The effects of supplementation of yeast (*Saccharomyces cerevisiae*) and postbiotic from *Lactobacillus acidophilus* on the health and growth performance of young Jersey heifer calves

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ABSTRACT. The study aimed to investigate the effects of a yeast/*lactobacillus* product, 'ZooLac Bovimix Milk', on the performance and health of Jersey heifer calves during the first month of life. The product contained live yeast (*Saccharomyces cerevisiae*) and postbiotic from *Lactobacillus acidophilus*. Danish Jersey heifer calves (n = 148) at birth were randomly allocated to a control diet (CON; 76 calves) or a diet supplemented with the yeast/*lactobacillus* product in the milk replacer (PRO; 72 calves). The average birth weight was 25.5 ± 0.3 kg and 25.4 ± 0.3 kg for CON and PRO groups, respectively. The yeast/*lactobacillus* product constituted 0.7% dry matter (DM) of the milk replacer for PRO group. Faecal samples were collected from 40 calves of each treatment around days 6–8 and 25–28, while blood was sampled from all calves around days 2–4 and 26–28. Additionally, 30 other faecal samples were obtained from some calves treated for diarrhoea. Significantly higher growth performance was observed in PRO group animals than in CON group ones, while no effect was found in the number of antibiotic treatments. The DM-% in manure from PRO group tended to be higher than for CON. Causative agents of diarrhoea were either *Cryptosporidium spp.*, rotavirus A or both. Serum metabolites were unaffected by the treatment, however CON group tended to have a higher IgA level. Thus, supplementation of the yeast/*lactobacillus* product into the milk replacer during the first month of life had a positive effect on calf growth performance but did apparently not affect the overall health.

Introduction

The production of good replacement heifers is essential to maintain a high production level in a dairy herd. Incidences of diarrhoea and respiratory diseases significantly decrease heifer growth in terms of height and weight (Heinrichs and Heinrichs, 2011). Additionally, diseases in the preweaning period have a significant effect on production during the first lactation. In meta-analyses by Soberon and Van Amburgh (2013) and Gelsinger et al. (2016) it was found that milk production during the first lactation is increased by 130–155 kg of milk for every extra 100 g of weight gain per day in the preweaning period. High morbidity also increases veterinary, feed and labour costs.

Additionally, incidences of diarrhoea are a major cause of calf mortality (Yong-Il and Kyoung-Jin, 2014). The young calf is especially susceptible to infection as it has not yet acquired immunological maturity, and thus, mainly being protected by the innate immune system and the passively transferred immunity from immunoglobulins in colostrum. As the calf ages, it becomes less vulnerable to diseases (Galvao et al., 2005).

Besides age, the breed is another factor affecting calf mortality. The calf mortality until day 180 amounts 6.6% among Danish Holstein (average of heifer and bull calves) and 12.5% among Danish Jersey (only heifers) (Fuerst-Waltl and Sørensen, 2010). Thus, it is especially important to enhance the health of Jersey calves, as they seem to be predisposed to have high mortality.

The treatment of diarrhoea is often associated with increased use of antibiotics. However, the use of antibiotics in animal production can be a threat to human health as it increases the risk of developing multiple antibiotic-resistant bacteria (Baynes et al., 2016). Therefore, it is relevant to find preventive strategies that have the potential to enhance the health of calves and thus limit the need to use antibiotics in calf production.

Preventive strategies to decrease the use of antibiotics in animal production are, among others, pro- and postbiotics. Probiotics are live non-pathogenic microorganisms that enhance the health of the host by improving the balance in the gastrointestinal tract (GIT) through molecular and cellular mechanisms. It is believed that probiotics can act by enhancing the innate immunity, disturbing the adhesion of pathogens, promoting intestinal epithelial cell survival, improving barrier function, decreasing pathogen-induced inflammation and/or improving protective intestinal responses (Ohland and MacNaughton, 2010; Alugongo et al., 2017a).

To enhance the health and performance of production animals, yeast products of *Saccharomyces cerevisiae* (SC) origin are commonly included in the diet (Alugongo et al., 2017a). The commercial products available on the market are classified as either live SC or SC fermentation products based on their content of live SC cells (Poppy et al., 2012). However, the two types of products might not have significant differences in their mode of action in the GIT (Alugongo et al., 2017a).

Postbiotics can be obtained from inactivated cell cultures of several probiotics. Postbiotics presumably contain functional bioactive compounds produced by probiotics which may be used to pro-mote the health of the GIT of the host (Wegh et al., 2019). 'ZooLac' (ChemVet; Silkeborg, Denmark) is a postbiotic product available on the market. The active components of the product are killed, but intact, whole bacterial bodies from a special strain of *Lactobacillus acidophilus*, lactic acids and lactic acid salts. It is believed that the product acts as a biofilm in the GIT, and thereby prevents the adhesion of pathogens and enhances the health of the host.

The aim of the current study was to investigate the effects of a product containing live yeast (*Saccharomyces cerevisiae*) as probiotic and a postbiotic product from *Lactobacillus acidophilus*, on the performance and health of Jersey heifer calves during the first month of life.

It was hypothesized that the supplementation of the yeast/*lactobacillus* products would improve the growth performance and reduce the prevalence of diarrhoea in Jersey heifer calves.

Material and methods

Animals, housing and diets

A production trial was conducted at a large Jersey dairy herd in Southern Denmark. All procedures involving animals were conducted in accordance with the guidelines of the Danish Ministry of Justice with respect to animal experimentation and care of animals under study (The Danish Ministry of Justice, 2014, LBK no. 474). The Danish Animal Experiments Inspectorate under the Danish Veterinary and Food Administration was consulted for guidance on required permissions and approved the project activities in writing without requiring further formal application and approval process.

In total, 148 Jersey heifer calves were randomly allocated to either a control diet (CON; 76 calves) or a diet with a yeast/*lactobacillus* product containing probiotic SC and postbiotic from *Lactobacillus acidophilus* added to the milk replacer (MR) from day 0 to 28 (PRO; 72 calves). The randomization was made simply by allocating every second calf born in the herd to either CON or PRO group. Some calves were crossbreds and therefore were not included in the data. Thus, the number of CON group were slightly higher in comparison to the number of PRO group. The calves were born from August to November 2019.

The calves were removed from the dam after 12 h. Time of birth, birth weight and colostrum allocation (time, quality and volume) were recorded for each calf. The average body weight at birth

(mean ± SEM) was 25.5 ± 0.3 kg for CON group and 25.4 ± 0.3 kg for PRO group. At birth, the calves received 2.5 l of colostrum with a BRIX-% of 20–32. CON group received colostrum with a BRIX-% of 24.4 ± 0.3 within 2.7 ± 0.4 h, and PRO group received colostrum with a BRIX-% of 24.5 ± 0.3 within 3.2 ± 0.4 h. The calves were also able to drink colostrum from the dams during the first 12 h of life.

The calves were housed individually in straw-bedded hutches for the entire period. Each hutch was supplied with a bawl for concentrate and a nipple-feeding bucket for milk replacer and water.

The calves were fed 2 l of MR (60% skimmed milk powder, 145 g MR/*lactobacillus* (L), 25.6% crude protein (CP), 10.9 MJ net energy (NE)/kg dry matter (DM)) twice a day, around 8:00 and 16:00 for the entire period. Besides skimmed milk powder, the MR was based on whey powder and vegetable oil. The calves allocated to CON diet were fed MR without any additives, while calves allocated to PRO diet were fed MR supplemented with 1 g of the product 'ZooLac Bovimix Milk' (ChemVet; Silkeborg, Denmark) (7.7 MJ NE/kg DM, 2.8% CP) per 145 g of MR. 'ZooLac Bovimix Milk' contained 55% 'Actisaf Powder' (living SC cells: Lesaffre proprietary strain: NCYC Sc 47/CNCM I-4407) and 45% 'ZooLac'. The inclusion rate of the yeast/*lactobacillus* product followed the manufacturer´s recommendations. The expected colony-forming unit (CFU) of SC in the MR was 6.0×10^9 CFU per kg of MR, and the content of SC constituted 0.4% per kg MR. 'ZooLac' is produced by fermentation of vinasse by the special strain of *Lactobacillus acidophilus*, which after fermentation is inactivated by thermal treatments, followed by lyophilization. According to the information provided by the manufacturer, the product consists of inactivated whole lactic acid bacteria, lactic acids and lactic acid salts. The number of thermostabilized *Lactobacillus acidophilus* was expected to be 2.9×10^9 per kg MR. 'ZooLac' constituted 0.3% per kg MR.

Water was supplied in the nipple-feeding buckets after every milk feeding. Concentrate was offered *ad libitum* from around week 2 of life. The ingredients and chemical composition of the concentrate are shown in Table 1.

Health

The usual treatment protocol in the herd was followed. Antibiotics administered to sick calves by the veterinarian or the farm-staff were recorded daily for each calf. No calves died during the production trial. The calves were vaccinated with 5 ml 'Bovilis

Table 1. Ingredient composition and nutrient content of the concentrate

Indices	Amount
Ingredients, %	
barley	29.0
maize grain	10.0
oat	5.0
soybean meals	21.0
sugar beet	10.0
green hay	5.0
molasses	5.0
rapeseed meals/cakes	5.0
sunflower meals	3.0
citrus	2.0
alfalfa	1.0
fat	1.0
vitamin-mineral pre-mix	3.0
Analysed nutrients per kg of dry matter (DM)	
crude protein, %	24.0
crude fat, %	3.6
starch,%	28.6
ash, %	0.9
net energy, MJ/kg DM	7.7

Bovipast RSP' (MSD Animal Health; Ballerup, Denmark) (BRSV, Pi-3 virus, and *mannheima hemolytica*) around week 3 of age.

Faecal samplings

Faecal samples were collected from the rectum from 40 CON and 40 PRO calves around days 6–8 and from the same calves again around days 25–28. All calves were individually handled. The faecal score was determined for each sample on a scale ranging from 0–2 (0 = semi-formed, pasty, 1 = loose, not formed, but stays in the hands; and/or with mucus, max 25% of the volume, 2 = watery; and/or with heavy amounts of mucus and/or fresh or coagulated blood) (modified from McGuirk (2008)). Afterward, the samples were stored at −20 °C until analysis. Faecal samples were analysed for DM by drying at 60 °C for 48 h.

Thirty other faecal samples were obtained from calves (12 CON and 18 PRO) that were treated with antibiotics for diarrhoea on the first treatment day. It was not possible to obtain samples from all calves treated with antibiotics for diarrhoea. The pathogens in the samples were tested according to Goecke et al. (2020); the chip used was 192.24 (192 samples and 24 assays).

Performance measurements

On day 29, records of final body weight were obtained. The average daily gain (ADG) of the calf was calculated by dividing the total growth with

the age of the calf at the end of the production trial. All calves drank the daily allocated milk replacer during the production trial. The intake of concentrate was not measured.

Blood samplings

Blood samples were collected around days 2–4 and 26–28 from all calves. The blood was sampled by puncture of a jugular vein using vacutainer serum tubes. After 1 h, the blood tubes were centrifuged at $3\,500 \times g$ for 8 min for separation of serum. The serum samples were stored at $-20\,°C$ until they were analysed.

The concentrations of glucose and urea were measured by a spectrophotometric assay following the manufacturer's guidelines (Siemens Medical Solutions, Tarrytown, NY, USA). Non-esterified fatty acids (NEFA) were determined using the Wako, NEFA C ACS-ACOD assay method. β-OH-butyrate (BHB) was determined as an increase in absorbance at 340 nm due to the production of NADH. The method involved oxamic acid in the media to inhibit lactate dehydrogenase as proposed by Harano et al. (1985). All analyses were performed using an autoanalyzer, ADVIA 1800 ®Chemistry System (Siemens Medical Solutions; Tarrytown, NY, USA).

Serum haptoglobin (Hp) concentrations were analysed by a spectrophotometric assay, according to manufactory guidelines (Tridelta Developments Ltd.; Kildare, Ireland). Serum amyloid A (SAA) concentrations were determined by ELISA (Tridelta Developments Ltd.; Kildare, Ireland) according to the manufacturer's guidelines. Concentrations of immunoglobulin G (IgG) and immunoglobulin A (IgA) were determined by ELISA (Bethyl Laboratories Inc; Montgomery, TX, USA) following the manufacturer's guidelines. Serum concentrations of tumor necrosis factor-alpha (TNFα) were assayed as per the manufacturer's protocol using a solid-phase sandwich ELISA validated for bovine TNFα (Thermo Fisher Scientific Inc; Roskilde, Denmark).

Statistical analysis

The data was analysed by using R (software version 3.5.3, 2019; Boston, MA, USA). The initial data was examined to discard any possible outliers. Outliers were defined as values more than mean $\pm$ 3 times the standard deviation. Data were also tested for normality of the residuals by evaluating the QQ-plots constructed in R, and the means were tested for homogeneity of the variance by using Bartlett's test.

A one-way ANOVA was used to test the effects of the yeast/*lactobacillus* product (CON vs PRO) on the performance of the calves, serum blood metabolites and DM content in the manure. The same model was used for all variables analysed. Minor differences in the BRIX-% of the colostrum and time difference from birth to colostrum feeding were initially included as covariates in the statistical model. However, there were no effects of the covariates on any parameter. This might be caused by the fact the calves were also able to drink colostrum from the dam during the first 12 h of life. Thus, the BRIX-% and time difference from birth to colostrum feeding were not included in the final statistical model. Fisher's exact test was used to test the faecal score and number of veterinary treatments.

Statistical significance was declared when $P \leq 0.05$ and statistical tendencies were declared when $0.05 < P \leq 0.10$. Results are presented as least squares means (LSM) and standard error of the mean (SEM).

Results

Health

The total number of antibiotic treatments did not differ between CON and PRO groups (Table 2). Diarrhoea was the main cause of morbidity, and 66% of the calves allocated to CON and 74% of the calves allocated to PRO received antibiotic treatments for diarrhoea during the first month of life. No calves were treated more than once for diarrhoea. The number of antibiotic treatments for pneumonia was also similar between the treatment groups.

Navel infection and calf diphtheria were called 'other diseases' and were defined by a veterinarian. One CON calf had a navel infection, and one PRO calf was treated against calf diphtheria.

Table 2. Number of antibiotic treatments in two groups[1] of Jersey heifer calves from birth to day 28

Indices	CON	PRO	*P*-value
Total number of treatments	53	56	0.474
Treatments for diarrhoea	50	53	0.289
Treatments for pneumonia	2	2	1.00
Treatments for other diseases	1	1	1.00

[1] calves (n = 148) were either fed a control diet (CON; 76 animals) or a diet supplemented with a product containing yeast (*Saccharomyces cerevisiae*) and postbiotic from *Lactobacillus acidophilus* (PRO; 72 animals) in the milk replacer

Faeces

The DM-% in the faecal samples did not differ at the first sampling ($P = 0.878$). The DM-% was 19.2 ± 1.32 in the samples from CON group and 19.5 ± 1.35 in the samples from PRO group. At the second sampling, the DM-% tended to be higher in the samples collected from PRO calves ($P = 0.094$). The DM-% was 17.7 ± 0.79 and 19.4 ± 0.72 for CON and PRO groups, respectively.

There were no differences in the faecal score between treatment groups. The frequency of samples with a faecal score above zero was similar at both samplings (first sampling: $P = 0.579$; second sampling: $P = 0.584$) (Table 3).

Table 3. The distribution of faecal scores in percentage of manure sampled from two groups[1,2] of Jersey heifer calves around days 6–8 (1st sampling) and 25–28 (2nd sampling) of life

Faecal score[3]	CON	PRO
1st sampling, %		
0	45.0	55.0
1	30.0	20.0
2	25.0	25.0
2nd sampling, %		
0	50.0	62.5
1	42.5	32.5
2	7.5	5.0

[1] the Jersey heifer calves (n = 148) were either allocated to a control diet (CON, 76 animals) or a diet supplemented with a product containing yeast (*Saccharomyces cerevisiae*) and postbiotic from *Lactobacillus acidophilus* (PRO, 72 animals) in the milk replacer from days 0–28 of life; [2] the same 80 calves were included in the dataset at both samplings: 40 calves received CON and 40 calves received PRO; [3] fecal score: 0 – semi-formed, pasty, 1 – loose, not formed, but stays in the hands; and/or with mucus, max 25% of the volume, 2 – watery; and/or with heavy amounts of mucus and/or fresh or coagulated blood (modified from McGuirk (2008))

The causative agents in the 30 diarrhoea faecal samples were either *Cryptosporidium spp.* or rotavirus A. Positive for both *Cryptosporidium spp.* and rotavirus A were 17 samples (CON: 8; PRO: 9), 9 samples turned out to be positive for only *Cryptosporidium spp.* (CON: 2; PRO: 7), and 4 samples turned out to be positive for only rotavirus A (CON: 2; PRO: 2).

Growth performance

The birth weight did not differ between the treatments (Table 4). After 28 days, the body weight of animals from PRO group tended ($P = 0.074$) to be higher in comparison to the CON group. The total growth and ADG were significantly higher for the calves allocated to PRO group in comparison to CON group.

Table 4. Body weight at birth and end of the production trial, total growth and average daily gain (ADG) of two groups[1] of Jersey heifer calves

Indices	CON	PRO	SEM	*P*-value
Body weight, birth, kg	25.5	25.4	0.328	0.845
Body weight, day 29, kg	37.2	38.2	0.422	0.074
Growth, kg	11.7	12.8	0.272	0.008
ADG, g/d	393	430	8.71	0.003

[1] the Jersey heifer calves (n = 148) were either allocated to a control diet (CON, 76 animals) or a diet supplemented with a product containing yeast (*Saccharomyces cerevisiae*) and postbiotic from *Lactobacillus acidophilus* (PRO, 72 animals) in the milk replacer from days 0–28 of life; SEM − standard error of mean

Blood metabolites

The concentrations of all measured blood metabolites did not differ between the treatments at birth, except the level of glucose which, for unknown reasons, tended ($P = 0.078$) to be higher in PRO group. At the second sampling, all the blood metabolites were also unaffected by the treatments, however in calves from CON group ($P = 0.083$) a higher level of IgA was noted (Table 5).

Table 5. Blood indices levels in the serum of Jersey heifer calves from two groups[1] on days 2–4 and 26–28 after birth

Indices	CON	PRO	SEM	*P*-value
1st blood sampling				
glucose, mM[2]	6.94	7.34	0.197	0.078
urea, mM[3]	4.09	4.12	0.231	0.989
BHB, mM[4]	0.54	0.54	0.009	0.907
NEFA, µM[4]	148	128	16.0	0.311
Hp, mg/ml[4]	0.092	0.080	0.012	0.551
SAA, µg/ml[4]	214	190	13.2	0.230
IgG, mg/ml[5]	20.6	22.6	1.22	0.248
IgA, mg/ml[6]	0.98	1.08	0.101	0.496
TNFα, ng/ml[4]	0.208	0.244	0.019	0.195
2nd blood sampling				
glucose, mM[4]	6.79	6.85	0.121	0.907
urea, mM[4]	2.78	2.86	0.055	0.297
BHB, mM[4]	0.54	0.54	0.004	0.821
NEFA, µM[4]	86.0	70.8	5.55	0.311
Hp, mg/ml[4]	0.20	0.24	0.036	0.278
SAA, µg/ml[4]	189	193	15.3	0.552
IgG, mg/ml[5]	14.5	13.5	0.43	0.779
IgA, mg/ml[7]	0.032	0.036	0.001	0.083
TNFα, ng/ml[4]	0.146	0.160	0.012	0.385

[1] the calves were either allocated to a control diet (CON) or a diet supplemented with a product containing yeast (*Saccharomyces cerevisiae*) and postbiotic from *Lactobacillus acidophilus* (PRO) in the milk replacer from days 0–28 of life; SEM − standard error of mean, BHB − β-hydroxybutyrate, NEFA − non-esterified fatty acids, HP − haptoglobin, SAA − serum amyloid A, IgG − immunoglobulin G, IgA − immunoglobulin A and TNFα − tumor necrosis factor-alpha; [2] 143 calves were included in the dataset: 74 receiving CON and 69 receiving PRO; [3] 143 calves were included in the dataset: 73 receiving CON and 70 receiving PRO; [4] 148 calves were included in the dataset: 76 receiving CON and 72 receiving PRO; [5] 147 calves were included in the dataset: 75 receiving CON and 72 receiving PRO; [6] 146 calves were included in the dataset: 75 receiving CON and 71 receiving PRO; [7] 139 calves were included in the dataset: 68 receiving CON and 71-receiving PRO

Discussion

The effects of this yeast/*lactobacillus* product on the health and performance of calves were presented till now only in the study of Thorsteinsson and Vestergaard (2020). However, the calves included in that study were one month old at the start of the trial. Thus, the amount of comparative literature is limited. However, it can be assumed that the SC in the tested product has a similar mode of action as the product consisted only of SC instead of SC and 'ZooLac'. The effects of 'ZooLac' alone were not published previousely. Additionally, the effects of postbiotics from *Lactobacillus spp.* in young ruminants were presented only in very few studies (Izuddin et al., 2019).

Health. The yeast/*lactobacillus* product did not affect the number of treatments for diarrhoea. The majority of the calves allocated to both treatments received an antibiotic treatment against diarrhea during the first month of life. This reflects a relatively high infection rate in the herd. Thorsteinsson and Vestergaard (2020) did also find that the yeast/*lactobacillus* product had no effect on the frequency of diarrhoea. However, the no-treatment effect seen in the aforementioned study was probably influenced by the very low frequency of diarrhoea.

The faecal score did not differ between the treatments. A tendency to higher DM-% in faecal samples was observed in PRO group in comparison to CON group. However, this tendency was not reflected in the number of treatments against diarrhoea or in the faecal score. It is important to emphasize that these faecal samples were sampled during the first two months of the entire three-month trial period, while the number of treatments was recorded during the entire trial period. Additionally, the DM content of the faecal samples only provides a snapshot of the digestion in these calves.

Both SC and postbiotic from *Lactobacillus spp.* have been found to lower the frequency of diarrhoea in production animals when fed separately (SC: Galvao et al., 2005; Magalhaes et al., 2008; Hill et al., 2009; Thu et al., 2011; Loh et al., 2013; Alugongo et al., 2017b) (postbiotic: Thu et al., 2011; Loh et al., 2013)). Hill et al. (2009) found that the supplementation of 4 g of SC per day to calves in the milk for 42 first days of life lowered the faecal score, which also indicates a lower frequency of diarrhoea. In the present study, the inclusion level of SC per kg MR was 0.4% corresponding to 2.4 g of SC per day. An inclusion rate of 0.5% postbiotic from *Lactobacillus spp.* has also been found to reduce the incidences of diarrhoea when fed to piglets (Loh et al., 2013).

In the current study, 'ZooLac' constituted 0.3% per kg of MR. Thus, the lack of treatment effect might be explained by a too low inclusion rate of the yeast/*lactobacillus* product even though it followed the recommendation provided by the manufacturer.

The causative agents of diarrhoea might also explain the lack of treatment effect. The 30 faecal samples taken from calves that were treated against diarrhoea showed that the causative agents were either rotavirus A, *Cryptosporidium spp*. or both. The carbohydrate fraction of the yeast cell wall contains mannan oligosaccharides (Hill et al., 2009). The attachment of pathogens to the epithelial cell is often mediated through the binding of pathogenic lectins to D-mannose-containing receptors on the epithelial cell (Eshdat et al., 1978). Thus, mannose residues from cell walls of SC can inhibit the adherence of pathogens as they bind to the residues instead of the receptor on the epithelial cells (Spring et al., 2000). Even though vira, bacteria, fungi and protozoa all express lectins on their surface, the types of lectin vary a lot (Nizet et al., 2017). This may have affected the affinity of the binding of pathogenic lectin to the mannose residues. The mannose residues from the cell wall of SC have been found to act as competitive binding sites for *Salmonella enterica* (Brewer et al., 2014; Harris et al., 2017) but this pathogen was not the one causing diarrhoea in the current study.

Postbiotics contain antimicrobial metabolites such as organic acids and bacteriocins. These metabolites have also been found to inhibit the colonization of pathogenic bacteria e.g., *Salmonella typhimurium, Escherichia coli, Listeria monocytogenes, Pediococcus acidilactici* and *Vancomycin-resistant Enterococci* (Izuddin et al., 2019). Thus, if the causative agents of diarrhoea had instead been related to some of these pathogenic bacteria, a treatment effect might have been detected.

Growth. Calves from PRO group grew significantly more in comparison to CON ones. A similar result was found by Thorsteinsson and Vestergaard (2020) when the yeast/*lactobacillus* product was supplemented to rosé veal calves in milk and concentrate from 4 to 10 weeks of age. The supplementation of SC and postbiotic from *Lactobacillus spp.*, when added separately, have also been found to increase the growth performance of calves (SC: Lesmeister et al., 2004; Galvao et al., 2005), piglets (postbiotic: Thu et al., 2011; Loh et al., 2013) and newly-weaned lambs (postbiotic: Izuddin et al., 2019).

Thorsteinsson and Vestergaard (2020) found that the yeast/*lactobacillus* product lowered (i.e. improved) the feed conversion ratio of rosé

veal calves. SC is known to increase the rumen digestibility of DM, organic matter and neutral detergent fibre in developed and adult ruminants (Khormizi et al., 2010). Similarly, supplementation of postbiotic from *Lactobacillus spp.* has been found to increase the digestibility of DM, CP and NDF in newly weaned lams (Izuddin et al., 2019). In the current study, the calves received the yeast/*lactobacillus* product in the MR. Thus, the product has presumably by-passed the rumen and only affected the digestion from the abomasum and in the intestinal segments of the GIT. To our knowledge, the effects of SC and postbiotic from *Lactobacillus spp.* on the intestinal digestibility in young calves are unknown. However, Shen et al. (2009) found that the supplementation of SC to weaned piglets increased the digestibilities of DM and CP. Similarly, Loh et al. (2013) found an increased protein digestibility when postbiotic from *Lactobacillus spp.* was supplemented to postweaning piglets. It could be speculated that the yeast/*lactobacillus* product has a similar effect on the digestion in young calves as seen in piglets because the stomach of a newborn calf is comparable to that of a monogastric animal due to its small and nonfunctional rumen (Alugongo et al., 2017a). This might have caused the higher growth performance of PRO group.

However, it is important to emphasize that the concentrate intake was not measured in the current study. Thus, in theory, the higher growth performance of PRO group could simply have been caused by a higher intake of concentrate, which was not possible to measure in this setting. The age of these Jersey calves and the milk feeding level used indicate that concentrate intake was unlikely to differ Quigley (1996) found that Jersey calves, fed a slightly lower milk feeding level than in the present study, consumed 66 g concentrate DM/day from week 1–4 of life. A similar concentrate intake of 3–4 weeks old Holstein calves were found by Jensen et al. (2020). If the concentrate intakes from Quigley (1996) or Jensen et al. (2020) are used (around 60–70 g/day), the potential energy contribution from concentrate to the total energy intake would correspond to 3–4% of the net energy in the present study.

Moreover, as discussed below, the BHB level in the blood serum did not indicate a difference in concentrate intake. It can be also precluded that the higher growth performance of PRO group was caused by a lower frequency of diarrhoea.

Blood metabolites. The levels of glucose, BHB and NEFA in blood serum can provide information on the nutritional status and energy metabolism in calves (Alugongo et al., 2017a).

Early in life, glucose is the main energy source for calves as rumen digestion is low and the rumen epithelium is undeveloped. This limits the production and utilization of volatile fatty acids (VFAs) (Baldwin et al., 2004). For unknown reasons, PRO group tended to have a higher concentration of glucose in blood serum on days 2–4 after birth, while no treatment effect was found at the second sampling around 26–28 days after birth. The concentration of glucose is influenced by the energy consumption of the calves (Magalhaes et al., 2008). Samples from both treatment groups were taken immediately after each other on all sampling days. Thus, the time difference from the last feeding to sampling cannot explain the tendency to increased concentration of glucose in PRO group at the first sampling. The similar levels of glucose between the treatments at the second sampling can be explained by a similar intake of MR. This complies with the findings by Thorsteinsson and Vestergaard (2020).

As the calf starts to consume a larger amount of concentrate, the rumen develops, and the contribution of VFAs to the energy requirements increases. The concentration of BHB in the blood is believed to be a result of increased ketogenesis in the ruminal epithelium (Galvao et al., 2005). A positive relationship with BHB and a negative relationship with glucose in the blood plasma of calves with increasing age and starter intake was previously reported (Quigley et al., 1991; 1994). For both treatments, the BHB levels were similar at the first and the second sampling, while the level of glucose was numerically (not tested) lower at second sampling in comparison to first one. This can be explained by the calves' young age at the end of the production trial. The rumen of a one-month-old calf fed a reasonably high amount of MR is still relatively undeveloped (i.e. 580 g of MR DM per day). Therefore, a swift in the main energy source cannot be seen yet. The BHB concentration was also unaffected by the supplementation of the yeast/*lactobacillus* product. Similar results were found by Thorsteinsson and Vestergaard (2020).

The serum urea level was also unaffected by the treatments. This indicates that the calves had a similar protein intake. The lack of treatment effect was also found by Thorsteinsson and Vestergaard (2020). The unaffected urea level in the blood serum in the present study and in they of Thorsteinsson

and Vestergaard (2020) implies that the yeast/ *lactobacillus* product does not affect the microbial activity and incorporation of ammonia into microbial protein.

A high concentration of NEFA indicates adipose tissue mobilization as the calf mobilizes NEFAs to maintain energy homeostasis during times of fasting. The similar concentrations of NEFA in blood serum indicate that the calves allocated to both treatments had a similar adipose tissue mobilization. This result complies with the findings by Thorsteinsson and Vestergaard (2020). In overall, the similar concentrations of glucose, BHB, urea and NEFA imply a similar consumption of concentrate by both treatment groups, suggesting that differences in concentrate intake might not explain the different ADG between the treatment groups.

Concentrations of acute-phase proteins, such as haptoglobin (Hp) and serum amyloid A (SAA), in blood, can be used as indicators of disease in the animal. Hp and SAA are absent or present at very low levels in healthy animals while their concentrations increase during bacterial or viral infections (Gånheim et al., 2007). Gånheim et al. (2003) found that healthy calves have levels below 0.13 mg/ml and 25.6 μg/ml of Hp and SAA, respectively. At both samplings, animals from PRO and CON groups had similar levels of Hp and SAA. The level of Hp at the first sampling was below the threshold described by Gånheim et al. (2003), and thus, the calves allocated to both treatment could be identified as healthy calves at 2–4 days of life, based on the level of Hp. From the first to second blood sampling, the level of Hp increased. Both treatment groups had levels above the threshold of healthy calves. This complies with the high morbidity during the production trial.

The level of SAA was almost 8 times as high as the threshold defined by Gånheim et al. (2003) at both samplings. The level of SAA in the blood seems more sensitive to stimulation in comparison to Hp as an increase in the blood level of SAA can be induced by other factors than diseases, such as stress (Gånheim et al., 2007). Thus, the concentration of Hp might be a better tool for discrimination between healthy and sick calves in comparison to SAA.

A pathogenic infection can initiate an immune response with the associated production of cytokines. TNFα has been proposed as the primary cytokine involved in responses associated with systemic infections with gram-negative bacteria (Basoglu et al., 2004). The concentrations of the measured TNFα were within a normal range for calves (Quigley et al., 2006; Nonnecke et al., 2009) even

though the morbidity was high. If the causative agents had been gram-negative bacteria, which it was not, an elevated concentration of TNFα might have been detected.

The levels of IgG at the first sampling exceeded 10 mg/ml for both treatment groups. Thus, the calves had a successful transfer of passive immunity (Godden et al., 2009). β-glucan in the cell wall of SC has been suggested as a potential immunomodulatory agent as it has been found to activate macrophages, neutrophils, natural killer cells, B and T lymphocytes, and increase phagocytosis and cytokine production in macrophages *in vivo* and *in vitro* (Kogan and Kocher, 2007; Jensen et al., 2008). Similarly, the supplementation of postbiotic from *Lactobacillus spp.* has also been found to increase the concentration of IgG in broilers (Humam et al., 2019) but the potential mode of action is, to our knowledge, still unknown. However, no treatment effect was found at the second sampling. This is opposite to the results found by Thorsteinsson and Vestergaard (2020) who found that the yeast/*lactobacillus* product significantly increased the blood serum level of IgG in rosé veal calves when supplemented from weeks 4 to 10 of life. The conflicting results might be explained by the young age of the calves in the present study. In neonatal calves, the immune cells in the innate immune system, such as the antigen-presenting cells, are functional, but they are present in lower numbers, are less chemotactic, and have lower enzyme activity in comparison to adult cattle. This limits the calves' ability to activate the adaptive immune system, and thus, production of antibodies (Kelly and Coutts, 2000).

The concentration of IgA tended to be higher in CON group in comparison to PRO group at the second sampling. However, the increased concentration of IgA in CON group could not be related to the health of the calves as the treatment groups had similar morbidity.

Conclusions

No effect on the health by the supplementation of the product, containing *Saccharomyces cerevisiae* and postbiotic from *Lactobacillus acidophilus*, in the milk replacer to Jersey heifer calves during the first month of life was found. Animals from control and experimental groups had a similar total frequency of antibiotic treatments and treatments against diarrhoea. However, the calves supplemented with the product containing probiotic and postbiotic had a significantly higher growth performance.

The serum concentrations of glucose, urea, β-OH-butyrate, non-esterified fatty acids, haptoglobin, serum amyloid A, immunoglobulin G and tumor necrosis factor α were unaffected by the supplementation of the product, while the control group tended to have a higher concentration of immunoglobulin A.

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