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INFLUENCE OF TOYOCERIN® (B*acillus cereus* var. *toyoi*) ON BREEDING PERFORMANCE OF PRIMIPAROUS RABBIT DOES

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Abstract: This study was carried out to evaluate the effects of *Bacillus cereus* var. *toyoi* (Toyocerin® 10^9) on performance of rabbit does during two reproductive cycles. A total of 102 does were assigned to three treatments: T0 (basal diet), T200 (basal diet + 0.2 g Toyocerin®/kg feed) and T1000 (basal diet + 1 g Toyocerin®/kg feed). The experimental diets contained a coccidiostat and were given *ad libitum*. No antibiotics were administered with the feed or water along the trial. Artificial insemination (AI) of does was performed 11 days (d) after kindling and kits were weaned at 35 d of age. Does, kits and feed were weighted at the beginning, AI, kindling, 18 d after kindling and weaning. Toyocerin® increased feed intake of lactating does, mainly between 18 d after kindling and weaning (P<0.05). When T200 was compared with T0 treatment, feed intake increased 76 g/d in the first cycle and 34 g/d in the second one. These increases allowed higher kit growth from 18th day to weaning and live weight at weaning (P<0.05). The weight of T200 kits was 43 g (4.9%) and 54 g (5.6%) higher than those of T0 kits in the first and second cycles, respectively. In the second cycle, Toyocerin® reduced kit mortality during the first 18 days of life from 18.5% with T0 to 11.1% with T200 and 9.9% with T1000 (P<0.05). Feed efficiency was not affected by treatments. No effects of Toyocerin level were observed on any recorded traits. The results of this experiment suggest that Toyocerin® can have a positive effect on productivity of rabbit does kept under an intensive system.

Key words: Rabbit does, probiotics, Toyocerin®, reproductive performance.

INTRODUCTION

Food safety has become one of the fundamental principles demanded by the European consumer. For this reason, European Community (EC) establishes a series of legislative measures and principles along the whole food chain, which goes from the farm - including the animal feeding - to the consumer. One of those measures was the prohibition of the antibiotic growth promoters in the animal feeding since January 1, 2006, in order to avoid the appearance of pathogenic microrganisms with crossed resistance to antibiotics used in human medicine. The incorporation in animal feeds of useful microrganisms (probiotics) or substances stimulating the growth of a positive gut microbiota (prebiotics), represent nowadays a possible alternative to antibiotic growth promoters in rabbit production (Mourão *et al.*, 2005).

The mode of action of probiotics is not clear but depends on proliferation and survival of microrganisms in the intestine. The possible effects of the probiotics include a) increase on intestinal metabolic activity, b) modification of intestinal microbiota by the exclusive competition with intestinal pathogenic bacteria (e.g.: *E. coli* and *salmonellas*) and c) modification of the structure and function of the intestinal epithelium, stimulating the immune system (Abbott, 2004). Another reported effect of probiotics is due to their action in the translocation of pathogens, which will be able to prevent enteric infections (Nakamura *et al.*, 2002).

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The husbandry of rabbits in intensive system can cause physiological and environmental stress, contributing to the development of digestive problems and consequent reduction of performance. The use of probiotics in rabbits reared in this condition has been evaluated, being most of the works carried out in growing rabbits (Abdel-Samee, 1995; Kamra *et al*., 1996; Esteve-Garcia *et al*., 2004; Kustos *et al*., 2004; Trocino *et al*., 2005). However, the information about the utilisation of probiotic in rabbit does is scarce. Maertens *et al*. (1994) observed an increase on kit and litter weight at weaning when does received a probiotic (Paciflor®) and the results of Nicodemus *et al*. (2004) suggest that the inclusion of a different probiotic (Toyocerin®) in the diet of lactating rabbit does reduce parturition interval and increase numerical productivity and feed efficiency. Mourão *et al*. (2005) and Falcão-e-Cunha *et al*. (2007) have recently presented literature reviews on the subject. At present there are only two microrganisms with permanent authorization to be used as additives in feeds for rabbit in the EC: *Bacillus toyoi*, EC no. E-1701 (Toyocerin®) and *Saccharomyces cerevisiae*, EC no E-1702 (Biosaf®).

The objective of this study was to evaluate the effects of diet supplementation with Toyocerin® on the reproductive performance and numeric productivity of rabbit does during their two first reproductive cycles.

MATERIAL AND METHODS

Housing

The study was carried out at the experimental rabbit house of the Animal Production Department of the Universidade de Trás-os-Montes e Alto Douro (UTAD) in Vila Real, Portugal, between October 2005 and March 2006. Animals were tattooed and housed in individual mother-cages (40×100×35-height cm). Heating and forced ventilation systems maintained environmental temperature between 10 and 20ºC. Rabbits received daily 12 hours of light and all the standard conditions of a commercial rabbitry. The trial was carried out in accordance with Portuguese rules on animal welfare in Experimental Research, and recommendations of the European Group for Rabbit Nutrition (EGRAN; Fernández-Carmona *et al*., 2005) for rabbit nutrition trials.

Product tested and diets

Toyocerin® is a feed additive containing viable spores of the microrganism *Bacillus cereus var. toyoi* NCIMB 40112/CNCM I-1012 (EC No. E 1701), at a minimum concentration of 10^{10} CFU/g. The product used in this test was the commercial premixture "Toyocerin® 10^9", containing a concentration of 10^9 viable spores of *B.cereus toyoi* per gram.

Three experimental treatments were applied to a common basal feed: 1) T0 treatment, basal feed with no additives (control), 2) T200 treatment, basal feed with 0.2 g/kg of Toyocerin® (0.2×10^9 *B.cereus toyoi* CFU/kg feed) and 3) T1000 treatment, basal feed with 1 g/kg of Toyocerin® (1×10^9 *B.cereus toyoi* CFU/kg feed). The basal feed was formulated following the recommendations for reproducing does given by De Blas and Mateos (1998) and De Blas (2000) and contained a coccidiostat (Robenidine®). Growth promoters or therapeutic antibiotics were neither added to experimental diets nor administered in the water along the experiment. Experimental diets were given *ad libitum* on a granulated form and were prepared every three months in order to assure feed quality and spore viability. The major ingredients and chemical composition of the experimental diets are shown in Table 1.

Animals and experimental procedure

A total of one hundred and twenty female rabbits of a maternal line from UPV (Universidad Politécnica de Valencia, Spain), with 9 week of age were purchased from a commercial rabbitry. Between 9 and 16 weeks of age, the does were fed a rearing commercial feed without antibiotics and submitted to a

Table 1: Probiotic supplementation and chemical composition of experimental diets

		Diets[1]		
		T0	T200	T1000
Toyocerin® [2] supplementation (g/kg feed)		0	0.20	1.00
Bacillus cereus toyoi (10^9 CFU/kg feed)				
	Expected	0	0.20	1.00
	Analysis of the 1st cycle diet[3]	0	0.20	0.89
	Analysis of the 2nd cycle diet[3]	0	0.18	0.98
Chemical composition (% DM)				
	Dry matter (% as fed)	89.1	88.6	89.0
	Organic matter	92.1	92.0	92.2
	Ash	7.9	8.0	7.8
	Crude Protein	18.9	18.5	18.6
	Ether Extract	4.4	4.4	4.4
	Starch	18.4	18.1	18.6
	NDF	35.3	35.5	35.9
	ADF	21.7	21.5	21.8
	ADL	9.0	8.0	8.3
	Digestible energy (kcal/kg)[4]	2487	2480	2474

[1]Ingredients (g/kg feed): Wheat, 150; sunflower meal, 200; soybean, 26; maize germ, 45; wheat bran, 250; sugar cane molasses, 40; dehydrated alfalfa meal, 150; grape seed meal, 60; wheat straw, 18; sugar beet pulp, 40; calcium carbonate, 9.5; salt, 4; mineral and vitamin premix, 6.6; coccidiostat Robenidine®, 0.1. [2]Toyocerin® 10^9 -premixture- containing a minimum of 1×10^9 *B.c. toyoi*/g. [3]Analysed according to EU (2002). [4]Estimated value according to De Blas *et al.* (1992).

standard program of vaccinations (mixomatosis and hemorrhagic viral diseases) and antiparasitic treatments. At 16 weeks of age, 102 homogeneous rabbits were selected by rejecting does with abnormal weight, abnormalities, etc., and randomly assigned to treatments T0, T200 and T1000 (34 does by treatment).

The experimental period of 108 days begun at first artificial insemination (AI) (17 weeks of age) and consisted of the two first reproductive cycles: 1st cycle from 1st AI to until weaning of kits of the 1st kindling; 2nd cycle from 2nd AI until weaning of kits of the 2nd kindling, therefore the two cycles partially overlaped (from 2nd AI until weaning of kits of the 1st kindling). Does of each treatment have similar initial body weight (Table 2). Does were inseminated 11 d *post-partum* and those that were not pregnant were maintained in the experiment and remated 42 d after. The litters born in the first or second kindling were equalized according to the number and weight of kits, with limitation of litter size to 9 (80% of litters) or 10 (20% of litters). Litter equalization was performed within treatments and kits of died does were also placed to does within group. Kits with very low weight at birth were rejected. Kits were weaned at 35 days of age. Between kindling and AI, programmed lactation was made, separating the litters from their mothers and permitting kits to suckle only once a day.

Recordings

During the experiment, weight of does and feed distributed in each mother-cage were recorded at each AI, kindling, 18 d after kindling and weaning. The weight and size of litters were recorded at kindling (before and after litter equalization), 18 d after kindling and weaning. The litter weight and size are referred to the litters that were alive at each control. In the first and second reproductive cycles, female fertility was calculated as the ratio between the number of kindling and the number of AI and was determined only when females inseminated were alive at kindling. Dead does and kits

Table2: Influence of supplementation of diet with Toyocerin® on live weight (g) of rabbit does

	No.	Diets			SEM[1]	*P*-value	
		T0	T200	T1000		T0 *vs* (T200+T1000)	T200 *vs* T1000
Initial-1st AI	102	3775	3774	3786	37	0.90	0.82
1st kindling	100	3701	3767	3716	59	0.57	0.55
2nd AI	99	3994	4015	4016	51	0.73	0.99
18d after 1st kindling	99	4316	4353	4421	51	0.26	0.34
1st weaning	99	4451	4500	4499	51	0.43	0.99
2nd kindling	98	4034	3999	3998	70	0.67	0.99
18d after 2nd kindling	94	4404	4303	4341	66	0.31	0.69
Final-2nd weaning	94	4683	4531	4610	63	0.14	0.38

[1]Standard error of mean

were recorded and mortality determined in each reproductive cycle. The kits dead as consequence of death of their mother during lactation period were excluded in the calculation of kit mortality. Feed efficiency in the entire experimental period (from the first AI until the end of the experiment) was determined as the ratio of feed intake to the total live weight of weaned rabbits in this period. In the first or second lactation periods feed efficiency was determined as the ratio of feed intake to the total live weight of rabbits weaned in each lactation period. Due to mortality and infertility of does or loss of the entire litter, the number of does or litters controlled changed at different moments of the experiment.

Analytical methods

Chemical analysis of diets was made using the procedures of the AOAC (2000) for dry matter, starch, ether extract, ash and crude protein, and the recommendations of Van Soest *et al.* (1991) for NDF and ADF. The viable count of Toyocerin® spores in the feed was checked in feed samples of each feed lot after pelleting. The analysis method for evaluating the concentration of *B.cereus toyoi* in the rabbit feed was carried out following a quantitative standard microbiological method by using a selective plate agar medium for *B.cereus toyoi* (EU, 2002).

Statistical analysis

Data were analyzed by comparison between Control and Toyocerin® treatments (T0 *vs.* T200 + T1000) and between levels of Toyocerin® (T200 *vs.* T1000). The first contrast tested the efficacy of Toyocerin® respect to the control, independently from the level, and the second contrast tested the efficacy of the highest level. Data were analysed using the contrast analysis of general linear model procedures for continuous variables and the Pearson Chi-Square test for fertility and mortality. Statistical significance was accepted at $P<0.05$. Statistical analysis was performed with JMP 5.0.1 (2003).

RESULTS AND DISCUSSION

During the assay, eight rabbit does dead or were eliminated, corresponding to a mortality rate of 7.8%. The results of *post mortem* pathological exam of died does revelled that the causes of death were pasteurellosis (1 doe with T1000), broken vertebral column (2 does with T1000) and pregnancy toxemia (1 doe with T200 and 3 does with T1000). Mortality was not affected by treatments (data not shown) and was lower to that reported by Nicodemus *et al.* (2004), which observed 20% of mortality.

Table 2 shows the weight and daily weight gains of rabbit does from 17 weeks of age (initial weight) until the weaning of kits born in the second reproductive cycle. The results were not affected by treatments and were similar to the ones observed by Nicodemus *et al.* (2004). The evolution of the

Table 3: Influence of dietary supplementation with Toyocerin® on feed intake (g/d) per mother-cage (doe+litter)

	No.	Diets			SEM[1]	*P*-value	
		T0	T200	T1000		T0 *vs.* (T200+T1000)	T200 *vs.* T1000
All rabbit does							
1st insemination-1st kindling	100	177	196	199	6	0.08	0.79
1st kindling-18d of 1st lactation	99	335	358	352	7	0.18	0.75
18d of 1st lactation-1st weaning	99	489	543	556	20	0.14	0.79
1st weaning-2nd kindling	98	252	247	268	11	0.79	0.44
2nd kindling-18d of 2nd lactation	94	338	359	349	8	0.35	0.66
18d of 2nd lactation-2nd weaning	94	530	639	615	18	0.01	0.62
Only lactating does							
1st kindling-18d of 1st lactation	78	359	379	377	5	0.09	0.92
18d of 1st lactation-1st weaning	78	568	644	646	11	<0.001	0.93
2nd kindling-18d of 2nd lactation	81	366	362	377	5	0.80	0.41
18d of 2nd lactation-2nd weaning	81	620	654	689	8	0.04	0.22

[1]Standard error of mean

weights of does during the experimental period agree with the one reported by Cervera *et al*. (2001) and Pinheiro *et al*. (2005).

The feed intake by does, with or without litters, in the adaptation and experimental periods is shown in Table 3. The contrast analysis showed significant effects of Toyocerin® (T0 *vs.* T200 + T1000) on feed intake from 18 d of lactation until weaning of first cycle (only in lactating does) and second cycle (in all does and in lactating does) and did not show effects of probiotic level (T200 *vs.* T1000). The feed intake was higher in the Toyocerin® groups than in T0 ($P<0.05$) and the effects were more evident when only lactating does are considered. This result disagree with those obtained by Nicodemus *et al*. (2004), which did not observed effects of diet supplementation with 0.2 g/kg of Toyocerin® on feed intake of adult females. The observed increase of feed intake between 18 d of lactation and weaning might be due to an increase of feed intake by kits, since in this period they also eat solid feed. This increase may result of a better health state of kits, as consequence of a stabilisation and optimisation of intestinal microflora induced by Toyocerin® (although this trait was not measured in this study). In this way, Hattori *et al*. (1984) showed that the addition of 1×10^6 or 5×10^6 spores of *Bacillus cereus var. toyoi* (Toyocerin®) per gram of feed decreased the intestinal population of *Escherichia coli*.

The effects of probiotic supplementation on reproductive performance of rabbit does are shown in Table 4. Doe fertility was similar (1st AI) or better (2nd AI) than the fertility commonly observed in rabbit commercial farms. On the overall, during the experiment the diet supplementation with Toyocerin® had no significant effects on fertility. However, in the second reproductive cycle Toyocerin® females

Table 4: Influence of dietary supplementation with Toyocerin® on fertility rate[1] (%)

	Diets			*P*-value	
	T0	T200	T1000	T0 *vs.* (T200+T1000)	T200 *vs.* T1000
1st cycle	85.7	88.2	93.8	0.39	0.44
2nd cycle	82.3	97.1	89.7	0.08	0.23

[1]No. rabbit does at kindling/No. rabbit does at AI. [2] χ^2 test probability

showed a tendency to increase fertility relatively to T0 ones (P=0.08). This effect agreed with the shortened interval from parturition to effective mating and between parturitions with Toyocerin® observed by Nicodemus *et al.* (2004). The increase on fertility could be due to a better health status of females as result of Toyocerin® supplementation.

The data of lactating kit performances in the two reproductive cycles are shown in Table 5. Generally, the performances during the lactation period were in accordance with expected values and with the

Table 5: Influence of dietary supplementation with Toyocerin® on kit performances during the first and second reproductive cycles

		No.	Diets			SEM[1]	*P*-value	
			T0	T200	T1000		T0 *vs* (T200+T1000)	T200 *vs* T1000
1st cycle								
Litter size	total born	89	11.1	10.3	10.4	0.3	0.16	0.87
	born alive	89	10.2	10.0	9.4	0.3	0.45	0.43
	at 18d	78	8.2	8.4	8.0	0.2	0.94	0.25
	at weaning	77	8.3	8.3	7.8	0.2	0.57	0.28
Weight of litter (g)	at birth[2]	87	486	517	474	14	0.75	0.21
	after equalization	87	449	473	466	6	0.13	0.65
	at 18d	78	2246	2364	2168	58	0.87	0.17
	at weaning	77	7273	7586	7213	159	0.71	0.34
Mean weight of kits (g)	at birth	87	45	51	46	1	0.14	0.06
	after equalization	87	49	51	51	1	0.13	0.59
	at 18d	78	270	281	272	5	0.50	0.41
	at weaning	77	875	918	925	10	0.02	0.76
Kits weight gain (g/d)	birth to 18d	77	13.3	13.5	13.0	0.2	0.85	0.40
	18d to weaning	77	33.2	35.4	36.3	0.4	<0.01	0.31
2nd cycle								
Litter size	total born	87	10.4	9.6	9.9	0.3	0.30	0.67
	born alive	87	9.8	9.2	8.8	0.3	0.29	0.68
	at 18d	81	7.9	8.3	8.3	0.2	0.28	0.88
	at weaning	81	7.4	8.0	7.9	0.2	0.25	0.78
Weight of litter (g)	at birth[2]	83	569	548	608	17	0.81	0.15
	after equalization	85	541	561	569	9	0.22	0.71
	at 18d	81	2227	2495	2508	66	0.05	0.93
	at weaning	81	6907	7840	7956	202	0.02	0.81
Mean weight of kits (g)	at birth	83	57	61	65	1	0.02	0.24
	after equalization	85	58	61	62	1	0.19	0.63
	at 18d	81	283	303	302	5	0.06	0.94
	at weaning	81	932	986	1016	13	0.01	0.33
Kits weight gain (g/d)	birth to 18d	81	12.5	13.5	13.3	0.2	0.08	0.79
	18d to weaning	81	38.2	40.1	42.0	0.5	0.01	0.17

[1]Standard error of mean. [2]Weight of litter without dead kits

ones obtained by Cervera *et al.* (2001) and Pinheiro *et al.* (2005). No effects of treatments on litter size at kindling, 18 d post kindling and weaning or on number of kits born alive were observed. Also Nicodemus *et al.* (2004) observed identical number of kits born alive or dead with 0.2 g/kg of Toyocerin®. However, these investigators observed a trend for a greater number of weaned kits which Toyocerin®. In the second cycle the mean live weight of kits at birth in T0 group was lower than in Toyocerin® groups (P<0.05), probably as consequence of a higher litter size at birth (although statistically not significant). After equalisation the litter size and weight did not differ between treatments.

Kit weight at weaning was higher (P<0.05) in Toyocerin® treatments than in control treatment in both reproductive cycles. In second cycle, the litter weight at 18 d of age and at weaning was also higher in Toyocerin® treatments than in T0 treatment (+280 g or +12.6% at 18 d of age, and +1049 g or +15.2% at weaning, in T200). These results are in accordance with the study of Maertens *et al.* (1994), in which a probiotic (Paciflor®) increased litter weight at weaning, but differ from that of Nicodemus *et al.* (2004), that did not observed differences. Also growth rate between 18 d and 35 d of age was higher in Toyocerin® treatments. The effects of Toyocerin® on kit growth rate and weight were less evident in the first 18 days of age, when kits do not eat solid feed and weight gain is directly related to the maternal milk production. Like that, these results show a possible lack of effects of Toyocerin® on milk production. From 18 d of age to weaning the growth performances of kits are also dependent on solid feed intake. In this period feed supplementation with Toyocerin® increased significantly the daily weight gain of kits (P<0.05) and also, as referred previously, the feed intake of does and their litters (Table 3). Consequently, we can attribute the increase on growth rate between 18 d and weaning, and thus the highest weaning weight, to the increase on feed intake and to a better health state of kits. As has been referred previously, Toyocerin® has beneficial effects on the digestive microflora of kits during the lactation period (Hattori *et al.* 1984).

Influence of Toyocerin® on feed efficiency of rabbit does is shown in Table 6. Contrast analysis did not show effects on feed efficiency of rabbit does during the entire experiment neither in first lactation period. However, in the second lactation period, Toyocerin treatments showed a higher feed efficiency than control (P=0.03) and T200 treatment shower better values, but no significative, when compared with T1000 (P=0.08). This result agrees with the increase of feed efficiency observed by Nicodemus *et al.* (2004) with Toyocerin® and is related to the observed increases in weigh gain of kits and litter weight at weaning.

Table 7 shows the mortality of kits during the first and second lactation periods. In the first lactation, there were no effects of treatments on mortality. In the second lactation, the mortality of kits from 0 to 18 days old differed significantly between treatments. Control kits showed a higher mortality (18.5%) than Toyocerin® treatments (11.1 and 9.9%; P<0.05). A decrease on mortality (25% to 0%) and a higher daily weight gain of the kits fed diets with Toyocerin® were also reported by Hattori *et al.* (1984). The level of Toyocerin® did not affect the mortality rate of kits.

Table 6: Influence of dietary supplementation with Toyocerin® on feed efficiency (weaned kits/ingested feed) of rabbit does

	No.	Diets			SEM[1]	P-value	
		T0	T200	T1000		T0 *vs.* (T200+T1000)	T200 *vs.* T1000
Experimental period	102	0.269	0.312	0.281	0.011	0.25	0.25
Lactation period of 1st cycle	99	0.322	0.336	0.329	0.018	0.80	0.87
Lactation period of 2nd cycle	94	0.313	0.424	0.353	0.017	0.03	0.08

[1]Standard error of mean

Table7: Influence of dietary supplementation with Toyocerin® on kit mortality[1] (%) during the lactation period

	Diets			*P*-value	
	T0	T200	T1000	T0 *vs.* (T200+T1000)	T200 *vs.* T1000
1st lactation					
From birth to 18d	16.7	17.4	20.4	0.48	0.38
From 18 to 35d	3.3	1.8	1.5	0.18	0.84
2nd lactation					
From birth to18d	18.5	11.1	9.9	<0.01	0.65
From 18 to 35d	5.2	3.0	5.5	0.50	0.20

[1]Only kindling rabbit does alive during the whole lactation period. [2] $\chi 2$ test probability

CONCLUSIONS

Data obtained in this experiment suggest that the main effects of Toyocerin® are the increase on feed intake by does with litters between 18 d of lactation and weaning, weight gain of kits in this period and weight of kits at weaning, and also the reduction of kits mortality. These effects seem to be related one to the others and result of an increase on health status. These results suggest that the addition of Toyocerin® to the diets of rabbit does can have positive effects on productivity of intensive rabbit production. The addition of 0.2 g Toyocerin® per kg feed has similar effects to 1 g/kg.

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