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PERFORMANCE RESPONSE OF DOE RABBITS TO TOYOCERIN® (*Bacillus cereus* var. Toyoi) SUPPLEMENTATION

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Abstract: The efficacy of Toyocerin® was evaluated on doe rabbit performance. Two isonutritive diets were formulated corresponding to 2 levels of Toyocerin®: 0 (diet T1) and 200 mg (diet T2) of Toyocerin®/kg of feed. Two experiments were conducted to evaluate the efficacy of Toyocerin® on rabbit doe performance in two different experimental periods of twelve and six months, respectively. Thirty-eight breeding cages were assigned to each treatment. No interaction was found between dietary treatment and experimental period. As a consequence, the results of the two trials were treated as a whole. Inclusion of Toyocerin® shortened interval from parturition to effective mating (15.0 *vs* 10.4 d, $P = 0.05$) and between parturitions (47.0 *vs* 42.2 d, $P = 0.05$) and tended to improve litter size at weaning (by 9.9%, $P = 0.09$). Consequently, numerical productivity increased from 59.6 to 71.2 weaned rabbits per cage and year ($P = 0.01$). Litter weight at weaning (25 d) also tended to be higher for diet supplemented with Toyocerin® (3673 *vs* 3952 g, $P = 0.10$). As treatment had no effect on daily feed intake (370 g as an average) of rabbits does during lactation, feed efficiency (kg of weaned rabbits per kg of feed intake) increased ($P = 0.01$) from 0.272 to 0.314 with Toyocerin® supplementation. Treatment did not affect doe body weight at parturition or at weaning, the mortality of rabbit does and the mortality of young rabbits during lactation. These results suggest the interest of the inclusion of 200 ppm Toyocerin® in the diet of lactating rabbit does to reduce parturition interval and increase feed efficiency and numerical productivity.

Key words: Toyocerin® supplementation, probiotic, rabbit does, performance.

INTRODUCTION

The restriction in the use of antibiotics in rabbit nutrition has increased the interest of using alternative natural products that allow maintenance of high productivities and reduction of morbidity and mortality in intensive farms. Probiotics as feed additives might potentially benefit the host animal by improving its intestinal

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microbial balance (FULLER, 1989). The action mode of probiotics is not clear. The reduction of pathogenic microbial species in the intestine could be due to a direct action of the probiotic or the indirect result of the stimulation of the beneficial bacteria. At present, it has not been demonstrated that these microorganisms can be established in the intestine and it is possible that their presence might depend on feed supplementation.

Several probiotics (Paciflor®, Toyocerin®, Biosaf® and Lacto-Sacc) have already been evaluated in rabbits (HATTORI *et al*., 1984; DE BLAS *et al*., 1991; MAERTENS and DE GROOTE, 1992; GIPPERT *et al*., 1992; YAMANI *et al*., 1992; MAERTENS *et al*., 1994; ABDEL-SAMEE, 1995; KAMRA *et al*., 1996). The results of these studies have shown that the effect of probiotics on productive traits are variable and the responses are higher when the conditions of the animal farms are worst. At present, there are only

Table 1: Ingredients (%) of basal diet.

Alfalfa dehydrated	28.4
Barley grain	20.0
Wheat bran	19.2
Sunflower meal 30% CP	17.5
Soybean meal 44% CP	5.1
Wheat grain	4.0
Pork lard	3.0
Calcium carbonate	1.45
Salt	0.5
DL-methionine	0.076
L-lysine 78	0.164
Threonine	0.085
Premix[1]	0.50

[1] Premix included: 0.40% of vitamin & mineral premix and 0.10% of Robenidine 6.6%.

two probiotics authorised to be used in diets for rabbits in the European Union: *Bacillus cereus* var. *toyoi* (Toyocerin®) and *Saccaromyces cerevisiae* (Biosaf®) (REPORT (EEC) nº 220/2001).

The aim of the present investigation was to study the effect of the inclusion of 200 ppm of Toyocerin® in the diet on several productive parameters of rabbit does. Toyocerin® is a probiotic composed of viable spores of *Bacillus cereus* var. *toyoi* CNCMI-1012/NCIMB 40112, which is not a habitual resident of the intestine and it is necessary to add it to the feed.

MATERIAL AND METHODS

Diets

A basal diet (T1) was formulated according to the nutrient recommendations of De Blas and Mateos (1998). The ingredient composition of the basal diet is shown in Table 1. Another diet (T2) was formulated by adding 200 ppm of Toyocerin® to the basal diet. The concentration of *B. c. toyoi* in the commercial product is 1 x 10^9 *B. c. toyoi* spores/g of Toyocerin®. The spores are resistant to high temperatures and to dry, acid conditions. Both diets were manufactured without any growth promoter or antibiotic, but included Robenidine® as coccidiostat. Diets were formulated every three months in order to assure the quality of the feed and the viability of the spores and were pelleted (3.5 mm diameter). The average chemical composition of the two diets is shown in Table 2.

Animals

Seventy-six cages (38 per treatment) with New Zealand x Californian rabbit does were assigned to the experimental treatments (T1 and T2), so that the treatment groups were broadly similar in terms of number of parturitions (1.05 *vs* 1.03, respectively) and weight of does (4229 *vs* 4173 g, respectively). Treatments were assigned to cages to give a random distribution to eliminate any effect of cage location in the building.

A 30-days adaptation period was allowed before recording rabbit performance in each assay. During this period the rabbits had *ad libitum* access to the experimental feeds. In late pregnancy (from 28 d onwards) and throughout lactation, does were given *ad libitum* access to feed. The rest of the cycle does were restricted (140 to 150 g/d of feed). Young rabbits had free access to their mother's feed. Does were mated 7 days after parturition and kits were weaned at 25 days of age. The experimental observations started when does were mated. Rabbit performance was recorded from parturition to weaning in two different experimental periods, to increase the number of replicates and to cover a longer period of time due to the cyclic incidence of mucoid enteropathy, over a 12 and 6-months production cycles, respectively, and was calculated by cage.

During the trials, animal that died or were discarded for different reasons (illness, unfertility or low prolifacy) were immediately replaced by nulliparous does (7 and 13 rabbits for T1 and T2 treatments, respectively). Natural mating was performed and males were included to give a female:male ratio of 8:1 throughout the experiments. Ten days after mating, the pregnancy was tested by abdominal palpation. Does that failed to mate, to conceive or lost their pups were immediately given the

Table 2: Chemical composition of the experimental diets (% DM).

	Diets	
	T1	T2
Dietary Toyocerin® content (ppm)	0	200
Dry matter	91.5	91.2
Crude protein	20.7	20.8
Neutral detergent fibre	33.8	34.0
Acid detergent fibre	16.9	17.1
Acid detergent lignin	4.46	4.52
Ash	8.56	8.62
Gross energy (MJ/kg)	18.7	18.7

opportunity to remate. Reproductive traits (parturition and parturition-effective mating interval, prolificacy and pup and does mortality) and productive traits (litter weight at 21 d and at weaning, feed efficiency and numerical productivity) were recorded for single rabbit does throughout the experiments and in each were expressed by cage. Feed consumption and weight of does were recorded between parturitions and during lactation.

Housing

Animals were housed in flat-deck cages measuring 600×500×330 mm high. Building heating systems and forced ventilation allowed the temperature to be maintained at 21 ± 4º C in the two experiments. Rabbit does were kept under 16 h of light and 8 h of darkness. Animals were handled according to the principles for the care of animals in experimentation published by the Spanish Royal Decree 223/88.

Analytical Methods

Chemical analysis of diets was made using the sequential procedure of Van Soest *et al*. (1991) for neutral detergent fibre, acid detergent fibre and acid detergent lignin. Procedures of the AOAC (2000) were used for dry matter, ash, and crude protein. Gross energy was determined by adiabatic pump calorimetry.

The viable count of Toyocerin® spores in the feed was checked in feed samples of each feed lot after pelleting obtaining as average 0.197×10^6 colony forming units/g, which is close to the value expected according to the dose used (0.200×10^6 cfu/g). The analysis method for evaluating the concentration of *B. c. toyoi* in the rabbit feed was carried out following a quantitative standard microbiological method and by using a selective plate agar medium for *B. c. toyoi* (Asahi Vet S.A., Barcelona, Spain, unpublished method).

Statistical Analysis

Data were analysed as a completely randomised block design with dietary treatment and experimental period as the main sources of variation and number of previous parturitions as block effect by using the General Linear Model (GLM)

procedure of SAS (1990). The cage was considered the experimental unit (n = 38 per treatment).

RESULTS

The effect of the dietary Toyocerin® content on several productive traits of doe rabbits is shown in Table 3. No interaction was found between dietary treatment and experimental period. As a consequence, the results of the two trials are treated as a whole. Does fed the diet containing Toyocerin® showed a shorter interval from parturition to effective mating (by 4.6 days, $P = 0.05$) and between parturitions (by 4.8 days, $P = 0.05$) and tended to improve litter size at weaning (by 9.9%, $P = 0.09$) compared to does fed with the control diet. Consequently, numerical productivity (weaned rabbits per cage and year) also increased (by 19.5%, $P = 0.01$). Litter weight at weaning tended to be higher for animals fed diet supplemented with Toyocerin® (by 7.6%, $P = 0.10$). As treatments had no effect on daily feed intake of rabbits does during lactation (370 g as an average), feed efficiency (expressed as kg of rabbit weaned per kg of feed intake) increased (by 15.4%, $P = 0.01$) with Toyocerin® supplementation. The treatments did not affect either the doe body weight at parturition or at weaning (4269 and 4591 g as average, respectively), the mortality of rabbit does (27.7% on average), or the mortality of young rabbits during lactation (13% on average). The lack of differences on mortality of rabbit does might be due to the reduced number of experimental units (cages) used and more data is required to conclude properly on this effect. Mortality of rabbit does were mainly related to problems around parturition and to mucoid enteropathy.

The number of previous parturitions did not influence any of the traits studied.

DISCUSSION

The effect of probiotics on reproductive performance of rabbit does have not been reported previously in the literature. Most of the studies have been conducted

Table3: Effect of Toyocerin on doe rabbit and lactating kits performance.

	Diets					
	T1	T2	SEM[1]	P_T[2]	P_A[3]	$P_{T\times A}$[4]
Average doe weight at parturition (g)	4328	4211	51.4	0.15	0.001	0.49
Average doe weight at weaning (g)	4633	4549	57.7	0.37	0.02	0.58
Doe weight gain from partum to weaning (g)	305	338	30.1	0.45	0.22	0.90
Litter weight at birth (g)	526	557	14.9	0.27	0.37	0.12
Litter weight at weaning (25 d) (g)	3673	3952	116	0.10	0.07	0.97
Number of kits born alive per litter	8.75	9.26	0.29	0.30	0.02	0.47
Number of kits born dead per litter	0.61	0.44	0.09	0.32	0.08	0.36
Litter size at weaning	7.37	8.10	0.28	0.09	0.21	0.64
Kits mortality from 0 to 25 days (%)	14.1	12.0	2.33	0.48	0.27	0.80
Daily feed intake of does+kits 0-25 days (g)	363	377	8.32	0.16	0.12	0.30
Daily feed intake of does between parturitions (g)	300	307	7.43	0.38	0.27	0.22
Feed efficiency (g kits weaned per g feed)	0.272	0.314	0.01	0.01	0.60	0.22
Numerical prouctivity (no. weaned kits per cage per year)	59.6	71.2	3.02	0.01	0.69	0.54
Parturition interval (d)	47.0	42.2	1.63	0.05	0.52	0.95
Parturition to effective mating interval (d)	15.0	10.4	1.61	0.05	0.37	0.96
Mortality of does (%)	19.6	35.9	8.16	0.22	0.09	0.56

[1] SEM = Standard error mean (n =38 cages), [2] P_T =*P*-value of treatment, [3] P_A = *P*-value of assay, [4] $P_{T\times A}$=*P*-value of treatment assay.

in growing rabbits or in productive traits of rabbit does. In our study, a shortened interval from parturition to the effective mating and between parturitions (by 30.6 and 10.2%, respectively, $P = 0.05$) was found with Toyocerin® supplementation. In this sense, a research study on pregnant sows (UCAAB, 1990) showed a significant reduction of the interval from weaning to the next gestation (7.12 *vs* 13.6 days) with inclusion of Toyocerin®. The results of this study also showed a trend to increase litter weight (by 7.6%, $P = 0.10$) and litter size at weaning (by 9.9%, $P = 0.09$) in animals fed the diet with Toyocerin®. This is in agreement with previous studies (Maertens *et al*., 1994 and Nguyen *et al*., 1988), in which the inclusion of probiotics (Paciflor®) increased litter weight at weaning (by 8%, $P< 0.05$). Probiotic treatments had not effect on daily feed intake of does during lactation. Consequently, feed efficiency (measured as g of young rabbits weaned per g feed) increased with Toyocerin® inclusion (by 15.4%, $P = 0.01$). This result might be explained by a positive effect of Toyocerin® on the development of an optimum bacteria flora in the gastrointestinal tract that allowed an improvement of feed utilization, although this trait has not been measured in this study. In this way, Hattori *et al.* (1984) showed that the addition of 1 or 5×10^6 spores per g of feed of Toyocerin® in the diet decreased the *Escherichia coli* population in the intestine. They related it to a lower mortality (25%, 5% and 0% corresponding to 0, 1 and 5×10^6 spores per g of feed, respectively) and to a higher daily gain of the young rabbits (by 20%). In this study, no differences among diets were found in the mortality of rabbit does, or young rabbits during lactation (27.7 and 13%, on average, respectively).

The results of this study suggest the interest of the inclusion of Toyocerin® at 200 ppm in commercial feeds for lactating rabbit does and young rabbits to reduce parturition interval and increase feed efficiency and numerical productivity.

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