. Prevention mostly consists of the incorporation of an anticoccidial product in the feed. At the time of writing, only two products were registered for use in rabbits under the current EU system of Brand Specific Approvals for anticoccidials. The active principle of one of these products, Cycostat® 66G, is robenidine hydrochloride. Robenidine hydrochloride can be used in fattening and breeding rabbits at a dose range of 50-66 ppm in complete feedstuffs. The objective of this study was to evaluate the efficacy of robenidine hydrochloride under battery cage conditions after inoculation with a mixed isolate of rabbit *Eimeria* species composed of *E. magna* and *E. media*, the most dominant species isolated in professional rabbit husbandry today (Coudert *et al.*, 2003). Comparison was made with an infected diclazuril treated group, acting as positive control, an infected non treated control group and a non infected non treated control group.

MATERIALS AND METHODS

Animals

A total of 192 weaned rabbits were enrolled in the study. These rabbits had been treated with 1 g/l sodium sulfachlorpyridazine in drinking water before weaning from 21 to 29 days of age to eliminate natural contamination with coccidia. After weaning, rabbits were housed in battery cages in groups of four. Rabbits from different litters were homogeneously distributed over 4 experimental treatment groups using a completely randomized block design:
Group 1: non infected non treated control group (NINT)
Group 2: infected non treated control group (INT)

Group 3: infected treated robenidine hydrochloride group (IR) (66 ppm robenidine hydrochloride)
Group 4: infected treated diclazuril group (ID) (1 ppm diclazuril)

Inoculum

A recent field isolate (S-05-008) from a small scale farm was multiplied in specific pathogen free rabbits at the INRA institute in France to obtain an inoculum containing approximately 30,000 oocysts/ml (13,000 *E. media* and 17,000 *E. magna).* Animals were inoculated with 0.23 ml of this inoculum, *i.e.* about 3,000 *E. media* and 4,000 *E. magna* oocysts per rabbit.

Recordings

Rabbits were clinically observed once daily during the entire study period and mortality and morbidity was noted. The individual body weights were measured at 7 time points during the study (study days -4, 0, 4, 10, 14, 21 and 35). Feed consumption was also monitored. Faecal samples from 4 different cages within each group were taken daily from day 7 till day 10 after inoculation to determine the number of oocysts per gram of faeces (OPG).

The schedule of the events is reported in Table 1.

Production data of rabbits were submitted to a 2-factorial analysis (treatment, block). LSD multiple range tests were used to separate means that were statistically different (Statistica 7 program).

Table 1: Schedule of events

Study Day	Age (days)	Procedure
-12	21	Sulfachlorpyridazine medication for does and offspring
-4	29	Weaning
		Stop sulfachlorpyridazine medication
		Body weight determination
		Start anticoccidial medication for groups 3 and 4
0	33	Body weight determination
		Inoculation of all groups (except NINT)
4	37	Body weight determination
7	40	Faecal samples collection from 4 cages
8	41	Faecal samples collection from 4 cages
9	42	Faecal samples collection from 4 cages
10	43	Body weight determination
		Faecal samples collection from 4 cages
14	47	Body weight determination
21	54	Body weight determination
35	68	Body weight determination

RESULTS AND DISCUSSION

Weight gain and feed consumption

Due to the coccidial challenge, weight gain was significantly lower in the INT rabbits during the 10 days period following the inoculation ($P<0.01$) (Table 2). During d4–d10, the weight gain of the NINT, IR and ID rabbits was significantly higher than in the INT animals ($P<0.01$) but weight gain in the ID group was significantly lower compared with the NINT and IR groups ($P<0.01$). There was still a small negative effect on weight gain between d10 and d14 post inoculation in the INT rabbits. However, from d14 post inoculation onwards, a significant compensatory growth was observed in the INT rabbits compared to the NINT group ($P<0.05$), indicating that the surviving rabbits did no longer suffer from the infection. However, for the total period there still was a significantly lower weight gain in the INT rabbits compared to the three other groups ($P<0.05$). IR rabbits had the highest numerical weight gain compared to the NINT and diclazuril treated rabbits (ID), although these differences were not statistically significant.

Table 2: Total weight gain and average daily weight gain (g) in the different periods

	NINT	INT	IR	ID	SEM	P
Start weight	694	700	692	713	4.2	0.27
End weight	2419	2349	2444	2441	15.9	0.14
d-4-d0	42.0	41.8	41.3	43.0	0.7	0.81
d0–d4	50.0 ^{A}	36.8 ^{B}	47.5 ^{A}	48.3 ^{A}	0.7	<0.001
d4–d10	52.0 ^{A}	27.0 ^{B}	51.3 ^{A}	40.8 ^{C}	0.7	<0.001
d10–d14	45.8 ^{ab}	42.0 ^{b}	48.5 ^{a}	46.3 ^{ab}	0.8	0.048
d14–d21	44.0 ^{a}	49.7 ^{b}	47.1 ^{ab}	50.1 ^{b}	0.8	0.032
d21–d35	39.9 ^{a}	46.2 ^{b}	40.3 ^{b}	41.6 ^{b}	0.7	0.006
Weight gain d-4–d35	1729 ^{a}	1639^{b}	1750 ^{a}	1727 ^{a}	13.9	0.037

SEM: standard error of the mean A,B,C: P<0.01 a,b,c: P<0.05

The amount of feed consumed by the animals of each individual cage was measured at days -4, 4 14, 21 and 35 and the results are represented in Table 3. Feed usage for the period was defined as the cumulative weight of feed introduced to the cage, minus the weight of unconsumed feed in the feed bin retrieved immediately before making the assessment. Thus feed usage included wasted as well as consumed feed. INT rabbits had a significantly lower feed intake compared with NINT and robenidine and diclazuril treated rabbits, which was most pronounced between d4 and d14 post inoculation (P<0.01). The following week a significantly higher feed intake was observed for INT, IR and ID groups compared with the NINT (P<0.05). In spite of this recovered feed intake from day 14 onwards, for the overall period, a significantly lower feed intake was observed in INT rabbits compared to the 3 other groups (P<0.05).

Table 3: Mean feed consumption data

	NINT	INT	IR	ID	SEM	P
d-4–d4	684 ^{a}	641 ^{b}	681^{a}	698 ^{b}	6.8	0.035
d4–d14	1218 ^{A}	861 ^{B}	1259 ^{A}	1098 ^{C}	12.3	<0.001
d14–d21	932^{a}	1015^{b}	992^{b}	1004^{b}	9.5	0.020
d21–d35	2136	2208	2165	2256	23.3	0.30
Total period d-4–d35	4970^{a}	4724^{b}	5097^{a}	5057^{a}	37.1	0.005

A,B,C: P<0.01; a,b,c: P<0.05

Mortality

Mortality was low, with one dead animal in the NINT, IR and ID groups and two dead rabbits in the INT group.

Oocyst excretion

OPG determination (Table 4) revealed *E. magna*, *E. media* and *E. perforans* oocysts. As *E. perforans* was not present in the inoculum, rabbits must have been cross contaminated with this species in the research unit. NINT rabbits also shedded oocysts, indicating a cross contamination of this group, something which is not so unusual to happen in inoculation trials.

Table 4: Mean oocyst excretion data and identification of oocysts

Group	Total OPG	% of *Eimeria* species		
		E. perforans	*E. media*	*E. magna*
NINT	2.7 x 10$^{3\,c}$	31^{a}	38^{a}	31^{b}
INT	9.6 x 10$^{6\,a}$	27^{ab}	28^{ab}	45^{b}
IR	4.6 x 10$^{3\,c}$	11^{bc}	22^{ab}	67^{ab}
ID	1.8 x 10$^{6\,b}$	2^{c}	5^{b}	93^{a}
Significance	P<0.001	P=0.016	P=0.089	P=0.019

a,b,c: P<0.05

The results show that the challenge infection was adequate as there was a more than thousand fold higher excretion of *E. magna* and *E. media* oocysts in the INT group in comparison with the NINT group. Both groups treated with anticoccidials showed significantly lower OPGs than INT animals

(P<0.001). In this trial, no significant difference between NINT and robenidine hydrochloride group could be noted. Total OPG in the ID group was significantly higher than in the IR group (1.8×10^6 versus 4.6×10^3, P<0.001). This relatively higher oocyst excretion in ID rabbits was most outspoken for *E. magna*.

In the field of poultry coccidiosis, a sharp distinction is often made between chemical and ionophore anticoccidials. Under very strict experimental conditions, chemical anticoccidials are typically characterized by a complete block of coccidial multiplication, which is in contrast with ionophore anticoccidials which always allow for a limited multiplication known as coccidial leakage (Chapman and Johnson, 1992). As both robenidine hydrochloride and diclazuril are chemical anticoccidials, one would expect them to be equally effective at blocking the multiplication of coccidia. However, total OPG in the robenidine hydrochloride treated rabbits was significantly lower than in the diclazuril group (P<0.001). A possible explanation for this difference could be that the *Eimeria* used for inoculation had previously been exposed to diclazuril (for a long period of time), leading to resistance to the latter molecule, something which has been described for robenidine hydrochloride as well (Coudert, 2004). However, such a previous exposure is improbable as diclazuril was not registered for use in rabbits at the time of the trial. Also, diclazuril had not been used for experiments in the research unit since more than ten years, so possible cross contamination with resistant *Eimeria* from the environment is very unlikely.

CONCLUSIONS

In the present trial robenidine hydrochloride adequately prevented the potential weight gain decrease caused by challenge infection with a mixed field isolate of *E. magna, E. media* and *E. perforans* in fattening rabbits. Although not blocking it completely, robenidine hydrochloride significantly reduced the excretion of oocysts.

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