Evaluation of Toyocerin, a Probiotic Containing *Bacillus toyoi* Spores, on Health Status and Productivity of Weaned, Growing and Finishing Pigs

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ABSTRACT : The aim of the study was to assess the efficacy of Toyocerin, a probiotic containing *Bacillus toyoi* spores, on the health status and productivity of pigs, during nursery, growing and finishing phases. On a commercial farrow-to-finish farm in Greece, 3 experimental groups were formed, each of 72 weaned piglets. The pigs of the first group (T1 group: negative controls) received normal feed with no antimicrobials or probiotics, the pigs of the second group (T2 group) received the same type of feed but supplemented with 1.0×10^9, 0.5×10^9 and 0.2×10^9 spores per kg of feed at weaning, growing and finishing stage, respectively, and the pigs of the third group (T3 group) were fed with Toyocerin at the dose of 1.0×10^9 spores per kg of feed during the entire fattening period (weaning, growing and finishing stages). The results have shown that, compared to the controls, Toyocerin treated pigs had reduced incidence of post-weaning diarrhoea ($p<0.05$). Enterotoxigenic strains of *Escherichia coli* were detected in faecal samples of 0% to 25% of pigs of the treated groups, but in 33.5% to 50% of pigs of the non-treated group ($p<0.05$). Over the negative controls, a significant improvement of weight gain (4.5% and 8.3% for T2 and T3 groups, respectively), and of feed conversion ratio (6.6% and 13.0% for T2 and T3 groups, respectively) was observed. The 76.5% of the carcasses of the T3 group was classified in the top three categories of the EUROP scale (S, E and U), whilst the respective figures were 47.8% for T2 group and only 10.5% for T1 group ($p<0.05$). ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 9 : 1326-1331)***

Key Words : Probiotics, Pig, Diarrhoea, Productivity

INTRODUCTION

The continuous demand of consumers for low cost pork resulted in the intensification of pig production methods during the last decades, and the extensive administration of antimicrobials for growth promoting purposes (NRC, 1980; Fuller, 1992). The antibiotics as growth promoters appear to act by reducing the pathogenic bacteria and modifying the microflora in the gut of the animal (Radostits et al., 1994). However, concern regarding the presence of drug residues in edible animal products and the potential transfer of antibiotic resistance to human pathogens has lately been raised by such administration. For these reasons, alternative solutions, aiming at both the safety of the consumer and the profitability of the farmer, are required.

Probiotics are live cultures of harmless bacteria or yeast species (e.g *Lactobacillus, Streptococcus, Saccharomyces* etc.) that equilibrate intestinal microflora to the benefit of the animal (Fuller, 1989; Ferencik et al., 2000). They may have a growth promoting activity by competing with harmful gut flora, and by stimulating the immune system of the animal and therefore increasing body's resistance to infectious agents (Tannock, 1980; Fuller, 1989; Khajarern and Khajarern, 1994). The positive effect of probiotics on the control of certain pathogens in animals has been shown in few only studies, where they appear to control enteric diseases associated with *Escherichia coli* or other enteric pathogens, one of which is post-weaning diarrhoea syndrome (PWDS) in pigs (Kozasa, 1983; Khajarern and Khajarern, 1994; Kyriakis et al., 1999). Therefore, it is reasonable to assume that, due to their ability to modulate gut microflora in favour of the animal, probiotics may also exhibit a growth enhancing activity (Lyons, 1987).

The purpose of the present study was to assess the effect of Toyocerin, a probiotic containing *Bacillus toyoi* spores, on the health status and the productivity of weaned, growing and finishing pigs under field conditions.

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MATERIALS AND METHODS

Description of probiotics

Toyocerin (Toyo Jozo Co., Japan) is a probiotic containing live *Bacillus toyoi* spores in a dry powder form (at least 10^{10} *Bacillus toyoi* spores per kg of product), which is incorporated in the feed. This strain was registered in the E.U under annex II in1994 for use in piglets (Kozasa, 1986).

Trial farm

The study was carried out from July 1998 to January 1999 on a commercial farrow-to-finish pig farm in Macedonia, Greece, with a breeding stock of 900 sows and an annual production of 16,000 fatteners (Dalland hybrids). The farm had its own feed mill and slaughterhouse. The piglets in the farm were weaned in weekly batches of approximately 320±50 animals each, at the age of 23±3 days. In flat decks, pigs were separated in pens of 24 animals each, and remained until the age of 63±3 days. After that age, the animals were moved into the fattening unit, keeping the same block design as in the flat-deck unit, where they remained until slaughter at the age of 161±3 days (approximate body weight of 95 kg). In the fattening unit, pigs were fed *ad libitum* two different dry meal rations, one up to the age of 112 days (growing stage feed) and a second one from 113 day to the slaughter age (finishing stage feed).

The farm had a previous history of post-weaning diarrhoea syndrome due to an uncomplicated *E. coli* infection as evidenced by appropriate microbiological and histopathological examinations presented elsewhere (Kyriakis et al., 1999).

All breeding animals were vaccinated against Aujeszky's disease, swine influenza, parvovirus infection, erysipelas, atrophic rhinitis and clostridial enteritis. For the control of endo/ectoparasites, all adults were treated with ivermectin twice a year.

From the 7th day of age until weaning all piglets received creep feed free from any antimicrobial, performance enhancer, probiotic or acidifier.

Experimental design

At one weaning, 216 (108 male and 108 female) piglets were moved from the farrowing house to the flat-deck unit. All piglets were individually weighted, ear-tagged and randomly allocated according to their bodyweight and sex into 9 pens, each pen including 12 non-castrated male and 12 female piglets. A randomised complete block design with three experimental groups and three replicates (pens) per group was used. Thus, each experimental group had three pens each of 24 piglets, making a total of 72 piglets. The average bodyweight of the pigs between the groups did not differ significantly at the beginning of the trial ($p>0.05$).

From this day up to the age of slaughter, each group of piglets was administered different doses of Toyocerin in feed, as follows:

T1: No addition of Toyocerin to feed from weaning up to the age of slaughter (negative control group).

T2: Addition of Toyocerin at the dose of 1.0×10^9, 0.5×10^9 and 0.2×10^9 spores per kg of feed at weaning, growing and finishing stage, respectively (Low dose group).

T3: Addition of Toyocerin at the dose of 1.0×10^9 spores per kg of feed during the entire fattening period (weaning, growing and finishing stages) (High dose group).

No antibiotics and/or antibacterials were added in the feed of any of the groups. Specification of the piglet feeds (on an air-dry basis) was the following: (a) Weaner feed: Digestible energy 14.6 MJ/kg, crude protein 20.5%, fibre 3.5%, lysine 1.4%, calcium 0.85% and total phosphorus 0.95%, (b) Grower feed: Digestible energy 13.9 MJ/kg, crude protein 19%, fibre 4%, lysine 1.05%, calcium 0.85% and total phosphorus 0.85% and (c) Finisher feed: Digestible energy 13.6 MJ/kg, crude protein 17.5%, fibre 4.5%, lysine 0.9%, calcium 0.75% and total phosphorus 0.85%.

Observations and sampling

All trial pigs were monitored daily for signs of disease. During the weaner stage (age of 23 to 63 days), all piglets were examined daily for the appearance of diarrhoea. A diarrhoea score was calculated on pen basis, by using the following formula: Pen diarrhoea score=[($Pig_1\times$days of scouring)+ …+($Pig_{24}\times$days of scouring)]/(40 days).

Rectal swabs were collected at the day of weaning and on post-weaning days 7, 14, 21 and 28 from two male and two female (always the same) piglets of each pen e.g. 12 piglets per experimental group. The faecal samples were examined for the presence of: a) coccidial oocysts by faecal flotation, b) *Cryptosporidium spp.* by modified Ziehl-Neelsen staining and c) enterobacteriaceae by culturing on MacConkey and Salmonella-Shigella agars, further subculturing to Columbia blood agar, and haemolytic species were identified by API 20E kit (BioMerieux, France). Those strains identified by the API 20E as *E. coli* were further serotyped using the Fimbrex K88, Fimbrex K99 and Fimbrex 987P latex agglutination kits, employing monoclonal antibodies against F4 (K88), F5 (K99) and F6 (987P) fimbrial antigens, respectively (Veterinary Laboratory Agency, UK).

For every dead pig, its weight and the date of death were recorded, autopsy was performed and appropriate samples were directly sent to the Laboratories of Microbiology and Pathology of the Veterinary School of the University of Thessaloniki for further examination as elsewhere described (Kyriakis et al., 1999).

Apart from the initial weighing at weaning, the pigs were individually weighed also at the ages of 63, 84, 112, 140 and 161 days. Feed consumption per pen was recorded during the periods 23 to 63 days of age, 64 to 84, 85 to 112, 113 to 140 and 141 to 161 days of age, and the average daily gain (ADG), the average daily feed intake (ADFI) and the feed conversion ratio (FCR) were calculated. The carcasses of the pigs were evaluated at abattoir by using the system EUROP (SEUROP) currently applied in European Union.

Table 1. Diarrhoea parameters of weaner pigs (age 23-63 days)

Parameter	T1 Negative controls	T2 (1.0, 0.5, 0.2×10^9 spores)	T3 (1.0, 1.0, 1.0×10^9 spores)
No. of pigs with diarrhoea up to the end of period	17/72 (23.6%)^a	10/72 (13.9%)^b	9/72 (12.5%)^b
Diarrhoea score (mean±SD)	0.773^a±0.11	0.370^b±0.04	0.353^b±0.07

$^{a, b}$ Figures within the same row with different superscripts differ significantly (p<0.05).

Table 2. Presence of ETEC strains in rectal swabs of pigs at different ages

Day post-weaning	No of rectal swabs positive to ETEC strains / total swabs examined (%)		
	T1 Negative controls	T2 (1.0, 0.5, 0.2×10^9 spores)	T3 (1.0, 1.0, 1.0×10^9 spores)
0	0/12 (0.0)^a	0/12 (0.0)^a	0/12 (0.0)^a
7	5/12 (41.7)^a	1/12 (8.3)^b	2/12 (16.7)^b
14	6/12 (50.0)^a	3/12 (25.0)^a	3/12 (25.0)^a
21	6/12 (50.0)^a	1/12 (8.3)^b	0/12 (0.0)^b
28	4/12 (33.5)^a	0/12 (0.0)^b	0/12 (0.0)^b

^{a,b} Means or percentages within the same row with different superscript differ significantly (p<0.05).

During the trial, pooled samples of each batch of feed were collected from the farm mill and sent to ASAHI VET S.A in order to assess the quality and to confirm the proper spore content of the feed.

Statistical evaluation

Each recorded parameter per pen as experimental unit was subjected to one-way analysis of variance using the General Linear Models (GLM) procedure of the SAS system (Site No:84912001/SAS Institute Inc. Cary, NY 27513, USA). Duncan's multiple range test was used to compare means and chi-square test to compare frequencies. The experimental unit was the individual pig, except the parameters involving feed consumption (ADFI, FCR).

RESULTS

In pigs of all trial groups, transient post-weaning diarrhoea lasting 1 to 3 days was a frequent finding. Stools were pasty or watery in consistency and greyish to brown in colour, without blood or mucous. Occasionally, some of the diarrhoeic animals in all groups were found dead with signs of dehydration, particularly during the first period of observations (23 to 63 days of age). This diarrhoeic syndrome resulted in animal growth retardation during the first 10 days after weaning. Although death rates were essentially the same between the three groups of pigs (8.3%, 6.9% and 5.6% for T1, T2 and T3 groups, respectively), more pigs of the control group developed scours compared with the T2 and T3 pigs treated with Toyocerin (Table 1) (p<0.05). Also, the diarrhoea score of the non-treated group was twice as that of the low (T2) and high dose groups (T3) (p<0.05). Autopsy of 4 animals died after the 63 day of age (1, 2 and 1 pigs from groups T1, T2 and T3, respectively) had revealed lesions of respiratory infection, but not of enteric disease.

Post-mortem examination of moribund or dead weaners has shown inflammation of the mucosa of the small intestine. Haemorrhagic or necrotic enteritis, or thickening and polyp-like mucosa, findings which are indicative of infections by *Clostridia, Salmonella* spp., various spirochaetes (including *Brachyspira hyodysenteriae*), *Cryptosporidium parvum* and *Lawsonia intracellularis* were not observed. Histopathology did not reveal any major morphological changes in the small or large intestine. Moreover, examination of the intestines of the pigs of all groups at slaughterhouse did not show any pathological findings.

All faecal samples examined for coccidia and *Cryptosporidium* sp. were negative at the time of examination. Neither were the samples positive for enterobacteriaceae but only for *E. coli*. Examinations of these samples have shown the presence of F4 or/and F5 enterotoxigenic strains of *E. coli* (ETEC strains) in all trial groups, but not of the 987P antigenic group. As presented in Table 2, ETEC strains were identified in more pigs of the control group and for longer time compared to the Toyocerin-treated groups (p<0.05). The highest positive percentage of rectal swabs was seen around day 14 in all groups of pigs, but a rapid reduction was subsequently observed only in the pigs of the T2 and T3 groups.

Growth performance parameters such as ADG and FCR were significantly improved after the administration of Toyocerin (Table 3). The ADFI was similar in all groups during all trial subperiods (p>0.05).

The ADG of the T2 group was significantly higher than that of the T1 controls (p<0.05), but only during the nursery subperiod (Table 3). Pigs treated with high doses of Toyocerin (T3 group) gained significantly more weight throughout all fattening subperiods compared with the pigs of the control group (p<0.05). When ADG was calculated

Table 3. Growth performance in the groups of pigs at different stages

Days of age	T1 Negative controls	T2 (1.0, 0.5, 0.2×10^9 spores)	T3 (1.0, 1.0, 1.0×10^9 spores)
		Average bodyweight (kg) ± SD	
At weaning	6.32^a±0.71	6.39^a±0.74	6.30^a±0.71
At slaughter	89.40^a±6.51	93.27^b± 6.35	96.31^c±7.17
		Average daily gain (kg) ± SD	
23-63	0.336^a±0.07	0.376^b±0.09	0.370^b±0.08
64-112	0.604^a±0.10	0.617^a±0.08	0.666^b±0.11
113-161	0.814^a±0.10	0.842^{ab}±0.12	0.866^b±0.12
23-161	0.602^a±0.05	$0.629\ ^b$±0.05	0.652^c±0.05
		Average daily feed intake (kg feed) ± SD	
23-63	0.618^a±0.07	0.585^a±0.01	0.545^a±0.04
64-112	1.559^a±0.13	1.510^a±0.09	1.413^a±0.03
113-161	2.604^a±0.05	2.531^a±0.04	2.469^a±0.22
		Feed conversion ratio± SD	
23-63	1.84^a±0.19	1.55^b±0.06	1.51^b±0.07
64-112	2.57^a±0.04	2.45^a±0.05	2.13^b±0.14
113-161	3.21^a±0.20	3.01^a±0.19	2.84^a±0.11

^{a,b} Means within the same row with different superscript differ significantly ($p<0.05$).

Table 4. Carcass classification according to the EUROP (SEUROP) system of the EU

Class	Lean (%)	Pigs in category/Total pigs examined (%) T1 Negative controls	T2 (1.0, 0.5, 0.2×10^9 spores)	T3 (1.0, 1.0, 1.0×10^9 spores)
S	>60	0/66 $(0)^a$	0/67 $(0)^a$	1/68 $(1.5)^a$
E	55-60	1/66 $(1.5)^a$	2/67 $(3.0)^a$	12/68 $(17.6)^b$
U	50-54	6/66 $(9.1)^a$	30/67 $(44.8)^b$	39/68 $(57.4)^b$
R	45-49	38/66 $(57.6)^a$	27/67 $(40.3)^a$	13/68 $(19.1\%)^b$
O	40-44	15/66 $(22.7)^a$	5/67 $(7.5)^b$	2/68 $(2.9)^b$
P	<40	4/66 $(6.1)^a$	1/67 $(1.5)^a$	0/68 $(0)^a$

^{a,b} Means within the same row with different superscript differ significantly ($p<0.05$).

for the entire fattening period, the pigs of the T3 group performed better than those of the T2 group, and particularly of the control group ($p<0.05$).

As compared to the control group, the FCR in the T3 group was significantly improved during the nursery and growers' stages, whereas that of the T2 group had been improved only during the nursery stage ($p<0.05$). Throughout the finishing period, a tendency for better feed utilisation was observed in the Toyocerin-treated pigs and particularly in those of the high dose group (Table 3).

The results of carcass classification, presented in Table 4, clearly show the positive effect of the administration of Toyocerin on meat quality. Indeed, carcasses of the Toyocerin-treated pigs, and particularly those of the T3 group, were of better quality compared to those of the control group. The 76.5% of the carcasses of the T3 group was classified in the top three categories of the EUROP scale (S, E and U), whilst the respective figures were 47.8% for T2 group and only 10.5% for T1 group ($p<0.05$).

DISCUSSION

During the critical post-weaning period, pigs are subjected to stressors that reduce their immune response and disturb the equilibrium of the microflora in their gut, so make them susceptible to several enteric pathogens. Microorganisms such as ETEC strains, Salmonella spp., clostridia, spirochaetes (including *Brachyspira hyodysenteriae*), *Lawsonia intracellularis, Cryptosporidium parvum,* rotavirus and coronaviruses are agents potentially causing scours from weaning up to the end of fattening period (Taylor 1995). The post-weaning problem seen in the present farm had epidemiological, clinical and post-mortem characteristics indicative for ETEC strains or rotavirus, both known to contribute to PWDS (Kyriakis, 1989). Additional support of this diagnosis was provided by the results of the laboratory examinations.

It is known that probiotics have a positive effect on PWDS (Kyriakis et al., 1999), as well as on other diarrhoeas (Breton and Munoz, 1998; Candy et al., 2000; Marcin et al., 2000). In-feed *B. toyoi* has presently reduced the incidence and severity of diarrhoea in weaned pigs (Table 1) and this can be explained by the decrease of pathogens locally in the gut, brought about by the ingestion of the probiotics, which did not allow increased proliferation of harmful bacteria (Vandevoorde et al., 1992;

Nouisiainen and Setala, 1993; Alvarez et al., 1996). Indeed, the number of pigs that were ETEC-positive was significantly diminished after treatment with the bioregulator (Table 2). The latter finding could also imply that pigs fed with Toyocerin are likely to excrete faeces resembling their gut microflora e.g. less pathogenic *E. coli* and high numbers of *Bacillus* spores. These faeces are spread in the pen creating a better (or even helpful) environment for the newly weaned pig. The exact mechanism of action of *B. toyoi* in the gut still remains unclear e.g. whether this was due to competition for receptors on gut mucosa, competition for nutrients, production of antibacterial substances, stimulation of immunity or/and other mechanisms (Fuller, 1989; Perdigon et al., 1990; Rodriguez-Ropon et al., 1998).

The beneficial effect of the bioregulator has also extended to the growing and finishing stages. An important index for evaluating a product, and one, which is of utmost importance for the farmer, is the productiveness of the animal. In the present study, several efficiency parameters of treated animals, particularly those of T3 group, were superior to those seen in controls ($p<0.05$). For example, ADG in T2 and T3 groups was 4.5% and 8.3% higher than that of the controls at the slaughter age ($p<0.05$). These figures are of particular significance for the farmer considering that, the continuous use of growth promoters during weaning-to-slaughter period is not expected to confer improvement in efficacy parameters higher than 5% (Buttery, 1993). A possible explanation for this benefit could be that microflora balance in the gut of these animals is optimised -as is the case in weaners -and a better utilisation of nutrients is taking place, thereby leading to faster metabolism and transformation of feed in body mass (ADG FCR), and transformation in lean meat.

IMPLICATIONS

The present study has shown that the administration of *Bacillus toyoi* can prophylactically reduce the incidence and the severity of scours caused by enterotoxigenic strains of *Escherichia coli* in recently weaned piglets, a finding confirming previous observations. It has also shown that, by this treatment, growth performance and carcass characteristics can be significantly improved to the economic benefit of the farmer. Finally, considering that probiotics are generally considered harmless, the findings of this study further support the view that the use of antimicrobials both for competing scours problems in pigs and for promoting growth of the animals can be reduced, minimizing this way the risks for public health (drug residues, antibiotic-resistant strains of bacteria).

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