World Rabbit Science

World Rabbit Sci. 2012, 20: 223 - 230
doi:10.4995/wrs.2012.1232

ACTIVITY OF DICLAZURIL AGAINST COCCIDIOSIS IN GROWING RABBITS: EXPERIMENTAL AND FIELD EXPERIENCES

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Abstract: The efficacy of diclazuril in growing rabbits was investigated under experimental and field conditions. In a first experimental trial, the susceptibility of recent isolated French *Eimeria* field strains to in-feed use of diclazuril, salinomycin and robenidine was studied in fattening rabbits. Rabbits were challenged at the age of 31 d with a mixed inoculum of *Eimeria magna, E. media* and *E. perforans*. Production data and oocyst excretion were compared with an infected-untreated control group and an uninfected-untreated control group. Infection resulted in significantly lower production data and higher oocyst excretion in the infected-untreated control group. Salinomycin and diclazuril treated rabbits were able to control the infection, demonstrated also by comparable weight gain and final weight to those of the uninfected-untreated control rabbits and significantly higher than those of the infected-untreated control rabbits. Based on the production data and oocyst excretion, robenidine was not able to control the infection adequately. Economic performance (weight gain, feed intake, feed conversion) and oocyst excretion were significantly worse than in the uninfected-untreated controls. In a second trial, a 1 yr longitudinal study was carried out in Italy to evaluate the excretion of coccidia in growing rabbits from 8 meat farms applying a 2-phase anticoccidial programme (diclazuril and robenidine). Parasitological parameters (oocyst counts and species identification) were measured monthly. Seven of the 11 known coccidial rabbit species were identified. Variable levels of oocysts per gram were detected in the farms, but on all farms lower oocyst per gram and a reduced number of *Eimeria* spp. in rabbit faeces were recorded in the 8-mo treatment period with diclazuril.

Key Words: coccidiosis, *Eimeria* spp., rabbit, diclazuril, robenidine, salinomycin.

INTRODUCTION

Coccidiosis in rabbits might be responsible for considerable losses in the rabbit industry (Peeters, 1987; Licois and Marlier, 2008). Rabbits can be infected by 11 different coccidial species, all of the genus *Eimeria,* of which 8 are of economic importance. Their replication site and virulence are variable (4 virulence classes), so determination of the economic impact benefits from both a quantitative and qualitative diagnosis (Coudert *et al.*, 1995). The control and prevention of coccidiosis in rabbits is based on hygiene and the prophylactic inclusion of anticoccidial drugs in the feed. At present, in Europe only Clinacox® (active diclazuril) and Cycostat 66G® (active robenidine) are authorised as feed additives for rabbit production, at 1 and 55-66 ppm, respectively.

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http://dx.doi.org/10.4995/wrs.2012.1232

Studies of the incidence of drug resistance in the field and in experimental research have shown that use of only a single coccidiostat in mono-programmes often results in severe drug resistance of coccidia (Peeters *et al.*, 1987; 1988). The reduced sensitivity of *Eimeria* spp. to any anticoccidial drug induces the development of subclinical or subacute coccidiosis. Even low levels of infection usually cause intestinal malabsorption, increased feed conversion rate and delayed growth. The most effective method proven to slow down the development of anticoccidial resistance consists of switching between anticoccidials which are chemically unrelated (Peeters and Geeroms, 1992). To optimise these programmes, the sensitivity of field coccidia strains against the anticoccidial drugs commercially available for rabbit production should be known. The periodic determination of drug-sensitivity profiles of coccidia isolates is important, taking their historical use into consideration.

In this study, the susceptibility of French *Eimeria* field strains to diclazuril was investigated and compared with the efficacy with of robenidine and salinomycin. In a second trial, the efficacious use of diclazuril in the field was researched under current commercial conditions in 8 industrial rabbit farms in Italy.

MATERIALS AND METHODS

Experimental trial

The experimental infection was conducted in Belgium at the ILVO trial facility. The housing management, feeding and husbandry conditions are regarded as representative of a modern commercial farm in Europe. Rabbits were housed in flat-deck cages measuring 0.70×0.46×0.50 m (length×width×height). At the age of 28 d, 180 animals were allocated to the 5 different treatments. Each treatment consisted of 12 replicates of 3 rabbits housed in one cage. To have a completely randomised block design, only litters with 5 homogenous weanlings were used. The pelleted feed contained 9.25 MJ/kg of digestible energy and 16.0% of crude protein. The feed of the different treatments was identical except for the inclusion of the anti-coccidials.

Treatments consisted of an uninfected-untreated control group (UUC), an infected-untreated control group (IUC), an infected group receiving in-feed treatment with diclazuril (Clinacox®) at 1 ppm (I-Dicla), an infected group receiving in-feed treatment with salinomycin (Sacox®) at 25 ppm (I-Salino) and an infected group receiving in-feed treatment with robenidine (Cycostat® 66G) at 66 ppm (I-Roben).

In feed supplementation with diclazuril, salinomycin and robenidine started after allocation of the animals to the different treatments at 28 d of age. Feed samples of each treatment were analysed for presence of the coccidiostat. At the age of 31 d, all animals in the infected treatment groups (IUC, I-Dicla, I-Salino, I-Roben) were challenged by gavage, with a mixed inoculum containing in total 50000 sporulated oocysts of *Eimeria magna, E. media* and *E. perforans*. The inoculum originated from commercial French rabbitries, from samples taken in 2008.

Weight and feed conversion were measured every 7 d till the end of the trial at the age of 59 d. A Mettler Toledo precision balance (SB16000) was used, equipped with a dynamic weighing application for animals.

Before the coccidiosis challenge, the absence of oocysts was checked by coprological examination. Oocyst excretion per treatment group was determined on days 0, 7, 11, 15, 21 and 28 by the analysis of pooled faeces per treatment group. The samples were collected using buckets placed under the cages between –3 to 0, 5 to 7, 9 to 11, 13 to 15, 19 to 21 and 26 to 28 d post infection (dpi). Oocysts of samples of 0 dpi and from 11 to 28 dpi were allowed to sporulate for further species identification.

All production parameters were subjected to a factorial ANOVA analysis including in the model the factors treatment (1-5) and block (1-12), using the Statistica release 8 program. The cage was considered as experimental unit.

Field trial

A longitudinal study was performed in Italy, monitoring 8 meat rabbit farms which belonged to the same vertical integration industry. Farms were located in Veneto Region, in the provinces of Padova (3 farms), Treviso (3), Vicenza (1) and Verona (1). The number of growing animals on the different farms ranged between 3300 and 13000. In each farm, pooled faecal samples were collected from growing rabbits for faecal oocyst (FO) counts and species determination. Faecal samples were taken monthly, in the same week and during the same hours, by taking a pool of 100 g of faeces from 60 growing rabbits from 12-15 cages sampled between the age of 34 and 71 d. Monthly sampling of these farms was performed for a 1 yr period from September 2009 till August 2010. Breeders and growing rabbits on all farms received the same in-feed anticoccidial products. Before the survey, rabbits received robenidine in the feed at a concentration of 66 ppm. From 17th September 2009 to 15th May 2010, the anticoccidial programme on the farms consisted of inclusion of diclazuril in the feed at 1 ppm. After the 15th of May, rabbits switched back to a programme with robenidine in the feed at a concentration of 66 ppm till the end of the trial period.

The 96 faecal samples were transferred to the laboratory in controlled cold conditions (+4 to 8°C) and stored there in a refrigerator (+4°C) till the analysis performed within the next day. Faecal samples were homogenised through a Stomacher machine and examined microscopically using a McMaster chamber for quantification. Five g of fresh faeces of each sample were added to 70 mL of flotation fluid (P.S. 1.300) and mixed thoroughly with a wooden blade. The solution was then poured through a double layer of cheesecloth into a test tube and centrifuged at 1500 r.p.m. for 5 min. The faecal suspension was taken with a Pasteur pipette and put in one compartment of the McMaster counting chamber. The counting chamber was allowed to stand for 5 min; oocysts on the demarcation lines were counted as 0.5. To determine the number of oocysts/g of sample, the number of oocysts under one etched area were multiplied by 100.

The microscopic identification of coccidial species was done after sporulation in 2.5% w/v $K_2Cr_2O_7$ solution, by using morphological parameters (Eckert *et al.*, 1995). The frequency of the different species was determined as percentage following the visualisation of 100 oocysts.

RESULTS

Experimental trial

Detected values of the in-feed supplemented products were in line with the expected concentrations (±20% of intended values).

Average weight and weight gain for the 5 treatment groups in the different periods are presented in Table 1. Weanlings in the different treatment groups had a weight around 750 g, and rabbits were equally randomised in weight. The weight of the rabbits at 28 dpi, was significantly (P<0.05) reduced in the IUC and also in the I-Roben group, i.e. –11.5 and –13.9%, respectively, compared to the UUC. This was mainly the result of the growth depression (–61% for IUC and –66% for I-Roben) observed in the first week post inoculation and to a lesser extent also in the second week. Although not significantly better than the UUC, the I-Salino group had the highest finishing weight and weight gain. Diclazuril treatment resulted in reduced weight gain

Table 1: Body weight (g) and weight gain (g/d) of growing rabbits in the different periods of the experimental trial.

	Treatment[1]						
	UUC	IUC	I-Dicla	I-Salino	I-Roben	SEM	*P*-value
Weaning weight	752	737	730	748	758	8.1	0.450
Weight 28 dpi	2181[a]	1931[b]	2151[a]	2234[a]	1878[b]	27.3	0.000
Weight gain:							
–3 to 0 dpi	142	137	135	137	134	2.1	0.812
0 to 7 dpi	349[a]	136[c]	277[b]	364[a]	117[c]	14.3	0.000
8 to 14 dpi	305[b]	292[b]	357[a]	353[a]	281[bc]	8.0	0.001
15 to 21 dpi	314	306	317	307	275	9.3	0.521
22 to 28 dpi	318	323	315	324	313	6.1	0.974
–3 to 28 dpi	1428[ab]	1194[c]	1402[b]	1486[a]	1120[c]	24.0	0.000

[a,b,c]: Means not sharing superscript were significantly different at P<0.05. dpi: day post infection. [1]Treatments: UUC, uninfected-untreated control; IUC, infected-untreated control; I-Dicla, infected treated with diclazuril; I-Salino, infected treated with salinomycin; I-Roben, infected treated with robenidine. SEM: standard error of the mean.

(*P*<0.05) compared to the UUC in the first week post infection, although the highest weight gain, even significantly higher than UUC rabbits, was observed in the second week.

For the overall period, the weight gain in the I-Salino group was the highest, significantly higher than in all other treatment groups and comparable to the control rabbits (UUC). Body weight gain in the robenidine treated rabbits was comparable with those in the IUC rabbits. In the week following inoculation, a very high feed conversion (*P*<0.05) was observed in both the IUC and robenidine treated rabbits. In the following weeks, no clear differences were observed. However, for the overall period, feed conversion in the robenidine treated rabbits was significantly less efficient than in the salinomycin or diclazuril treated rabbits (see Table 2).

FO counts demonstrated low levels of oocysts (<2.4×10^3) on the day of inoculation in all the experimental groups. Identification revealed the presence of *E. magna*, *E. media* and *E. perforans*. After inoculation, the excretion of the control group (UUC) varied slightly, according to the day of sampling, with highest levels at 7 and 11 dpi, which corresponded with the peak of excretion for *E. media* and *E. magna* at, respectively, 6-7 and 8-9 dpi after experimental infection (see Table 3). In infected untreated rabbits (IUC), the inoculation resulted in a strong increase of the FO counts at 7 and 11 dpi compared to the day of inoculation. From 15 dpi, the FO counts returned to levels comparable with those of the UUC group. The FO count of the I-Roben group presented an evolution very similar to that of the IUC group throughout the experiment.

Table 2: Feed conversion of growing rabbits in the different periods of the experimental trial.

	Treatment[1]						
	UUC	IUC	I-Dicla	I-Salino	I-Roben	SEM	*P*-value
–3 to 0 dpi	1.56	1.55	1.58	1.59	1.50	0.02	0.634
0 to 7 dpi	2.02[a]	4.05[b]	2.12[a]	1.92[a]	3.65[b]	0.25	0.007
8 to 14 dpi	2.58	2.37	2.37	2.43	2.33	0.04	0.147
15 to 21 dpi	2.99	2.87	2.79	2.80	2.84	0.06	0.858
22 to 28 dpi	3.47	3.14	3.56	3.47	3.57	0.07	0.436
–3 to 28 dpi	2.61[ab]	2.65[bc]	2.58[ac]	2.51[a]	2.71[b]	0.02	0.042

[a,b,c]: Means not sharing superscript were significantly different at P<0.05. dpi: day post infection. [1]Treatments: UUC, uninfected-untreated control; IUC, infected-untreated control; I-Dicla, infected treated with diclazuril; I-Salino, infected treated with salinomycin; I-Roben, infected treated with robenidine. SEM: standard error of the mean.

Table 3: Faecal oocyst counts in growing rabbits at the different time points of the experimental trial.

	Day post inoculation					
Treatment[1]	0	7	11	15	21	28
UUC	2.40E+03	1.23E+04	3.00E+03	2.63E+03	2.75E+02	1.20E+03
IUC	1.73E+03	9.41E+05	3.16E+05	4.30E+03	1.75E+02	6.75E+02
I-Dicla	1.80E+03	1.46E+05	2.36E+04	4.00E+02	0.00E+00	0.00E+00
I-Salino	9.00E+02	2.28E+03	1.00E+02	2.50E+01	0.00E+00	0.00E+00
I-roben	1.03E+03	6.59E+05	4.15E+05	2.88E+03	1.73E+03	2.50E+02

[1]Treatments: UUC, uninfected-untreated control; IUC, infected-untreated control; I-Dicla, infected treated with diclazuril; I-Salino, infected treated with salinomycin; I-Roben, infected treated with robenidine.

Group I-Dicla had FO counts slightly lower (less 1 logarithm of difference) compared with those of group IUC at 7 and 11 dpi. From 15 dpi, excretion of the oocysts was controlled perfectly, since the values were lower than those of IUC and even the UUC groups, and no oocysts were found at 21 and 28 dpi (Table 3). Finally, for group I-Salino, a strong reduction in FO counts was observed compared to the animals from the IUC group: more than 2 logarithms at 7 dpi and more than 3 at 11 dpi. Starting from 15 dpi, very low levels of excretion were observed.

Species identification 11 dpi demonstrated that *E. magna* remained the main species in IUC and I-Roben groups, with 70 and 86% respectively. For IUC and I-Dicla groups, the percentage of *E. magna* was 43 and 43.2%, respectively. In the I-Salino group the FO counts was too low to differentiate the species. At 15 dpi, *E. magna* represented 42.8, 23.2 and 44.3% of oocyst excretion, respectively, for UUC, IUC and I-Roben groups. For the other 2 groups, the oocyst counts were too low to give a valid percentage.

Field trial

In the field trial, 8 farms in Italy were sampled over a period of 1 yr. An overview of the FO counts results for the 1 yr period on the different farms is shown in Figure 1.

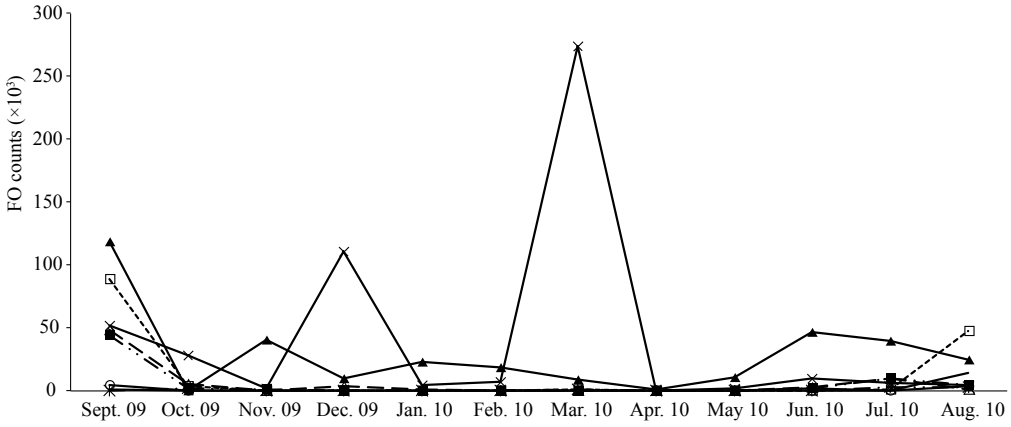

Figure 1: Global faecal oocyst (FO) counts throughout the monitoring period for the 8 commercial farms: —— A, -⊟- B, —▲— C, —×— D, —○— E, ·—✳—·F, · △ G, ·—■—·· H.

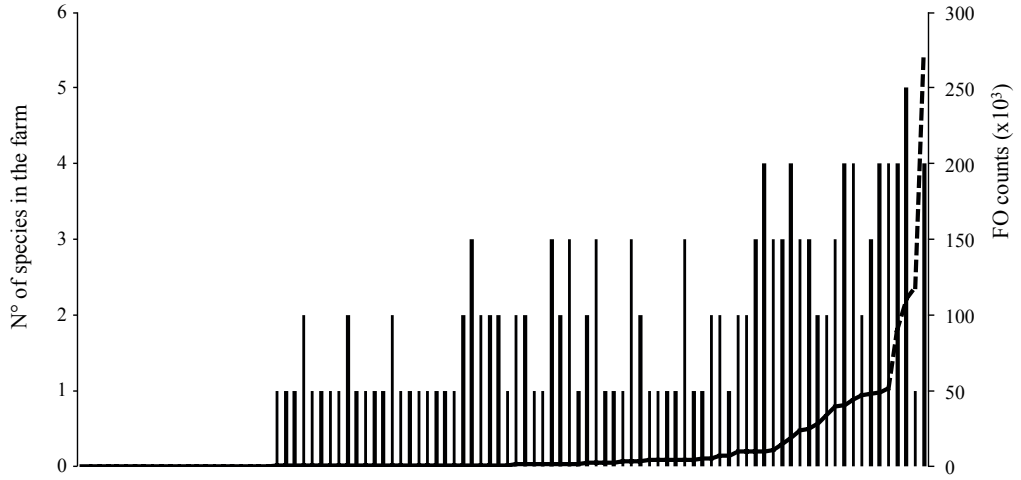

Figure 2: Number of species identified (in bars) in relation to the faecal oocyst counts (the line) at the time of sampling.

From September 2009 onwards, all farms received diclazuril in the feed. In this month, very low numbers of FO counts were counted in all the samples collected on the different farms. In 23% of the investigated samples, no oocysts were detected. Considering the arbitrary classification of FO counts levels according to Coudert *et al.* (2003), levels >10000 (not for the highly pathogenic strains of *E. intestinalis* and *E. flavescens*) are deemed a health risk. Rates >10000 FO counts were only counted on 2 of the 8 farms during the diclazuril supplementation in feed. On 1 farm (D), very high rates of FO counts (274000) were recorded in March. One month later, only 200 FO counts was recorded for this same farm, in the absence of any anticoccidial treatment.

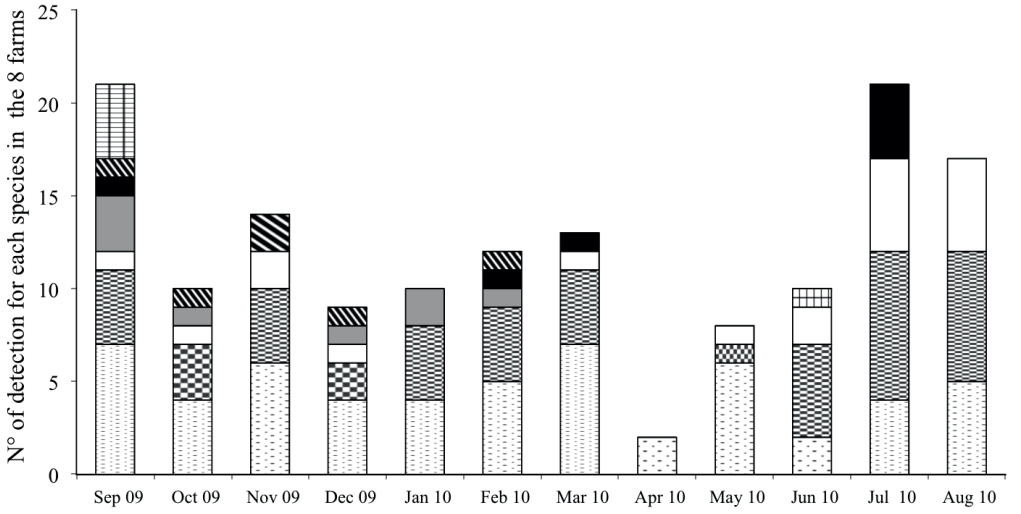

Figure 3: Monthly global results of *Eimeria* spp. faecal oocyst counts in the 8 studied farms. *E. media, E. perforans, E. magna, E. stiedai, E. exigua, E. coecicola, E. irresidua.*

Identification of the different species in the farms revealed the presence of *E. media, E. perforans, E. magna, E. stiedai, E.exigua, E. coecicola* and *E. irresidua.* The pathogenic species *E. intestinalis* and *E. flavescens* were not detected. The most frequently diagnosed species were *E. media, E. perforans* and *E. magna* (38.8, 30.6 and 12.9% respectively). *E. stiedai,* responsible for liver coccidiosis, was detected in 5.4% of the investigated samples. *E. exigua*, *E. coecicola* and *E.irresidua* were identified in 4.8, 4.1 and 3.4%, respectively, of the samples analysed. The number of species identified per farm, and in the farms over time are shown in Figure 2 and 3, respectively. The number of different species per farm proportionally increased with higher FO counts levels. At the start of the trial in September 2009, a high number of different strains were present on the different farms. The number of different strains decreased over time, to be the lowest in the month April, when only 2 farms had counts of *E. media*. After changing back to robenidine in the feed in May 2010, the number of different *Eimeria* species increased again. There was no relation between the age of the rabbits at the moment of sampling and the FO counts.

DISCUSSION

In the experimental trial, weaned rabbits at 31 d of age were challenged with a mixed inoculum originating from French rabbitries. Since conventional rabbits were used for the trial, it could not be avoided that low levels of *Eimeria* were detected in all the trial groups at the start of the study. Inoculation with a mixed inoculum of *E. magna, E. media* and *E. perforans* at the age of 31 d resulted in significantly lower production data in the infected-untreated control group compared to the uninfected-untreated control. Rabbits receiving feed which contained salinomycin and diclazuril were able to control the infection. This was demonstrated by weight gain and final weights, which were comparable with the uninfected-untreated control rabbits and significantly higher than the infected-untreated control group. Rabbits that received diclazuril in the feed showed an irregular weight evolution, being lower in the first week post infection but higher in the second week post infection. No reason for this evolution could be found. Diclazuril slightly reduced the FO counts on the peak of excretion; the strongest reduction however was seen during the second period of the experiment.

Salinomycin was the most effective in reducing oocyst excretion significantly after the experimental infection. Based on the production data, robenidine was not able to control the infection adequately. Weight gain, feed intake and feed conversion of robenidine treated rabbits were significantly worse than in the uninfected untreated controls. Furthermore, FO counts demonstrated levels of FO counts similar to the infected untreated control group, which confirmed that in-feed prevention with robenidine was ineffective to control the infection in this experiment, in contrast to a recent study with breeding rabbits (Maertens and Van Gaver, 2010). An explanation for the different response to an experimental infection could be in the different origin of the inoculum. In the latter study, the inoculum was based on faecal samples collected on small farms in the Czech Republic, whereas the inoculum of the present trial originated from commercial French rabbitries. In the French rabbitaries, rabbits were usually treated with Cycostat®66G, and this probably resulted in development of resistance.

The field trial demonstrated the activity of diclazuril under practical conditions in Italy. After supplementation of diclazuril to the feed, the FO counts decreased in all the sampled farms. On six farms, there was a very good control of coccidiosis during the 8 mo supplementation period with diclazuril, with FO counts levels below 5000. On 2 farms, there was higher pressure of coccidiosis with values >10000 FO counts on different sampling points. The levels of higher

FO counts were alternated with low FO counts levels and no reason for the sudden increase could be detected.

The most frequent species diagnosed in the field study in Italy were *E. media, E. perforans* and *E. magna*. This is in line with the findings of the horizontal study conducted in France by Coudert *et al.* (2003). In the same French study, only these 3 species were identified, while in our work 7 different species were identified. The highest number of different species was detected in farms with high FO levels, so a direct correlation might exist between rates and types of coccidian infections.

It can be concluded that diclazuril was able to control coccidiosis in rabbits under experimental and field conditions. Out of the field trial it was shown that diclazuril was still able to control the infection when used as supplement in the feed for a period of 8 mo. However, to avoid resistance it is advisable to also implement rotation programmes in rabbits.

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