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The impact of probiotics in the nutrition of calves on live weight gain and on health status

Vliv probiotik ve výživě telat na hmotnostní přírůstky živé hmotnosti a zdravotní stav

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ABSTRACT

This paper aims to monitor the impact of *Lactobacillus sporogenes* (LS), *Saccharomyces cerevisiae* (SC), the combination thereof *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* (CLS) on the health status and the live weight gain in calves compared to a control group (C). The experiment took place in the period from March 2022 to March 2023. 100 Holstein heifers in the age from 1 to 56 days were included in the experiment. The differences in live weight gain were significant when the live weight gains were compared in the first 14 days after birth between the CLS vs C group (63,36.72 ± 4.81 vs 59.55 ± 4.55, $P < 0,05$) and in 56 days after the birth between the CLS vs C group, LS vs C group and SC vs C group (87,34 ± 4.95 kg vs 83.15 ± 5.32 kg, $P < 0,01$; 86.41 ± 5.34 kg vs 83.15 ± 5.32 kg, $P < 0.05$ an 85.92 ± 5.86 kg vs 83.15 ± 5.32 kg, $P < 0.05$). The differences in live weight gain between the experimental groups were not proved statistically $P > 0.05$. The impact on decrease and duration of diarrhea was not proved statistically $P = 0.0634$. However, a tendency to decrease the occurrence and duration thereof was proved. The impact of feed additives on the transmission of passive immunity in calves in their first week of life was not proved as statistically significant.

Keywords: *Lactobacillus sporogenes*, *Saccharomyces cerevisiae*, dairy calves, diarrhea, feed additives

ABSTRAKT

Cílem této studie bylo sledovat vliv *Lactobacillus sporogenes* (LS), *Saccharomyces cerevisiae* (SC) a jejich kombinaci *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* (CLS) na zdravotní stav a přírůstek živé hmotnosti telat oproti skupině kontrolní (C). Pokus se uskutečnil v období březen 2022 až březen 2023. Do pokusu bylo zařazeno celkem 100 holštýnských jalovičeк ve stáří 1 až 56 dní. Rozdíly v přírůstku živé hmotnosti byly významné, pokud byly porovnány hmotnostní přírůstky ve 14. dech po narození mezi skupinou CLS vs C (63,36.72 ± 4.81 vs 59.55 ± 4.55, $P < 0.05$) a v 56 dnech po narození mezi skupinu CLS vs C, LS vs C a SC vs C (87.34 ± 4.95 kg vs 83.15 ± 5.32 kg, $P < 0.01$; 86.41 ± 5.34 kg vs 83.15 ± 5.32 kg, $P < 0.05$ a 85.92 ± 5.86 kg vs 83.15 ± 5.32 kg, $P < 0.05$). Rozdíly v přírůstku živé hmotnosti mezi pokusnými skupinami nebyly statisticky prokázány $P > 0.05$. Vliv na snížení výskytu a trvání průjmových onemocnění nebyl statisticky prokázán $P = 0.0634$, ovšem byla zde prokázána tendence ke snížení jejich výskytů a době trvání. Statisticky významný nebyl prokázán vliv krmných aditiv na přenos pasivní imunity u telat v prvním týdnu života.

Klíčová slova: *Lactobacillus sporogenes*, *Saccharomyces cerevisiae*, mléčná telata, průjem, krmná aditiva

INTRODUCTION

The gastrointestinal tract (GIT) of ruminants faces the challenge of protecting the host from its birth from luminal content and from pathogenic microorganisms and is forced at the same time to support absorption and nutrient metabolism to ensure the growth and development of the individual. The GIT of a calf undergoes the fastest microbial and structural changes documented in nature. These abruptly ongoing changes in the GIT functioning make the calf susceptible to diseases or to the occurrence of GIT malfunctions. Despite these predispositions, the GIT of a calf disposes of a certain degree of flexibility and is able to react to the supply of nutrients and the bioactive component (Meale et al., 2017; Godden et al., 2019).

The authors (Cho et al., 2014; Klein-Jobstl et al., 2014) state that diarrhea is the most common and most detrimental health problem in calves in their postnatal period. Diarrhea is one of the most common causes of mortality in newborn calves (Katsoulos et al., 2020; Maier et al., 2022). It affects almost 19% of the animal population (Smulski et al., 2020). Diarrhea is a complex multifactorial disease with many infectious and noninfectious factors, such as calf dyspepsia. Dyspepsia is characterized by impaired secretion, resorption and motility of mucus and intestines with subsequent loss of appetite, diarrhea and rapidly developing dehydration (Constancis et al., 2022). The factors having an influence on the pathogenesis of diarrhea consist of the exposition to pathogens, conditions of the environment (feed and hygiene), management (management of calving, stagnation and management of colostrum administration), nutritional status and the status of the immune system (Klein-Jobstl et al., 2014).

The increase in growth, higher daily weight gain, the increase in dry matter intake and the optimal development of rumen can be gained by an increase of nutrient supply consisting of the increase of fed milk, adding fodder or feed additives in starter mixes (Bahmanpour et al., 2023).

There are many feed additives applied in the diet of animals such as probiotics, prebiotics, synbiotics, phytobiotics and food industry waste which have a positive impact on the health condition of farm animals (Gálik et al., 2023; Rolinec et al., 2023; Hanušovský et al., 2020). For example, lactic acid bacteria are used for silage fermentation, other microorganisms can be used as a source of proteins or for providing amino acids, vitamins, etc. (Wenk, 2000).

Probiotics are defined as living microorganisms that positively impact their host by improving their intestinal microbial balance (Gibson et al., 1995). Lactic acid bacteria are used most frequently as probiotics, especially the genera *Lactobacillus*, *Streptococcus*, *Enterococcus* and *Lactococcus* (Ouwehand et al., 2002). Next, members of the genus *Bifidobacterium* or the yeast cells *Saccharomyces cerevisiae* are applied. The yeast cells *Saccharomyces cerevisiae* can influence the production of volatile fatty acids in rumen (Doležal et al., 2012). Probiotics improve resistance against infectious diseases, increase growth abilities and production, improve the conversion of feed, and promote digestion of feed and absorption of nutrients. Effective probiotics stimulate beneficial microorganisms in the GIT and suppress pathogens by means of competitive exclusion (Dawson et al., 1990).

MATERIALS AND METHODS

Animal ethics

All methods and processes described in this paper were approved in compliance with the "Act on protection of animals used for scientific purposes" of the Czech Republic, which is in compliance with the directive 2010/63 / EU on the protection of animals used for scientific purposes by the decision of Ministry of Agriculture of the Czech Republic no. 22036/2019-MZE-18134.

Animals and basic feed ration

The experiment took place from March 2022 to March 2023 in a commercial dairy farm (N 49° 55′; E 14° 21′) in the Czech Republic. 100 Holstein heifer cows aged from 1 to 56 days were included in the experiment. The feed ration was adjusted according to the nutrition

demands. The calves were immediately after their birth placed in outdoor individual boxes to stay there until they reached the age of 56 days. The calves were fed colostrum at the latest two hours after their birth, at a temperature of 38 °C. The colostrum had a minimum volume of immunoglobulins 21% Brix, measured with an optical refractometer Hadex ATC 0-32% Brix. The calves were fed with thawed colostrum of an exactly set level of immunoglobulins twice a day in the amount of 3-4 liters per feeding. The calves were fed colostrum and subsequently a milk replacer in plastic buckets with nipples. These were placed in the individual outdoor boxes 45 cm above the ground. From the 3rd day after calving the calves were fed with a dairy feed mixture twice a day in the amount of 4 to 5 liters per one feeding with *ad libitum* access to drinking water, a granulated starter mixture, and hay. The calves were offered the starter feed since their 3rd day. They were fed with a dairy feed mixture up to their 10th day. Then they were fed native milk. They were continuously used to roughage by adding hay after their 3rd day. All calves were micro-chipped with an ear chip by the company Alltex controlled by the programme SenseHub to monitor regularly the movement activity of the calves.

The formula of the dairy feed mixture: a mixture of vegetable oils (palm and coconut oil), dried whey protein, calcium carbonate and hydrolysed wheat glutein. Analytical components: crude protein 23%, crude oils and fats 18%, crude fibre 0,15%, crude ash 7,5%, calcium 0,9%, sodium 0,4%, phosphorus 0,7%. Nutritional additives: vitamins A 25 000 m.j./kg, vitamin D3 10 000 m.j./kg, vitamin E 500 m.j./kg, potassium iodide – 0,25 mg/kg, manganese sulphate monohydrate – 40 mg/kg, copper sulphate pentahydrate – 10 mg/kg, sodium selenite – 0,4 mg/kg, ferrous sulphate monohydrate – 100 mg/kg, zinc sulphate monohydrate – 50 mg/kg. Antioxidants E321 BHT 150 mg/kg. Preservative: citric acid – 1000 mg/kg. The formula of the starter: extracted soybean meal without GMO 24,5%, barley 20,14%, corn 17%, dry matter 10%, wheat bran 9%, oat 8%, wheat 5%, complete mineral and vitamin premix 0,2%, sugar 1,5%, vegetable oil 1,5%, lime stone 1,45%, salt 0,48%, vitamin A - 145 000 m.j./kg, vit. D3 – 2 700 m.j./kg, zinc oxide – 85 mg/kg, vitamin E in the form of alphatocopherol – 70 mg/kg, manganese oxide – 60 mg/kg, copper sulphate pentahydrate – 25 mg/kg, anhydrous calcium iodate – 1,30 mg/kg and sodium selenite 0,50 mg/kg.

The formula of the hay (g/kg of dry matter): dry matter 849,0; nitrogenous substances 133,5; soluble nitrogenous substances 24.8; non-degradable feed protein actually digestible in the small intestine 92,0; net energy of lactation 5,04; neutral detergent fiber 576,0; acidophilic detergent fiber 325,0; starch 0,0; sugars: 78,5; fat 27,5.

Care of calves after calving

Each calve was provided basic care after calving. The viability of each calf was checked and after securing the vital functions the naval was dipped in disinfection. To treat the naval the Pederipra Spay (a chlorotetracycline spray for treating surface wounds) was applied. A treated and cared for calf was placed in a clean, disinfected individual outdoor box, with straw bedding. A veterinarian dehorned the calves aged 3 to 4 weeks by means of a gas cautery in compliance with the Act 246/1992 Coll. On the protection of animals against cruelty. To monitor whether a sufficient amount of colostrum was consumed the calves collected blood from vena jugularis between the 3rd and 5th day after calving. The blood was centrifuged at 2000 rotations/min. The total level of protein was controlled in the blood plasma by means of a digital refractometer.

Experimental design

One hundred Holstein heifers from a closed herd were included in the experiment. These were randomly divided into 4 groups – 25 in the group *Lactobacillus sporogenes* (LS), 25 in the group *Saccharomyces cerevisiae* (SC), 25 in the group of a combination thereof *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* (CLS) and 25 in the control group (C). The calves in the experimental group LC received orally 5 g of *Lactobacillus sporogenes* (4,1 x 10^8 CFU / g) in colostrum and subsequently in their dairy feed mixture from the 1st up to the 14th day after calving. The calves in the experimental group SC received

orally 3 g of *Saccharomyces cerevisiae* in colostrum and subsequently in their dairy feed mixture from the 1st up to the 14th day after calving. The group CLS received 3g orally of (4,1 x 10^8 CFU / g) and 3g of *Saccharomyces cerevisiae* in colostrum and subsequently in their dairy feed mixture from the 1st up to the 14th day after calving. The experimental groups were fed these additives once a day (at the first feeding). The control group became a basic feed dosage without additives.

All calves were weighed within 2 hours after they were born, then on their 14th, 21st and 56th day. To evaluate and detect diarrhea a classical method according to Larson et al. (1977) was applied. Excrement monitoring and monitoring of health status were evaluated twice a day at the same time by measuring temperature in the rectum at feeding periods. The respiration status was evaluated according to the types of symptoms (normal, rhinitis, labored breathing and cough – humid or dry). Respiratory diseases were evaluated as random, intermittent, or persevering. The caretakers monitored the state of hair and eyes (dull or bright) and marks of dehydration (sunken eyes, inelastic skin, exhaustion).

Weighing the calves

The calves were weighed when transported from the calving barn to individual outdoor boxes, at least 2 hours after calving, and then on the 14th, 21st, and 56th day when transported to group boxes in the calf shed. To weigh and transport a double wheel cart with an inbuilt tensometric scale with an accuracy of 2 tenths was used. The cart was also provided with a fixing barrier and was also used to dehorn the calves. No calf died during the experiment and no twin calves were ranged in any group.

Statistical analysis

The data were analyzed using a General Linear Model ANOVA (four ways with the interactions) of the statistical package STATISTICS 10 (Analytical Software, Tallahassee, FL, USA). Factors were evaluated of the treatment group (1 – LS, N = 25; 2 – SC, N = 25; 3 - CLS, N = 25 and 4 – C, N = 25); Normality of data distribution was evaluated by Wilk-Shapiro/Rankin Plot procedure. All data conformed to a normal distribution. Significant differences between groups were tested by Comparisons of Mean Ranks. Values are expressed as means ± SD and differences were considered significant at $P < 0.05$.

RESULTS

The impacts of probiotic feed additives on live weight, weight gain, and diarrhea of calves are shown in Table 1. The calves from the CLS group showed the highest live weight in 56 days. Compared with group C (87.34 ± 4.95 kg vs 83.15 ± 5.32 kg, $P < 0.01$) the differences were significant. The experimental groups LS and SC presented a statistical significance compared to group C (86.41 ± 5.34 kg vs 83.15 ± 5.32 kg, $P < 0.05$ an 85.92 ± 5.86 kg vs 83.15 ± 5.32 kg, $P < 0.05$). The impact on decrease of occurrence and duration of diarrhea was not proved statistically $P = 0.0634$, there was, however, a tendency to decrease its occurrence and duration.

All experimental groups showed slightly higher values of the total protein compared to the control group. However, a statistically significant difference was not proved (Table 1) (CLS - P = 0.54; LS - P = 0.68; SC - P = 0.73). All values were within the reference range of 50 – 70 g/l.

Table 1. The impact of probiotic feed additives on live weight gain, diarrhea and the level of total amount of protein in blood serum in calves

Variables	N	Treatment groups				*P*	Significance
		CLS	LS	SC	C		
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD		
birth BW (kg)	100	42.75 ± 4.89	41.14 ± 5.27	41.95 ± 4.71	42.85 ± 4.25	NS	
BW on 14th day (kg)	100	54.63 ± 4.56	53.98 ± 5.21	54.74 ± 4.77	53.12 ± 4.43	NS	
BW on 21h day (kg)	100	63,36.72 ± 4.81	61,84.23 ± 4.95	61.12 ± 4.89	59.55 ± 4.55	0,038	1:4*
BW on 56th day (kg)	100	87,34 ± 5.31	85,41 ± 5,34	85,92 ± 5,86	83,15 ± 4.86	0.0072**	1:4**, 2:4*, 3:4*
ADG from birth to 56th day (g)	100	796.3 ± 58.0	790.5 ± 57.3	785.2 ± 4.32	719,6.0 ± 85.6	0.0032**	1:4**, 2:4*, 3:4*
Duration of diarrhea (in days)	100	4.15 ± 0.43	4.25 ± 0.36	4,49 ± 0.55	5.01 ± 0.51	NS	
Total number of diarrheas	100	0.18 ± 0.06	0.18 ± 0.09	0.21 ± 0.08	0.25 ± 0.09	NS	
ACTP	100	65,48 ± 4,85	64,12 ± 5,45	63,89 ± 4,66	63,02 ± 5,43	NS	

*P < 0.05; **P < 0.01; NS = non significance; SD = standard deviation; ADG = average daily gains; BW = body weight; P = significance; N = number (CLS – *Lactobacillus sporogenes* and *Saccharomyces cerevisiae*, N = 25, LS - *Lactobacillus sporogenes*, N = 25; SC - *Saccharomyces cerevisiae*, N = 25; C – control, N = 25); ACTP = average content of total protein in blood serum of calves.

DISCUSSION

The inclusion of probiotic feed additives in the nutrition of dairy calves was manifested in the increase of live weight only. A statistically significant impact on the decrease of diarrhea was not proved as stated by Bayatkouhsar et al. (2013). In papers Soto et al. (2014), Frizzo et al. (2011), Timmerman et al. (2005) and Zábranský et al. (2022) the positive impact on the increase in weight of calves was disclosed.

On the other hand, Simon et al. (2001), Uyeno et al. (2015) and Renaud et al. (2019) argue that the improvement in weight increase and feed conversion are isolated and the impact of probiotics on the improvement of the health status is inconclusive and they consider research on this issue insufficient. The results of this paper indicate that adding probiotics in feed dosage has no statistically significant impact on the decrease in the occurrence of diarrhea in calves as published by He et al. (2017) but due to a decrease in duration and the frequency of occurrence of diarrhea, the live weight in experimental groups increased. Diarrhea in calves is a serious problem as shown in the papers by Cho et Yoon (2014), Smulski et al. (2020) and Katsoulos et al. (2020). The health status of calves included in the experiment was evaluated based on counting the occurrence of diarrhea and evidence of the course thereof. The results prove that the administration of probiotic preparations had an impact on the frequency of diarrhea, especially when combinations of probiotic strains were administered. The papers by Liu et al. (2022) and Wu et al. (2021) agree on the positive effect of probiotics.

However, Alawneh et al. (2020) are critical of this statement and recommend studying hematological parameters and the development of the rumen. In the same way, Pinos-Rodríguez et al. (2008) do not recommend feeding yeast cultures to ruminant calves because it did not improve their productive yield despite increased intake of dry matter and improvement in rumen fermentation.

According to Cho et al. (2014), the basic concept of decreasing the occurrence of diarrhea consists of decreasing pathogen prevalence in the environment, i.e. the purity of the environment. Maier et al. (2022) agree with the previous statement and they suggest supporting the preventive measures with vaccination.

Soto et al. (2011) and Ülger (2019) state that a regular, preventive administration of probiotics can result in an improvement in the health state of calves. Also, Alawneh et al. (2020) affirm that there is enough research and evidence proving that a more or less significant improvement of parameters in calf yield resulted from probiotics supplementation.

Morril et al. (2015) argue that the colostrum quality check preceding the administration thereof is a prevention from poor quality feed. Sharon et al. (2020) add thereto that GIT diseases are common in calves insufficiently fed with colostrum of quality. The control of determination of the level of antibodies from the blood of the calves is more important than the quality check of colostrum. Measuring the total protein concentration during the first week can be used as an indirect indicator of colostrum supply.

According to Deelen et al. (2014), the border values of a balanced indication of the level of colostrum-induced immunity in Holstein's calves are the values 8,3% Brix. On average all groups have passed the indicated border value. Gaspers et al. (2014) mention a negative correlation between the serum immunoglobulin G and the birth weight of the calves. Therefore calves having a higher birth weight can have a lower concentration of the serum immunoglobulin G than calves with a lower birth weight in the first 24 hours.

Shivley et al. (2018), Godden et al. (2019), Lombard et al. (2020) and Poborská et al. (2021) argue that a successful transfer of passive immunity is achieved when the concentration of total protein in blood serum exceeds the value of 50 g/l. An unsuccessful transfer of passive immunity is considered a value lower than 50 g/l. To reach this level calves are recommended to be administered colostrum in the volume of 10% of birth weight, i.e. 3 to 4 liters. In this volume, the calves should consume at least 150 to 200 g of immunoglobulin G within 2 hours after birth. A later feeding has a negative impact on the absorption of IgG.

CONCLUSION

Application of probiotic feed additives CLS, LS and SC in colostrum and subsequently in the dairy feed mixture had a proven impact on an increase in live weight gains in calves from the 1st to the 56th day. The tested feed additives had no statistically applicable impact on the decrease of the occurrence of diarrhea in calves to the 56th day after their birth. However, there was a tendency to decrease the occurrence of diarrhea. The results show a more frequent occurrence of diarrhea in control group C, fed only with a basic feed dosage compared to the experimental groups CLS, LS and SC. All calves were fed only high-quality colostrum, therefore the colostrum quality was not studied in this paper as to whether it had, in varying concentrations, an impact on live weight gains and diarrhea. Monitoring these parameters together with other categories, such as the blood profile of calves or microbiological analyses would be a useful supplemental issue to study in further papers.

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