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EVALUATION OF THE ANTICOCCIDIAL EFFICACY OF CYCOSTAT® IN EXPERIMENTALLY INFECTED BREEDING RABBITS HOUSED UNDER CONVENTIONAL CONDITIONS

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Abstract: The present study was designed in order to evaluate the efficacy and safety of Cycostat® 66G in the prevention and control of coccidiosis in breeding does after experimentally induced infection with common *Eimeria* spp. at the age of 18 wk. The inoculum was prepared based on faecal samples collected on farms in the Czech Republic. A total of 94 young and pregnant does were divided into 4 groups and enrolled in the study. The zootechnical, clinical and parasitological results of 2 control groups were compared, a non-infected non-treated (NI-NT) and an infected non-treated (I-NT), and 2 infected and dietary supplemented groups, with 50 (I-50) and 66 mg/kg (I-66) robenidine, respectively. Does in the I-NT group showed a significant drop in weight gain and feed intake during the first 14 d after inoculation. No treatment related effects on the weight development of the does were observed, although does in the I-NT group always had approximately ± 200 g lower body weight. Litter size (live born) was not significantly different at either the first or second parturition and amounted to 9.0, 8.2, 10.1 and 9.8 (1st litter) and 11.3, 10.4, 10.1 and 11.0 (2nd litter) in NI-NT, I-NT, I-50 and I-66 does, respectively. At weaning, both in the 1st and 2nd cycle, the highest litter weight was obtained in the I-50 and I-66 groups. Litter weaning weight values for I-66 (litter 2) were higher but not significant (P=0.069) compared to the NI-NT litters. The lowest young mortality was also observed in the I-66 litters, both in the first and second cycles. Between 6 to 11 d after inoculation, the oocyst excretion of both supplemented groups was reduced by more than 90% in comparison to the I-NT. Supplementation with Cycostat® 66G diminished the excretion of *E. flavescens* and *E. intestinalis*, the most pathogenic species, by 100%. The excretion of *E. magna* was reduced by >90 and 100% for the I-50 and I-66 groups, respectively. The reduction in oocyst excretion of *E. media* was less pronounced. It was concluded that Cycostat® 66G supplemented in the feed at a concentration of 50 or 66mg/kg was able to prevent coccidiosis in breeding does after experimental inoculation.

Key Words: coccidiosis, rabbit does, Cycostat®, performance, oocyst excretion.

INTRODUCTION

Coccidiosis still represents the main parasitological infection in rabbit units (Coudert *et al*., 2003; Licois and Marlier, 2008). Rabbits can be infected by a large number of species; however, only 11 species have been isolated in pure culture and characterised (Licois and Marlier, 2008). All are of the genus *Eimeria* of which eight are of economic importance (Bhat *et al*., 1996). The most dominant *Eimeria* species isolated today in professional rabbit husbandry are intestinal species: *E. magna* and *E. media* (Coudert *et al*., 2003). Even low levels of infection usually cause intestinal malabsorption, increased feed conversion and

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delayed growth (Coudert, 1997). The importance of the disease increased even more with the onset of Epizootic Rabbit Enteropathy (Coudert *et al*., 2000).

The most applied method of control and prevention of coccidiosis in intensive rabbit production, except for farm management (all-in, all-out), hygiene and disinfection, is the prophylactic inclusion of anticoccidials in the feed. Cycostat® 66G is a coccidiostat containing 6.6% robenidine hydrochloride and is well known for its efficacy against coccidiosis in rabbits (Peeters and Halen, 1980). A regular update of the efficacy and safety provides useful information on the performance of the product under current housing conditions (Vancraeynest *et al*., 2008) and is legally required in view of the authorization process.

The study objective was to evaluate the efficacy of two different dietary supplementation regimes of Cycostat® 66G for the prevention of coccidiosis in conventional breeding rabbits under practical housing conditions and husbandry available today. For this purpose, commercially obtained young rabbit breeders were orally infected with recent isolates of rabbit specific *Eimeria*.

MATERIAL AND METHODS

Animals and procedures

One hundred and twenty young does were purchased at a commercial farm at the age of 10-11 wk. They belonged to the progeny of the Institutes' dam line and were later on inseminated with males of the Institutes' sire line (Maertens, 1992). Animals were vaccinated at arrival against Rabbit Hemorrhagic Disease (RHD) (Hebovak 88T, Eurovet Animal Health). These young does were dietary treated with salinomycine (20 mg/kg) for 2 wk to obtain coccidia-free animals for the study.

The females were uniquely identified with ear tags and randomised into 4 experimental groups one week before the start of the trial (Table 1). At study start (0 d) the does were 16-17 wk old and inseminated for the first time with mixed semen.

Only the pregnant does were retained after the control of gestation on 13 d of the trial. Because the overall gestation success was rather low (62%) and lower than expected, the intended 20 pregnant females/group were not reached. Therefore, it was decided to inseminate the non-pregnant females a second time on study 20 d; this is 20 d after the first unsuccessful insemination. These females were handled in exactly the same way according to the experimental design and schedule with the only difference being the time delay of 20 d. In total, 94 young pregnant females were observed during the study.

At parturition, after counting the original litter size, litters were equalised intra-group at 7-9 kits/litter or 9-10 kits/litter depending of the number of kits born alive, for litter 1 and 2, respectively. Since the females were inseminated on the same day, 31 d was considered as the day of parturition and all litters were weaned another 31 d later. Females were rebred 15 d after the first parturition (Table 2).

Table 1: Experimental design.

Group	Infection	Treatment (Cycostat® 66G)	Active Ingredient	Dosage (active ingredient)
A =NI-NT	−	−	NA[1]	NA[1]
B =I-NT	+	−	NA[1]	NA[1]
C =I-50	+	+	Robenidine	50 mg/kg
D =I-66	+	+	Robenidine	66 mg/kg

NI-NT: a non-infected non-treated, I-NT: and an infected non-treated, I-50: infected and dietary supplemented with 50 mg/kg robenidine, I-66: infected and dietary supplemented with 66 mg/kg robenidine.
[1]NA = Not Applicable,

Table 2: Scheme of main activities.

Day of study	Activity
0	Insemination 1. Collection of faecal samples
13	Inoculation
19-24	Collection of faecal samples
31	Parturition 1
38-39	Collection of faecal samples
46	Insemination 2
55-57	Collection of faecal samples
62	Weaning litter 1
77	Parturition 2
84-86	Collection of faecal samples
101-103	Collection of faecal samples
108	Weaning litter 2

The experimental protocol was approved by the Use and Care Committee of the ILVO Research Institute in September 2007.

Housing

The trial took place in one of the experimental rabbit houses of the ILVO Animal Science Unit and started on 21 September 2007. The rabbit house compartments had a net floor space of 95 m² (9.5 ×10 m) and contained 96 flat-deck cages measuring 0.70 ×0.46 and 0.50 m height. Before the experiment, all cages were cleaned intensively and heat-treated (gas burner). The wire cages were of zinc-coated wire with a 3 mm diameter and rectangular slats of 75×15 mm. Twice a week, the droppings were removed.

Females were housed individually and each cage was equipped with a feeder and a nipple drinker. The cages were aligned in 6 rows (16 cages): 2 against the walls and 2 double rows in the middle of the compartment. Each cage was also equipped with an outside nestbox (30×28 cm). Four days before parturition, females were allowed to enter their outside placed nest box filled with wood shavings.

Infected treatment groups were housed together in the same compartment. The infected untreated animals (I-NT) were housed on 2 rows separated from the 2 infected and treated groups (I-50 and I-66). The non-infected untreated (NI-NT) does were housed in a separate compartment, equipped with identical cages, to avoid contamination from the infected rabbits.

The trial site was equipped with dynamic (over-underpressure) ventilation with 2 air inlets at the door side of each compartment and air extraction at the other side of the room. The ventilation rate varied between 1 and 2 (winter conditions) m^3/kg BW/h, depending on the outside temperature and occupation of the house. The incoming air was heated before the inlet of the experimental rooms and distributed over the different compartments. With this acclimatisation system, a temperature of 17±2°C was maintained throughout the experiment.

The rabbit house was windowless. Illumination was provided by electric bulbs, placed regularly in the centre of each row. Females received a lighting schedule of 16h of light per day throughout the entire experimental period. Illumination was automatically regulated by means of a 24 h-clock.

Table 3: Ingredient composition and calculated nutrient composition of the diet.

Ingredients		Calculated nutrients (g kg^{-1})[1]	
Alfalfa meal 15	26.0	Crude protein	180
Wheat shorts	22.1	Ash	105
Sunflower meal 27	14.0	Ether extract	46
Wheat	11.0	Crude fibre	155
Beet pulp	8.0	NDF	290
Soybean meal 44	3.0	ADF	180
Full-fat soybeans	6.0	ADL	43
Flax chaff	3.0	DE (MJ/kg)	10.5
Fat	1.0		
Molasses	3.0		
Minerals and vitamins	2.8		
Amino acids	0.10		

[1]According to Maertens *et al*. (2002)

Feeding

Breeding does always received their respective experimental diet *ad libitum* from the first insemination onwards. Each female had a bucket on top of the cage allowing individual feed control as scheduled between insemination 1, inoculation, 28 d and when the kits were 1 and 3 wk old.

All the diets were prepared and pelleted (3 mm diameter and 0.8 cm long) at the ILVO-Animal Science feed mill. The diet was formulated to have an energy content of 10.5 MJ/kg and a crude protein content of 18.0%. The feed composition and the main nutrients calculated are presented in Table 3. No growth promoter was added to the experimental diets. The test substance (Cycostat® 66G) was mixed into the meal before the pelleting process.

Supplemented groups were given either 50 mg/kg or 66 mg/kg robenidine hydrochloride in their feed. These doses correspond to the lowest and highest recorded supplementation regimen for Cycostat® 66G in rabbits. The control groups received blank feed from the same batch. Kits received the same feed as their mothers, as they used the same feeder till weaning age.

A sample of each of the experimental diets was taken to determine the robenidine hydrochloride content. In total, 2 feed batches were prepared of each experimental diet.

Drinking water was supplied by an internal water system network stemming from a water well. Each cage was connected with this system by one nipple drinker.

Inoculation

The inoculum was provided by Dr Dominique Licois from the INRA Tours (France). The inoculum was based on faecal samples collected by Michal Pakandl (Biology Centre AS CR, Institute of Parasitology, Branišovská 31, 37005 České Budějovice, Czech Republic) on farms in the Czech Republic in June 2007. The oocysts from these faecal samples were extracted and the *Eimeria* spp. were propagated in coccidia free rabbits. The derived inoculum was equilibrated with historical strains of Eimera spp. isolated from rabbit farms in France in order to obtain an acceptable pathogenic inoculum with the following composition: *E. magna*: 29%; *E. media*: 26%; *E. perforans*: 8%; *E. intestinalis*: 19% and *E. flavescens*: 18%.

The inoculum was administered orally with a plastic syringe at an inoculation dose of 500µl/doe. Each doe was inoculated with approximately:

1015 oocysts of *E. magna*

910 oocysts of *E. media*

280 oocysts of *E. perforans*

665 oocysts of *E. intestinalis*

630 oocysts of *E. flavescens*

The inoculation was done on Day 13 of the experiment. Non-pregnant females were also inoculated 13 d after the second insemination, if pregnant.

Clinical and parasitological parameters

Faecal samples were collected by placing a bucket under the cages of the rabbits. After homogenising, a representative sample was taken from each bucket. Faecal samples were only collected from females that were pregnant after the first artificial insemination and followed the initial time schedule.

Immediately after collection, the samples were transported for oocyst counts determination and/or differentiation to the DGZ-Flanders laboratory in Torhout (Belgium).

At the start of the study (Day 0), four pooled faecal samples from does, each consisting of 3 individual samples, were collected per group and the oocyst counts (OPG) were determined. On 19-24 d (control of artificial infection), 38-39 d (±7 d after the first kindling) and 84-86 d (±7 d after the second kindling) faecal samples were gathered. The OPG was determined on 5 pooled samples per group, each consisting of at least 3 individual samples. At the same time, differentiation was performed on 2 pooled samples per group, each consisting of ± 8 individual samples. On the samples collected on 55-57 d (±25 d after the first kindling) and 101-103 d (±25 d after the second kindling) only OPG counts were determined (5 pooled samples per group).

Statistical analysis

Zootechnical parameters (prolificacy, weight development and feed intake) were analysed using the Kruskal Wallis Test. If a significant difference ($\alpha<0.05$) was observed between groups, further statistical analysis was performed using the Mann-Whitney U test. Data concerning mortality or fertility were analysed by the χ^2 test.

No statistical analysis was performed on the OPG values. Differences of more than 0.5 log were considered to be significantly different (i.e. reduction of the OPG by 50%).

RESULTS AND DISCUSSION

Concentration of robenidine hydrochloride in the experimental diets

The results of the dietary concentrations of robenidine are presented in Table 4. The analysed concentration in each sample was somewhat higher than intended. However, with the exception of the concentration found in sample C2, the difference between the analysed and intended inclusion rate was less than 10%. The difference between the analysed and intended rate for sample C2 was ±20%. Overall, it may be concluded that the difference between the intended and analysed concentrations was within an acceptable range.

Table 4: Analysed dietary concentration of robenidine in the different feed batches.

		Concentration robenidine, mg/kg		
Period	Sample ID	Intended	Result	Description
First batch	Sample A1	0	1,6	Feed 1 groups NI-NT and I-NT
	Sample C1	50	53,8	Feed 1 group I-50
	Sample D1	66	70,6	Feed 1 group I-66
Second batch	Sample A2	0	<1,0	Feed 2 groups NI-NT and I-NT
	Sample C2	50	60,3	Feed 2 group I-50
	Sample D2	66	67,6	Feed 2 group I-66

Zootechnical performance of the does and their offspring until weaning

It is a well known fact that the zootechnical performance (such as prolificacy, milk yield, feed intake,…) of breeding does is strongly influenced by the parity order (Maertens *et al*., 2006). For this reason, the cycle 1 and 2 results are presented separately.

Cycle 1

The effect of the inoculation on the weight development of the does was clearly demonstrated in the inoculated non-treated group (I-NT group). The weight gain during the first 2 wk post infection was significantly different (P=0.048) between experimental groups (Table 5). Mean weight gain was 280, 197, 289 and 240g in the NI-NT, I-NT, I-50 and I-66 does, respectively. This indicates that the objective of a quite severe infection was reached. Moreover, only females in the I-NT group showed signs of diarrhoea or wet faeces (prevalence >80%) between the 10th and 12th d post inoculation. These signs were not observed in the other experimental groups.

Table 5: Evolution of the mean body weight and weight gain of the does during their first cycle.

Treatment	NI-NT	I-NT	I-50	I-66	SEM	P-value
Number of does	22	25	23	24	-	
Mean body weight (g)						
At insemination (1)	3828	3795	3910	3853	34	0.587
At inoculation (2)	4263	4202	4341	4307	35	0.549
14 d post inoculation (3)	4543	4400	4629	4548	37	0.168
7 d post kindling (4)	4553	4443	4610	4619	40	0.508
21 d post kindling (5)	4615	4503	4665	4678	44	0.612
At weaning (6)	4550	4563	4676	4749	47	0.396
Mean weight difference (g)						
Interval (1) – (2)	435	408	431	454	16	0.800
Interval (2) – (3)	280^{a}	197^{b}	289^{a}	240^{ab}	15	0.048
Interval (3) – (4)	10	44	-19	71	18	0.226
Interval (4) – (5)	63	60	54	59	20	0.964
Interval (5) – (6)	-65^{a}	60^{b}	11^{ab}	71^{b}	18	0.017

Means with different superscripts in the same row differ from each other (P<0.05).

Table 6: Mean feed intake of does during the first cycle.

Treatment	NI-NT	I-NT	I-50	I-66	SEM	*P*-value
Number of does	22	25	23	24	-	-
Mean feed intake (g/day/doe)						
Period 1*	208	200	203	201	2.6	0.771
Period 2**	166[a]	139[b]	163[ab]	198[c]	4.6	< 0.000
Period 3***	317	320	325	315	3.6	0.636

*Insemination-inoculation ** Inoculation-14 d post-inoculation ***14 d post inoculation- 21 d post parturition.

Differences in mean body weight were not significant at any of the 6 time points ($P>0.05$). Although not significantly different, the mean body weight of the I-NT group generally remained lower than that of the other groups during the first cycle. Unexpectedly, females from the NI-NT group lost weight (−65g) in the week before weaning (Figure 1); this can be explained by the presence of a natural and unintended infection with coccidiosis occurring in this period. Oocyst excretion during this period supports this hypothesis (see further).

Average feed intake during the 14 d post infection was significantly lower ($P<0.05$) in I-NT does compared to controls and I-66 females (Table 6) and reached nearly the significance level ($P=0.101$) with I-50 females. These results confirm the impact of coccidiosis on the general health status and weight development of the breeding does in the first 14 d after infection. Afterwards, feed intake was comparable between the different experimental groups.

The mean litter size at birth (total born/litter and live born/litter) was not significantly different between the different supplementation groups, although there was a tendency ($P=0.103$) towards a lower litter size at birth in the I-NT group and a higher litter size in the I-50 and I-66 groups (Table 7). After standardising (intra-group) of the litter size at 9 young, especially in the I-50 and I-66 groups, a considerable number of young were still available, so it was decided to use them partially to enlarge the litter size of I-NT litters. This explains why the litter size in I-NT litters after standardising (8.9) was higher than the initial live

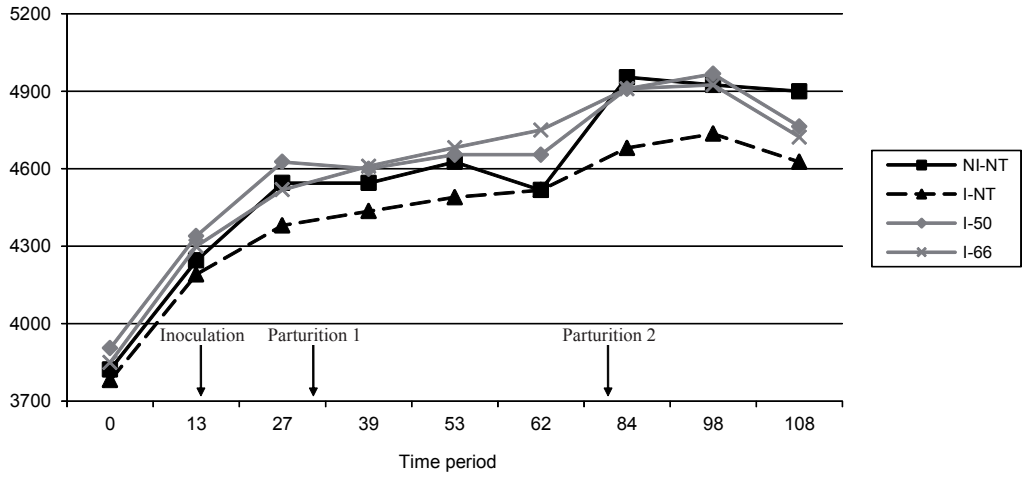

Figure 1: Weight development of does during the trial (g).

Table 7: Litter size during the first cycle.

Treatment	NI-NT	I-NT	I-50	I-66	SEM	*P*-value
Number of does	22	25	23	24		
Total born/litter	9.1	8.3	10.2	9.9	0.32	0.103
Born alive (n/litter)	9.0	8.2	10.1	9.8	0.33	0.123
Standardised at birth	8.7	8.9	9.0	9.0	0.03	
Litter size after 1 wk	8.5	8.7	8.6	8.8	0.07	0.334
Litter size after 3 wk	8.2^{a}	8.4^{a}	8.3^{a}	8.8^{b}	0.09	0.027
Litter size at weaning	7.8^{a}	8.3^{ab}	7.8^{a}	8.7^{b}	0.12	0.017
Mortality 1 wk – weaning (%)	8.0^{c}	4.5^{c}	8.5^{c}	1.0^{d}	-	0.003

Means with different superscripts in the same row differ from each other ($P<0.05$).

born young (only 8.2). The advantage of this approach is that the development of the young amongst the different groups became more comparable.

The differences between the mean litter sizes were only significant 3 wk after kindling and at weaning ($P<0.05$). At the 3 wk time point, only the result from the I-66 group (8.8) differed significantly ($P<0.05$) from the other groups (8.2, 8.4 and 8.3 for NI-NT, I-NT, I-50, respectively). The mortality in the I-50 group was mainly caused by the high mortality in one litter and can therefore be considered as not treatment related. The mortality in the NI-NT group is more difficult to explain and could have been caused by the unintended natural infection with coccidiosis during this period, as is supported by the oocyst excretions during this period (See further).

The mean litter and young weight must be interpreted in relation to the litter size. The litter size after the first week was comparable amongst the different supplementation groups, as shown in Table 7. At this time point, the litter weight of both supplemented groups was significantly ($P<0.05$) higher than the I-NT group (Table 8). This was not reflected in the mean young weight, although there was a tendency ($P=0.119$) towards a lower young weight in the I-NT group compared to both supplemented groups.

After 3 wk, the litter size was significantly higher in the I-66 group and comparable to the other groups (Table 7). This clearly resulted, as expected, in a higher mean litter weight and a lower mean young weight in the I-66 group compared to the I-50 and NI-NT groups, although this difference was not statistically

Table 8: Mean litter weight and mean young weight during cycle 1.

Treatment	NI-NT	I-NT	I-50	I-66	SEM	*P*-value
Number of does	22	25	23	24		
Litter weight after 1 wk (g)	1279^{ac}	1273^{a}	1373^{b}	1364^{bc}	21	0.037
Litter weight after 3 wk (g)	2580	2530	2710	2765	45	0.175
Litter weight at weaning (g)	5089	5240	5489	5706	107	0.133
Litter weight gain 1 wk - weaning	3810	3966	4116	4343	94	0.180
Young weight after 1 wk (g)	151	146	159	155	2.2	0.119
Young weight after 3 wk (g)	320	300	328	314	4.9	0.324
Young weight at weaning (g)	659^{ab}	629^{a}	699^{b}	654^{a}	10.0	0.033

Means with different superscripts in the same row differ from each other ($P<0.05$).

significant. Mean litter and young weight in the I-NT group were lower than in the other groups, but the difference is not significant.

The results at weaning are more difficult to interpret. At this time point, the NI-NT, I-NT and I-50 group had a comparable litter size (Table 7). The litter weight one week post parturition and the mean young weight at weaning in the I-50 group were significantly higher ($P<0.05$) than in the I-NT group and comparable to the NI-NT group (Table 8). The presence of an unintended natural infection in the NI-NT group during this time period, as is shown by the oocyst excretions during this period (see further), could also have influenced the results in this group. The I-66 group had a significantly higher ($P<0.05$) litter size than the NI-NT and I-55 group, which resulted in a lower mean young weight and a higher mean litter weight in comparison to the I-50 group, although this difference was not significant ($P=0.133$). The results in the I-66 group at weaning were not significantly different from those in the NI-NT or I-NT groups.

Cycle 2

The weight evolution of the does was very comparable (Figure 1), although the does in the I-NT group always had a ± 200 g lower body weight. The only significant result was the loss of body weight in all groups at the end of cycle 2 ($P=0.049$). The NI-NT group had the lowest loss in body weight, followed by the I-NT group and both supplemented groups. This can be explained by the higher mean litter weight at weaning for the I-NT, I-50 and I-66 groups, although this difference was only marginally significant ($P=0.069$).

Litter size was high and exceeded 10 young/litter in all groups (Table 9). No differences in litter size were observed during cycle 2. The somewhat higher mortality in the NI-NT and I-50 groups was due to some exceptionally mortality in a limited number of litters (2 in both groups) and not relevant. A lack of milk was probably the cause.

Mean litter and young weight were comparable amongst the different supplementation groups, although litter weaning weight values for I-66 (litter 2) were higher but no significant ($P=0.069$) compared to the NI-NY litters. Differences in fertility (n° inseminated/n° parturitions) were not significantly different ($P=0.309$, data not shown). From the females that started the experiment, respectively 81.8, 88.0, 95.6 and 95.8% of the NI-NT, I-NT, I-50 and I-66 group gave birth to second litters. Although the current trial was not powerful enough (limited n° females/group) to demonstrate a significant effect, such a difference of around 10% is not negligible in practice.

Table 9: Overview of the performances during the 2nd cycle.

Treatment	NI-NT	I-NT	I-50	I-66	SEM	*P*-value
Number of does at parturition	18	22	22	23	-	-
Total born/litter	11.4	10.6	10.5	11.3	0.34	0.649
Born alive (n/litter)	11.3	10.4	10.1	11.0	0.36	0.669
Litter size after 3 wk	8.8	9.3	8.6	9.5	0.16	0.251
Litter size at weaning	8.4	9.0	8.4	9.3	0.18	0.403
Litter weight at weaning (g)	6533	7002	7043	7419	141	0.069
Young weight at week 3 (g)	394	383	398	392	5.7	0.945
Young weight at weaning (g)	782	775	810	803	8.4	0.319
Mortality after week 1 (%)	9.2	4.5	8.1	4.2	-	0.163

Oocyst output and differentiation

Mean oocyst excretion during the first cycle is summarised in Figure 2. Results are presented as natural logarithms of the geometrical mean. At D0 of the study, all the oocyst counts were below the detection limit (<50). Thus, at the start of the trial the does were free of coccidia.

On 13 d of the study, all the pregnant does were inoculated with the inoculum and faecal samples were collected from 6 to 11 d after the inoculation (period 19-24), which corresponds to shortest cycle (*E. media* and *E. magna*: 5-6 d) and the longest cycle (*E. maxima* and *E. flavescens*: 10-11 d) of the *Eimeria* spp. The oocyst excretion of both supplemented groups was reduced by more than 90% in comparison to the I-NT group, as shown in figure 2 (reduction by 1 logarithm). The NI-NT group initially remained free of coccidia, but apparently became infected approximately one week after kindling. The peak excretion of the NI-NT group was probably situated between D40 and D55.

Results from the species identification are presented in Figure 3. The excretion of *E. flavescens* and *E. intestinalis*, the 2 most pathogenic species, was reduced by 100% in both supplemented groups. Excretion of *E. magna* was reduced by 100% in the I-66 group and with more than 90% in the I-50 group. The reduction in oocyst excretion of *E. media* was less pronounced. The results of the OPGs and the species identification are in line with the clinical observation of diarrhoea in the I-NT group 10-12 d after infection. During this period, the NI-NT group remained free of coccidial infection.

Oocyst excretion 7 to 9 d after kindling (period D38-40) was overall low, but higher in the supplemented groups than in the I-NT. The results from the species identification demonstrate that the higher oocyst excretion in the supplemented groups is only due to the low pathogenic *E. perforans* and therefore of low importance.

In the I-NT group the very pathogenic *E. intestinalis* and the pathogenic *E. magna* were still present during this period. In this period, the NI-NT group became naturally infected with *E. magna*, probably by cross-contamination.

In the last period of the first cycle, the oocyst excretion of the supplemented groups was practically zero. However, the infection remained present in the NI-NT group. During the second cycle, the oocyst excretions were very comparable between the different groups (counts ≤200) and no differentiation could be performed. The low infection pressure during the second cycle can be explained by the good hygienic conditions in this experimental facility and the development of immunity against the different *Eimeria* spp. Consequently, the results were very comparable amongst the different groups.

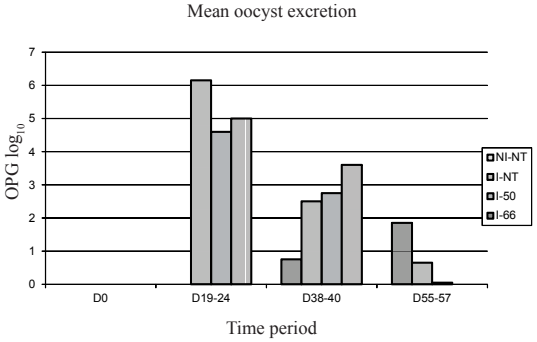

Figure 2: Mean (geometric) oocyst excretion (OP $\log_{10}$) during the first cycle.

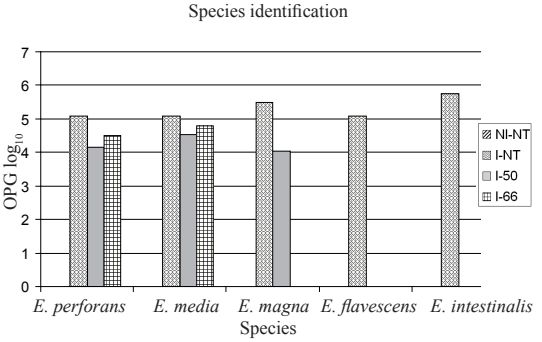

Figure 3: Mean (geometric) oocyst excretion (OPG $\log_{10}$) per species for period 19-24 d.

CONCLUSIONS

Cycostat® 66G supplemented in the feed at a concentration of 50 or 66 mg robenidine HCl/kg was able to prevent coccidiosis in breeding does after experimental inoculation with different rabbit specific *Eimeria* spp. No negative effects on the overall zootechnical performance (prolificacy, fertility, longevity and weight development) of breeding does and their progeny, nor on the mortality in the females and their young was observed by the dietary inclusion. On the contrary, the highest litter size at birth and at weaning was observed in females fed the diet with 66 mg. Supplementation with Cycostat 66G reduced the excretion of *E. flavescens* and *E. intestinalis*, the 2 most pathogenic species, by 100%, and caused a partial reduction of *E. magna* oocyst excretion, while the reduction in oocyst excretion of *E. media* was less pronounced.

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