Effects of probiotic dietary supplementation on diarrhoea patterns, faecal microbiota and performance of early weaned calves

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ABSTRACT: Twenty Lithuanian Black-and-White calves (10 bulls, 10 heifers) were used to evaluate the effects of the supplemental probiotic product, *Enterococcus faecium* M74 (2.4 g/day/calve), added to fresh milk and skimmed milk in a 56 day-study. The probiotic was administered by dietary supplementation to first group of calves and their respective pens (probiotic group), whereas the second group (control group) received no probiotic supplementation. The results of this trial indicate positive effects of the probiotic product *Enterococcus faecium* M74. The actual percentage of calves with diarrhoea was reduced from 50 % to 20% among the calves fed the pre-and probiotic diet. Probiotic supplementation reduced the faecal count of clostridia and enterococci. The calves fed *Enterococcus faecium* M74 weighed more at 20, 40 and 62 days of age by 4.9%, by 9.7% ($P < 0.05$) and by 9.4% ($P < 0.01$), respectively, than the control calves. The calves fed *Enterococcus faecium* M74 had increased daily weight gains compared with the calves not fed a probiotic product. The average weight gain and the daily weight gain of the probiotic-supplemented calves were by 7.8 kg ($P < 0.01$) and by 0.14 kg higher ($P < 0.01$) compared with the control calves. The calves given the *Enterococcus faecium* M74 also had forage and total DM intakes that were numerically higher than those fed the control diet, without any additive. During the 56 days experimental period, the average feed conversion rate was improved by 12.9% in the probiotic-treated group.

Keywords: dairy calves; *Enterococcus faecium*; growth rate; clostridia

In recent years, producers worldwide have been looking for management techniques and products to improve the performance as well as the health of weanling piglets and calves. With the EU ban on the use of antibiotic feed additives, substitutes such as antibiotic growth promoters, (e.g. probiotics) must be found for the entire field of animal husbandry in general, and particularly for young animals, such as weaned piglets and calves. The main effects of these feed additives are improved resistance to colonization with pathogenic bacteria and enhanced host mucosa immunity resulting in a reduced pathogen load and improved health status of the animals (Choct, 2009). Intestinal bacteria are an integral component of the intestinal immune system. The intestine, particularly the large intestine, is inhabited by a diverse population of bacteria that perform a variety of functions such as acting as a barrier to pathogens and macromolecules of the digestive epithelium. When the homeostatic control is disturbed, chronic inflammation, diarrhoea and disease may occur. A normal intestinal bacterial flora is critical for maintaining health. A key part of the function of the flora is to "outcompete" the pathogenic bacteria and keep them from becoming established in the gut (Donohue et al., 2002). When an animal is exposed to significant stress, it is possible for the growth of these normal enteric bacteria to become impaired. This allows for the growth of potential pathogens, thereby increasing the risk of disease (Nabuurs et al., 2001; Soderholm and Perdue, 2001).

In bovine production systems, the critical stage of growth is the transition from the monogastric condition, when fed with milk, to the herbivore condition, in which their pre-gastric fermentative cameras must be completely active to effectively digest fibrous intake (Davis and Drackley, 1998; Bloom, 2006). Probiotics are a general category of dietary products that can be included in animal rations to enhance performance and/or reduce pathogenic bacteria (Fuller, 1989; Collins and Gibson, 1999). The studies of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria, even if the word "probiotic", used to denote substances produced by microorganisms which promoted the growth of other microorganisms, was not coined until 1960 (Lilly and Stillwell, 1965). A probiotic was initially defined as a 'live microbial feed supplement which beneficially affects the host animal by improving intestinal microbial balance' (Fuller, 1989). A similar formulation was proposed by Havenaar and Huis in't Veld (1992) who defined a probiotic as "a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora". A more recent, but probably not final definition is that of "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma, 1998). Nowadays, probiotics have been classified as zootechnical feed additives (EC regulation 1831/2003) and (FAO/WHO, 2001) expert Consultation on Health and Nutritional properties of powder milk with live lactic acid bacteria suggest that probiotics, live microorganisms administered in adequate amounts that confer a health effect on the host, are emerging as significant dietary ingredients in the field of nutrition. In recent years, the technical definition of probiotics has been broadened to include products containing microbes or their end products (i.e., fermented dairy products, etc.) and the effect is not necessarily restricted to a change in colonization *per se*. A proposed definition for probiotics is 'a preparation or a product containing viable, defined micro-organisms in sufficient numbers, which alter the micro-flora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host' (Schrezenmeir and de Vrese, 2001). The use of probiotics is expected to improve BW gain, feed conversion and health of livestock because probiotics promote the establishment of a beneficial gut flora and inhibit the growth of pathogenic bacteria in the intestine. Many researchers have studied the use of lactobacilli and streptococci as probiotics (Fuler, 1989), especially enteroccocus probiotics (Fleige et al., 2007; Samli et al., 2007 Vahjen et al., 2007). Some probiotic compounds are claimed to have formulae designed to provide suitable conditions in the alimentary tract so as to minimize the incidence of diarrhoea, therefore effecting improvements in body weight gain, body height and general health condition. *Enterococcus faecium* is a lactic acid bacterium that is a normal inhabitant in the gut and that shows effects against enteropathogens (Willard et al., 2000; Benyacoub et al., 2005). Studies on the efficacy of probiotics and prebiotics in animals and man have often produced contrasting results: these can derive from the heterogeneity of the experimental protocol utilized and the experimental conditions. Therefore, this study was undertaken to investigate diarrhoea incidence, growth performance and feed efficiency characteristics of Lithuanian Black-and-White calves fed fresh milk and skimmed milk diets supplemented with probiotic bacteria *Enterococcus faecium* M74 under farm conditions.

MATERIAL AND METHODS

As recommended by the Scientific Committee on Animal Nutrition (SCAN), the efficacy of the probiotic product was assessed according to Directive No. 87/153/EEC. The feed mixture and milk did not contain any medicated additives. The experiment was arranged and conducted in due form using animal number in groups and number of groups that are satisfactory for establishing the minimum claimed response. The experiment was conducted under good hygienic conditions and the feeding trial was performed in pursuance with the Lithuanian animal care, management and operation legislation (No 8-500, 28 November 1997, No. 108).

Experimental conditions. The trial was carried out in a calf house with separated pens, each of which was equipped with a feeding trough and a watering trough as required for calves. All pens were located in the same calf house and the calves were randomly allocated. The total area per pen was 8.5 m^2. The calf house was equipped with controlled ventilation and the bedding in the pens was chopped straw. Manure was removed daily and chopped straw was once again given to all pens. Temperature, air humidity and concentration of ammonia in the calf house were monitored. Average temperature was 14.5 ± 2.0 °C, relative humidity was 75.0 ± 5.0% and ammonia concentration averaged at 6.5 mg/m^3.

Experimental animals. Lithuanian Black-and-White calves were taken from their dams at six days of age and 20 calves on the basis of initial weight and sex were selected for the trial. All calves were marked with earmarks after birth and dehorned with an electric dehorner. The actual trial period started at weaning (day 0) when the calves were six days old and stopped 56 days later. At weaning the calves were divided into two analogous groups according to weight and each group was moved to a pen in the calf house. The gender of the calves was also considered. There were 10 calves in the experimental group and 10 calves in the control group. Each group consisted of five male calves and five female calves**.** The initial average weight of the calves in the two groups was identical, i.e., 41 kg. Each group was further divided into two groups of five calves and placed into two separate pens. To avoid infection caused by *E. coli* all calves were vaccinated. The health status of the calves was monitored daily with particular attention paid to the occurrence of diarrhoea. All calves were given a diarrhoea score according to the following scale: 0 = firm, no signs of diarrhoea, 1 = soft, slightly loose faecal consistency and 2 = liquid, very loose faecal consistency. For each calf the daily scores and the number of days with liquid faeces (score 2) were summed into an index of the severity of the diarrhoea.

Feeds and feeding. During the entire trial the calves were given fresh milk and skimmed milk two times a day and had free access to meal feed, hay and grass silage. One and the same meal feed was used for the whole trial period. The feed mixture contained no growth promoters. Hay and silage were made at the agriculture enterprise and were of high quality. The composition and the analyzed nutrient content of the offered feeds are provided in Table 1.

Feed mixture, hay and silage were supplied daily and refusals were recorded (Table 2). The total consumption of feed per day was recorded per pen.

Table 1. The composition and analysed nutrient content in the diet fed to the calves over 56 days from six days of age

Composition (%)	Compound feed	Fresh milk	Skim milk	Hay	Silage
Barley	33.0				
Wheat	25.0				
Soybean meal	14.0				
Rapeseed cake	14.0				
Fish meal	10.0				
Dicalcium phosphate	2.7				
Sodium chlorine	0.3				
Mineral and vitamin supplement*	1.0				
Analyzed content					
Dry matter (%)	87.8	12.5	10.0	83.0	33.4
Crude fibre (g/kg)	48.0	–	–	26.0	80.7
Crude protein (g/kg)	196.0	34.4	35.2	79.0	42.7
Fat (g/kg)	32.0	37.6	13.0	8.0	11.8
Calcium (g/kg)	11.9	1.3	1.4	2.4	2.5
Phosphorus (g/kg)	8.1	1.1	1.2	2.3	0.8
Zinc (g/kg)	–	–	–	–	–
Lysine (g/kg)	10.0	–	–	–	–
Methionine + cystine (g/kg)	8.3	–	–	–	–
Metabolizable energy (MJ/kg)	11.7	2.4	2.0	6.3	2.8

*Provided (pp/kg): iron 121.3, manganese 74.5, iodine 0.40, vitamin A 12 000 IU/kg, vitamin D_3 1200 IU/kg, vitamin E 40 IU/kg

Table 2. Feeding programme

Age		Live weight at the end of period	Feed per calf per day (kg)				
			milk		compound feed	hay	silage
month	10 days		fresh milk	skim milk			
I	1		6	–	–	–	–
	2		6	–	0.1	–	–
	3	51	4	2	0.2	0.1	–
II	4		4	2	0.3	0.2	–
	5		3	3	0.5	0.3	0.2
	6	72	2	4	0.8	0.4	0.5
Total per 2 months			250	100	19	10	7

The milk, feed mixture, hay and silage used to feed the experimental as well as the control calves had the same nutritional value and quality. The only difference was that the milk feed for the experimental calves contained the probiotic content of live *Enterococcus faecium* M74 at 50 × 10^9 CFU/gram. *Enterococcus faecium* M74 was added to the milk to the final concentration of 20 × 10^9 CFU/kg of milk. Milk samples were analyzed with regard to the content of *Enterococcus faecium* M74 in CFU/kg at the Lithuanian National Veterinary Laboratory (Table 3).

Weighing of the calves. All calves were weighed at the start (0 day) of the trial, i.e., at weaning (six days of age), at day 14 of the trial (20 days age), at day 34 of the trial (40 days of age) and at the end (56^{th} day) of the experiment when the calves were 62 days of age.

Monitoring of health. As a routine on the farm, all occurrences and treatments of disease and injuries were noted individually. Faecal samples were collected from three to five calves from each group on the first day as well as during the last week of the trial and microbial groups were cultivated in the State Veterinary Laboratory.

Statistical evaluation. A one-way generalized linear model (GLM) analysis was used in a randomized complete block design with feed-additive treatment as the main factor. When feed intake and feed conversion rates were statistically analyzed, one pen containing five calves was used as the experimental unit. This was done because feed-intake was only determined for a group of calves. For weight and weight gain respectively, each calf within a pen was used as the experimental unit. This was done because weights could be determined for each individual calf. Due to the limited number of observations in the study, no adjustments for differences in initial weights of calves were made. The Fisher's least significant difference (LSD) procedure at 1% and 5% significance level was used to determine differences in treatment means. The results were analysed using GLM of SAS.

Table 3. The content of *Enterococcus faecium* M 74 (in CFU/kg) in milk for calves

10 days	Milk without probiotic	Milk with probiotic supplementation (0.4 g/1 l of milk)
1	–	19 × 10^9
2	–	20 × 10^9
3	–	18 × 10^9
4	–	21 × 10^9
5	–	20 × 10^9
6	–	19 × 10^9

RESULTS AND DISCUSSION

Diarrhoea

No calves died during the experimental period. Diarrhoea was seen during the first three weeks after weaning for calves fed the untreated diet, but only during the first week after weaning for calves fed the diet with *Enterococcus faecium* M74. The diarrhoea score calf per day was lower for the *Enterococcus faecium* M74 calves as compared with that of the untreated calves (Figures 1 and 2). Our results with respect to health status and mortality are in agreement with the findings of Gill et al. (1987), Abe et al. (1995) and Abu Tarboush et al. (1996) and confirm the beneficial effects of the probiotics on the health condition and vitality under our experimental conditions.

The dietary *Enterococcus faecium* M74 content as shown in Fig.3 influenced the percentage of calves that had diarrhoea. The percentage of calves with diarrhoea for two or more days was reduced from about 50% to 20% among the calves fed the *Enterococcus faecium* M74 diet. Similarly, Hooper (1989) reported that probiotics decreased by 37.3% the incidence of diarrhoea.

Gorgulu et al. (2003) reported that with respect to occurrence of diarrhoea the probiotic-fed calves were healthier to the control group. They concluded that probiotic administration could improve calf health and decrease mortality and medication cost, the same results as in the present study. Marcin et al. (2003) and Ohya et al. (2001) arrived at the same conclusion for piglets and calves. These results suggest that improvements in the growing performance of calves by probiotics could depend on rearing conditions and on the calf. In our trial, the health status of the calves was improved by probiotics, while it also had positive effects on growth performance. The results obtained in the present experiment suggest that probiotics based on *Enterococcus faecium* improve the health status of calves and can decrease medication costs under these experimental conditions.

The effects of the probiotic on the microflora in the faeces of the calves are presented in Table 4. Probiotic supplementation reduced the stock of clostridia and increased enterococci in faeces.

Weight and weight gains of the calves

The average weight of the calves in the control and experimental groups at the start (six days of age) was comparable, i.e., 41.00 kg in the control group and 40.80 kg in the experimental group. A positive ef-

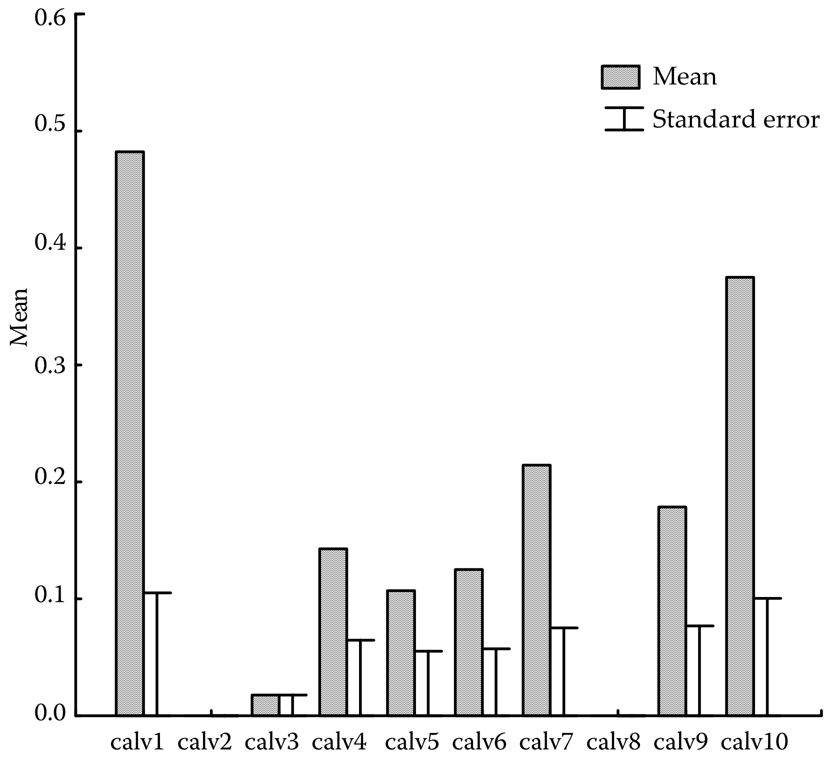

Figure 1. The diarrhoea score calf per day during the experimental period (56 days), control calves

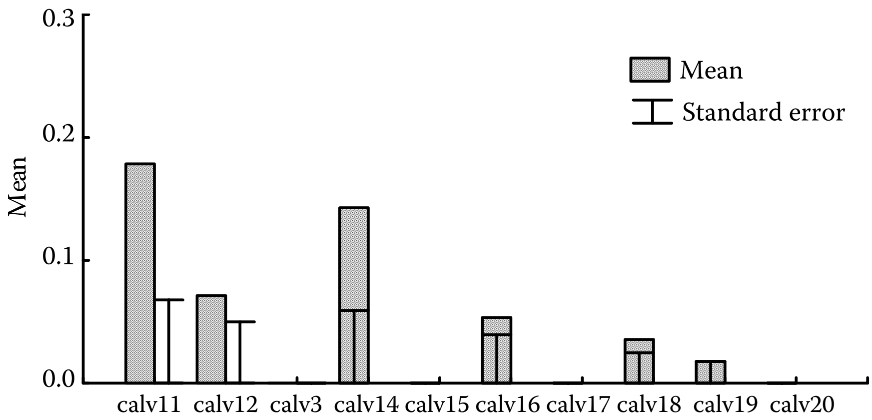

Figure 2. The diarrhoea score calf per day during the experimental period (56 days), *Enterococcus faecium* M74 calves

fect on the growth produced by the probiotic became evident when weighing the calves after 20 days of the trial. The average weight of the calves in the experimental group was 4.9% (i.e.2.30 kg) ($P < 0.01$) higher than the control. During the following 20 days the difference increased to 9.7%, i.e. 6.00 kg ($P < 0.05$). During the next 22 days, or at the end of the 56-day-experiment the weight of the experimental group of calves was by 9.4%, i.e. 7.60 kg higher ($P < 0.01$) in comparison with the control calves (Table 5).

During the 56-day-trial period (from six days to 62 days) the average weight gain for the calves was 40.1 kg in the control group and 47.9 kg in the experimental group, i.e., the weight gain over the course of the whole trial in the experimental group was therefore 7.8 kg higher compared with

Table 4. Population of faecal micro-organisms

	Faeces	
	calves	
	untreated	*Enterococcus faecium* M74 supplementation 0.4 g/kg feed
Clostridia	1.3×10^5	< 300
Coliforms	not found	not found
Enterococci	4.0×10^3	1.4×10^8
Salmonella spp.	not found	not found
Campylobacter	not found	not found
*E. coli**	+	+

*No pathogenic strains of *E. coli* were found in the faeces of the calves. Toxic strains of *E. coli* were unspecified

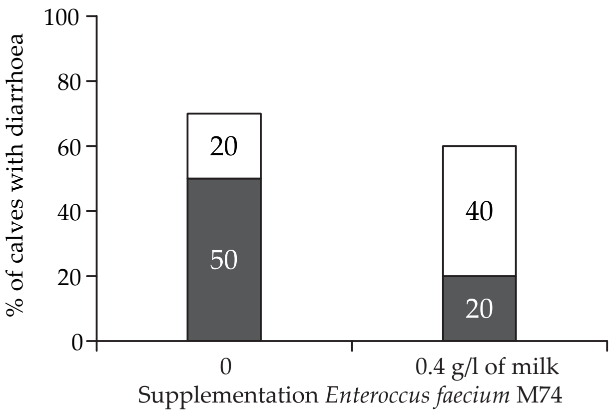

Figure 3. Effects of *Enterococcus faecium* M74 supplementation on the percentage of calves with diarrhoea. The percentage of calves with diarrhoea for one day (blank plus shaded column) and for two or more days (shaded column)

Table 5. Body weight, average daily gains, feed intake and feed conversion rate of calves with probiotic supplemented to their diet

	Supplement *Enterococcus faecium* M74 per basic diet		$LSD_{0.05}$	SE	*P*-value
	0	0.4 g/1 l milk			
Number of calves	10	10			
Body weight (kg/calves)					
at start (6 days)	41.0	40.8	4.00	1.341	ns
at 20 days of age	46.9	49.2	3.76	1.261	ns
at 40 days of age	61.9	67.9	4.93	1.653	0.05
at 62 days of age	81.1	88.7	4.87	1.632	0.01
Daily gain (kg/calves)					
6–20 days	0.42	0.60	0.174	0.058	0.05
21–40 days	0.71	0.89	0.111	0.037	0.01
41–62 days	0.91	0.99	0.069	0.023	0.05
6–62 days	0.72	0.86	0.070	0.024	0.01
Feed intake (kg DM/calf)					
6–20 days	11.16	11.32	2.033	0.113	ns
21–40 days	20.69	22.43	14.739	0.820	ns
41–62 days	36.45	37.42	13.342	0.742	ns
6–62 days	68.3	71.17	3.431	0.191	ns
Feed conversion rate (kg DM feed/kg gain)					
6–20 days	1.92	1.35	2.964	0.165	ns
21–40 days	1.40	1.20	1.904	0.106	ns
41–62 days	1.90	1.80	0.126	0.007	ns
6–62 days	1.71	1.49	1.418	0.088	ns

the control group and this difference was statistically significant ($P < 0.01$).

The experimental calves also grew at a faster rate throughout all the periods of the experiment: from six to 20 days (2.5 kg, $P < 0.05$); from 21 to 40 days (3.7 kg, $P < 0.05$); and from 41 to 62 days (1.6 kg, $P < 0.05$) compared with the control calves.

Table 5 shows that the daily weight gain of the calves fed the probiotic-supplemented diet was significantly higher than that of the calves fed the probiotic-free diet in all trial periods: from six to 20 days by 42.4% ($P < 0.05$); from 21 to 40 days by 25.3% ($P < 0.05$); from 41 to 62 days by 8.7% ($P < 0.05$), and from 6 to 62 days by 19.4% ($P < 0.01$). Abe et al. (1995) reported that probiotics had a beneficial effect on the body weight of newborn calves until 25 days of age. Al-Saiady (2010) reported a significant increase in body weight of calves at five weeks and during the entire experimental period in the group fed probiotics. Morrill et al. (1995) did not observe a significant difference in the body weight of calves fed probiotics during a six week-trial, and likewise neither (Kamra et al., 2002) nor (Gorgulu et al., 2003) reported a significant difference in body weight gain for calves fed probiotics. In the present study, at the end of the experiment the probiotic group's body weight was by 9.4 % ($P < 0.01$) higher than the control group, which is in agreement with Higginbotham and Bath (1993), Abdula et.al. (2002), Hossaini et al. (2010).

Feed intake and conversion rate

Feed consumption in each of the four pens was monitored separately. The milk intake was identical for all 20 calves in both groups, i.e., 6 kg of milk per calf per day. Total dry matter intake was numerically higher in the experimental group in all trial periods compared with the control group. For the entire trial the calves in the experimental group consumed on average 0.22 kg less feed dry matter per kg of weight gain than the calves in the control group (Table 5).Thus, during the 56 day-experimental period, the average feed conversion rate was improved by 12.9% in the probiotic-treated group. Fleige et al. (2007) reported that a high dose of lactulose in combination with *E. faecium* affects the intestinal immune function. Consequently, the calves might be more resistant to diseases and have improved performance. Administration of probiotic strains separately and in combination has been shown by several groups to significantly improve feed intake, feed conversion rate, daily weight gain and total body weight in calves, chicken and piglets (Timmerman et al., 2006; Adams et al., 2008; Awad et al., 2009;). The administration of probiotics reduced the necessity for antibiotic treatments against digestive diseases, decreased medication costs and improved calf performance under the experimental conditions described here. Further studies with calves are required to confirm these data. Such experiments should look at the underlying mechanisms between the diet, gut micloflora and host factors (e.g., immune system). The effect of factors such as microbial species composition (e.g., single or multistrain) and viability, administration level, application method, frequency of application, overall diet, animal age, overall farm hygiene and environmental stress factors on the efficacy of probiotic applications need to be thoroughly assessed. Providing the nutrients that specifically support the growth of beneficial bacteria may be the way forward.

CONCLUSIONS

A study on 6-day-old dairy calves indicated that administration of probiotic *Enterococcus faecium* M74 may have beneficial effects of calf health. Body weight and daily weight gain were significantly improved by probiotic treatment over the entire trial period from six to 62 days. The addition of *Enterococcus faecium* M74 to milk also improved faecal scores. Probiotic treatment reduced the incidence of diarrhoea and the average number of diarrhoeic days. Probiotic research is set to grow in the future and much work lies ahead in terms of advancing our understanding of the remarkably complex dynamics of the animal gut ecosystem and the multifactorial dynamics of the efficacy of probiotic application.

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