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Short communication

Effect of rearing systems and diets composition on the survival of probiotic bifidobacteria in the digestive tract of calves

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ABSTRACT

The effect of rearing systems and diets composition on the survival of administered probiotic bifidobacteria in the digestive tract of calves was examined. Two bifidobacteria strains of calf origin with suitable physiological properties, which were identified as *Bifidobacterium animalis* ssp. *animalis* and *Bifidobacterium longum* ssp. *suis*, were administered to 8 Charolais calves reared in an extensive farming system fed the full-milk diet and 8 Holstein calves from an intensive system fed the combined diet. Skim-milk fermented by rifampicin-resistant bifidobacteria variants of the *B. animalis* ssp. *animalis* and *B. longum* ssp. *suis* strains were administered once to 2-day-old calves. Survival of the administered bifidobacteria and the numbers of other bacterial groups in faecal samples was monitored by culturing. Probiotics administered to Charolais calves survived at higher counts than 10^7 CFU/g in the digestive tract for at least 26 days. Significantly lower bifidobacteria survival rate was observed in the Holstein calves. Three days after administration of bifidobacteria were detected in counts 10^7 CFU/g; however, their numbers rapidly dropped reaching a value of about 10^2 CFU/g on day 26 after administration. Bifidobacteria dominated the faecal flora of 5-day-old calves in both groups. Significantly higher lactobacilli counts were detected in the Charolais calves than in the Holstein calves. Our results showed that administration of probiotics is more effective in calves fed the full-milk diet reared in an extensive farming system. To achieve a probiotic effect in intensively reared animals, repeated application would probably be required, because the tested bifidobacteria were not able to colonise the digestive tract of calves fed the combined diet from an intensive rearing system.

1. Introduction

Microorganisms are first introduced into the sterile gastrointestinal tract (GIT) of new-born calves during birth, both from the faeces and vagina of their mothers and from the environment. Colonisation patterns during early life are unstable, and new-born animals are susceptible to environmental pathogens. This initial colonisation is very important to the host because bacteria can modulate gene expression in epithelial cells to establish a favourable habitat for themselves (Siggers et al., 2007). The intestinal microbiota is stabilised after the first weeks of life, and high numbers of beneficial bacteria, such as bifidobacteria and lactobacilli, are desirable. Balance of the intestinal ecosystem in calves can be negatively impacted by rearing in intensive farming systems due to separation from their mothers, feeding with milk replacers and elimination of the benefits of cow's milk, inadequate colostrum intake, stressful situations, and the use of antibiotics (Soto et al., 2011). Other factors, such as physiological and physical stresses, immune deficiency, infections, and the intake of certain dietary components, have also been shown to contribute to intestinal dysbiosis (Hawrelak and Myers, 2004; Stecher et al., 2013). These stressors lead to intestinal barrier dysfunction and increased intestinal permeability and can impact the microbial composition of the gut and increase susceptibility to enteric pathogens (Gareau et al., 2009). One strategy to improve the gastrointestinal microbiota of animals is the use of probiotics. A significant effect has been reported when probiotics were included in the diet of animals, particularly during stressful periods (Chaucheyras-Durand and Durand, 2010). Microorganisms used in animal feed including livestock are mainly bacterial strains of gram-positive bacteria belonging to genus *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Streptococcus*, and strains of yeast belonging to the *Saccharomyces cerevisiae* species and *Kluyveromyces* ([Anadon et al., 2006; Gaggìa et al., 2010]); however, interest in bifidobacteria is growing. Bifidobacteria dominate the intestinal microbiota of

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many mammals, especially during the milk-feeding period because they are supported by the mother's milk components, and their presence in high numbers is associated with good health (Chiu et al., 2014; Russell et al., 2011; Sanchez et al., 2013). The bifidobacterial species that are most commonly used for animal feed are *Bifidobacterium animalis* ssp. *animalis*, *B. animalis* ssp. *lactis*, *Bifidobacterium longum* ssp. *longum*, *Bifidobacterium pseudolongum* ssp. *pseudolongum*, and *Bifidobacterium thermophilum* (Gaggìa et al., 2010). Some bifidobacteria are host-specific, whereas others are common in many hosts (Bunesova et al., 2014). Bacteria that are intended for use as probiotics must meet a number of requirements. For example, they must survive passage through the upper part of the intestinal tract and remain active in the colon long enough to achieve a probiotic function. Therefore, it is important to use strains belonging to indigenous populations that were isolated from the host species for which the inoculum is intended, because they are adapted to the environment of the host GIT (Soto et al., 2011). Furthermore, administration of probiotic bacteria of bovine origin may favour the establishment of stable, balanced intestinal microbiota which would improve the health of the calf (Abe et al., 1995; Soto et al., 2011). However, the origin of the strain is not the only factor affecting its survival and activity in the intestine; the viability of administered bacteria in the colon may be affected by various exogenous and endogenous factors. Therefore, the aim of this study was to observe the effect of rearing system and diet composition on the survival of calf-origin probiotic bifidobacteria in the digestive tract of calves.

2. Material and methods

2.1. Bifidobacterial strains

The bifidobacterial strains administered to calves in this study were isolated in our previous experiments (Bunesova et al., 2012; Vlkova et al., 2010). Briefly, the bifidobacteria were isolated from calf faecal samples obtained during the milk-feeding period. Bifidobacteria selected for calves treatment showed auto-aggregation and antimicrobial activity, and were bile and acid tolerant; they exhibited a decrease in viability < 1 log CFU/ml after incubation for 3 h for bile tolerance and 2 h for low pH tolerance. Ten strains with suitable physiological properties were identified by 16*S rRNA* gene sequencing, and were used to prepare rifampicin-resistant mutants (RRBs) using a gradient plate technique. No differences in physiological characteristics were found between RRBs and original bifidobacteria. A mixture of 10 RRBs was fed to 2-day-old suckling calves. The survival of the administered strains was monitored in faeces by cultivation on selective agar containing rifampicin. The strains that survived in the intestinal tract of calves for at least 40 days at greater than 10^6 CFU/g were re-isolated and identified to the strain level by fingerprinting techniques. For the present study, we chose 2 of these strains with the best physiological properties, which were evaluated both *in vitro* and *in vivo* as described above. These strains were identified as *B. animalis* ssp. *animalis* and *B. longum* ssp. *suis*.

2.2. Animals, bacteria administration, and sampling

Probiotics bifidobacteria were administered to eight 2-day-old Charolais calves from a local farm named “Chov Charolais” (Slabce, Czech Republic) and to 8 Holstein calves of the same age from farm named “Dvorec” (Vrčeňská zemědělská, Vrčeň, Czech Republic). Rifampicin-resistant variants (RRBs) of *B. animalis* ssp. *animalis* and *B. longum* ssp. *suis* were sub-cultured in Wilkins-Chalgren broth (Oxoid). Each RRB was inoculated into 100 mL of 10% skim milk and cultivated in an anaerobic atmosphere at 37 °C overnight. The number of bacteria in the fermented milk was approximately 10^8 CFU/mL as determined by cultivation. Each calf was fed a single dose (200 mL, containing approximately 2×10^{10} bifidobacteria) containing a mixture of both strains. None of the experimental animals was on antibiotics, and the calves were fed different diets at the two farm.

The Charolais calves were reared in an extensive farming system, and were housed with their dams and suckled. Water was available *ad libitum*. The Holstein calves were reared in an intensive farming system. These calves were removed from their dams and housed in individual pens. They were fed colostrum for 3 days and then switched to cow's milk. The milk (6–8 L) was supplied twice a day. From the age 7 days, the calves were fed a mixture of granulated feed (ČOT-S BK GP 3, 40%; Mikrop Čebín, Czech Republic) and crimped oats (60%) *ad libitum* as a starter feed. Water was also available. Granulated feed contained soybean meal, alfalfa meal, yeasts, barley, vitamin and mineral supplement and was composed from 350 g/kg of crude proteins, 57 g/kg of crude fibre, and 61 g/kg of crude fat, dry matter of the feed was 85%.

The survival of the administered RRBs and other bacterial groups was monitored in faeces by cultivation on appropriate media. Faecal samples were taken from the rectum using sterile gloves, transferred to a tube with Wilkins-Chalgren broth (Oxoid), and transported to the laboratory within 2 h. Initial samples were taken from 2-day-old calves before probiotic administration, and additional samples were obtained at 5, 9, 14, 21, and 28 days of age.

2.3. Microbiological assays

Samples were serially diluted in Wilkins-Chalgren broth (Oxoid) under anaerobic conditions. RRBs were enumerated using Wilkins-Chalgren agar (Oxoid) supplemented with soya peptone (5 g/L, Oxoid), L-cysteine (0.5 g/L, Sigma), Tween 80 (1 mL/L, Sigma), mupirocin (100 mg/L, Merck), rifampicin (80 mg/L, Sigma) and glacial acetic acid (1 mL/L). Total bifidobacterial counts were determined on the same medium without rifampicin supplementation (Rada and Petr, 2002), and total anaerobes were cultivated on Wilkins-Chalgren agar (Oxoid). Anaerobic bacteria were incubated in an anaerobic jar (Anaerobic Plus System, Oxoid) at 37 °C for 72 h. To enumerate lactobacilli, Rogosa agar (Oxoid) adjusted to pH 5.4 ± 0.2 with acetic acid was used, and the plates were incubated under microaerophilic conditions at 37 °C for 48 h. To create microaerophilic conditions, the first agar layer was covered with a second layer of Rogosa agar before incubation. Petri dishes containing TBX agar (Oxoid) for enumeration of *Escherichia coli* and presumptive coliforms were inoculated with 0.1 mL of an appropriate culture dilution and spread with a sterile glass rod. Plates were incubated aerobically at 37 °C for 24 h.

2.4. Statistical analyses

The mean and standard deviation of the bacterial counts were calculated. The significance of differences in bacterial counts between the Charolais and Holstein calves at the same age was evaluated by independent *t*-test. The one-sample Kolmogorov–Smirnov test of composite normality was used to test for a normal distribution. The analysis of variance (ANOVA) was applied to determine the statistical significance among bacterial counts within each group over time with 95% confidence interval. Shapiro–Wilks test was used for testing of normality. The results were processed in software Statistica (Statistica 12.0, Tulsa, USA).