

Performance of Holstein calves fed milk-replacer and starter mixture supplemented with probiotic feed additive

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ABSTRACT

The aim of a study was to determine the efficacy of the microbial feed additive containing spores of *Bacillus licheniformis* and *Bacillus subtilis* (BioPlus 2B®) on performance and health status of rearing calves. Sixty four female Holstein calves, aged on average 16.7±4.5 days, were randomly assigned into two groups of 32 animals: control (C) and BioPlus 2B® (BP). Milk replacer (MR) and starter mixture (SM) fed to BP group contained 1.32×10^9 (±3.2%) and 1.13×10^9 (±11.5%) spores of *Bacilli* strains, respectively. Each calf was fed with 2.25 l of MR two times a day for eight weeks, up to the age of approximately 10 weeks. Starter diet (SD) offered to calves consisted of SM and whole maize grain, which were mixed in ratio 50:50 (wt/wt). Intake of MR was equal in both groups, but SD intake was higher in the whole experiment in the BP group (1075 vs 951 g/d; P<0.01). The BP calves grew faster than C ones in the whole trial (P=0.05), but especially in weeks 3-4 (P<0.05). At the end of the trial the BP calves were about 2.9 kg heavier than the C ones (P<0.001). Feed efficiency, calculated as consumption of ME (MJ) or crude protein (g), was not different between treatments. There were also no differences in the health status and faecal score between treatments. The results of this study suggest the beneficial effect of microbial feed additive containing spores of *Bacillus licheniformis* and *Bacillus subtilis* for rearing calves.

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KEY WORDS: calf, probiotic, weaning, gain, health

INTRODUCTION

The ban of growth promoting antibiotic usage in animal nutrition in the European Union countries from January 1, 2006, forces to looking for effective alternatives. Among them, the probiotics seem to be the most promising (Abe et al., 1995; Morrill et al., 1995; Abu-Tarboush et al., 1996; Cruywagen et al., 1996; Strzetelski et al., 1998; Timmerman et al., 2005). If probiotics effectively colonize the gastrointestinal tract, they will inhibit pathogen proliferation, neutralize the enterotoxins and stimulate intestinal immune system (Krehbiel et al., 2003).

From an economical perspective calves should be weaned from an expensive milk or milk replacer to a less expensive solid diet as soon as possible. That is why a so called “early weaning system” (as described by Kertz et al., 1979) is getting more common in dairy herds. Early weaning, for example at 35-45 days of age, is possible only when the digestive tract is morphologically and functionally adapted for an utilization of solid feeds. Inadequate forestomach development can be a main cause of health problems and can delay the weaning age (Beharka et al., 1998).

It has been already accepted that solid feed intake promotes rumen development (Harrison et al., 1960; Beharka et al., 1998), especially when the butyrate and propionate are the main products of fermentation in the rumen (Tamate et al., 1962; Lane et al., 2000). According to this concept, an early weaning program is based on *ad libitum* intake of starter diet, which promotes both butyrate and propionate fermentation in the rumen. In modern calf feeding programmes the starter diets often consist of starter mixture (source of starch) and whole cereal (maize, oats) grains, which stimulate the development of rumen muscles. On the other hand, a high rate of milk or milk replacer feeding reduces starter intake and can delay rumen development (Hubert et al., 1984). Thus, restricted amounts of milk or milk replacer (8-10% of birth weight) are generally accepted for early weaned calves (NRC, 2001).

Intensive calf rearing systems require a specific prophylactic programme (Morrill et al., 1995). For many years such programmes have been based on the use of antibiotic growth promoters. The question whether the probiotics can now effectively replace antibiotic growth promoters in such intensive conditions is still open. In this context, the aim of a study was to investigate the use of the probiotic feed additive, a product containing spores of *Bacillus licheniformis* and *Bacillus subtilis* (BioPlus 2B®; Chr. Hansen A/S, Denmark; EC Reg. No. E 1700 (2005 year)) on productivity and health responses of rearing Holstein calves.

MATERIAL AND METHODS

Animals and management

Sixty four female Holstein calves healthy, well-eating, were selected from the own dairy herd for the trial. Before the onset of the trial the animals followed the routine procedure of new-born calf management, including ingesting the colostrum (fed within 4 h after birth), full milk feeding up to 7th day of age and then feeding with commercial milk replacer. *Ad libitum* feeding with starter diet started from 5-7th days of age.

The calves were introduced into the trial in 5 blocks of 14, 10, 20, 10 and 10 animals and within each block the analogues were then assigned randomly into two groups, control (C) and BioPlus 2B[®] (BP), resulting in 32 calves for each treatment. One calf from the BP group was excluded from the trial because of a leg injury.

At the start of trial the calves were 16.3 ± 4.4 and 17.4 ± 4.6 days of age (on average 16.7 ± 4.5 days), in C and BP group, respectively. The initial weight was in a range of 32.0-54.0 kg. Each calf was in the trial over a period of 8 weeks until an age of approximately 10 weeks. The experimental period covered important changes around possible weaning in early age (35-45 days of age), but due to the feeding organization at the farm the calves received milk replacer up to 10 weeks of age. To avoid confusions in the whole paper the data will be based on weeks of experiment.

The calves were kept in individual pens of 1.5 m (length) x 1.2 m (width), on concrete floor, covered with straw. There were two empty pens left between the groups to minimize the possible group effect (contamination by spores).

Feeds and feeding

The animals were fed individually. During the whole experimental period the calves were fed with 450 g/d of milk replacer powder (MR; Economilk, Koudijs, Poland), divided into two equal portions of 225 g. This amount was dissolved in warm water (about 40°C) in the ratio of powder to water as 1 to 9, so the MR was offered in the amount of 4.5 l per day. The MR powder contained no (CMR) or 400 g/t of BioPlus 2B[®] (BPMR). BP was introduced into the BPMR by the producer of milk replacer. BPMR contained 1.28×10^9 spores of *Bacillus licheniformis* and *Bacillus subtilis* per kg of powder. Both MR did not contain any antibiotics. MR was fed from a bucket at approximately 8.00 and 15.00 h. Amount of MR refused by a single calf was measured at each feeding. When each MR meal was finished, a bucket of fresh water was provided.

A commercial pelleted (diameter of 3 mm) starter mixture (SM; Starter Concentrate KcalfF, Koudijs, Poland) was mixed with whole maize grain (50/50;

wt/wt) and offered as a starter diet (SD) once daily *ad libitum*, beginning on the first day of the trial. The SM offered to C (CSM) or BP (BPSM) group contained no or 400 g/t of BioPlus 2B[®], respectively. In the BPSM preparation it was equivalent to a concentration of 1.28×10^9 spores per kg (as fed). As above the BioPlus 2B[®] was introduced into the BPSM by the producer of SM. Both SM did not contain any antibiotics.

The SD was offered from a bucket once daily at 9.00 h. Refusals were collected daily. A sample of refusal from each calf were kept separately for checking a preference in consuming of SM or whole maize grain. Refusals from each two week period were pooled for each calf and the 1 kg sample was then divided by sieving into two fractions: SM or whole grain. Both fractions were then weighed.

Samples of MR powder, SM and maize grain were collected weekly. Weekly samples were then pooled and analysed for ash, crude protein, ether extract, crude fibre using standard procedures (AOAC, 1995).

Before the start of trial the representative samples of MR and SM (about 1 kg of each) were checked for the final concentration of spores. This investigation (Verbandsmethode VDLUFA 28.2.2) was conducted by Bayerische Landesanstalt für Gesundheit und Lebensmittelsicherheit, Oberschleissheim (Germany).

Measurements

The calves were individually weighed on day 0 (start), 14, 28, 42, 56 (end) of the trial. The weighing of the animals was always started from the C group to avoid possible contamination by spores.

The health status of calves was checked daily. It was also scored at the start of the trial and at every week using the health status scores: very good - 5, good - 4, sufficient - 3, bad - 2, very bad - 1. Every abnormal condition as well as every veterinary treatment used were documented, with the date of first observation, duration and nature of any treatment administered and outcome. Additionally, the quality of bedding in each pen was evaluated weekly using the faecal score: normal - 1, soft - 2, watery (diarrhoea) - 3.

Calves with diarrhoea were treated with a commercial electrolyte solution and Duphalyte (Fort Dodge Veterinaria SA, Spain). Antibiotic treatment was used as recommended by veterinarian surgeon only when it was absolutely essential. The antibiotic treatment (Micotil; Bayer, Germany) in this trial was only related to the respiratory diseases.

Faeces grab samples were individually taken from 10 calves in each group (10 from control and 10 from BP) on the first days of the trial (4-7) and on day 29. The samples of faeces (10-20 g) were then transferred into a small sterile box and then immediately frozen until they were used for microbial analysis (spore counting of

BioPlus 2B®). The analysis was conducted at the laboratory of Chr. Hansen A/S (Denmark).

Statistical analysis

Data (energy, protein, starter diet and maize grain intake, liveweight, body weight gain, feed conversion ratio and faecal scores) were analysed as a linear model using PROC MIXED of SAS with repeated measures in time, according to different weeks (SAS, 1996). The statistical model included calf as a random effect, and diet and its interaction with time as fixed effects. The initial body weight of calf was taken as covariate. If the diet effect was significant, differences between treatments in particular weeks were determined using ESTIMATE statements of SAS (Littell et al., 1998). Additionally, means for above mentioned data as well as for health status of calves calculated for the whole experimental period were subjected to one-way analysis of variance using PROC GLM (SAS, 1996). The significance was declared at $P < 0.05$, but tendencies at $P < 0.10$. One calf from C group was detected as outlier by using the CookD test (SAS, 1996) and therefore it was eliminated from the statistical analysis. Thus finally, the statistical analysis was based on the data of 31 C and 31 BP calves.

RESULTS

There were no differences between treatments in MR and SM chemical composition (Table 1). Both BPMR and BPSM contained spores of *Bacilli* strains in expected range. The SM and whole maize grain were mixed in the ratio 50:50 (wt/wt), so that the offered SD contained about 141 g of crude protein and 13.8 MJ ME per kg. Average viable *Bacilli* strains spore counts in faecal samples were 4.15×10^3 and 4.58×10^5 (CFU/g) for C and BP group calves, respectively ($P < 0.001$; data not shown).

Intake of MR was equal in both groups (about 4490 ml/day) and there were almost no refusals, except at the beginning of the experimental period (weeks 1-2). The consumption of SD during the whole trial was higher in the BP group than in the control one (Figure 1A; $P = 0.06$). Higher SD intake in the BP group was significant in weeks 5-6 and 7-8 of the experiment. The average daily SD consumption in the whole trial was about 120 g higher in BP group (Figure 1B; $P < 0.05$). Also energy intake was higher in the BP group during the whole trial (Figure 1C; $P = 0.04$), particularly in weeks 5-6 and 7-8. The BP calves also tended to consume more protein in the whole trial, however, this observation was not statistically significant.

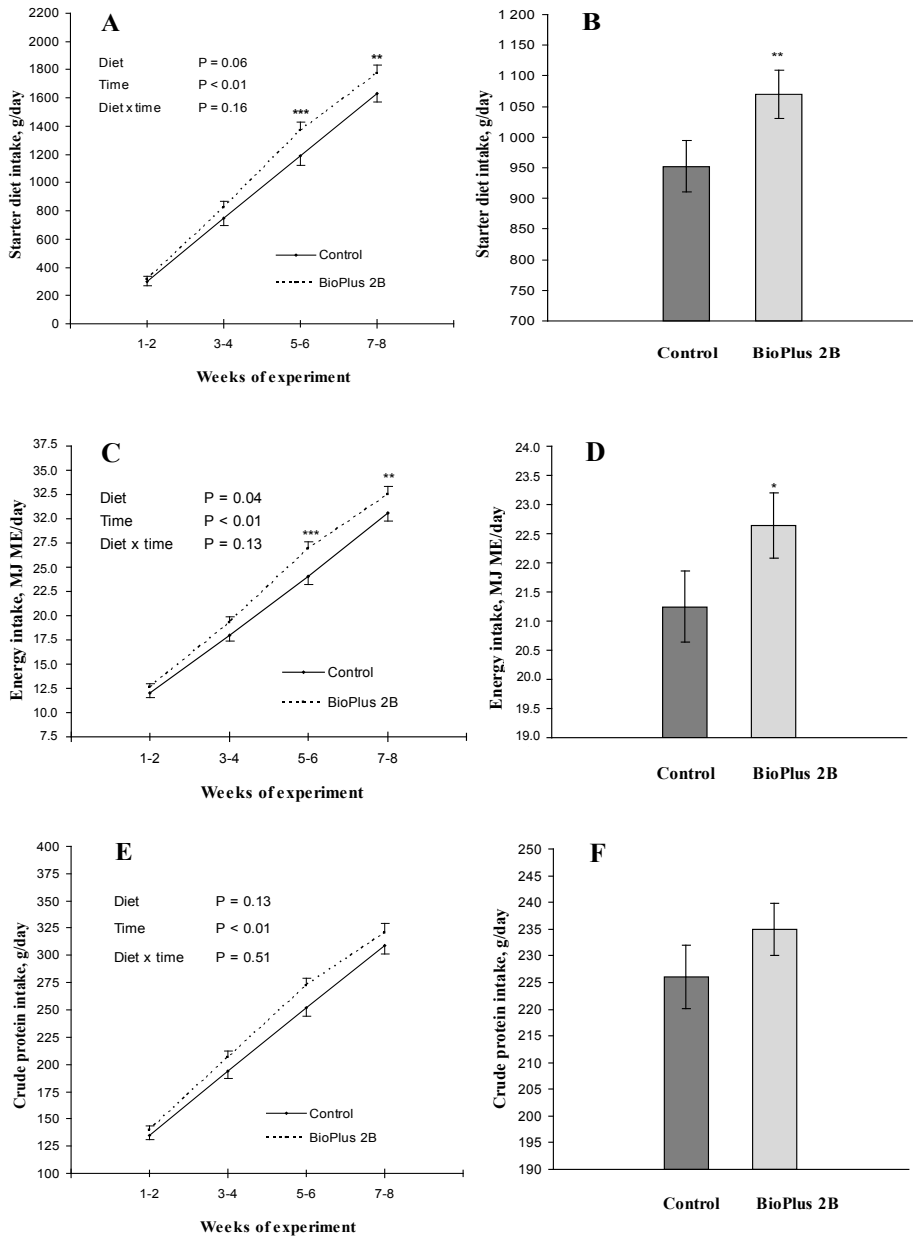


Figure 1. Starter diet, energy and crude protein intake (Figures 1A, 1C, 1E, respectively) and average daily intake in the whole experimental period (Figures 1B, 1D, 1F, respectively); *** P<0.01; ** P<0.05; * P<0.10

Table 1. Chemical composition of feeds. *Bacilli* strains spore counts in milk replacer powders and starter mixtures, CFU/kg

Item	Milk replacer powder ¹		Starter mixture ²		Maize grain
	CMR	BPMR	CSM	BPSM	
Dry matter, g/kg	976	976	895	896	873
Crude protein, g/kg	213	213	187	186	96
Crude fat, g/kg	151	153	34	34	51
Crude fibre, g/kg	6	5	57	61	14
Ash, g/kg	89	89	66	66	12
ME ³ , MJ/kg	18.28	18.28	13.18	13.18	13.80
<i>Bacilli</i> strains spore counts, CFU/kg	<10 ⁸	1.32 × 10 ⁹ ± 3.2%	<10 ⁸	1.13 × 10 ⁹ ± 11.5%	

¹ CMR - control milk replacer powder; BPMR - BioPlus 2B milk replacer powder; both consisted of whey powder, fat-whey carrier, wheat gluten, soyabean meal, mineral premix and vitamin premix

² CSM - control starter mixture; BPSM - BioPlus 2B starter mixture; both consisted of oats, maize, barley, wheat, dried brewer's grains, rye bran, soyabean, rape, sunflower, palm expeller, calcium phosphate, chalk, salt, magnesium oxide, vitamin premix and vanilla flavour

³ metabolizable energy, calculated from NRC (2001) as: ME (Mcal kg⁻¹) = [0.057 × CP % + 0.092 × fat % + 0.0395 × lactose %] × 0.97 + 0.96 for calf milk replacer (CMR and BPMR); ME (Mcal kg⁻¹) = [1.01 × DE - 0.45] + [0.0046 × (fat % - 3)] for starter mixture (CSM and BPSM) and maize grain, where DE (Mcal kg⁻¹) = (0.057 × CP % + 0.094 × fat % + 0.0415 × carbohydrates %) × 0.82 and carbohydrates % = 100 - crude protein % - crude fat % - ash %; accepted 1 Mcal as equal to 4.184 MJ

At the beginning of the trial calves in both groups consumed whole maize grain and SM in equal proportions (about 50%; Figure 2A), but older calves started to consume more maize grain, particularly those from the BP group. In

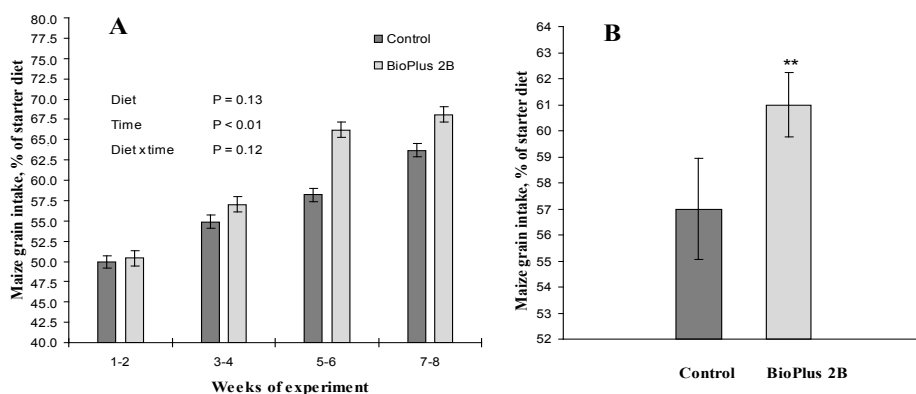


Figure 2. Maize grain intake as a % of starter diet intake (Figure 2A) and average intake in the whole experimental period (Figure 2B); *** P < 0.01; ** P < 0.05; * P < 0.10

the last two weeks of the trial the ratio of maize grain to SM consumed reached a level of 63.7:36.3 and 68.1:31.9 in C and BP group, respectively. On average in the whole trial the BP calves sorted daily more maize grain from SD (Figure 2B; $P < 0.05$).

The calves of the BP group were significantly heavier in weeks 4, 6 and 8 of the experiment (Figure 3A). At the end of the trial the BP calves were about 2.9 kg heavier than the C ones. The daily liveweight gain during the whole trial tended to be higher in the BP group (Figure 3C; $P = 0.1$), especially in weeks 3-4. There were also differences between treatments in daily body weight gain calculated for the whole experimental period (Figure 3D; $P < 0.1$).

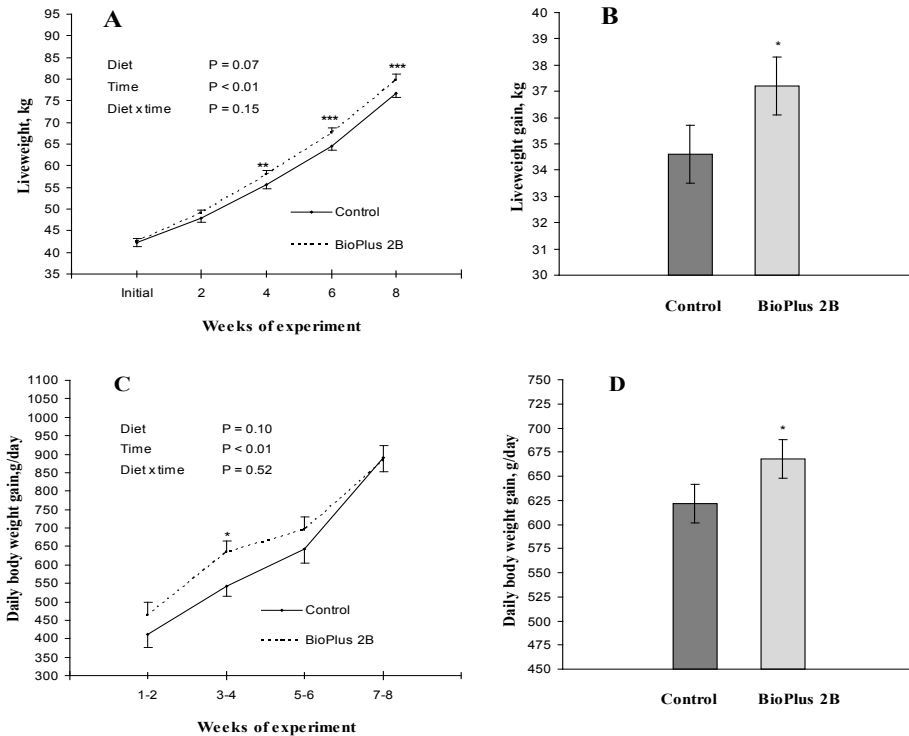


Figure 3. Liveweight and daily body weight gain (Figures 3A, 3C, respectively) and average liveweight gain and daily body weight gain in the whole experimental period (Figures 3B, 3D, respectively); *** $P < 0.01$; ** $P < 0.05$; * $P < 0.10$

Feed efficiency, calculated as intake of ME (MJ) or crude protein (g) per 1 kg of daily body weight gain, was not different between treatments (Figure 4).

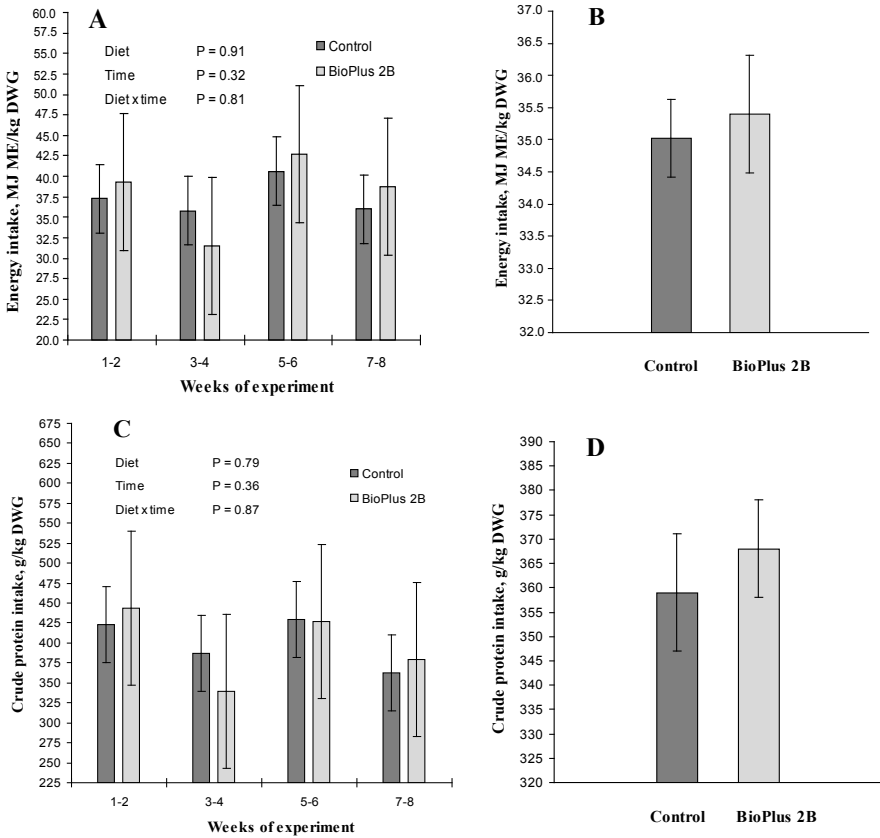


Figure 4. Feed conversion calculated as energy and crude protein intake per kg of daily body weight gain (Figures 4A, 4C, respectively) and average feed conversion in the whole experimental period (Figures 4B, 4D, respectively); *** P<0.01; ** P<0.05; * P<0.10

There were also no differences among treatments in the health status of the calves either at the start or end of the trial. The calves of both groups were judged as 4.9-5.0 (healthy) in the 5-point scale (data not shown). Weekly faecal score tended to be slightly lower in the BP calves, especially in week 2 (Figure 5A; P<0.01). These results are consistent with no mortality problems. Only two animals were excluded from calculations: one calf (BP group) because of leg injury and another one (control) as statistical outlier because of body weight. The use of electrolytes and antibiotics for respiratory problems was very limited and not statistically elaborated. There were no evident differences between treatments in the number of veterinary interventions.

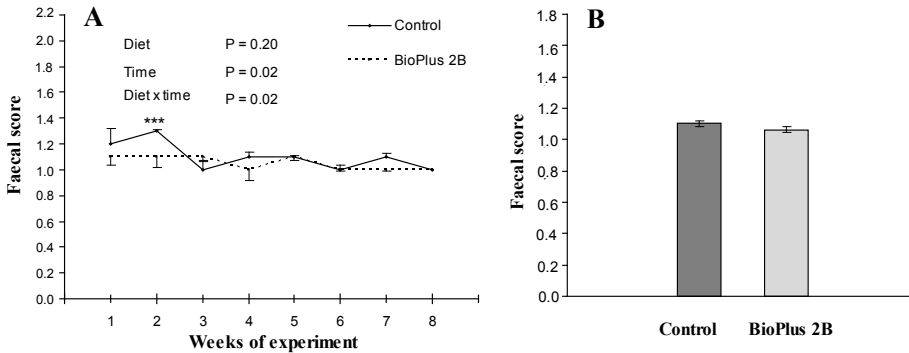


Figure 5. Faecal score during the whole trial (Figure 5A) and average faecal score in the whole experimental trial (Figure 5B); *** P<0.01; ** P<0.05; * P<0.10; normal - 1, soft - 2, watery (diarrhoea) - 3

DISCUSSION

The present study shows that the probiotic feed additive BioPlus 2B[®], containing spores of *Bacillus licheniformis* and *Bacillus subtilis*, added to milk replacer and starter diet can be an attractive alternative for antibiotic growth promoters for rearing calves.

In each experimental period, the amounts of energy and protein consumed daily by calves from both groups were above or close to NRC (2001) recommendations for Holstein calves. However, addition of BioPlus 2B[®] improved SD, energy and protein intake. This positive effect was particularly apparent in later stages of the trial (weeks 3-4), it means in older calves (from 5th week of age). Similar effect of probiotics on higher solid feed intake by calves was observed by others (Schwab et al., 1980; Ruppert et al., 1994; Abe et al., 1995), although in some experiments no effect (Jenny et al., 1991; Cruywagen et al., 1996; Abu-Tarboush et al., 1996; Donovan et al., 2002) or even negative effect (Morrill et al., 1977) was observed. Beneficial effect of BioPlus 2B[®] on SD should be especially appreciated in early weaning programmes, in which a time of weaning, usually in the age of 35-45 days, depends primarily on the consumption of solid feeds (Greenwood et al., 1997). It is worth to notice that the calves of both groups consumed 1160-1380 g/d of a SD in week 5-6 of a trial, when they were 7-8 weeks old, when early weaning usually takes place. It is enough to maintain good growth rate after possible weaning. Greenwood et al. (1997) showed that dry matter consumption of 1% of birth body weight (about 400 g/d) for 3 consecutive days is adequate to weaning.

The calves showed the ability to sort SM granules and whole maize grain. With increasing age, the animals of both groups consumed more maize grain and less SM. Choosing the grain by older calves might be due to the necessity to maintain the proper physical structure of the diet, since both C and BP diets did not contain hay. On the other hand, young calves (up to 4-5 weeks of age) had to consume relatively higher amounts of SM than older ones to cover their protein and energy requirements, since the daily amount of milk replacer was kept low (4.5 l/d) and constant throughout the whole experiment. The reason for higher selection of maize grain by BP calves is not clear, but it may indicate a positive influence of BioPlus 2B[®] on rumen fermentation, resulting in higher consumption of whole grain.

Average daily gains of both groups were well within the range expected for dairy female calves. But as a result of higher SD consumption, the BP calves grew faster than C ones and their average daily body weight gains in the whole trial was higher (617 and 668 g/d for C and BP, respectively). The differences between treatments were particularly apparent in early stages of the experiment. On the other hand, it is worth to note that at the end of a trial there were no differences between groups in daily weight gain, being about 870-880 g/d.

Our results are similar to those obtained by others who showed beneficial effects of probiotics on performance of rearing calves (Abe et al., 1995; Morrill et al., 1995; Cruywagen et al., 1996; Donovan et al., 2002; Timmerman et al., 2005), although in some studies no effect of probiotics was observed (Morrill et al., 1977; Jenny et al., 1991; Nakanishi et al., 1993; Abu-Tarboush et al., 1996). The reasons for such a discrepancy in opinions may result from the health status of the maternal herd, experimental conditions and management systems. The use of non host-specific species or strains might be also the reason for no response to the probiotics (Krehbiel et al., 2003; Timmerman et al., 2005). Because the basic mechanisms of probiotics action are not well defined and are not clearly understood (Krehbiel et al., 2003), there may be also many other reasons that may affect effect of probiotics.

In some studies, higher daily weight gains of calves that received probiotics were noticed mainly in the first two-three weeks of age (Jenny et al., 1991; Cruywagen et al., 1996; Timmerman et al., 2005). Thereafter, the advantages of using probiotics were not so apparent. It is possible that by using probiotics the calves can faster accommodate to the stress of the first weeks of life (Timmerman et al., 2005). Therefore, the probiotics should be provided as soon as possible after birth (Abe et al., 1995). In our study, although the calves were introduced into the trial when they were about two weeks old, the effect of BioPlus 2B[®] was also noticeable. It is possible that the inclusion of the probiotic feed additive to the starter mixture, not only to milk powder, prolonged the beneficial effect of BioPlus 2B[®] on calves. In majority of former research the probiotics have been added to milk replacers

(Morrill et al., 1995; Timmerman et al., 2005).

Faster growth of BP calves was rather a result from a higher solid feed intake than from increased feed efficiency, since there was no treatment effect on both energy and protein conversion rate. Higher solid feed intake by BP calves, resulting in higher daily weight gains (particularly at 3-4 weeks of a trial), might have been caused by improvement in absorption of nutrients from the intestines (Sissons, 1989) and/or faster rumen function development (Beharka et al., 1998). In contrast, others (Schwab et al., 1980; Jenny et al., 1991; Abe et al., 1995; Timmerman et al., 2005) showed improved feed efficiency by calves fed diets supplemented with probiotics. Jenny et al. (1991) observed a better feed efficiency in calves supplemented with *B. subtilis* in contrast to calves receiving mixed microbial concentrate containing *L. acidophilus* and *L. lactis*. It indicates the importance of considering the bacterial strain used as feed additive.

Although the BP calves ate more concentrate and grew faster than C ones there were no treatment differences between groups in the health status. These results are similar to those presented by Jenny et al. (1991) and Cruywagen et al. (1996). In contrast, many authors noticed a significant reduction in health problem incidences when calves received the probiotics (Abe et al., 1995; Abu-Tarboush et al., 1996; Timmerman et al., 2005). As stated earlier the reaction of calves on probiotics depends on the conditions in which the experiment is being conducted. A good health status of the control and BP calves speaks well about the sanitary and management conditions of the experiment. However, it is worth to notice that the BP calves had better faecal score in week 2 of a trial and this period coincides with better growth rate and starter diet intake.

CONCLUSIONS

It can be concluded that feed intake and heifer calf performance were improved by using the *Bacillus* strains (*Bacillus licheniformis* and *Bacillus subtilis*) in the probiotic feed additive BioPlus 2B®.

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