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Long-term use of yeast fermentation products in comparison to halofuginone for the control of cryptosporidiosis in neonatal calves

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ABSTRACT

The objective of this study was to compare the effect of non-GMO Saccharomyces cerevisiae fermentation products (SCFP) with that of a halofuginone treatment against Cryptosporidium parvum infection in pre-weaned calves on a commercial dairy farm. A total of 123 neonatal female calves, housed in individual hutches, were enrolled sequentially based on date of birth in 41 blocks of 3 animals each. Calves within each block were allocated to one of 3 treatments: remaining untreated, fed with SCFP (Diamond V SmartCare® at 1 g/d in milk and NutriTek® at 5 g/d in starter grain) for the first 63 days of life, or treated with halofuginone (0.1 mg/kg/d) for the first 7 days of life. Fecal samples collected on days 4–21 post-partum were examined for both Cryptosporidium oocysts and coproantigen. The presence and intensity of diarrhea were monitored by scoring daily for the first 4 weeks of life. Calves were weighed at 0, 21, 42 and 63 days of age. Almost all calves were Cryptosporidium-positive at least once during the study. Halofuginone significantly reduced the number of Cryptosporidium-positive fecal samples as compared to the two other groups. Based on the coproantigen scores, both halofuginone and SCFP feeding significantly reduced the intensity of Cryptosporidium infection as compared to the untreated group. Diarrhea was recorded in almost all calves at least once. Neither the proportion of diarrheic calves nor the intensity and duration of diarrhea differed among the 3 treatment groups significantly. The mean daily weight gain during the first 3 weeks of life was significantly lower in halofuginone treated calves than in both other groups; however, at the end of the study period the total weight gain did not significantly differ among the 3 treatment groups. In conclusion, the clinical results and weight gains of pre-weaning supplementation with the SCFP were neither better nor worse than the 7-day halofuginone treatment suggesting that the SCFP feeding may be from the clinical point of view a natural alternative measure, instead of halofuginone treatment, in bovine cryptosporidiosis.

1. Introduction

Cryptosporidium species are minute protozoan pathogens of vertebrates (mammals including man, birds, reptiles, fish and amphibians) with a worldwide occurrence (Fayer, 2008; Deplazes et al., 2016). They belong to the phylum Apicomplexa and have been recently reclassified from coccidian to gregarine parasites (Cavalier-Smith, 2014; Thompson et al., 2016). More than 30 Cryptosporidium spp. are known at present, the genotype B ('bovine type') of the C. parvum complex being a major enteropathogen in neonatal calves (Broglia et al., 2008; Feng et al., 2018; Holzhausen et al., 2019). Cryptosporidium spp. have a direct life cycle. In brief, infected hosts shed infectious oocysts with their feces, and new hosts become infected by oral ingestion of oocysts as host-to-host route, from their environment or via oocyst-contaminated drinking water or food. In mammals, Cryptosporidium spp. primarily infect the intestinal epithelium parasitizing the microvillous brush border of enterocytes. The endogenous life cycle includes a first asexual multiplication (merogony) followed by a sexual development (gamogony) and a second asexual multiplication (sporogony) resulting in huge quantities of infectious oocysts which are shed with feces. Oocysts can remain viable and infectious in the environment for months (Fayer, 2008; Deplazes et al., 2016).

Cryptosporidium infections are very common and endemic in many cattle farms worldwide, especially in large dairy enterprises (Joachim et al., 2003; Trotz-Williams et al., 2007; Al Mawly et al., 2015; Delafosse et al., 2015; Niine et al., 2018; Urie et al., 2018a, b). C. parvum is one of the four main causes of diarrhea in calves within the first 3 weeks of life (Gillhuber et al., 2014; Al Mawly et al., 2015;
2.3. Test materials

Two S. cerevisiae fermentation-based non-GMO feed additives (SCFP), SmartCare® and NutriTek™, were supplied by Diamond V. Both products were stored at room temperature and given according to the manufacturer’s instructions: 1 g SmartCare/calf/day was mixed in milk and given orally, and 5 g NutriTek/calf/day were mixed with a small amount of starter feed and offered directly for feeding, both at the same time each day on days 1–63.

Halofuginone (HALO; Halocur® 0.5 mg/ml, MSD Tiergesundheit, Germany) was given according to the manufacturer’s instructions: calves of 35–45 kg and 45–60 kg birth weight received 8 ml and 12 ml Halocur, respectively; lighter or heavier calves received 2 ml Halocur/10 kg (corresponding to a dose rate of 0.1 mg halofuginone/kg). The drug was orally administered using the product’s dispenser once daily after morning feeding on days 1–7.

2.4. Treatment groups

The study calves were sequentially enrolled in 41 blocks of 3 animals based on their date of birth, and calves within each block were allocated to one of 3 treatments: 1) remaining untreated (CON); 2) fed with SCFP; or 3) treated with HALO. After allocation any calf suffering from a disease other than diarrhea during its first 3 weeks of life as well as both calves in the same block were removed from the study and replaced by a new block of 3 calves.

2.5. Sampling and clinical methods

The calves were weighed using a scale on the day of birth as well as on days 21, 42 and 63. Each calf was examined daily by inspection for any clinical symptoms in the morning throughout the study period. Blood samples were collected from each calf by puncture of the Vena jugularis on day 4; serum was isolated by centrifugation and stored at −20 °C until examination. A total of 17 fecal samples were rectally collected from each calf (in the morning of days 4–14, 16, 18, 21, 28, 42 and 63), labeled and kept at +4 °C until examination. The consistency of fecal samples was estimated by scoring (solid = score 0, semi-liquid = 1, and watery = 2). Two of the four calves that died during the study were necropsied by an external laboratory.

2.6. Laboratory methods

2.6.1. Serum total protein concentration

To determine the efficacy of passive transfer of immunity serum total protein concentration (g/l) was estimated using a hand refractometer (Weaver et al., 2000).

2.6.2. Cryptosporidium

The negative carbol-fuchsin fecal smear staining method was used to detect Cryptosporidium oocysts (Heine, 1982; Potters and Van Esbroeck, 2010) in fecal samples of days 4–21. The intensity of oocyst shedding was estimated by scoring (scores 0–4) according to Castro-Hermida et al. (2002). For the detection of Cryptosporidium spp. coproantigen, a commercially available immunochromatographic in-situ rapid diagnostic kit (SmartStrips® Cryptosporidium-Bio K403 in combination with SmartStrips® App; both: BioX Diagnostics, Rochefort, Belgium) was used according to the manufacturer’s instructions. This test allows a semiquantitative estimation of the intensity of Cryptosporidium infection by scoring (scores 0–4).

For the molecular characterization of the Cryptosporidium species and C. parvum genotype present on the farm, DNA was isolated from 10 oocyst-positive fecal samples of different calves using QIAamp DNA
Stool kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s protocol. The small-subunit ribosomal RNA (SSU rRNA) gene was partially amplified by PCR, and restriction fragment length polymorphism (RFLP) analysis was performed as described by Xiao et al. (1999). To identify the *C. parvum* subtype, DNA was isolated, and a 400 bp-fragment of the glycoprotein 60 (gp60) gene was amplified by nested PCR using primers AL3532 and AL3533 in the first PCR and AL3532 and LX0029 in the second PCR as described by Sulaiman et al. (2005). The amplicons were purified and directly sequenced using the primers of the second PCR through an external service provider (LGC Genomics GmbH, Berlin, Germany). The gp60 sequences obtained were assembled and a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) of the GenBank database was performed. Subtypes of *C. parvum* were designated according to the nomenclature described by Sulaiman et al. (2005), i.e., the number of the tandem trinucleotide repeats TCA (A), TCG (G), and TCT (T) coding for the amino acid serine and the number of the repeat ACATCA (R) immediately after the nucleotide triplets.

### 2.6.3. Rotavirus, Coronavirus and Escherichia coli K99

For the qualitative detection of Rotavirus, Coronavirus and/or *E. coli K99* coproantigens in all fecal samples of days 4–21, a commercially available immunochromatographic rapid diagnostic kit (Rainbow Calf Scours-Bio K288; BioX Diagnostics, Rochefort, Belgium) was used according to the manufacturer's instructions.

### 2.6.4. *Entamoeba*, *Giardia* and other parasites

On day 63, a fecal sample of each calf was qualitatively examined for *Entamoeba* spp., *Giardia* sp. and any other parasite stages, e.g., *Entamoeba* cysts and *Strongyloides* eggs, using the modified formalin-ether sedimentation technique (Young et al., 1979). Additionally for *Giardia* spp. coproantigen, a commercially available qualitative enzyme immunoassay (Prospect™ *Giardia* Microplate Assay, Virotech Diagnostics GmbH, Rüsselsheim, Germany) was used according to the manufacturer’s instructions.

### 2.7. Statistical analyses

Statistical analyses were performed using the statistical program packages BMDP (Dixon, 1993) and BLAS (Ackermann, 2010). For the description of parameters the respective arithmetic mean, standard deviation, minimum and maximum were calculated. Differences of the detection of any pathogen between the 3 treatment groups were analyzed for significance using Chi²-test (dichotomous or nominal scaled variables) or Kruskal-Wallis test with Bonferroni corrected Dunn’s post-test (ordinal scaled parameters). To get a global basis for evaluation the intensity of the *Cryptosporidium* infection was estimated using the ‘area under the curve’ (AUC) of both the oocyst shedding and coproantigen scores over the time. In a first step, the AUC of the respective scores were calculated for each calf during days 4–21. In a second step, a statistical comparison was done for the individual AUC values using the one-way analysis of covariance with repeated measures with respect to the treatment group, having the grouped number of parturitions of cows as a covariate; in case of an overall significant difference the scores were not metrically scaled and normally distributed, the results must be seen as exploratory ones. The scoring results of both methods used for the detection of *Cryptosporidium* infection. Additionally, the correlation of scoring results of both these methods was evaluated using Spearman’s rank correlation coefficient (rs) with respect to the scale of the parameters. All the statistical tests were two-tailed, and differences or effects with p-values ≤ 0.05 were considered as significant.

### 3. Results

#### 3.1. Lactation number of cows

The study calves were born from primiparous, secundiparous or multiparous cows. The average lactation number of cows was 1.15 ± 1.34 and did not differ significantly among the treatment groups (p = 0.822).

#### 3.2. Serum total protein concentration

The serum total protein concentration on day 4 reached or exceeded the value of 55 g/l in 118 calves; in 3 and 2 calves it was 50 g/l and 54 g/l, respectively. It was not influenced by the number of parturitions of dams and did not differ significantly among the treatment groups (CON: 63.3 ± 4.9 g/l; SCFP: 62.4 ± 6.1 g/l; HALO: 61.7 ± 5.5 g/l).

#### 3.3. *Cryptosporidium* infection

##### 3.3.1. Species, genotype and subtype

The *Cryptosporidium* species isolated from 10 different calf fecal samples was identified by PCR-RFLP analysis as *C. parvum*, genotype B, and based on the gp60 gene the isolates belonged to subtype IlaA15G2R1.

##### 3.3.2. Comparison of methods

For all 1713 fecal samples examined the results (positive vs. negative) of the coproantigen immunochromatography substantially agreed with those of the carbol-fuchsin fecal smear staining (kappa value: 0.85; 95% confidence interval: 0.80–0.89). The relative sensitivity and relative specificity of the staining method was 88.9% (86.5–91.0%) and 95.3% (93.7–96.5%), respectively, employing the immunochromatography as relative gold standard. The scoring results of both methods were highly correlated (rs = 0.867; p < 0.001).

##### 3.3.3. Prevalence

Almost all study calves, regardless of the treatment group, shed *Cryptosporidium* oocysts (98.4%) and were positive for coproantigen (99.2%) at least once during their first 3 weeks of life (Table 1). In total, 726 (42.4%) and 766 (44.7%) of the 1713 fecal samples examined on days 4–21 were positive for oocysts or coproantigen, respectively. The number of oocyst- and coproantigen-positive fecal samples in the treatment groups differed significantly (p = 0.033 and p = 0.006, respectively).

| Parameter      | Treatment group | N° calves | N° (%) fecal samples |
|---------------|----------------|-----------|---------------------|
| Oocysts       | CON            | 41/41*    | 259/571 (45.4)%     |
|               | SCFP           | 41/41*    | 258/572 (45.1)%     |
|               | HALO           | 40/41*    | 209/570 (36.1)%     |
| Coproantigen  | CON            | 40/41*    | 263/571 (46.1)%     |
|               | SCFP           | 41/41*    | 278/572 (48.6)%     |
|               | HALO           | 40/41*    | 225/570 (39.9)%     |

Values of a parameter with different superscripts in a column are significantly different (p < 0.01); in all other cases values in a column are not significantly different (p > 0.05).

1 Number of positive calves or samples/total number of calves or samples.
respectively): it was significantly lower in HALO treated calves than in those of the CON (p = 0.003 and p = 0.029, respectively) and SCFP fed calves (p = 0.005 and p = 0.002, respectively). There was no significant difference between CON and SCFP (Table 3).

### 3.3.4. Onset and duration

Cryptosporidium oocysts and coproantigen were first detected in feces on day 6 and 4, respectively. There was a trend of a later onset of oocyst shedding and coproantigen positivity in HALO treated calves than in those of the other treatment groups (Table 2). The individual duration of oocyst shedding or coproantigen positivity ranged from 0 to 16 days and 0 to 18 days, respectively, without significant differences among the treatment groups (Table 2).

### 3.3.5. Intensity

The AUC values of both the oocyst shedding and coproantigen scores over time were used to estimate the intensity of the Cryptosporidium infection. The overall comparison of both AUC values showed a significant difference among the 3 treatment groups (oocyst shedding: p = 0.013; coproantigen: p = 0.0062). In the subsequent pairwise comparison, the AUC values of oocyst shedding scores were significantly lower in HALO treated calves (0.77 times, p < 0.01) than in CON ones, and the AUC values of coproantigen scores were significantly lower in both HALO treated (0.77 times, p < 0.01) and SCFP fed calves (0.85 times, p < 0.05) than in CON ones (Table 3).

The two-way analysis of covariance did not show any significant effect of the age of cows (first parturition vs. second parturition, or first parturition vs. > 2 parturitions) on the Cryptosporidium oocyst shedding scores. However, the mean oocyst shedding score was significantly associated with the treatment (p < 0.001), and time (p < 0.001), and there was a significant treatment by time interaction (p < 0.001). This implies that the overall average score was different among the treatment groups, and the scores varied as calves aged. The score curves of CON and SCFP were similar and started earlier and ran higher than that of HALO (Fig. 1), however, overall means of AUC between SCFP and HALO were similar (Table 3). Very similar results were obtained for coproantigen scores (data not shown).

There was no statistically significant correlation between the AUC values of Cryptosporidium oocyst shedding scores (r = 0.074, p = 0.427) or coproantigen scores (r = 0.148, p = 0.111) of each calf and the serum total protein concentration measured on day 4.

### 3.4. Rotavirus, Coronavirus and E. coli

Rotavirus, Coronavirus or E.coli coproantigen was found at least once in 30.3%, 2.4% and 21.3%, respectively, of calves and in 3.5%, 0.2% and 2.3%, respectively, of the fecal samples examined on days 4–21. There was no significant difference among the treatment groups (Table 4).

**Table 2**

First and last day as well as duration of detection of Cryptosporidium oocyst shedding and coproantigen in calves.

| Parameter          | Treatment group | First detection on day | Last detection on day | Duration (days) |
|--------------------|-----------------|------------------------|-----------------------|-----------------|
|                    |                 | Mean (SD)2             | Min-max3              | Mean (SD)2      | Min-max3      |
| Oocysts            | CON             | 9.9 (2.5)a              | 6–18                  | 17.7 (2.2)a     | 13–21         | 8.5 (2.5)a   | 4–16          |
|                    | SCFP            | 9.7 (2.1)a              | 6–16                  | 16.8 (2.1)a     | 12–21         | 8.1 (2.0)a   | 3–13          |
|                    | HALO            | 11.1 (2.3)a             | 6–18                  | 17.9 (2.2)a     | 13–21         | 7.7 (2.7)a   | 0–16          |
| Coproantigen       | CON             | 9.3 (2.9)               | 4–18                  | 17.6 (2.5)a     | 13–21         | 9.0 (3.3)a   | 0–16          |
|                    | SCFP            | 9.4 (2.3)               | 4–16                  | 17.4 (2.4)a     | 12–21         | 9.0 (2.6)a   | 3–13          |
|                    | HALO            | 11.0 (2.9)              | 6–18                  | 18.2 (2.4)a     | 13–21         | 8.0 (3.3)a   | 0–16          |

Values of a parameter with the same superscript in a column are not significantly different (p > 0.05).

1 Day 0 and 21 were the day of birth and last day of fecal examination, respectively.

2 Arithmetic mean (standard deviation).

3 Minimum–maximum.

**Table 3**

Intensity of Cryptosporidium infection in calves estimated by AUC values of oocyst shedding scores and coproantigen scores during days 4–21 post-partum.

| Treatment group | Mean (SD)1 of AUC values |
|-----------------|-------------------------|
|                 | Oocyst scores | Coproantigen scores |
| CON             | 17.0 (6.2)a      | 17.1 (6.5)a          |
| SCFP            | 14.7 (5.6)ab     | 14.6 (4.2)b          |
| HALO            | 13.1 (6.3)b      | 13.1 (5.0)b          |

Values with different superscripts in a column are significantly different (p < 0.05 and p < 0.01 for oocysts and coproantigen, respectively).

1 Arithmetic mean (standard deviation).

**Fig. 1.** Course of mean Cryptosporidium oocyst shedding scores (0–4) of calves daily treated with halofuginone on days 1–7 (HALO), daily fed with S. cerevisiae fermentation products on days 1–63 (SCFP) or remaining untreated (CON).
3.5. Mortality

Four calves having diarrhea died during the study period (3.3% mortality): one calf from each treatment groups CON and SCFP (on day 15 and 18 day, respectively), and two HALO treated calves (on days 14 and 42). The SCFP calf and one of the HALO calves were post-mortem examined by an external laboratory; the pathological diagnosis was ‘bacteria- and/or virus-caused enteritis’ and ‘Candida-caused enteritis’, respectively.

3.6. Diarrhea

3.6.1. Frequency, duration and intensity

Diarrhea (diagnosed if a sample had not solid consistency) occurred in 121 (98.4%) of the 123 calves at least once during days 4–21. In total, 29.9% of all fecal samples showed a semi-liquid or watery consistency, and the treatment groups did not differ significantly. Diarrhea lasted for 5.5–5.7 days on average (Table 5). The majority of fecal samples with altered consistency was seen between days 8 and 18 (Fig. 2). The number of days with diarrhea was not significantly correlated with its serum total protein concentration on day 4 (r = 0.053, p = 0.566). The AUC values of the number of days with diarrhea did not differ significantly among the treatment groups (Table 5).

3.6.2. Diarrhea and Cryptosporidium infection

The intensity of Cryptosporidium infection measured by the individual mean AUC value of coproantigen scores was significantly correlated with the number of days with diarrhea during days 4–21 (r = 0.243, p = 0.007): the higher the AUC values the more days with diarrhea were observed in mean. For oocyst shedding this correlation was not statistically significant (r = 0.046, p = 0.621).

3.7. Bodyweight

The individual birth weight of the calves ranged from 26.0 to 55.4 kg, but the mean values did not differ significantly (p = 0.306) among the treatment groups (Table 6). There was a highly significant group difference of the daily weight gain during the first 3 weeks of life (p = 0.009): HALO treated calves had a significantly lower average daily gain (ADG) than SCFP fed (p = 0.029) and CON calves (p = 0.031). There was no significant difference between SCFP and CON calves. However, no significant difference in ADG was observed for the rest of the study period. At the end of the 9-week study period the individual total weight gain ranged from 15.5 to 41.1 kg, and the ADG was 581 ± 84 g; it was slightly but not significantly lower in HALO treated calves than in calves of the other treatment groups (Table 6).

3.8. Eimeria, Giardia and other parasites

On day 63, 15 (12.9%) of the 116 calves examined were positive for Eimeria (mainly E. bovis) oocysts, 32 (27.6%) for Giardia (cysts or coproantigen) and 35 (30.2%) for Entamoeba cysts without any significant differences among the treatment groups. No stages of other parasites were found.
protein concentration, the intensity of Cryptosporidium. Therefore, there was no impact of the age of dams on the serum total protein concentration reached or exceeded the value of 55 g/l on day 4, regardless of the treatment group. This indicates a sufficient transfer of immunoglobulins by colostrum to calves (Weaver et al., 2002; Niine et al., 2018; Urie et al., 2018a). It is also similar to data from experimental infections (Zambriski et al., 2013) showing that SCFP had been shown to have a positive effect on the intestinal histological alterations in the intestinal mucosa corresponding with the endogenous multiplication of the parasite would result in less pathological alterations in the intestinal mucosa corresponding with the endogenous multiplication of the parasite would result in less patho-

### 4. Discussion

Currently, halofuginone is the only drug approved by the authorities in Europe for the use in newborn calves against Cryptosporidium infection. However, its prophylactic effect against cryptosporidiosis is equivocal (Silverlås et al., 2009). Therefore, there is a demand for alternatives. The results of an experimental study had suggested a re-
duction of histopathological alterations in the small intestines caused by Cryptosporidium in calves when SCFP had been fed (Vázquez Flores et al., 2016). The present longitudinal study was performed to evaluate, for the first time under field conditions, the effects of a long-term feeding with two non-GMO SCFP against Cryptosporidium infection in neonatal calves in comparison with both a prophylactically HALO treated and an untreated group. As main results, the HALO treatment showed a partial anticytrsporidial activity but not a significant clinical effect. The same findings were recorded for SCFP feeding, which was, in other words, neither better nor worse than the HALO treatment.

The calves enrolled in the study had a very similar history: all were females, from the same herd, of the same breed, kept under same management conditions and received the same basic feed. The age of their dams did not differ significantly among the treatment groups. Therefore, there was no impact of the age of dams on the serum total protein concentration, the intensity of Cryptosporidium infection (estimated by AUC values of oocyst shedding or coproantigen scores) nor the intensity of diarrhea (estimated by AUC values of the number of days with diarrhea) of the respective calves. In almost all calves the serum total protein concentration reached or exceeded the value of 55 g/l on day 4, regardless of the treatment group. This indicates a sufficient transfer of immunoglobulins by colostrum to calves (Weaver et al., 2000), which is principally necessary to protect calves from morbidity (Urie et al., 2018c). It was, therefore, not surprising that the serum total protein concentration was not correlated with the intensity of Cryptosporidium infection (measured by AUC values of oocyst shedding or coproantigen scores) or the number of days with diarrhea in this study.

#### 4.1. Cryptosporidium infection

A Cryptosporidium infection was diagnosed in almost all calves in all treatment groups. In untreated control calves nearly half the fecal samples collected during the first 3 weeks of life were positive for oocysts and coproantigen. In contrast, co-infections with other enteropathogens (Rotavirus, Coronavirus and E. coli) were detected in a smaller number of calves, and most of them were positive on one day only, resulting in only few positive fecal samples. This is in accordance with results from previous studies (e.g., Gillhuber et al., 2014; Al Mawly et al., 2015; Anonymous, 2017). Therefore, Cryptosporidium was considered to be the main enteropathogen and responsible for the episodes of diarrhea in the present study. In untreated calves Cryptosporidium oocyst shedding started 9.9 days after birth on average, which is in accordance with previous field observations (e.g., Castro-Hermida et al., 2002; Niine et al., 2018; Urie et al., 2018a). It is also similar to data from experimental infections (Zambriski et al., 2013) showing that the study calves became infected shortly after birth. The species was identified as C. parvum genotype B, subtype IlaA15G2R1 that is commonly found in neonatal calves in Germany (Broglia et al., 2008; Holzhausen et al., 2019) and elsewhere (Feng et al., 2018).

The sensitivity of the direct fecal smear staining was lower compared with a coproantigen detection method used as gold standard, a finding already reported previously (e.g., Chartier et al., 2013). However, the results of both methods substantially agreed indicating that the fecal smear staining can be used as a simple, fast and inexpensive method for the Cryptosporidium diagnosis, at least in endemically infected cattle farms.

#### 4.2. Effect of halofuginone on Cryptosporidium infection

The 7-day prophylactic HALO treatment neither prevented the Cryptosporidium infection nor reduced the number of Cryptosporidium-positive calves. However, HALO treatment resulted in a significant reduction of the number of Cryptosporidium-positive fecal samples as compared with both the CON and SCFP fed calves. There was also a trend, being not significant, for a later onset of oocyst and coproantigen positivity in HALO treated calves than in the two other groups. Finally, the intensity of the Cryptosporidium infection (estimated by AUC values of oocyst shedding or coproantigen scores) was significantly reduced in the HALO group in comparison with CON but not with SCFP. All in all, these results indicate some but not fully prophylactic anticytrsporidial effect of HALO, as already observed in previous studies (e.g., Silverlås et al., 2009; Trotz-Williams et al., 2011; Delafosse et al., 2015; Niine et al., 2018).

#### 4.3. Effect of S. cerevisiae fermentation products on Cryptosporidium infection

Daily feeding with the two SCFP for 9 weeks reduced neither the number of Cryptosporidium-positive calves nor the number of positive fecal samples. It did also not delay the onset or shorten the duration of oocyst and coproantigen positivity in comparison to CON. However, it was interesting to note that the intensity of the infection estimated by AUC values of coproantigen scores (but not of oocyst shedding scores) was significantly lower in SCFP fed calves than in CON ones. This finding may indicate some, although partial effect of the SCFP on the endogenous multiplication of Cryptosporidium, possibly resulting from a modified intestinal environment. Assuming this is correct, a reduced endogenous multiplication of the parasite would result in less pathological alterations in the intestinal mucosa corresponding with the histological findings by Vázquez Flores et al. (2016), albeit obviously without clinical improvement. In this context it is of interest to mention that SCFP had been shown to have a positive effect on the intestinal development of preweaned calves (Xiao et al., 2016) and a positive immunomodulatory effect in animals (Park, 2014; Chou et al., 2017). Of course, it warrants to further investigate the mechanism of action of SCFP.

#### 4.4. Bodyweight gain and diarrhea

In the present study relatively low daily weight gains, ranging from...
566 to 589 g in group average, were recorded at the end of the 9-week period. This may be a negative consequence of the diarrheal episodes during the first several weeks of life in more or less all calves. The number of diarrheal days was significantly correlated with the intensity of the Cryptosporidium infection indicating that the parasite was responsible for diarrhea in the present study. In contrast, a considerably higher ADG (700 g) at an average weaning age of 66 days was reported for dairy calves in a large-scale US study; however, here clinical signs had been seen in only 38% of the animals, including 21% with digestive signs (Urie et al., 2018b).

Although the HALO treatment showed a partial anticryptosporidial activity, it had no clinical effect in the present study: it did not significantly affect the onset, nor the frequency, duration and intensity of diarrhea, the main clinical symptom of cryptosporidiosis. These results are consistent with observations in previous studies reporting a non-significant association between its prophylactic use and the incidence, duration or intensity of diarrhea (Silverlås et al., 2009; Trotz-Williams et al., 2011; Almawly et al., 2013; Meganck et al., 2015). The same findings were recorded for SCFP feeding, which was, in other words, neither better nor worse than the HALO treatment.

A non-significant difference of weight gains between HALO treated and CON calves had been reported by others (Trotz-Williams et al., 2011), and in a more recent study the HALO treatment even had a negative effect on the daily weight gain after 3 months (Niine et al., 2018). It should be stressed that in the present study the 7-day HALO treatment clearly resulted in a negative impact on the weight gain during the first 3 weeks of life as compared SCFP or CON calves. This temporarily insufficient weight development can be explained by the known toxicity of HALO that can induce reversible gastro-intestinal temporarily insufficient weight development can be explained by the known toxicity of HALO that can induce reversible gastro-intestinal activity, it had no clinical effect in the present study: it did not significantly affect the onset, nor the frequency, duration and intensity of diarrhea, the main clinical symptom of cryptosporidiosis. These results are consistent with observations in previous studies reporting a non-significant association between its prophylactic use and the incidence, duration or intensity of diarrhea (Silverlås et al., 2009; Trotz-Williams et al., 2011; Almawly et al., 2013; Meganck et al., 2015). The same findings were recorded for SCFP feeding, which was, in other words, neither better nor worse than the HALO treatment.

4.5. Eimeria, Giardia and other parasites

Some calves from each treatment group were positive for Eimeria spp. oocysts, Giardia and/or Entamoeba cysts at 9 weeks of age. Patent Eimeria infections are occasionally seen at an age of 3 weeks but still considered clinically inapparent (e.g., Faber et al., 2002). Severe Eimeria infection are first observed in older, group-housed calves (Niine et al., 2018) and may then result in morbidity (Bangoura et al., 2012). Giardia spp. infections are very common in cattle farms worldwide. Calves usually become infected before the 5th week of life, and prevalence peaks were reported in 1- to 6-month old animals (Geurden et al., 2010 2012; Gillhuber et al., 2014; Niine et al., 2018; Urie et al., 2018a). Giardia spp. seem to be able to induce diarrhea (Gillhuber et al., 2014) and may reduce weight gain (Urie et al., 2018a) mainly in calves older than one month. Entamoeba bovis infections are not uncommon in cattle but are considered less pathogenic (Matsubayashi et al., 2018).

5. Conclusions

A pre-weaning supplementation with the two S. cerevisiae fermentation feed additives showed very similar clinical results and weight gains in Cryptosporidium infected newborn calves as a 7-day halofuginone treatment under the conditions on this farm. This suggests that feeding with these S. cerevisiae fermentation products may be from the clinical point of view a natural alternative measure, instead of halofuginone treatment, in bovine cryptosporidiosis.

Conflict of interest statement

This study was funded through a grant by Diamond V, Cedar Rapids, Iowa, USA. PZ and IY are current employees of Diamond V.

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