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EFFECT OF DIET SUPPLEMENTATION WITH TOYOCERIN® (*Bacillus cereus* var. *toyoi*) ON PERFORMANCE AND HEALTH OF GROWING RABBITS

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ABSTRACT: Two trials were performed to evaluate the effect of a dietary supplementation with *Bacillus cereus* var. *toyoi* on performance and health of growing rabbits. The studies were conducted in two commercial farms using the same experimental diets. In the first trial, 216 rabbits were controlled from 35 d (weaning) until 70 d of age. In the second trial, 180 rabbits were controlled from 37 until 79 d of age. At weaning, rabbits were put into bicellular cages, divided into three groups and fed the experimental diets: diet C, diet T1 and diet T2 supplemented with 0, 200 ppm ($2x10^5$ spores/g diet) and 1000 ppm ($1x10^6$ spores/g diet) of Toyocerin® (concentration: $1x10^9$ *B. cereus* var. *toyoi* spores/g), respectively. The diets did not contain antibiotics or growth promoters and presented similar chemical composition (CP: 17.4% DM, NDF: 40.8% DM; ADL: 5.2% DM, starch: 16.5% DM). The differences in growth performance between the two trials depended mainly on the different final age of rabbits. Weight gain (42.0 *vs* 36.5 g/d) was lower and feed conversion (3.12 *vs* 3.96) higher in the second trial. Mortality (13.0% *vs* 21.7%) and morbidity (2.8% *vs* 25.0%) were significantly higher in the second trial. The probiotic supplementation (diet C *vs* diets T1+T2) significantly increased final live weight (2,517 *vs* 2,580 g; *P*=0.02) and daily weight gain (38.2 *vs* 39.8 g/d; *P*=0.01) and improved feed conversion (3.63 *vs* 3.50; *P*=0.01). Morbidity was significantly lower with supplemented diets (18.2 *vs* 10.3%; *P*=0.03), while mortality and sanitary risk were not affected by dietary treatment. No effect of probiotic inclusion rate (diet T1 *vs* diet T2) and no significant interaction between dietary treatment and trial were measured. In conclusion, the supplementation of *Bacillus cereus* var. *toyoi* improved growth performance and reduced morbidity of rabbits reared in farms with or without severe health problems. Increasing probiotic inclusion rate from $2x10^5$ to $1x10^6$ spores/g diet did not improve rabbit growth performance and health.

Key words: rabbits, probiotic, *Bacillus cereus* var. *toyoi*, growth performance, health status.

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INTRODUCTION

The spread of enteric diseases and in particular epizootic rabbit enteropathy (ERE) has negatively affected the health status in rabbit farms and largely increased antibiotic use as a preventive method (Licois *et al.*, 2000; Duperray *et al.*, 2003). However, on the base of consumer demand, European legislation is banning the use of antibiotics as growth promoters and is working towards a reduction of therapeutic antibiotics for all livestock production, to avoid crossed resistance in humans and improve food safety.

Probiotics and prebiotics appear as possible alternative feed additives to modulate intestinal microflora and improve animal health (Williams and Newbold, 1996; Bosi *et al.*, 2001; Medina *et al.*, 2002). In rabbits, live yeast supplementation provided some positive effects on growth performance and health status, especially when animals were kept under sub-optimal environmental and sanitary conditions with high stocking density and low hygiene control (Maertens and De Groote, 1992; Tedesco *et al.*, 1994; Maertens and Ducatelle, 1996). Lactic-acid bacteria could modify caecal microflora composition (Canzi *et al.*, 2000), but seems to have weak effects on growth performance and mortality of growing rabbits (Yamani *et al.*, 1992). Various strains of *Bacillus* sp. were tested with contrasting results (De Blas *et al.*, 1991; Zoccarato *et al.*, 1995; Bonanno *et al.*, 1996). Few studies were performed under experimental conditions testing the action and efficacy of *Bacillus cereus* var. *toyoi* (Hattori *et al.*, 1984; Nicodemus *et al.*, 2004).

In the present study, two trials were performed to evaluate the effect of a dietary supplementation of *Bacillus cereus* var. *toyoi* on performance and health status of growing rabbits kept in bicellular cages in two typical Italian commercial farms.

MATERIALS AND METHODS

Trial location and equipment

Two trials were performed in two commercial farms located in the North-East of Italy (Padova province), characterised by a similar housing and management conditions. In both farms, the buildings were made of concrete. The trials were performed in the autumn-winter period, but the environmental conditions were controlled by an automatic heating system regulated to maintain a minimum temperature of 16°C during the whole trial. Forced ventilation was assured by extracting fans. A natural photoperiod (about 10-12 h light and 12-14 h dark) was used.

Bicellular flat-deck cages for fattening (28 x 40 x 30 cm) made of galvanized wire net equipped with automatic nipple drinkers were used. The cage top gates were modified to permit the use of manual feeders to measure separately the consumption of each cage.

Experimental diets were always provided *ad libitum*. The animals, kept in couples (bicellular cages), were managed as usual in the two farms, apart from recordings of weight, feed consumption and health status.

Animals and diets

In the first trial, 216 hybrid rabbits of a Hyla line (Hycole Sarl, Ribecourt La Tour, France) were controlled from weaning (35 d) until 70 d of age. In the second trial, 180 hybrid rabbits of a Grimaud line (Grimaud Frères Selection, France) were controlled from weaning (37 d) until 79 d of age.

At weaning, rabbits were chosen from among those born the same day in the farm from multiparous does, moved from the maternal sector to the fattening sector and put in bicellular cages. The two rabbits in each cage came from different litters and had similar live weight (maximum weight difference: 100 g). Rabbits were divided into three groups, homogeneous in terms of average live weight and variability and fed three experimental diets: diet C, control, without probiotic supplementation; diet T1 and diet T2 supplemented with increasing concentrations of *Bacillus cereus* var. *toyoi* ($2x10^5$ spores/g diet and $1x10^6$ spores/g diet, respectively) by the inclusion of 200 ppm or 1,000 ppm of Toyocerin® (concentration: 1×10^9 *B. c. toyoi* spores/g of Toyocerin®). The additive, produced by Asahi Vet. S.A. (Barcelona, Spain) in powder form, was added to the vitamin and mineral premix in substitution of the premix support (wheat middlings) and then included in the diet T1 and diet T2 before pelleting. The diets were pelleted to a diameter of 3.5 mm and a length of 1.0-1.1 cm. The temperature during the pellet conditioning did not exceed 60°C and no water was added (humidity 9-10%).

The diets were similar to common commercial diets for growing rabbits and formulated using commercial raw materials currently adopted by Italian feed producers. They did not contain antibiotics, additives, growth promoters or coccidiostatics.

The diets presented similar chemical composition (average value of the two trials), as expected, since differing only for the probiotic inclusion (Table 1). Very little differences in composition could be attributed to the method of diet preparation, (with three successive stages), ingredient loading, mixing and pelleting (according to the sequence: diet C, diet T1 and diet T2). In general, the chemical composition of the diets was in accordance with current requirements for growing rabbits (De Blas and Mateos, 1998).

Recordings

At weaning, the rabbits were given identification marks on the ear, put into bicellular cages and fed the experimental diets. Individual live weight and cage feed intake were recorded weekly. Mortality was controlled daily throughout the experimental period. Daily feed intake was calculated taking into account the effective number of animals per cage per day, thus excluding the intake of dead animals.

The general health status of rabbits was controlled daily by the breeder with a visual inspection to detect the presence of dead or ill animals, these latter showing sign of diarrhoea or prostration. All rabbits were examined individually the day of weighing to detect the occurrence of digestive or respiratory problems. The rabbits were considered ill when evidencing clear signs of diarrhoea or a reduction of live weight and/or a 20% decrease of daily feed intake compared to the previous week. In the calculation of morbidity, the ill rabbits were counted only once, independently of the duration of illness. The dead animals were not considered in the morbidity calculation. The health risk was calculated as the sum of morbidity and mortality (BENNEGADI *et al.*, 2000). The animals were kept under control by the official veterinary service of the farm. The dead animals were submitted to veterinary inspection at the Istituto Zooprofilattico Sperimentale delle Venezie (Legnaro, Padova, Italy) to determine the causes of death.

In the first trial, mortality amounted to 8 rabbits per diet C, 8 rabbits per diet T1 and 12 rabbits per diet T2. The number of cages (with one or two rabbits) considered

Table 1: Chemical composition and nutritive value of experimental diets (mean values of the two trials).

	Diet C	Diet T1	Diet T2
Dry matter, %	89.9	89.7	89.6
Crude protein, % DM	17.4	17.4	17.5
Ether extract, % DM	3.6	3.6	3.6
Crude fibre, % DM	17.8	17.2	17.3
Ash, % DM	8.6	8.8	8.6
NDF, % DM	41.1	40.7	40.7
ADF, % DM	21.1	20.8	20.8
ADL, % DM	5.3	5.2	5.2
Starch, % DM	16.3	16.7	16.6

Calculated value for all diets: Lysine, 0.81% DM; Methionine+cistine, 0.66% DM; Digestible energy (DE), 10.88 MJ/kg DM; Digestible protein/DE ratio, 11.5 g/MJ.

for statistical analysis was 35, 35 and 33 for diet C, diet T1 and diet T2, respectively. In the second trial, mortality was 12 rabbits per diet C, 15 rabbits per diet T1 and 12 rabbits per diet T2. The number of cages considered for statistical analysis was 28, 28 and 29 for diet C, diet T1 and diet T2, respectively.

Chemical analysis

The diets were analysed by AOAC (2000) methods following the European harmonised procedures (EGRAN, 2001). Ether extract was determined after acid-hydrolysis treatment. Fibre fractions were determined by Goering and Van Soest method (1970) as modified by Robertson and Van Soest (1981). NDF determination was performed in the presence of a heat-resistant amylase (Thermamyl L120, Novo Nordisk, Denmark). Starch was determined by HPLC (Methods 996.11 and 979.10, AOAC, 2000; University of Florida, 2000) after enzymatic treatment (Boehringer Mannheim, Starch determination, cat. no. 207748).

Statistical analysis

Data were analysed using the GLM procedure of the Statistical Analysis Systems (SAS, 1991) according to a 2x2 factorial arrangement with the type of diet, the trial and their interaction as the main sources of variation and the cage as the experimental unit. Comparisons among means of the three dietary treatments were performed using the Bonferroni test and the contrast "control *vs* probiotic supplementation": C *vs* T = C *vs* (T1+T2). Differences in mortality and morbidity of rabbits according to the dietary treatment, the trial and their interaction were tested using the CATMOD procedure of SAS.

RESULTS AND DISCUSSION

Growth performance of rabbits are reported in Table 2. The effects of the main experimental factors are described and discussed separately, since no significant interaction was measured (P>0.10).

Table 2: Growth performance of rabbits (cage data).

	Diet			*P*-value		Trial		*P*-value		
	C	T1	T2	Diet	C *vs* T	1	2	Trial	Diet x Trial	RSD
Cages (no.)	63	63	62			103	85			
Initial live weight (g)	1052	1058	1057	0.89	0.63	1017	1095	<0.001	0.85	73
Final live weight (g)	2517[a]	2586[b]	2573[ab]	0.05	0.02	2488	2630	<0.001	0.24	166
Weight gain (g/d)	38.2[a]	40.0[b]	39.6[ab]	0.04	0.01	42.0	36.5	<0.001	0.11	4.0
Feed intake (g/d)	137	138	137	0.98	0.97	131	143	<0.001	0.43	12
Feed conversion	3.63[b]	3.47[a]	3.52[ab]	0.03	0.01	3.12	3.96	<0.001	0.49	0.33

C *vs* T: probability of the contrast diet C *vs* diet T1+diet T2.
RSD: residual standard deviation.
Means within a row with different superscript differ ($P<0.05$).

Differences in growth performance between the two trials depended mainly on the different final age (70 and 79 d). Growth performances in the two trials were also affected by the different health status of animals (Table 3). Mortality (13.0% *vs* 21.7%) and morbidity (2.8% *vs* 25.0%) were significantly higher during the second trial. Health problems were mainly due to diarrhoea or mucoid enteritis with the presence of *E. coli* and often *Clostridium* sp. In the first trial, health problems appeared at the end of the first week of trial and reduced by the beginning of the third week. In the second trial, health problems appeared in the second week and persisted until the beginning of the fourth week.

Regardless of the health condition, the effect of the probiotic inclusion was observed in both farms (no significant interaction). On average, *B. cereus* supplementation significantly increased final live weight (+2.5%) and daily weight gain (+4.2%) and improved feed conversion (-3.7%) (C *vs* T, $P<0.01$). Daily weight gain during the second week (average of the two trials) was significantly lower ($P<0.01$) in rabbits fed the control diet (29.9 g/d) than diet T1 and diet T2 (35.4 and 37.0 g/d). The performance of animals fed diet C significantly differed from those of animals fed diet T1, showing a positive effect of the lowest supplementation, while performance of rabbits fed diet T2 was intermediate. Similarly, morbidity was significantly lower with the administration of the diet T1, while mortality and health risk were not affected by the supplementation level (Table 3).

Various species of *Bacillus* have been tested as probiotics in growing and reproducing rabbits. In comparison with other probiotics, *Bacillus* supplementation is easier to carry out from a technical point of view, due to the resistance of the spores during storage and feed processing. Competition has been shown to exist between *Bacillus* sp. and the pathogenic flora at the gastro-intestinal level, which could help in maintaining a positive flora and good health condition. In particular, when including *B. subtilis,* Cristofalo *et al.* (1980) found reduced incidence of enteric lesions in dead rabbits and decreased mortality. Hattori *et al.* (1984) recorded a reduction of *E. coli* in the gastro-intestinal tract with increasing dietary concentration of *B. cereus* var. *toyoi*.

Table 3: Percentage of mortality, morbidity and sanitary risk of rabbits.

	Diet			*P*-value		Trial		*P*-value	
	C	T1	T2	Diet	C *vs* T	1	2	Trial	Diet x Trial
Mortality	15.1	17.4	18.2	0.78	0.49	13.0	21.7	0.02	0.51
Morbidity	18.2[a]	7.6b	12.9[ab]	0.03	0.03	2.8	25.0	<0.001	0.41
Health risk	33.3	25.0	31.1	0.27	0.32	15.7	46.7	<0.001	0.55

C *vs* T: probability of the contrast diet C *vs* diet T1+diet T2.
Means within a row with different superscript differ (P<0.05).

In terms of productive performance, the inclusion of *B. subtilis* did not always improve growth or health status of growing rabbits (CRISTOFALO *et al.*, 1980; LAMBERTINI *et al.*, 1990). On the other hand, when *B. subtilis* was associated with *B. licheniformis*, growth rate, feed conversion and digestive efficiency increased (ZOCCARATO *et al.*, 1995; BONANNO *et al.*, 1999). In the case of unfavourable sanitary farm conditions (average mortality: 23%), mortality was reduced to 5% with the highest dietary level of *Bacillus sp.* (BONANNO *et al.*, 1996). The supplementation of *B. cereus* var. *toyoi* at $2x10^5$ spores/g diet in rabbit does and suckling kits gave higher values for litter weight (3.673 *vs* 3.952 g; P=0.10) and litter size (7.37 *vs* 8.10; P=0.09) at weaning (25 d) (NICODEMUS *et al.*, 2004).

According to our results, the supplementation with $2x10^5$ spores *B. cereus*/g diet slightly decreased the digestive problems and morbidity, while no significant effect was observed with a higher inclusion rate ($1x10^6$ spores/g diet). On the contrary, HATTORI *et al.* (1984) observed increased body weight and a substantial reduction of diarrhoea when increasing the inclusion rate of *B. cereus* var. *toyoi* from $1x10^5$ to $5x10^6$ spores/g diet.

CONCLUSIONS

The supplementation of *B. cereus* var. *toyoi* at the dose of $2x10^5$ spores/g diet by means of Toyocerin® improved moderately the growth performance, and reduced only the morbidity, but not the mortality or the health risk of rabbits kept in commercial farms both in the absence and presence of severe health problems. Increasing *B. cereus* supplementation until 1x10^6 spores/g diet did not produce any improvement of performance or health status.

Probiotic and prebiotic supplementation in rabbit feeding represents a promising way of reducing antibiotic utilization and offers safer meat to the consumer. More studies are needed, however, to evaluate the action and test the efficacy of commercial products as well as to identify and develop new additives.

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