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AGAINST COCCIDIOSIS IN FATTENING RABBITS.**

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# THE ANTICOCIDIAL ACTIVITY OF CYCOSTAT 66G AGAINST COCCIDIOSIS IN FATTENING RABBITS.

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## ABSTRACT

The anticoccidial CYCOSTAT 66G (robenidine hydrochloride 6.6%) was tested in three separate infection trials in young New Zealand White rabbits using an infection of  $5 \times 10^3$  *Eimeria magna* and *Eimeria media* (trial 1), an infection of  $5 \times 10^3$  *Eimeria flavescens* and *Eimeria intestinalis* (trial 2) and a single infection of  $5 \times 10^3$  or  $5 \times 10^4$  *Eimeria stiedai* (trial 3). An uninfected, unmedicated and an infected, unmedicated group served as controls. After intestinal coccidial infections (trials 1 and 2) the continuous medication of 66 ppm robenidine hydrochloride in the feed was able to completely prevent mortality and diarrhea. Moreover a severe ( $P < 0.001$ ) reduction of the average body weight gain was seen in the non treated control groups during the critical phase of the coccidial infection. After infection with *E. stiedai*, no mortality nor diarrhea was observed. A significant drop in ABWG was observed between day 7 and day 10 in the untreated group inoculated with  $5 \times 10^4$  oocysts. The liver weight/eviscerated weight ratio was significantly ( $p < 0.001$ ) higher in all non treated inoculated groups. The number of livers affected with apparent lesions and the severity of the lesions was highly correlated with the inoculation dose in non treated groups but were markedly reduced in the treated groups and decreased from 9 to 2 (5000 oocysts/dose) and from 9 to 4 (50000 oocysts/dose).

## INTRODUCTION

CYCOSTAT 66G (robenidine hydrochloride 6.6%) has been widely used in the EU as an anticoccidial drug since 1982 and is still one of the leading anticoccidials in commercial rabbit production. Several authors have demonstrated the anticoccidial efficacy of CYCOSTAT 66G in fattening rabbits against the most relevant *Eimeria spp.* using laboratory and field trials (COUDERT *et al.*, 1979; LICOIS *et al.*, 1980; NIEDZWIADK *et al.*, 1989; PEETERS *et al.*, 1979, 1982; REID, 1970; VARGA I., 1982; YIN P., 1988). Nowadays, mainly *E. magna* and *E. media* are present in commercial rabbitries where they still cause economic losses due to subclinical infections affecting feed conversion and favouring colibacillosis by increasing caecal pH (LICOIS, 1980). Coccidial infections thus enhance the development of the enteritis complex (PEETERS, 1991). Although years of intensive use of CYCOSTAT 66G have led to the disappearance of *E. intestinalis* and *E. flavescens* from most commercial rabbitries, they are still present in traditionally reared domestic rabbits causing diarrhoea with high mortality. Also, the incidence of hepatic coccidiosis has almost disappeared from commercial rabbitries but rabbits are still affected in small farm or in non conventional rearing (bio, label, ...). Domestic rabbitries form thus a constant threat to the commercial rabbitries for intestinal and hepatic coccidiosis

The present study was initiated to re-examine the anticoccidial activity of CYCOSTAT 66G against *Eimeria magna*, *Eimeria media*, *Eimeria flavescens*, *Eimeria intestinalis* and *Eimeria stiedae* in three separate infection trials.

## MATERIAL AND METHODS

Three separate infection trials were performed in our own research facilities. In each trial, young New Zealand White rabbits (strain INRA 1077) were used from litters of SPF does

from our rabbitry (COUDERT et al. 1988). The repartition took place at the day of weaning (28 days) on which rabbits from a same litter were allocated to the different treatment groups. The animals were randomised into groups with 12 rabbits each (6 cages of 2 animals) for trials 1 and 2 or 9 rabbits (3 cages of 3 animals) for trial 3 and housed in conventional conditions (VIARD-DROUET et al., 1983).

In each trial, all rabbits were fed a standard fattening diet used in intensive rabbit production. In treated groups, the feed was supplemented with CYCOSTAT 66G (1 kg premix/ton or 66mg/kg robenidine HCl). The correct concentration of CYCOSTAT 66G in feed was confirmed by analysis. Diets and drinking water were provided *ad libitum* during the experiment.

At 5 weeks of age, the animals were individually challenged per os. The oocysts used for challenge inoculation were originating from pure isolates cultured in our laboratory and used as reference strains by many European Investigators. The strains were multiplied before the start of the experiment in SPF rabbit of 4 weeks of age and were sporulated at  $26 \pm 1^\circ\text{C}$ . The rabbits were challenged with either a mixture of *E. media* + *E. magna* (trial 1), *E. flavescens* + *E. intestinalis* (trial 2) or *E. stiedai* (trial 3). The inoculum in trial 3 was chosen to provoke a moderate ( $5 \times 10^3$  oocysts/dose) or a serious ( $5 \times 10^4$  oocysts/ dose) hepatic coccidiosis. In each trial, one group was used as an uninfected untreated control group. Table 1 gives information on the infections and inoculation doses.

**Table 1: Description of the infection trials and inoculation doses:**

Trial N°	Eimeria species	Inoculation dose/animal (n° of sporulated oocysts)
1	<i>E. magna</i> or <i>E. media</i>	$4 \times 10^4$
2	<i>E. flavescens</i> or <i>E.intestinalis</i>	$5 \times 10^3$
3	<i>E. stiedai</i>	$5 \times 10^3$ or $5 \times 10^4$

In all three trials, individual body weights and clinical examination were registered twice a week after inoculation. Autopsy was performed on all dead animals. In trial 3, relative liver weight (=liver weight /live body weight - visceral weight) and liver lesions were determined after slaughter (on day 22). The lesions were scored macroscopically according to the index in table 2.

**Table 2: Score index of macroscopically observed lesions**

Score	Lesions
0	No apparent lesion; normal liver aspect
1	A few pointed lesions; normal aspect
2	Numerous lesions; jaundiced liver
3	Innumeros coalescent lesions; degeneration

Data were interpreted by a 1-factorial analysis of variance (treatment) using Systat 5.04. Means were compared with Tukey HSD test.

## RESULTS

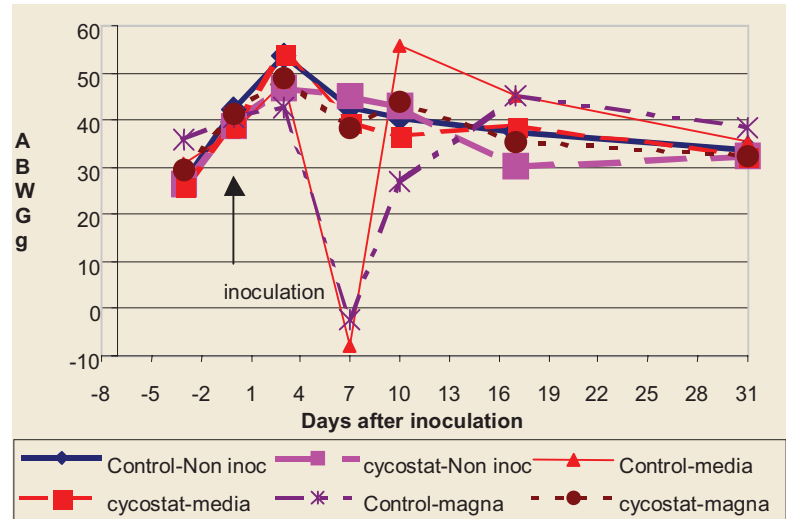
### Trial 1: Infection with *E. media* and *E. magna* (Figure 1)

As expected with these 2 *Eimeria* sp. there was few mortality but an acute and short growth depression. At D31, mortality was 2/12 (*E. media* non supplemented) and 3/12 (*E. magna* non supplemented) versus 1/12 in all other groups.

From the weaning (day -7) to the third day after inoculation the growth was similar in all groups.

From the 3<sup>rd</sup> to the 10<sup>th</sup> day after inoculation the effects of coccidiosis were only seen in the 2 inoculated groups without medicated feed. The statistical analysis of the ABWG confirm that it was the only significant difference ( $P < 0.001$ ).

Figure 1: Inoculation with *E. media* and *E. magna*; effect of CYCOSTAT 66G on the Average Body Weight Gain (ABWG)



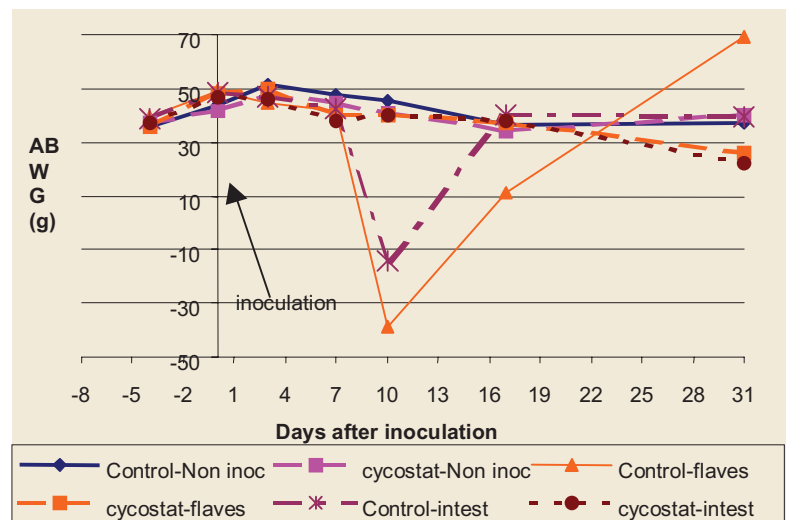
### Trial 2: Infection with *E. intestinalis* and *E. flavescens* (Figure 2)

Until D17 mortality occurred only in the non treated group inoculated with *Eimeria flavescens*: 4/12 versus 0/12 in other groups ( $\chi^2$ :  $P < 0.01$ ).

Diarrhoea was observed in all non treated animals. Necropsy of the 4 dead animals revealed typical lesions of *Eimeria flavescens* on the caecum.

From the day of weaning (day -8) to the 7<sup>th</sup> day after inoculation the growth was similar in all groups ( $F < 0$ ). From the 7<sup>th</sup> to the 17<sup>th</sup> day after inoculation, the effect of coccidiosis on ABWG was only seen in the 2 inoculated groups (*Eimeria intestinalis* or *Eimeria flavescens*) fed without medicated feed. It was the only significant difference ( $P < 0.001$ ). From the 17<sup>th</sup> until the 31<sup>st</sup> day the ABWG was similar in all groups and any symptoms of disease were observed.

Figure 2: *E. intestinalis* and *E. flavescens*; effect of CYCOSTAT 66G on the Average Body Weight Gain (ABWG)



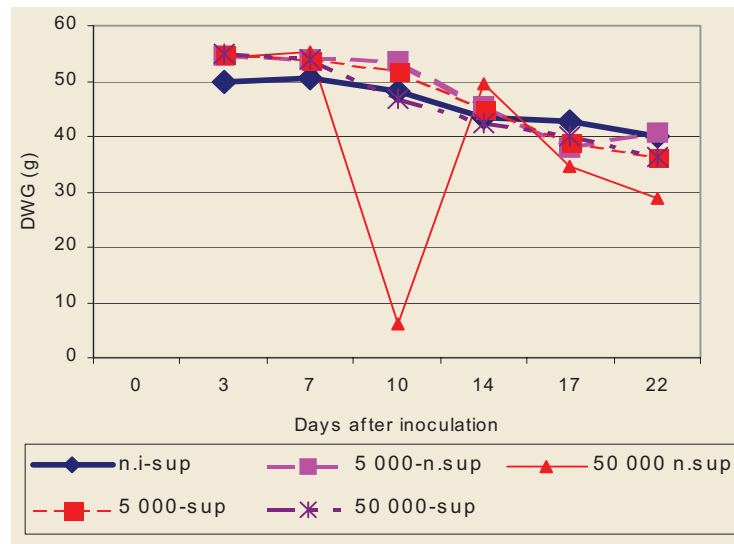
The very high ABWG of the group inoculated with *Eimeria*

*flavescens* but not supplemented correspond to a physiological rehydration following the previous episode of diarrhoea.

### Trial 3: Infection with *E. stiedai* (Figure 3)

There was no mortality nor diarrhoea during the experiment. At day 0 no significant differences in ABWG were observed between the different groups. A significant drop in ABWG was observed between day 7 and day 10 in the untreated group inoculated with  $5 \times 10^4$  oocysts. This drop corresponds with the inflammation phase noticed in case of severe hepatic coccidiosis. From day 10 - 14 onwards, the ABWG in this group recovered up to the same level observed in the other groups. However, in practice

**Figure 3: *E. stiedai*; effect of CYCOSTAT 66G on the Average Body Weight Gain (ABWG)**



the ABWG is a very poor criterion for this kind of study as from days 10 – 14 the hypertrophy of the liver artificially compensated the substantial muscular shrinking.

### Lesion scores and hepatic hypertrophy:

The number of animals with hepatic lesions is summarized in table 3.

The liver of all non-supplemented rabbits showed several (5000 oocysts/dose: index 2) or numerous lesions (50000 oocysts/dose: index 3).

After inoculation with 5000 oocysts, the total number of affected livers was 9 in the non treated group compared to 2 in the treated group with just a few lesions (index 1). After inoculation with 50000 oocysts, 9 livers were affected in the non treated group with index 3 whereas 4 livers were affected in the treated group of which 2/9 showed lesions with index 1 and 2/9 livers showed lesions with index 2.

**Table 3: Hepatic lesions**

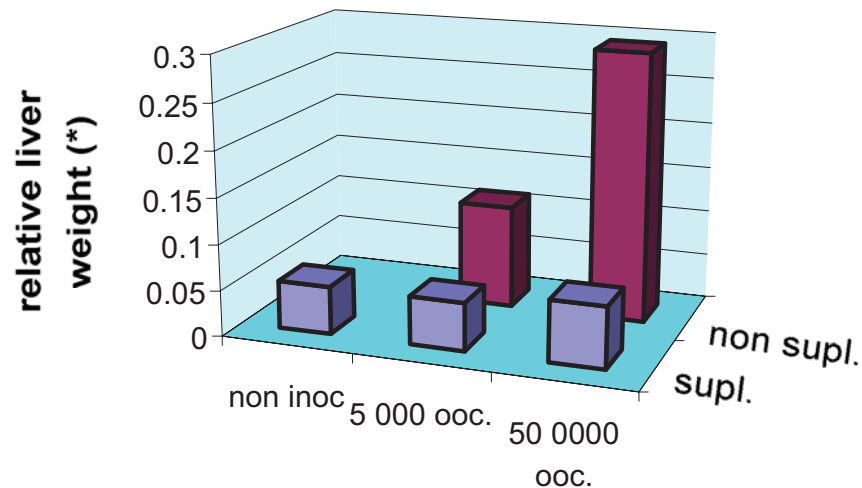
Experimental group		Index (number of animals)				
Treatment	Inoculation dose	0	1	2	3	Total affected
T	Not inoculated	9	0	0	0	0
NT	5000	0	0	9	0	9
T	5000	7	2	0	0	2
NT	50 000	0	0	0	9	9
T	50 000	5	2	2	0	4

T: treated with CYCOSTAT 66G (66 ppm robenidine hydrochloride)

NT: not treated

The most reliable criterion for this study is the liver weight/ eviscerated weight ration (figure 4). This ratio was significantly ( $p<0.001$ ) higher in the two groups not treated with CYCOSTAT 66G.

**Figure 4: *Eimeria stiedai*: relative hypertrophy of the liver**



(\*) liver weight/eviscerated carcass weight ratio

After infection with *E. stiedai*, the supplementation of the feed with CYCOSTAT 66G, allowed a growth of the inoculated groups identical to the non-inoculated control rabbits. Especially the results from the treated group inoculated with 50000 oocysts were favorable as compared to the non-supplemented group. After treatment, no inflammation was observed from Day 10 to Day 22 and no apparent lesions 5 times out of 9. In all CYCOSTAT 66G treated groups, the liver weight/eviscerated carcass weight ratio was not significantly different from the non-inoculated control group.

## CONCLUSION

The results obtained in trial 1 and 2, confirm the continuous efficacy of CYCOSTAT 66G against intestinal coccidiosis in fattening rabbits caused by *E. magna*, *E. media*, *E. intestinalis* and *E. flavescens*, even at high inoculation doses. Also, favorable results were obtained after infection with *E. stiedai* although a complete prevention of disfigured livers could not be obtained. CYCOSTAT 66G should thus be used systematically in commercial rabbit feed to avoid the risk of an outbreak of intestinal or hepatic coccidiosis associated with major economic losses due to increased prevalence of enteritis and disfigured livers.

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