Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 3, 418-427

# LASALOCID : TOLERANCE FOR THE RABBIT AND ACTIVITY AGAINST E. flavescens AND E. intestinalis.

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## Introduction

In many countries, the more or less systematic use of an efficient anticoccidian, Robenidine (Coudert 1978, Coudert 1979, Peeters et al 1979, Varga 1982, Galazzi et al 1983, Krylov et al 1981) has momentarily reduced the extent of coccidiosis (Peeters 1983, Peeters et al 1986) during the past few years.

Nevertheless, in some countries, it is still a topical problem (Tassi and Cringoli 1986, Madsen 1986, Hervouet and Nouaille 1986, Sambeth 1986, Dousek et al 1987).

Some ionophore antibiotics are now widely used as anticoccidian in poultry breeding, but most of them prove to be toxic for rabbits (Coudert 1981). Lasalocid has rarely been studied in rabbits and the results did not enable a definite conclusion to be established as to whether the inoculum did not contain very pathogenic coccidia (Sambeth and Raetber 1980), or that the tests were carried out without contamination (Pakandl 1986).

We carried out tests to determine on the one hand the level of toxicity of the product and on the other hand its efficacy in the prevention of the most pathogenic coccidiosis in the rabbit.

## Materials and methods

The activity of lasalocid was assessed in two successive experiments :

1) - Objectives and methods of the first experiment (A)

The objective was on the one hand to assess the effect of a feed supplemented with incerasing doses of lasalocid on the growth of healthy rabbits, and on the other hand to measure the anticoccidian effect of these different doses on E. flavescens and on E. intestinalis. The experimental design consists of 18 treatments of 12 animals per treatment (Table 1 (A)).

2) - Objectives and methods of the second experiment (B)

In view of the results of the first experiment, a doubt remained with regard to the action of lasalocid on E. intestinalis.

It was essential to check that the non reduction of oocyst output was not due to a particular resistance to the strain of E. intestinalis used.

Hence, as well as our control strain used in experiment A, we isolated into pure strains 5 other strains of E. intestinalis from different sources, and unlikely to have a common origin.

The activity of lasalocid (50 and 90ppm) on the six strains was tested in two successive trials with strains 1, 2 and 3, then 4, 5 and 6 according to the experimental design consisting of 12 treatments of 12 animals (Tab 1(B)).

3) - Experimental conditions

The two previous experiments were carried out in the same conditions.

3.1 - Animals

The 360 animals used came from the INRA 1077 strain (New Zealand) and originated from a standard colony at INRA. They are weaned at 30 days and divided up immediately into the experiment room.

3.2 - Experimental rooms and grouping

Three identical rooms each containing a number of wire cages on two levels were used. The rooms are desinfected with pressurized steam and gazeous formol before each experiment. The watering valves and water pipes are autoclaved. The weanlings are split up at random into similar weight groups with regard to the different treatments (2 (A) or 4 (B) per cage). On arrival the animals are given non-supplemented diet for seven days, this week of observation used as a period for the weanlings to adapt to their surroundings and weaning. After this period, they are given medicated feed. They are inoculated 7 days later, i.e. two weeks after being installed.

3.3 - Coccidia and experimental infestation

The strains used for the experimental infestation are E. intestinalis and E. flavescens which are the two intestinal coccidia which are the most pathogenic in the rabbit. These pure strains of Eimeris are remultiplied and put into sporulation at 26°C just before the infestation.

The infestation (Day0), i.e. 1/2 ml parasitic suspension is given orally. Each infested animal is given 8000 (A) or 5000 (B) sporulated oocysts.

3.4 - Parameters evaluated

The animals are weighed at weaning, then twice a week for the 5 week duration of the experiment (Day-16 to Day21).

The oocyst output is evaluated by counting in the totality of faeces excreted between Day8 and Day13 for each cage. Autopsies are carried out on all the dead animals.

3.5 - Analysis

The weight gain and feed consumption are subject to variance analysis on several points (Bachacou et al 1981).

For this analysis, we only took into account the animals which were still alive at the end of the experiment (Day21).

The oocyst output is expressed by the total number of oocysts produced per animal in each treatment group (i.e. the average of 6 measures (6 cages) for (A) and 3 measures for (B), which corresponds to a maximum of 12 animals for the two experiments (A) and (B) when there were no deaths.

## Results

1) - Results of the first experiment (A)

1.1 - Lasalocid tolerance for the rabbit

This assessment can be made from the entire group of animals between Day-7 (beginning of treatment) and Day+7 (beginning of coccidiosis).

In the total group of animals, between Day-7 and Day+7, that is to say before the interaction dose - coccidium became significant (Tab. 3), the same phenomenon can be noted ; moreover it is visible that the drop in weight is proportional to the dose administered. The feed consumption of animals receiving doses 1, 2, 3 and 4 is intermediate between dose 0 and dose 5, but there is no longer any difference between the intermediary doses.

1.2 - Efficacy of lasalocid on E. flavescens

In non treated animals, there were no deaths due to infestation between Day7 and Day11 (Tab. 2). On the other hand, the animals were very ill with a weight loss of 265g between Day7 and Day11, and a very slow growth rate after this.

The oocyst output was on average $4.10^8$ oocysts (Fig. 1) per animal, which corresponds to the highest amount possible for this coccidia (Coudert et al 1988).

It should be noted here that the non-inoculated, non medicated control animals did not excrete oocysts.

In treated animals, the study of the growth rate shows that the illness is completely controlled with 25ppm.

The excretion of oocysts (Fig. 1) is also well controlled as soon as the first doses are administered but 100ppm are necessary to halt the output of parasites.

1.3 - Efficacy of lasalocid against E. intestinalis

In non treated animals, seven out of twelve animals died of coccidiosis (Tab. 2). The illness evolved in a normal manner with a heavy weight loss between Day7 and Day11, followed by a rapid recovery. The total output was $2.6x10^9$ oocysts (Fig. 1), which also corresponds to the maximum excretion possible (Coudert et al 1988).

In treated animals, the control of this coccidiosis is much less distinct than in the previous case. With 25ppm, the effect is practically inexistant, both on mortality (Tab. 2), growth. The higher doses control the mortality but are insufficient to completely prevent weight loss, although with the dose of 75ppm, a satisfactory result is achieved. This moderate effect on E. intestinalis is confirmed by the oocyst output (Fig. 1) : even with the highest dose, there is no detectable control of parasitic multiplication. This important difference of the activity of lasalocid on E. flavescens and E. intestinalis could have been due to a spontaneous resistance of one strain, hence we carried out a second experiment with other strains of E. intestinalis.

2. Results of the second experiment (B)

2.1 - Lasalocid tolerance for the rabbit.

This tolerance can be assessed in a study of the entire group of animals as was done previously (Day-7 to Day+7). During this period, a significant depressive effect (Tab. 4) with the supplemented feed.

2.2 - Efficacy of lasalocid on the six strains of E. intestinalis

In non treated animals. The infestation with 5000 oocysts caused little mortality (S1 = 1 death ; S2 = 3 deaths ; S3 = 4 deaths ; other treatments caused no deaths). This allowed us to correctly assess the oocyst output which is similar for the six strains (Fig. 2). The pathogenic capacity of the different strains is also similar within each of these trials (S1 = S2 = S3) and (S4 = S5 = S6) (Tab. 5).

In treated animals. In the two trials, the supplement had a significant effect on the weight gain between Day7 and Day11 (Tab. 4) but for 4 strains out of 6, the feeds at 50ppm had no detectable effect (Tab. 5). The effect of the supplement is thus practically due only to the feeds at 90ppm. In trial 1, it can nevertheless be noted that this dose is insufficient to completely control the illness.

As far as the oocyst output is concerned, in all the strains we observed (Fig. 2) a "dose effect" which was slight but evident.

If the effect on the supplement on the oocyst output is underiable, it remains very slight on the whole and highly insufficient even at 90ppm.

## Discussion

Although it is better tolerated than other ionophore antibiotics, in our experimental conditions, we show that lasalocid causes growth rate to slow down. This is particularly noticeable during the first two weeks of supplementing for the lowest doses but constantly persists with 125ppm.

The anticoccidian efficacy of this product is very different for the two species of coccidia used. Lasalocid is extremely effective against the coccidiosis due to E. flavescens as soon as 25ppm are administered but only completely controls parasitic output when given in doses over 75ppm.

On the contrary, the efficacy against coccidiosis due to E. intestinalis is particularly moderate. Only the dose of 75ppm seems to have been effective : below this level the incidence of illness remains high and above this level the interaction with the depressive effect due to the product does not allow a clear interpretation of the results. The fact that lasalocid does not permit the control of multiplication of E. intestinalis is far more serious. This resistance seems to be linked to this species, as the six strains which were used are of completely different origins.

With 90ppm the results are particularly interesting, as the illness, assessed on the weight gain and mortality, is completely controlled while the oocyst output remains practically unchanged. This result is close to the one obtained with E. flavescens at 50ppm.

If the oocyst output is not always a good criterion to assess the activity of an anticoccidian on the illness, it remains an essential criterion as far as epidemiology is concerned. Indeed, if an anticoccidian does not permit the effective control of the production of coccidia of such pathogenic types as E. flavescens or E. intestinalis, one can easily imagine that this uncontrolled excretion is highly likely to pollute the colony with a very pathogenic strain, which will cause a catastrophic situation if ever the anticoccidian is not administered. This eventuality with regard to the breeding colony is applicable to the whole of the rabbit breeding in Latin countries (Spain, France, Italy) in view of the frequency of commercial exchanges in the field of reproduction and the pyramidal structure of distribution of breeds of rabbits in these countries.

## Acknowledgements

The authors wish to thank Hoffmann-La Roche (CH-4002 BASLE, Switzerland) for the contribution to these experiments.

Moreover, the originality of this study is partly due to Madame BOIVIN, whose infinite patience enabled us to count the oocysts.

Tab. 1 - EXPERIMENTAL DESIGN

EXPERIMENT A

| | MEDICATED FEED (dose in ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 25 | 50 | 75 | 100 | 125 |
| Control (T) not inoculated | 6 cages x 2 rabbits | id | id | id | id | id |
| Inoculated (Fl) E. flavescens | " | " | " | " | " | " |
| Inoculated (In) E. intestinalis | " | " | " | " | " | " |

EXPERIMENT B

| | | MEDICATED FEED (dose in ppm) | | |
|---|---|---|---|---|
| | | 0 | 50 | 90 |
| | Control (T1) | 3 cages x 4 rabbits | id | id |
| | Strain 1 (S1) | " | " | " |
| Trial n° 1 | Strain 2 (S2) | " | " | " |
| | Strain 3 (S3) | " | " | " |
| | Control (T2) | " | " | " |
| | Strain 4 (S4) | " | " | " |
| Trial n° 2 | Strain 5 (S5) | " | " | " |
| | Strain 6 (S6) | " | " | " |

( ) Contraction used in the text.

Tab. 2 - NUMBER OF DEAD ANIMALS ACCORDING TO Eimeria species AND TO THE CONCENTRATION OF LASALOCID IN FEED (experiment A)

| | DAY OF DEATH AFTER INOCULATION | CONTROL NOT INOCULATED 0 1 2 3 4 5 (1) | INOCULATED WITH 8000 E. flavescens 0 1 2 3 4 5(1) | INOCULATED WITH 8000 E. intestinalis 0 1 2 3 4 5(1) |
|---|---|---|---|---|
| Day of death | 1 à 6 | 3 3 2 0 0 1 | 2 0 0 1 0 0 | 0 0 0 1 0 1 |
| after | 7 à 11 | 0 1 0 0 0 0 | 0 0 0 0 0 1 | 5 2 0 0 0 1 |
| inoculation | 12 à 21 | 1 0 0 0 0 1 | 1 0 0 0 0 0 | 2 0 0 0 0 0 |
| | Total (2) | 4 4 2 0 0 2 | 3 0 0 1 0 1 | 7 2 0 1 0 2 |

(1) 0 = Non medicated feed (0ppm) 1 = 25 ppm 2 = 50 ppm 3 = 75 ppm 4 = 100 ppm 5 = 125 ppm
(2) There are 12 animals in each of the 18 treatments

Tab. 3 - GROWTH OF RABBITS INOCULATED WITH E. flavescens or E. intestinalis AND RECEIVNG GRADED DOSES OF LASALOCID IN FEED(1) (experiment A)

| | INTERVAL BETWEEN WEIGHINGS (2) | | | |
|---|---|---|---|---|
| DOSE IN FEED | D-7 to D7 | D7 to D11 | D11 to D21 | D0 to D21 |
| 0 ppm | 615 | - 96 | 276 | 474 |
| 25 ppm | 475 | 46 | 415 | 714 |
| 50 ppm | 452 | 87 | 420 | 739 |
| 75 ppm | 401 | 123 | 369 | 717 |
| 100 ppm | 368 | 84 | 355 | 662 |
| 125 ppm | 292 | 103 | 374 | 629 |
| Dose effect (3) | ** | ** | ** | ** |
| Inoculation effect (3,4) | | ** | * | ** |
| Interaction (3) | | ** | ** | ** |

(1) 24 animals inoculated : 12 with E. intestinalis + 12 with E.flavescens
Only weights of animals surviving on D21 are analysed.
(2) D0 = Day of inoculation
(3) Statistical significance ; * = P < 0,05 ** = P < 0,01
(4) Difference between animals inoculated with E. flavescens and E.intestinalis

Tab. 4 - GROWTH OF RABBITS INOCULATED WITH 6 STRAINS OF E intestinalis AND FED WITH TWO CONCENTRATIONS OF LASALOCID (experiment B) (4)

| | | INTERVAL BETWEEN WEIGHINGS (2) | | | |
|---|---|---|---|---|---|
| | DOSES IN FEED | D-7 to D7 | D7 to D11 | D11 to D24 | D0 to D24 |
| TRIAL n° 1 | 0 ppm | 579 | - 101 | 453 | 630 |
| E.intestinalis | 50 ppm | 500 | - 36 | 462 | 686 |
| Strains 1 to 3 | 90 ppm | 438 | 81 | 401 | 731 |
| | Dose effect (3) | * | ** | | * |
| | Inoculation (3) | | ** | | ** |
| | Interaction (3) | | ** | | |
| TRIAL n° 2 | 0 ppm | 523 | 39 | 501 | 745 |
| E.intestinalis | 50 ppm | 463 | 93 | 482 | 803 |
| Strains 4 to 6 | 90 ppm | 322 | 151 | 461 | 777 |
| | Dose effect (3) | ** | ** | | |
| | Inoculation (3) | | ** | | |
| | Interaction (3) | | ** | | |

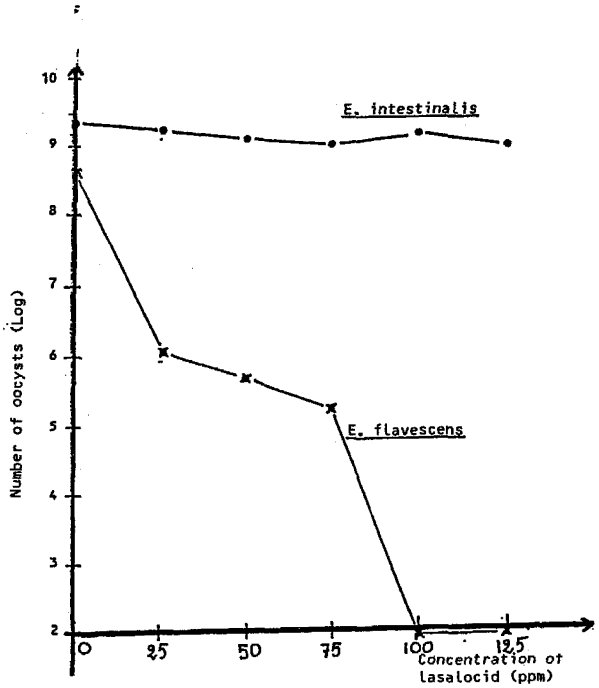
(1) Only weights of animals surviving on D21 are analysed.
(2) D0 = Day of inoculation
(3) Statistical significance ; * = P < 0,05 ** = P < 0,01

Tab. 5 - GROWTH OF RABBITS INOCULATED WITH SIX STRAINS OF E. intestinalis AND FED WITH DIFFERENT CONCENTRATIONS OF LASALOCID (Experiment B)

| | EXPERIMENTAL GROUP | FEED 0 ppm | 50 ppm | 90 ppm |
|---|---|---|---|---|
| TRIAL n° 1 | Control 1 | 135 a | 149 a | 157 a |
| | Strain 1 | - 161 c | - 119 c | - 27 b |
| | Strain 2 | - 212 c | - 35 b | 93 a b |
| | Strain 3 | - 167 c | - 140 c | 45 b |
| TRIAL n° 2 | Control 2 | 170 d | 150 d e | 144 d e |
| | Strain 4 | 55 d e f | 79 d e f | 172 d |
| | Strain 5 | - 59 f | 143 d e | 123 d e |
| | Strain 6 | - 7 e f | 0 e f | 164 d |

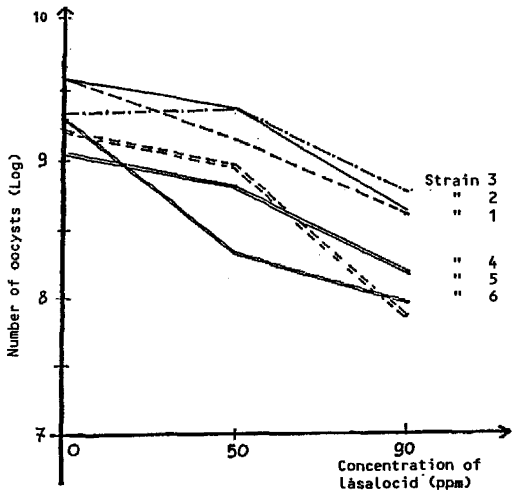
Weight gain in grams during the acute period of coccidiosis (D7 to D11)

Fig. 1 - TOTAL OOCYST OUTPUT OF RABBITS INOCULATED WITH E. flavescens OR E. intestinalis AND FED WITH DIFFERENT CONCENTRATIONS OF LASALOCID (1)




(1) Mean values of 6 cages of 2 animals for each treatment. The whole faeces are collected between day 8 and day 13 after inoculation.

Fig. 2 - TOTAL OOCYST OUTPUT OF RABBITS INOCULATED WITH 6 STRAINS OF E.intestinalis AND FED WITH DIFFERENT CONCENTRATIONS OF LASALOCID (1)




(1) Mean values of 3 cages of 4 animals for each treatment. The whole faeces are collected between day 8 and day 13 after inoculation.

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## ABSTRACT

**LASALOCID : TOLERANCE FOR THE RABBIT AND ACTIVITY AGAINST E.flavescens AND E. intestinalis**

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Lasalocid is a coccidiocid drug widely used against poultry coccidiosis. Two trials were performed to test its activity against the most pathogenic coccidia of the rabbit. Because of the susceptibility of this animal to ionophores, toxicity of lasalocid incorporated in rabbit peletted feed (25 to 125 ppm) has been evaluated within the same trials. In our experimental environment, non medicated or inoculated animals have a daily weight gain of 44 grams. In those conditions, lasalocid has a growth-depressive effect. This adverse effect disappears after two weeks with the lowest dose but persists with 125 ppm. Efficacy against caecal coccidiosis induced by E. flavescens is very good and even the lowest concentration (25 ppm) has a noticeable prophylactic effect. Nevertheless 100 ppm are necessary to suppress oocyst output of E. flavescens. This drug is much less efficient against the intestinal coccidiosis induced by E. intestinalis. The most satisfactory result on control of illness was obtained with 75-90 ppm of lasalocid in the ration but even with 125 ppm there was no control of oocysts output. To confirm that this unexpected lack of efficacy was not due to a special resistance of our strain of E. intestinalis we made a second trial with five other strains of this species coming from different countries. The first results were confirmed. The practical and theorical conclusions of this study are discussed, especially the usefulness and the limits of validity for the rabbit of oocyst output as criteria to test anticoccidial drugs.

## RESUME

**TOLERANCE DU LASALOCIDE PAR LE LAPIN ET EFFICACITE CONTRE E. flavescens ET E. intestinalis**

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Deux expérimentations ont été réalisées avec des doses de lasalocide allant de 25 à 125 ppm dans l'aliment pour juger de son innocuité pour le lapin d'une part et de son efficacité contre les deux coccidies les plus pathogènes du lapin d'autre part. Dans des conditions expérimentales, telles que les témoins ont un GMQ moyen de 44 g par jour, on observe un effet dépressif sur la croissance plus ou moins marqué selon la dose. Aux doses faibles, l'effet dépressif disparaît après deux semaines d'accoutumance mais persiste avec 125 ppm. L'efficacité contre la coccidiose due à Eimeria flavescens apparaît dès la dose de 25 ppm. Il faut néanmoins 100 ppm pour contrôler entièrement l'excrétion d'oocystes. L'efficacité contre la coccidiose due à E. intestinalis est beaucoup moins évidente et il faut au moins 75 ppm pour obtenir un résultat satisfaisant, mais même avec 125 ppm il n'y a aucune réduction de l'excrétion d'oocystes. Nous avons vérifié l'inefficacité de ce produit sur 5 autres souches d'E.intestinalis provenant de différents pays. Un produit peut donc être très actif sur une coccidie (E. flavescens) et pas du tout sur l'autre (E. intestinalis). Il est également discuté de la limite de validité de l'excrétion d'oocystes comme critère d'efficacité dans ce type d'expérimentation ; en effet, chez le lapin, un anticoccidien peut permettre le contrôle de la maladie sans provoquer aucune réduction de l'excrétion d'oocystes.