EFFECT OF INCLUSION OF PROBIOTICS ON MICRO-ORGANISMS CONTENT, HEALTH AND PERFORMANCE OF FATTENING RABBITS: 1. STUDY IN A COMMERCIAL FARM WITH INTERMEDIATE HEALTH STATUS

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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%, 1680 rabbits in 2 treatment groups (4 weaning batches, 105 cages per treatment, each cage of 8 rabbits) were fed with: a) a basic feedor; b) the same basic feed supplemented with probiotic from the 4th day postweaning (41th days of age) up to 88th day of age. The rabbits were slaughtered on an average of 93 days. Clinical signs, microbiological status (enumeration of *E. coli* and *C. perfringens* and presence of *P. multocida*) 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 control ones was observed during 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 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 and increased sensitivity to diseases. 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 (Kritas and Morrison, 2005; 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 such as probiotics. 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 (Cross, 2002; O'hara and Shanahan, 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; 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 performance has been investigated mainly in rabbit farms with high mortality rates throughout the fattening period (13-22%), where 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* and *B. subtilis* 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

Animals and experimental design

The trial was performed in a commercial rabbitry of 1,000 does. Weaning of the rabbits took place at 35-38 days of age, and each week 550-650 animals were placed in wire fattening cages of 8 animals until slaughter at 90-93 days of age. 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 23ºC. The flow of fattening rabbits in the farm was continuous. The fattening rabbits were crosses of New Zealand x California does and Boscat x New Zealand bucks. Does were vaccinated for viral hemorrhagic disease.

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. From ten days before weaning until 4 days post-weaning, antibiotics (sulphadiazine/trimethoprim 375/75 ppm and tiamulin 100 ppm) were incorporated in the pelleted feed in order to keep morbidity and mortality low. In addition, 60 ppm Robenidin was included in the fattening feed as coccidiostat up to 5 days before to slaughter. Fresh feed was supplied daily.

Experimental substance

BioPlus® 2B (Chr. Hansen A/S, Denmark) is a thermostable probiotic containing *B. licheniformis* and *B. subtilis* spores in a 1:1 ratio. It contains 3.2 x 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 1680 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, while those of the second treatment (group P or probiotic group) the same feeds, but including BioPlus 2B from 4 days postweaning (41 day of age on average) up to the age of 88 days. All other aspects of management and feeding of rabits before and after the experimental period were common to both groups. Each cage (8 rabbits per cage) served as experimental unit.
Appropriate biosecurity measures to minimize risk of carry over effects or transfer of active agents among treatment groups were considered such as separate preparation and storage of feeds, washing and disinfecting hands of the responsible personnel, using different equipment, no contact between groups (cages located in separate rows).

Measurements and sampling procedures

A composite sample from each feed was analysed before the start of the experiment, to ensure that both feeds were similar in their chemical composition. All rabbits were monitored daily for clinical signs of any disease. 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 (38, 63 and 93 days of age on average, respectively). Feed refusals in feeders of individual cages were recorded on 73 control and 73 probiotic cages. 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 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. These animals were autopsied, and cotton swabs were used to collect the samples. Enumeration of *E.coli* (feces) and *Clostridium perfringens* (mucosal scrapings) was performed in all samples (setting arbitrarily a threshold of 10^7 CFU per g), while lung samples were cultured on sheep blood agar for determining merely the presence *P. multocida*. 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 ^{o}C until later shipment to Chr. Hansen A/S (Denmark) for microbiology.

Statistical analysis

The Pearson chi square test was used for the evaluation of mortality, death causes and microbiological counts, and the independent samples T-test analysis for productive parameters (SPSS Inc., USA).

RESULTS AND DISCUSSION

The microbiological examinations in feed samples confirmed the presence of probiotic bacteria 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 ceacal 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. 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. 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 1). 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* ($P>0.05$).

Table 1: Presence of *Escherichia coli* (>10^7 cfu/g), *Clostridium perfringens* (>10^7 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 (%)		
		Control-group	Probiotic-group	P*
Growing (38-62 days)	*E. coli*	50/840 (6.0)	29/840 (3.5)	0.02
	C. perfringens	46/840 (5.5)	28/840 (3.3)	0.03
	P. multocida	18/840 (2.1)	10/840 (1.2)	0.13
Finishing (63-93 days)	*E. coli*	20/784 (2.6)	14/805 (1.7)	0.26
	C. perfringens	23/784 (2.9)	14/805 (1.7)	0.11
	P. multocida	24/784 (3.1)	19/805 (2.4)	0.39
Total (38-93 days)	*E. coli*	70/840 (8.3)	43/840 (5.1)	0.01
	C. perfringens	69/840 (8.2)	42/840 (5.0)	0.01
	P. multocida	42/840 (5.0)	29/840 (3.5)	0.11

* Pearson chi square (P=0.05)

The results regarding the growth, feed intake and feed conversion ratio of rabbits are shown in Table 2. 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 two 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).

Table 2: Growth performance in the groups of rabbits at different fattening stages

Period (age)	Control-group	Probiotic-group
Average bodyweight (g) ± SD	n=105	n=105
At weaning (38 day)	1025^a ± 162	1019^a ± 153
End of growing (63 day)	1892^a ± 123	1946^b ± 126
End of finishing (93 day)	2689^a ± 142	2812^b ± 140
Average daily gain (g) ± SD	n=105	n=105
Growing (38-62 days)	34^a ± 5	37^b ± 4
Finishing (63-93 days)	27^a ± 5	29^b ± 5
Total fattening (38-93 days)	30^a ± 3	33^b ± 3
Feed conversion ratio± SD	N=73	n=73
Growing (38-62 days)	2.90^a ± 0.46	2.67^b ± 0.41
Finishing (63-93 days)	5.15^a ± 1.38	4.66^b ± 0.84
Total fattening (38-93 days)	4.01^a ± 0.72	3.65^b ± 0.41

* Different superscripts in the same row denote statistically significant difference (P≤0.05)

Probiotics 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 *et al*. (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. Similarly, some probiotics were also shown to reduce *C. difficile* – associated disease in humans (McFarland, 2006).

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 (Kritas and Morrison, 2007). 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.

CONCLUSIONS

The results of this study showed that administration of the probiotic BioPlus 2B at 400 g/T of feed to fattening rabbits from 4 days post weaning until 5 days before the slaughter age reduces mortality and the presence of *E. coli* and *C. perfringens* in the faeces, and improves growth performance (ADG and FCR)..

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