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The effects of supplementing prickly pear (*Opuntia ficus-indica*) powder on dairy calves' health and growth performance

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Abstract

This study aimed to evaluate the effects of dietary prickly pear (*Opuntia ficus-indica*) powder and *Lactobacillus* supplements on calf growth and faecal pathogen counts. Prickly pear leaves were harvested, sun-dried for 14 days, and oven-dried at 105 °C, before grinding into a powder. Twenty-four female Holstein dairy calves (39.03 ± 0.75 kg live weight) were randomly assigned to each of the four treatment groups. The four treatments were: T1: the control group, calves fed a basal diet (milk plus calf meal), without supplementation; T2: calves fed the basal diet plus *Lactobacillus* at 5 g/day; T3: calves fed the basal diet plus prickly pear powder at 2.5 g/day; and T4: calves fed the basal diet plus prickly pear powder at 5 g/day. These treatments were applied from four days of age until weaning. Calf starter feed and clean water were provided *ad libitum* for the entire study period. The dietary treatments affected the feed dry matter intake, feed conversion ratio, average daily gain, weaning weight, faecal total coliform count, faecal *Escherichia coli* count, and faecal *Enterobacteriaceae* count, compared to the control group. It was concluded that the dietary supplementation of *Lactobacillus* or prickly pear powder improves feed efficiency and body weight gain, as well as reducing faecal pathogen counts in Holstein dairy calves.

Keywords: body weight, cactus powder, enteric pathogens, growth, *Lactobacillus*

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Introduction

Newborn calves are generally more susceptible to intestinal diseases (Frizzo *et al.*, 2011), because of their immature and developing immune systems. In addition, the balance of intestinal microbes in calves is primitive, leading to a series of diseases, if no proper management practices are incorporated into the production system (Jiang *et al.*, 2020). These factors lead to inefficient digestion and the poor absorption of nutrients (Morshedy *et al*., 2020). On intensive dairy farms, gastrointestinal infections in calves inhibit nutrient metabolism, delay growth, and predispose calves to diarrhoea and dehydration. Diarrhoea is the main disease causing calf mortality in dairy enterprises, and a number of enterobacteria can cause diarrhoea (Frizzo *et al.*, 2011; Kodithuwakku *et al.*, 2021). The high calf

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mortality rate in cattle production systems in sub-Saharan Africa is a subject requiring immediate attention (Scott *et al.*, 2019). In traditional dairy farming systems, large quantities of antibiotics, in conjunction with electrolyte therapy, are used to reduce calf mortality rates (Cangiano *et al*., 2020; Casper *et al.,* 2021; Kodithuwakku *et al.,* 2021; Davies *et al.*, 2022). However, there is a current push against the extensive use and misuse of antibiotics, because of the risks of antimicrobial resistance (Cangiano *et al*., 2020). Worldwide, antimicrobial resistance is a subject for debate, as it has detrimental effects on both human and animal health, and the spread of antibiotic-resistant genes via contaminated milk or meat products cannot be ignored (Mohammed *et al.*, 2019). Therefore, the use of probiotics and growth-promoting substances as alternatives to antibiotics has been promoted (Frizzo *et al*., 2011; Cangiano *et al*., 2020; Casper *et al*., 2021; Kodithuwakku *et al*., 2021; Davies *et al*., 2022).

As reported by Guo *et al.* (2022), probiotics stimulate intestinal goblet cell mucus production, which lubricates the intestinal epithelium and protects the host animal from invasion and colonisation by pathogens. Tropical and sub-tropical environmental conditions promote the development of multiple enteric pathogens, with potentially devastating results (Busanello *et al*., 2021). The success of a dairy enterprise relies on the maintenance of a healthy cow-calf unit (El-mostafa *et al.*, 2014; Morshedy *et al.*, 2020), and the use of natural antimicrobial feed additives, particularly plant extracts, has therefore been on the rise (Marandure, 2016). The inclusion of agro-industry byproducts and medicinal plants in livestock diets is beneficial, as it can reduce the impact of livestock on the environment, reduce feeding costs, increase feed source alternatives, and offset human-animal competition for food (Morshedy *et al.*, 2020). Plant extracts are the most plausible natural alternatives to antibiotics, as they have antimicrobial properties covering a wide range of microbes (Owusu *et al.*, 2021).

Opuntia ficus-indica, commonly known as prickly pear, is a dicotyledonous angiosperm plant belonging to the *Cactaceae* family, and is remarkably well adapted to arid and semi-arid climates. Compelling evidence of the nutritional and health benefits of prickly pear has emerged in the last decade (El-neney *et al*., 2019; Moula *et al*., 2019; Morshedy *et al*., 2020; Busanello *et al*., 2021). Prickly pears are rich in amino acids, polyphenols, and polyunsaturated fatty acids, as well as in vitamins, compounds, and derivatives with biologically relevant activities. These activities include anti-inflammatory, antimicrobial, antioxidant, hypoglycaemic, and neuroprotective properties (El-mostafa *et al.*, 2014). However, the effects of dietary prickly pear on calf growth and health status have not been fully studied.

It is widely recognised that factors that can alter the gut microbial community can benefit calf health (Jiang *et al.*, 2020; Casper *et al.*, 2021; Guo *et al.*, 2022), and we therefore hypothesised that the addition of prickly pear to calf diets could promote a healthier gut. Consequently, the objectives of this study were to evaluate the effects of dietary *Lactobacillus* and spineless cactus (prickly pear) powder (CACP) supplementation on calf growth performance, as well as on the prevalence of selected faecal pathogens (*Escherichia coli* and *Enterobacteriaceae*), in female Holstein dairy calves.

Materials and methods

Ethical clearance for this research was granted by the Animal Care and Use Committee of the Agricultural Research Council, South Africa (ethical clearance number: 2018/CAES/136).

Study site

The study was conducted at the Agricultural Research Council's Animal Production unit on Irene campus, in the dairy calf section, which is located at 25° 55' S 28° 13' E, at an altitude of 1523 m above sea level, in south Pretoria, South Africa.

Source and preparation of prickly pear leaves

Fresh spineless prickly pear leaves (Figure 1) were collected from an Agricultural Research Council unit situated in Roodeplaat (east of Pretoria), at 25° 44' S 28° 45' E, and at an altitude of 1524 m above sea level. The prickly pear leaves were harvested and weighed following the procedures described by Zeeman (2005), and chopped into 10–15 mm strips using a knife. The strips were naturally dried on a clean, dry cement floor using direct sunlight for 14 days, with frequent turning to prevent moulding (Zeeman, 2005). Thereafter, they were oven-dried at 105 °C for four to five days until a constant mass was achieved. The dried prickly pear samples were ground into a fine powder through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA), and were placed in plastic bottles and kept at room temperature until further analysis.

Figure 1 Spineless prickly pear (*Opuntia ficus-indica)* grown at the Agricultural Research Council unit in Roodeplaat, South Africa.

Chemical analysis of CACP

The crude protein (CP), crude fibre (CF), ether extract (EE), and phosphorous contents of the CACP were analysed according to AOAC (2000) procedure numbers 968.06, 920.39, 962.09, and 965.17, respectively. A Perkin Elmer atomic spectrophotometer was used to determine the calcium content. The neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) contents were analysed according to the methods described by Van Soest *et al*. (1991). Briefly, 0.45 g samples were refluxed with detergent solutions for one hour using an ANKOM 2000 fibre analyser (ANKOM Technology, New York). Heat stable bacterial α-amylase and sodium sulphate were used for NDF analysis, and the fibre fractions are expressed in g/kg dry matter (DM), inclusive of residual ash (Table 2).

The flavonoid concentration of the CACP was determined according to method described by Zhang *et al.* (2009). A 100 g sample of CACP was suspended in 40%–80% ethanol at a ratio of solvent to raw material of 20–40, and was soaked for 4 hours. The mixture was then sonicated in an ultrasonic cleaner (model: KQ2200; rated power: 300 W; temperature: 40 °C; Kunshan Ultrasonic Instrument Co., Jiangshu, China) for 15–40 minutes. The resultant deep brown extract was filtered (Shoa Shong, Shanghai, China). The filtrate was passed through a D101 macroporous absorptive resin column (4.0 × 60 cm; Fubang Chemical Science Technologies Co., Tianjin, China) at a rate of 20 ml/minute. The column was eluted with distilled water until the liquid produced was colourless, and was then eluted with 80% ethanol. The eluant was collected and evaporated using a rotary evaporator (model: RE52AA; Yalong Biochemical Instrument Co., Shanghai, China) under reduced pressure at 40 °C to obtain the flavonoids. After extraction, the total flavonoid content was determined using the aluminium chloride colorimetric method (Zhang *et al*., 2009), and the results are expressed as milligrams of quercetin equivalents per gram of CACP (mg QE/g CACP).

Animal management and data collection

After birth, 24 Holstein calves from primiparous cows of approximately 27 months of age and 450 ± 15.23 kg body weight were separated from their dams and housed in single-roofed pens, the floors of which were covered with black rubber mats and hay for insulation. Soon after birth, the umbilical stumps were disinfected with an iodine solution to prevent bacterial infection. The calves were fed fresh colostrum from their respective dams within two hours of calving, and for the following three days. Each calf was fed 4 L of colostrum per day (2 L in the morning and 2 L in the afternoon), using an open 5 L plastic bucket. Thereafter, the calves were fed whole milk plus commercial starter meal (91.2% DM, 17.5% CP, 18.75% NDF, 8.69% ADF, 0.65% calcium, 0.44% phosphorous, 1.17% potassium, 0.22% magnesium, 250.16 ppm iron, 46.08 ppm manganese, and 10.28 ppm copper; Meadow Feeds, Randfontein, South Africa) until weaning, as shown in Table 1. The calves had *ad libitum* access to clean water throughout the study.

Table 1 Milk feeding regime of Holstein calves from four days old to weaning at 42 days of age

Time (days post-partum)	Morning feeding (09:00)	Afternoon feeding (15:00)
Day 4 to day 7	2 L	2 L
Day 8 to day 14	3 L	3 L
Day 15 to day 35	2 L	2 L
Day 36 to weaning	2 L	0 L

Only female calves were used in this study because the Holstein cows used were inseminated with sexed semen. The calves were weighed at birth to determine their initial body weights, and were thereafter weighed every seven days before morning feeding until weaning at 42 days of age. The average daily gain (ADG) was calculated from the weekly weight gain. The calves were given weighed amounts of calf starter meal each morning, with the left-overs being weighed and recorded the following morning, prior to adding new feed, to determine the daily dry matter intake (DMI). The feed conversion ratio (FCR) was calculated using the DMI, and expressed as the kg of DM consumed over the kg of weight gained.

Experimental design

Twenty-four Holstein dairy calves from the Agricultural Research Council's Animal Production unit were used in a completely randomised design, to determine the effects of four treatments on growth parameters and faecal pathogen counts. The calves were blocked according to order of birth, after which they were randomly allocated to one of the four treatment groups. The experimental treatments were as follows: T1: (the control group) calves fed the basal diet (milk and commercial starter meal) without supplementation; T2: calves fed the basal diet, with *Lactobacillus* (Biorem biological products for calves, Reg number G0958, Act 36 of 1947) supplemented at 5 g/day; T3: calves fed the basal diet, with CACP supplemented at 2.5 g/day; and T4: calves fed the basal diet, with CACP supplemented at 5 g/day. The calves supplemented with *Lactobacillus* (T2) or CACP (T3 and T4) were fed these supplements from day four post-partum until weaning. The two products were mixed into their daily milk allowance, with 50% of the daily supplemented amount provided during each feeding time, at 09:00 and 15:00. In recent years, the use of microbial feed additives has gained popularity in neonatal calf production (Cangiano *et al.*, 2020; Casper *et al.*, 2021), hence the inclusion of *Lactobacillus* as an alternative treatment in this study**.**

Faecal microbial analysis

Samples for faecal microbial analysis were collected within the first 10 days post-partum. Six calves (40.5 ± 3.5 kg body weight) were randomly allocated to each of the four treatments (T1–T4). Fresh faecal samples (50 g) were collected in triplicate from the rectum of each calf by massage, using disposable sterile gloves. Samples were collected before feeding at three, five, and 10 days post-partum. The samples were transferred into tubes with white plastic caps and immediately transported to the laboratory (Jacob *et al*., 2008), where they were stored at −20 °C until further analysis. The total

coliform count was determined according to ISO standard 4832 (2006), and the *E. coli* and *Enterobacteriaceae* counts were determined according to ISO 21528-1 (2004).

Statistical analysis

The daily feed intake and growth measurements were reduced to weekly means prior to analysis. Data were analysed using a one-way analysis of variance (ANOVA), within the general linear model procedure of SAS software (SAS, 2009). The model included calf as a random effect, and treatment and time (weeks) as fixed effects. Initial body weight was included in the model as a covariate. The Tukey (HSD) test was used for the mean comparison of treatments at the P <0.05 level. The statistical model used was:

$$Y_{cgt} = \mu + \alpha_g + \beta_t + (\alpha\beta)_{gt} + \gamma(\alpha)_{cg} + e_{cgt}$$

where:
Y_{cgt} = the observed value for body weight or DMI for calf c from treatment group g at time t;
μ = the overall mean for the population;
α_g = the fixed effect of group g, where g = T1 (control), T2 (5 g/day *Lactobacillus*), T3 (2.5 g/day CACP), or T4 (5 g/day CACP);
β_t = the fixed effect of time t, where t = week 1, 2, 3, 4, 5, or 6;
$(\alpha\beta)_{gt}$ = the fixed interaction of the effects of group g and time t;
$\gamma(\alpha)_{cg}$ = the random effect of calf c, nested within group g; and
e_{cgt} = the error associated with the measurement taken from calf c from group g at time t.

Results

The chemical composition of the CACP supplemented to the Holstein calves is presented in Table 2.

Table 2 The chemical composition of prickly pear (*Opuntia ficus-indica*) powder (CACP) supplemented to Holstein dairy calves

Chemical parameters	Composition
Dry matter	83.30%
Crude protein	2.24%
Ether extract	0.97%
Crude fibre	9.29%
Neutral detergent fibre	32.77%
Acid detergent fibre	12.91%
Acid detergent lignin	2.89%
Calcium	1.93%
Phosphorous	0.04%
Flavonoid (mg quercetin equivalent/g CACP)	3

The effects of the four dietary treatments on calf growth and DMI are presented in Tables 3 and 4. The calves' average DMI values and initial body weights did not differ (P >0.05) between the treatments. There were similarly no differences in DMI between the treatments in weeks one, two, four, five, and six (P >0.05) post-partum; however, the treatments did differ in week three, with T1, T3, and T4 having higher DMI values than T2 (P <0.05). The calves in the T2, T3, and T4 groups had higher (P <0.05) ADG values than the calves in the T1 group. The opposite was true for the FCR, with the T1 calves having a higher (P <0.05) FCR than the calves in the other treatment groups. The average body weight at weaning was higher for the T2, T3, and T4 calves than for the T1 calves (P <0.05).

Table 3 The effects of dietary supplementation with *Lactobacillus* or *Opuntia ficus-indica* powder (CACP) on the growth (kg) and feed efficiency of Holstein dairy calves, from birth until weaning at 42 days of age

Parameters	Treatments				SEM	*P*-value
	T1	T2	T3	T4		
Initial body weight (kg)	39.2	39.1	38.8	39.1	0.72	0.868
Weaning body weight (kg)	58.0^{b}	62.0^{a}	60.3^{a}	61.3^{a}	0.63	<0.001
Average daily DMI (kg)	0.38	0.35	0.34	0.35	0.02	0.429
Average daily gain (kg)	0.44^{b}	0.54^{a}	0.52^{a}	0.51^{a}	0.02	0.002
Feed conversion ratio	0.87^{a}	0.67^{b}	0.65^{b}	0.68^{b}	0.05	0.007

^{ab} Means in the same row with different superscript letters differed significantly (*P* <0.05). T1: negative control, T2: calves supplemented with 5 g *Lactobacillus*/day, T3: calves supplemented with 2.5 g CACP/day, T4: calves supplemented with 5 g CACP/day. SEM: standard error of the mean, DMI: dry matter intake.

The results for the weekly body weight measurements are presented in Table 4. There were no differences (*P* >0.05) between the treatments during weeks one and two. However, in weeks three and five, the T2 group had a higher (*P* <0.05) body weight than the T1 group, while the body weights of the T3 and T4 groups did not differ (*P* >0.05). In week four, the T2, T3, and T4 groups all had higher body weights than the T1 group (*P* <0.05).

Table 4 The effects of dietary supplementation with *Lactobacillus* or *Opuntia ficus-indica* powder (CACP) on the average body weights (kg) of Holstein dairy calves, from birth to five weeks of age

Weeks post-partum	Treatments				SEM	*P*-value
	T1	T2	T3	T4		
1	38.7	38.5	38.1	39.0	0.74	0.855
2	43.5	42.8	42.8	43	0.86	0.809
3	47.6^{b}	50.2^{a}	49.7^{ab}	50^{a}	0.85	0.012
4	51.3^{b}	54.9^{a}	52.2^{a}	52.8^{a}	0.71	0.003
5	55.0^{b}	57.4^{a}	56.0^{ab}	58.0^{a}	0.49	0.008

^{ab} Means in the same row with different superscript letters differ significantly (*P* <0.05). T1: negative control, T2: calves supplemented with 5 g *Lactobacillus*/day, T3: calves supplemented with 2.5 g CACP/day, T4: calves supplemented with 5 g CACP/day. SEM: standard error of the mean.

The effects of the dietary treatments on enteric microorganisms are presented in Figures 2, 3, and 4. The Kolmogorv-Sminov test was done to test for normality and *P*-values of 0.90, 0.930, and 0.838 were observed for the total coliform, *E. coli*, and *Enterobacteriaceae* counts, respectively, indicating that the distributions were normal.

Both the dietary treatments and the sampling day influenced (*P* <0.05) the total coliform counts in the calves' faecal samples. Coliform counts decreased over time in the T2, T3, and T4 groups, and the T2, T3, and T4 groups had lower counts than the control group (*P* <0.05). Coliform counts increased over time in the control group (*P* <0.05).

The dietary treatments also influenced the *E. coli* counts over time (*P* <0.05). A treatment × time interaction was significant (*P* <0.05) for the T1 and T2 groups, with the *E. coli* count in T1 increasing from day three to day five and then decreasing from day five to day 10, and the *E. coli* count in T2 decreasing from day three to day five and then increasing from day five to day 10. In the calves supplemented with CACP (T3 and T4), the *E. coli* counts decreased over time (*P* <0.05). The change in *E. coli* counts from day five to day 10 in the T3 and T4 groups was not significant.

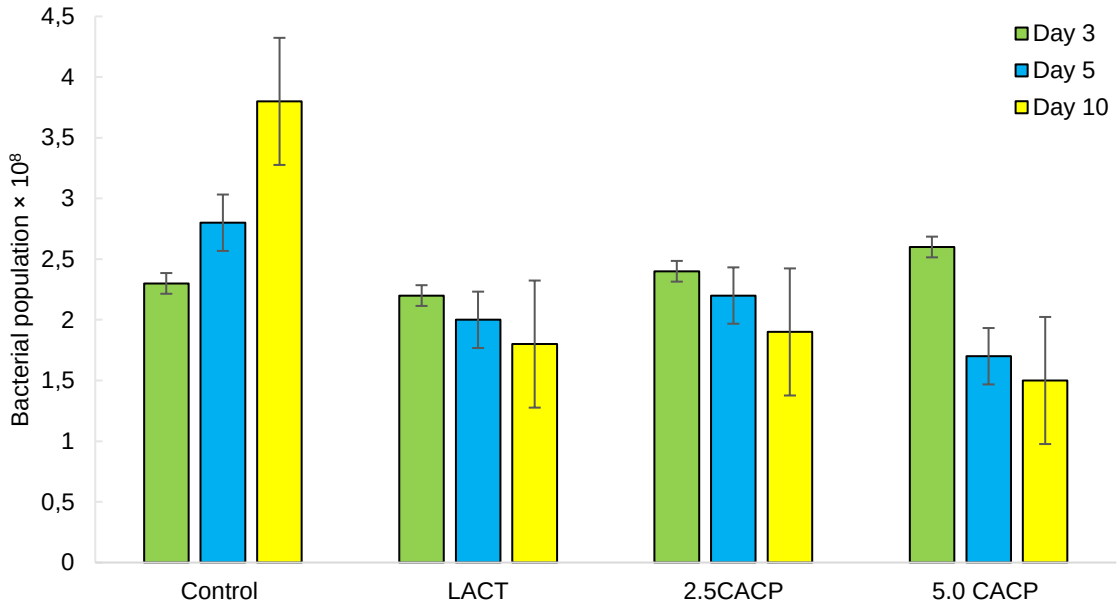

Figure 2 Calf total faecal coliform count, as affected by dietary supplementation with *Lactobacillus* (LACT: 5.0 g/day) or *Opuntia ficus-indica* powder (CACP, 2.5 g/day or 5.0 g/day).

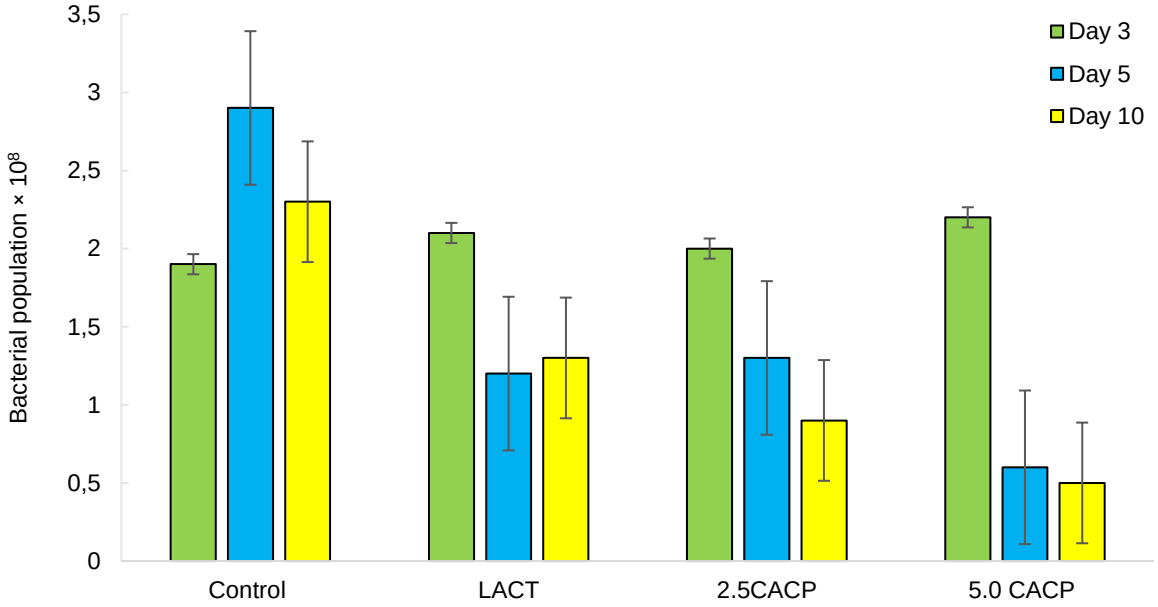

Figure 3 Calf faecal *Escherichia coli* count, as affected by dietary supplementation with *Lactobacillus* (LACT: 5.0 g/day) or *Opuntia ficus-indica* powder (CACP, 2.5 g/day or 5.0 g/day).

The faecal *Enterobacteriaceae* count decreased over time in the *Lactobacillus* and CACP-supplemented calves, but not in the control calves (P <0.05). Low *Enterobacteriaceae* populations were recorded for both the T3 and T4 calves (P <0.05). From day five to day 10, there were no significant changes (P >0.05) in the *Enterobacteriaceae* counts in the T3 and T4 groups; however, a marked change was observed in the T2 group (P <0.05).

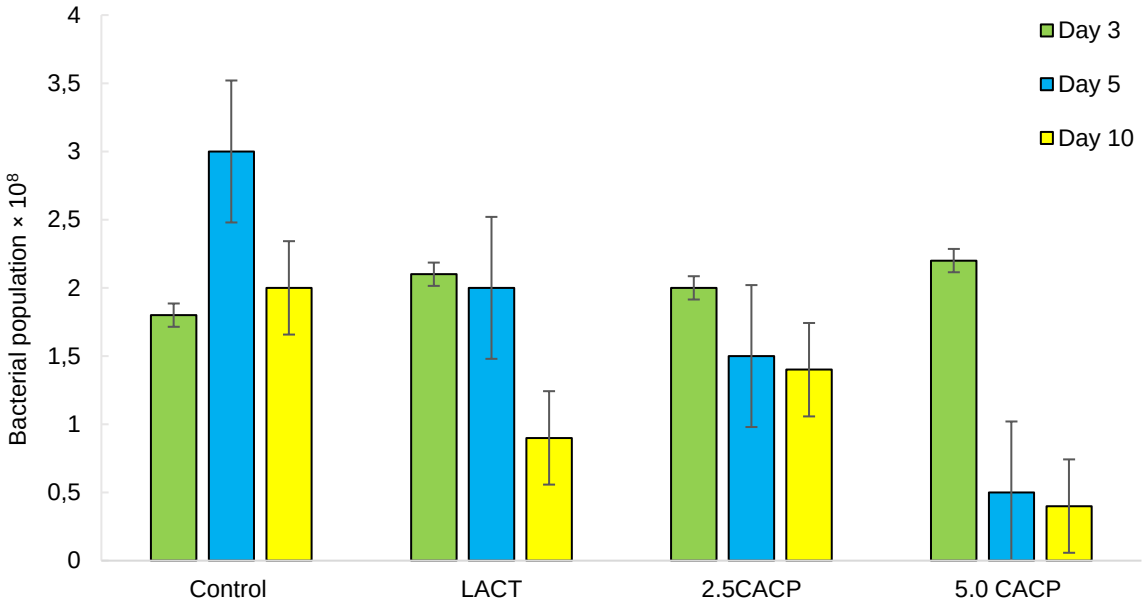

Figure 4 Calf faecal *Enterobacteriaceae* count, as affected by dietary supplementation with *Lactobacillus* (LACT: 5.0 g/day) or *Opuntia ficus-indica* powder (CACP, 2.5 g/day or 5.0 g/day).

Discussion

The prickly pear powder contained low concentrations of CP, fat, ADF, and ADL, but contained adequate DM, NDF, and calcium concentrations to support ruminant animal production (NRC, 2007). Similar results were reported by Ajith *et al*. (2017), and Pastorelli *et al.* (2022) demonstrated that cactus pear could be used as a substitute for pasture hay in ruminant diets without negative effects on production. The high ash and pectin contents of prickly pear could positively affect its utilisation as a livestock feed supplement (Pastorelli *et al.*, 2022), by making it a good source of dietary fibre and phytochemicals (Morshedy *et al.*, 2020). As reported by Medina *et al.* (2021), high-starch diets promote early ruminal microbiome development, and this could explain the effects of the dietary treatments on the growth and weaning weights of the calves. Abubakr *et al.* (2014) similarly postulated that dietary ingredients can boost microbial establishment and activity in calves, and consequently quicken the transition from a liquid to a solid diet, thereby hastening weaning. This may also be due to the decrease in pathogenic organisms that usually colonise the gastrointestinal epithelium of newborn calves (El-neney *et al.*, 2019).

The DMI at 42 days of age in the current study was below the recommended value of 1.4 kg/day (Medina *et al*., 2021); however, the consumption of 0.9 kg of calf starter per day is recommended for rumen development prior to weaning (NRC, 2007). The dietary inclusion of prickly pear has been previously shown to increase the DMI and decrease the water intake (Pastorelli *et al.*, 2022); our results differ from these reports, since neither CACP nor *Lactobacillus* supplementation had a significant effect on the average DMI. Nonetheless, a reduction in DMI has been previously reported for animals fed prickly pear (El-neney *et al.*, 2019; Moula *et al.*, 2019; Pastorelli *et al.*, 2022). This could be attributed to its low CP and NDF contents, which are known to regulate DMI (Washaya *et al.*, 2018; 2021). In other studies, supplementing calves with garlic powder (Kekana *et al.*, 2020) and papaya seed powder (Makoya, 2018) improved calf DMI, as a result of improving gut health. These studies also reported a reduced pathogenic bacterial population. These improvements may be a result of specific properties or a combination of plant secondary metabolites, and similar conclusions could be drawn from the results of the current study.

The *Lactobacillus* and CACP treatment groups had lower FCR values than the control group, secondary to ADG and BW values that were higher than those of the control group. Similar results were reported by Pastorelli *et al.* (2022) and Zhan *et al.* (2017), and this was attributed to the phytochemical components, particularly flavonoids, in the mulberry leaf meal used in these studies. The effects of CACP on live weight have also been reported in other species (Moula *et al.*, 2019; Salem *et al*., 2020). Higher ADG values in calves supplemented with *Lactobacillus* have been ascribed to an increased

abundance of *Bacteroidetes* in the intestine (Jiang *et al.*, 2020), which promoted nutrient absorption and thereby improved growth performance (Diao *et al.*, 2019; La *et al.*, 2019; Zhang *et al.*, 2019). Alternatively, the differences in ADG could be related to differences in propionate and butyrate concentrations. Butyrate stimulates the development of the rumen mucosal papillae and epithelial cells (Beiranvand *et al.*, 2014), and it is possible that there was a shift in fermentation dynamics as a result of the dietary supplements tested in this study. The effects of the flavonoids present in the CACP are not known; however, quercetin 3-methyl ether has been recognised as one of the most abundant flavonoids in prickly pear plants (El-mostafa *et al.*, 2014), and could be responsible for the effects observed, as the effects of phytochemicals on calf growth have been previously reported (El-mostafa *et al.*, 2014; Ali & Ali, 2017; Morshedy *et al.*, 2020). Dietary flavonoids have been found to regulate the secretion of growth hormones (Miksicek, 1993) through the stimulation of the hypothalamus-pituitary hormone axis, thereby accelerating protein synthesis, and resulting in muscle tissue growth and body weight gain. We believe that the same mechanism could have been responsible for the differences in calf growth between the control and treatment groups in the current study, and further studies should be conducted to investigate this hypothesis.

The use of probiotics to regulate calf growth has been extensively studied (Frizzo *et al.*, 2011; Cangiano *et al.*, 2020; Kodithuwakku *et al.*, 2021; Guo *et al.*, 2022; Wang *et al.*, 2022a); however, results have often been either inconsistent or contradictory (Wang *et al*, 2022a). Nonetheless, in this study, *Lactobacillus* supplementation increased the ADG and BW, at a lower DMI (at three weeks post-partum). This was possibly because the probiotics modified rumen fermentation patterns, and offset the normal proportions of volatile fatty acids in favour of butyrate production (Jiang *et al.*, 2020). During this study, the calves' diet consisted of mainly milk and calf meal, and consequently, more butyrate than normal was produced. This may have played a pivotal role in stimulating the development of the rumen mucosa (Wang *et al.*, 2022b). Besides modifying fermentation patterns, lactic acid bacteria are also known to trigger the immune system (Zhan *et al.*, 2017), leading to antibody production. Our results confirm the effects of dietary *Lactobacillus* and CACP on enteric pathogenic bacteria counts. The improvement in calf growth in the current study could therefore be related to the beneficial effects of *Lactobacillus* and CACP on the production of digestive enzymes, the development of the rumen microbiome, and the modification of volatile fatty acid production (Wang *et al*., 2022b).

It is generally accepted that newborn animals are more susceptible to diseases, resulting in high morbidity and mortality rates in calves (Wang *et al.*, 2022b). However, supplementation with probiotics has been found to reduce the relative risk of diarrhoea in calves (Wang *et al.*, 2022a). This was achieved by increasing the levels of circulating immunoglobulins and macrophagic activity (Uyeno *et al.*, 2015), and by competitively excluding enteric pathogens in the intestines. This results in a reduction in intestinal inflammation (Wang *et al.*, 2022b), with a concomitant antioxidant effect. Additionally, the antibiotic effects of probiotics reduce the adherence of pathogens (Wang *et al.*, 2022a). Lactic acid bacteria thus positively impact rumen development and intestinal health in calves, and thereby improve overall growth performance. This is achieved by stimulating the immune system, improving the intestinal microbial environment, and producing bacteriostatic effects against unfavourable microorganisms (Zhang *et al.*, 2019; Jiang *et al.*, 2020). These properties were possibly exhibited by CACP in the current study.

During the early stages of calf growth, calves should only consume milk, because solid food tends to bypass the undeveloped rumen, causing metabolic upsets. The neonatal calf is generally vulnerable to diseases, since they depend on passive immunity (Frizzo *et al.*, 2011; Cangiano *et al.*, 2020). It has been established that neonatal calves are more susceptible to *E. coli* colonisation, and are hence prone to diarrhoea within the first four days of life (Mohammed *et al.*, 2019; Prieto *et al.*, 2022). It was for this reason that we collected the faecal samples between three and 10 days post-partum. According to Young & Rood (2013), *E. coli*, *Salmonella*, and *Clostridium perfringens* types B, C, and D are the most important bacteria responsible for causing calf diarrhoea within the first few weeks of life. The results of the current study clearly show the effects of *Lactobacillus* and CACP supplementation on enteric pathogenic bacteria counts, and we therefore promote their use for better calf rearing in both extensive and intensive farming systems. The effects of CACP on faecal pathogens is attributed to its phytochemical properties, particularly its flavonoid content. Similar conclusions were drawn by Kekana *et al.* (2020) and Morshedy *et al.* (2020). The presence of dietary flavonoids has been previously found to reduce faecal pathogen counts (Bonelli *et al.*, 2018; Formato *et al.*, 2022), and both *Lactobacillus* and CACP supplementation lowered total coliform, *E coli*, and *Enterobacteriaceae* counts in this study.

The dietary supplementation of *Lactobacillus* and CACP may have inhibited the growth of adherent pathogenic intestinal microorganisms by lowering the intestinal pH (Jiang *et al.*, 2020), and thereby reduced the incidence of diarrhoea. Colonisation by lactic acid bacteria has been previously found to optimise the pre-weaning intestinal microbiome in calves (Takino *et al.*, 2017). In addition, Mengatto *et al.* (2015) found that feeding *Lactobacillus* decreased the relative abundance of faecal pathogenic bacteria, such as *E. coli, Salmonella, Vibrio cholera*, and *Helicobacter pylori.* Therefore, we propose that dietary supplementation with *Lactobacillus* and CACP has the ability to inhibit the growth of pathogenic microorganisms in the intestine, and thus decrease the incidence of intestinal diseases.

Conclusions

Dietary supplementation with *Lactobacillus* and CACP improved the ADG and weaning weight of dairy calves, and reduced the prevalence of faecal pathogens. Feeding 5.0 g CACP/day also reduced the faecal coliform, *E. coli,* and *Enterobacteriaceae* counts at five days post-partum.

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Authors' contributions

MPM: conception, development of the original hypotheses, supervision, and results interpretation. SAS: collection of data, collaboration in interpretation of the results, and writing of the initial draft of the manuscript. WS: designing of the experiments, conducting the statistical analyses, and finalisation of the manuscript. MMC: collaboration in interpreting the results and supervision. All authors reviewed and approved the final version to be published.

Conflict of interest declaration

There is no conflict of interest associated with this manuscript.

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