



## The Effect of Probiotics on Microbiology, Health and Performance of Fattening Rabbits

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**ABSTRACT** : The objective of the present trial was to determine the effect of a thermostable probiotic containing *Bacillus licheniformis* and *B. subtilis* on health and production parameters of fattening rabbits from weaning until slaughter. In a rabbitry with average post-weaning mortality of 5-9%, 1,680 rabbits were supplied with: a) a basic feed, or b) the same basic feed supplemented with probiotic from the 4th day postweaning (41st day of age) up to 88th day of age. Clinical signs, microbiological status and growth performance were recorded for two distinct fattening periods, growing and finishing. A significant decrease in mortality of probiotic-treated rabbits when compared to the controls was observed during the growing and entire fattening periods. Within these periods, *E. coli* and *C. perfringens* - but not *P. multocida* - were isolated at a lower frequency from probiotic-treated rabbits ( $p < 0.05$ ). Compared to the control animals, probiotic-treated rabbits were 54 g and 123 g heavier at the end of the growing and finishing phases, respectively, and had significantly higher average daily gain and better feed conversion ratio ( $p < 0.05$ ). (**Key Words** : Probiotic, Health, Production, Rabbits)

### INTRODUCTION

Weaning, a crucial period for all young animals, is associated with a lot of stress caused by the separation from the mother, the transfer into different environment with other mates and the nutritional alterations. This stress cannot be easily tolerated by a young animal, resulting in elevated cortisol levels in systemic circulation and hence immunosuppression and increased sensitivity to diseases (Kanitz et al., 2004; Otten et al., 2002; Morrow-Tesch and Andersson, 1994). Nutritional alterations, in particular, cause microflora disturbances at the level of intestine and disrupt local immunological mechanisms, leading to increased frequency of enteric diseases (O'hara and Shanahan, 2006). In addition, systemic immunological defence via impaired digestion and absorption of nutrients

can be greatly affected (O'hara and Shanahan, 2006).

Prevention or control of both preweaning and post weaning enteric diseases was shown to be achieved by the incorporation of antibiotics in the feed of the young animals (Kyriakis et al., 1996; Berge et al., 2005). However, the demand for withdrawal of antibiotics from the feed of farm animals represents a challenge for researchers to explore less harmful alternative ways particularly on prophylaxis level. On this direction, probiotics are live cultures of harmless bacteria or yeast species (e.g. *Lactobacillus spp.*, *Streptococcus spp.*, *Saccharomyces spp.* etc.) that equilibrate intestinal microflora to the benefit of the animal (Fuller, 1989; 1991). It has been shown that, they may have a growth promoting activity by competing with harmful gut flora, and stimulating the immune system. Therefore, they increase resistance to infectious agents (Fuller, 1989; Cross 2002; O'hara et al., 2006). The positive effect of probiotics on the control of certain pathogens in animals has been shown in several studies, where they appear to control enteric diseases associated with *Escherichia coli* or other enteric pathogens (Abe et al., 1995; Alvarez et al., 2001; Kritas and Morrison, 2005; Timmerman et al., 2005).

As in other species, weaned rabbits develop several ill conditions that may influence their entire fattening period. The effect of probiotic administration on their health and

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Received March 31, 2007; Accepted December 2, 2007

performance has been investigated to a limited scale, mainly in rabbit farms with high mortality rates throughout the fattening period (13-22%) (Kustos et al., 2004; Trocino et al., 2005). It was found that administration of probiotics in fattening rabbits had improved growth performance and morbidity or/and mortality (Kustos et al., 2004; Trocino et al., 2005). However, no studies exist regarding the possible relation between probiotic addition and digestive health in rabbits (Fortun-Lamothe and Boullier, 2007). The objective of the current experiment was to determine the effect of a thermostable probiotic containing *Bacillus licheniformis* (DSMZ 5749) and *B. subtilis* (DSMZ 5750) on health parameters (mortality, and gut and lung microbiology) and performance (growth rate, feed conversion ratio) for fattening rabbits from weaning until slaughter, reared in a rabbitry with moderate health status (5-9%).

## MATERIALS AND METHODS

### Farm and animals

The trial was performed in a commercial rabbitry of 1,000 does, with an average of 6.5 parturitions per doe per year and 8 weaned rabbits per litter. Weaning of the rabbits took place at 35-38 days of age, and each week 550-650 animals were placed in wire fattening cages until slaughter at 90-93 days of age. Each fattening cage was subdivided in 4 compartments of 2 rabbits each. All 8 rabbits of the cage had a central common feeder. The cages were organized in a pyramid structure (48 cages formed longitudinally a row, and 3 rows formed a pyramid). The pyramids were placed in a closed building under controlled and constant micro-environment. A standard light:dark hour pattern of 16L:8D was provided using artificial illumination to encourage maximum feed intake whereas temperature was maintained between 18 and 23°C. The flow of fattening rabbits in the farm was continuous.

The fattening rabbits were crosses of New Zealand×California does and BoscaxNew Zealand bucks. Does were vaccinated for viral hemorrhagic disease. The average annual post-weaning mortality was 5-9%, between 45 and 65 days of age, and it was considered as being intermediate among the Greek rabbitries.

### Feeds

During the regular operation of the farm, a basic feed with crude protein 17%, crude fat 4%, crude fiber 17%, ash 10%, moisture 12.5% and digestible energy 10.4 MJ/kg was fed to the rabbits up to slaughter age, based on the following raw materials: lucerne meal (28%), wheat bran (16.5%), barley (15%), soya bean meal (15%), sun flower meal (15%), mollasses (4%) and soya oil (3%). Ten days prior to 4 days post-weaning, antibiotics (sulphadiazine/trimethoprim 375/75 ppm and tiamulin 100 ppm) were

incorporated in the feed in order to keep morbidity and mortality low. In addition, 60 ppm Robenidin (Cycostat 66G from Alpharma AS) was included in the fattening feed as coccidiostat up to 5 days prior to slaughter. All feeds were in the form of 3 mm-pellets and prepared in pelletizing temperature of 72°C and steam pressure of 2 Atm. From the first day after weaning, rabbits were offered daily 200 g feed per cage, increasing to *ad libitum* consumption at the 7<sup>th</sup> day post-weaning and thereafter. Fresh feed was supplied daily.

### Experimental substance

BioPlus<sup>®</sup> 2B (Chr. Hansen A/S, Denmark) is a thermostable probiotic containing *B. licheniformis* (DSMZ 5749) and *B. subtilis* (DSMZ 5750) spores in a 1:1 ratio. It contains  $3.2 \times 10^9$  total colony-forming units/g of product. Both component microorganisms of BioPlus 2B are registered in Annex II of 70/524 European Union Directive as safe for use as feed additives when used according to the manufacturer's instructions and with the target animal categories specified. The product was incorporated during pelleting at the dose of 400 g/T feed.

### Experimental design

For this trial a total of 1,680 rabbits of 4 sequential weaning batches, further randomly allocated in 210 cages (30, 48, 54 and 78 cages per batch 1, 2, 3 and 4, respectively), were used in two experimental treatments (105 cages per treatment). The rabbits of the first treatment (group C, control or untreated group) were offered the basal feed as previously described. In the second treatment (group P or probiotic group) and for the same period of time, the rabbits were offered the same feeds as group C, but including BioPlus 2B from 4 days postweaning (41 day of age) up to the age of 88 days. All other aspects of management and feeding of rabbits before and after the experimental period were common to both groups.

The treatments were allocated at random within each of the blocks. Each cage (8 rabbits per cage) served as experimental unit. The initial replicates (cages) that were formed at the beginning of the experiment remained the same through slaughter.

### Biosecurity measures

In order to minimize risk of carry over effects or transfer of active agents among treatment groups the following recommendations were considered.

The feed of group C was prepared prior that of group P and was stored in sealed bags to avoid contamination with spores.

The personnel involved in the experiment followed a designated procedure of washing and disinfecting hands when working with animals of different treatments.

**Table 1.** Mortality of fattening rabbits after administration of probiotics

Period (age)	No of dead rabbits/No of total rabbits (%)		p*
	Control-group	Probiotic-group	
Growing (38-62 days)	56/840 (6.7)	35/840 (4.2)	0.02
Finishing (63-93 days)	25/784 (3.2)	20/805 (2.5)	0.40
Total fattening (38-93 days)	81/840 (9.6)	55/840 (6.5)	0.02

\* Pearson chi square (p = 0.05).

The equipment used for feeding was different for the different treatments.

Feeders were completely separated between cages, and there was not any contact between rabbits of side cages. The experimental rabbits were housed in cages located in separate rows. At all times, rabbits were maintained in their respective treatment groups (i.e. there was no mixing).

### Measurements and sampling procedures

A composite sample from each feed was obtained and analysed before the start of the experiment, to ensure that both feeds were similar in their chemical composition and non-limiting in mineral and vitamin content. All rabbits were monitored daily for clinical signs of any disease. When necessary only injectable medication was administered. If there was any dead animal, then its bodyweight and the possible cause of death, based on post-mortem examination, was recorded. Moreover, detailed microbiological examinations, depending on the time when the dead animal was found, were performed following its immediate shipment to the laboratory.

Weighing the rabbits of each cage was performed at weaning, at end of growing period and at the end of finishing period (37, 62 and 93 days of age, respectively).

Feed refusals in feeders of individual cages were recorded on 146 (73 control and 73 probiotic) out of the 210 cages. Fresh feed was added in each feeder daily. At the end of growing and finishing periods, the rest of the remaining feed was subtracted, and feed consumption of that period was calculated. If one or more animals in a cage had died, then their estimated feed intake for the respective period has been subtracted from the total amount of feed consumed by the cage animals during that period.

The average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for each period and for the entire duration of the experiment; such ratios were expressed on the basis of the remaining living rabbits.

### Microbiological examinations

Samples from the lungs (all lobes) and the small intestine (ileum) were collected from rabbits that had died recently or have been put down because of the severity of clinical symptoms associated with pneumonia or diarrhea, respectively.

Enumeration of *E. coli* (feces) and *Clostridium*

*perfringens* (mucosal scrapings) was performed in all samples, using sheep blood agar and MacConkey agar under aerobic conditions, and sheep blood agar under anaerobic conditions, respectively. API 20E and API 20A identification system (BioMerieux SA, Marcy l'Etoile, France) were used for biochemical identification of the isolates. Lung samples were cultured on sheep blood agar for *P. multocida* detection, while identification to the species level was performed using standard test (Quinn et al., 1994).

Feed samples were sent to Chr Hansen A/S (Horsholm, Denmark) to confirm the presence of *B. licheniformis* and *B. subtilis* spore content in the feed of P-group and the absence of spores in the feed of C-group. In order to document the recovery of *Bacillus* spores, caeca of 12 randomly selected rabbits per treatment, slaughtered at 93 days of age, were stored immediately at -20°C until later shipment to Chr. Hansen A/S (Denmark) for microbiology.

### Statistical analysis

The SPSS statistical program (Version 13.0 for Windows, 2004; SPSS Inc., USA) was used for the statistical analysis of the data. The Pearson chi square test was used for the evaluation of mortality and death causes, and the independent samples T-test analysis for productive parameters.

## RESULTS

The microbiological examinations in feed samples confirmed the presence of *B. licheniformis* and *B. subtilis* spores in the feed of P-group and the absence of spores in the feed of C-group. In addition, *Bacillus* spores were recovered from all ceecal samples of the treated rabbits and from none sample of the untreated rabbits.

In general, health status of the rabbits was satisfactory, and mortality was within the normal expected annual variations of the farm (Table 1). Nevertheless, mortality has been significantly reduced after treatment with probiotics, compared to the control rabbits during the growing period (4.2% and 6.7% respectively) (p<0.05). Such a beneficial effect was not seen during finishing stage, but it was observed during the overall fattening period (p<0.05).

The results regarding the presence of *Escherichia coli*, *Clostridium perfringens* and *Pasteurella multocida* in the faeces, intestine and lungs of fattening rabbits are presented

**Table 2.** Presence of *Escherichia coli* (>10<sup>7</sup> cfu/g), *Clostridium perfringens* (>10<sup>7</sup> cfu/g) and *Pasteurella multocida* in the faeces, intestine and lungs of fattening rabbits, respectively, after administration of probiotics

Period (age)		No of infected rabbits/No of total rabbits (%)		p*
		Control-group	Probiotic-group	
Growing (38-62 days)	<i>E. coli</i>	50/840 (6.0)	29/840 (3.5)	0.02
	<i>C. perfringens</i>	46/840 (5.5)	28/840 (3.3)	0.03
	<i>P. multocida</i>	18/840 (2.1)	10/840 (1.2)	0.13
Finishing (63-93 days)	<i>E. coli</i>	20/784 (2.6)	14/805 (1.7)	0.26
	<i>C. perfringens</i>	23/784 (2.9)	14/805 (1.7)	0.11
	<i>P. multocida</i>	24/784 (3.1)	19/805 (2.4)	0.39
Total (38-93 days)	<i>E. coli</i>	70/840 (8.3)	43/840 (5.1)	0.01
	<i>C. perfringens</i>	69/840 (8.2)	42/840 (5.0)	0.01
	<i>P. multocida</i>	42/840 (5.0)	29/840 (3.5)	0.11

\* Pearson chi square (p = 0.05).

**Table 3.** Growth performance in the groups of rabbits at different fattening stages

Period (age)	Control-group	Probiotic-group
Average body weight (g)±SD	n = 105	n = 105
At weaning (38 day)	1,025 <sup>a</sup> ±162	1,019 <sup>a</sup> ±153
End of growing (63 day)	1,892 <sup>a</sup> ±123	1,946 <sup>b</sup> ±126
End of finishing (93 day)	2,689 <sup>a</sup> ±142	2,812 <sup>b</sup> ±140
Average daily gain (g)±SD	n = 105	n = 105
Growing (38-62 days)	34 <sup>a</sup> ±5	37 <sup>b</sup> ±4
Finishing (63-93 days)	27 <sup>a</sup> ±5	29 <sup>b</sup> ±5
Total fattening (38-93 days)	30 <sup>a</sup> ±3	33 <sup>b</sup> ±3
Average daily feed intake (g feed)±SD	n = 73	n = 73
Growing (38-62 days)	99 <sup>a</sup> ±18	97 <sup>a</sup> ±15
Finishing (63-93 days)	135 <sup>a</sup> ±23	137 <sup>a</sup> ±21
Total fattening (38-93 days)	121 <sup>a</sup> ±22	120 <sup>a</sup> ±16
Feed conversion ratio±SD	n = 73	n = 73
Growing (38-62 days)	2.90 <sup>a</sup> ±0.46	2.67 <sup>b</sup> ±0.41
Finishing (63-93 days)	5.15 <sup>a</sup> ±1.38	4.66 <sup>b</sup> ±0.84
Total fattening (38-93 days)	4.01 <sup>a</sup> ±0.72	3.65 <sup>b</sup> ±0.41

\* Different superscripts in the same row denote statistically significant difference (p≤0.05).

in Table 2. During the growing phase, the main reason of mortality was severe diarrhoea. Isolation of *E. coli* and *C. perfringens* was most frequent from the dead control rabbits, followed by isolation of *P. multocida*. The addition of probiotics in the feed had significantly reduced isolation of both *E. coli* and *C. perfringens* (p<0.05), but not of *P. multocida* (p>0.05).

In finishing phase, the main reason of mortality was respiratory problems. Isolation of *E. coli* and *C. perfringens* from the dead rabbits was less frequent compared to that observed in the growing stage, while almost all dead rabbits of both groups harboured *P. multocida* (Table 2). The addition of probiotics in the feed did not have any significant effect in the frequency of isolation of any bacterium (p>0.05).

During the overall fattening period, a significant decrease in isolation of both *E. coli* and *C. perfringens* had resulted after probiotic administration (p<0.05), while no effect was observed on *P. multocida* (p>0.05).

The results regarding the growth, feed intake and feed conversion ratio of rabbits are shown in Table 3. The

average bodyweight between the 2 groups of rabbits was similar at the beginning of the trial. However, rabbits treated with probiotics (group P) were 54 g and 123 g heavier at the end of growing and finishing stage, respectively, compared to control rabbits (p<0.05). Although the average daily feed intake was similar between the 2 groups throughout each period separately and collectively, the average daily gain and the feed conversion ratio of the probiotic-treated rabbits was significantly improved during all the examined growth periods compared to the control rabbits (p<0.05).

## DISCUSSION

Due to the unique physiology of their digestive tract, rabbits usually show a fragile balance in their gut function. That is why they frequently suffer of postweaning alimentary disturbances. Probiotics, known to work well in other monogastric animal species (Abe et al., 1995; Alvarez et al., 2001; Kritas and Morrison, 2005; Timmerman et al., 2005), were also shown to improve growth performance

and morbidity or/and mortality in rabbit farms with high mortality rates throughout the fattening period (13-22%) (Kustos et al., 2004; Trocino et al., 2005). The results of the present study confirmed these observations. The relatively high level of mortality can be partly attributed to the continuous flow system of the farm that allows rapid spread of pathogens and improper disinfections. An attempt to record basic microbiological counts in the presence or absence of probiotics was additionally made in the present study. Major potential pathogens such as *E. coli* and *C. perfringens* were shown to get reduced in rabbits after probiotic treatment. It is possible that this is the result of optimization of enteric commensals over pathogenic bacteria. It is known that some probiotics may have an inhibitory effect in *E. coli* in the intestine in dose-dependent manner (Mattar et al., 2001). The reason for this is not known. It maybe that they promote changes on enteric microbiota, so that some pathogens cannot adhere effectively (Mattar et al., 2001). Lee and co-workers (2000) had shown that experimental neonatal rabbits receiving probiotics have reduced by 25% *E. coli* counts in their small intestine. In addition these rabbits had significantly decreased frequency of bacteria translocation in lymphnodes, spleen and liver, indicating a reduced possibility of systemic infection (Lee et al., 2000). Similarly, some probiotics were also shown to reduce *C. difficile* - associated disease in humans (Bengmark 2005; McFarland 2006; Yamano et al., 2006). It may be that probiotics can reduce adhesion of this pathogen on mucus (Rinkinen et al., 2003), or may displace pre-adhered *C. difficile in vitro* (Collado et al., 2006). Of course the effects against a pathogens greatly depend upon the probiotic microorganism. Thus for instance certain probiotic isolates were shown to be more effective against *C. perfringens* and less against *E. coli* in chickens (Kizerwetter-Swida and Binek, 2005).

Growth parameters are not only useful for monitoring economic performance but also for the evaluation of animal health, particularly in diseases of a chronic or mild character in which visible clinical signs are often absent (e.g. Kritas and Morrison, 2006). In the current study, bodyweight, ADG and FCR of treated rabbits were greatly improved compared to the untreated group. This suggests that the health status of the treated animals was better. The improvement range of the parameters was also economically important, since 4.7% higher bodyweight or 6.7% higher ADG have been observed in probiotic treated rabbits, a range that is expected in other species only after the continuous use of growth promoters from weaning to slaughter (Buttery, 1993).

The results of this study showed that that administration of the probiotic BioPlus 2B at 400 g /ton of feed to fattening rabbits starting 4 days post weaning up to 5 days prior the slaughter age reduces mortality and the presence

of *E. coli* and *C. perfringens* in the faeces, and improves growth performance characteristics (ADG and FCR).

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