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A randomized clinical trial to examine hazelnut skin extract's effects on diarrhea and bronchopneumonia incidence, growth rate, antioxidant status, and fecal microbiota in neonatal dairy heifers

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ABSTRACT

This study aimed to assess the impact of a 15-d administration of a polyphenol-rich extract from hazelnut skin (HS) on the incidence of neonatal calf diarrhea (NCD) and bronchopneumonia (BP) in Holstein heifers. Additionally, the study investigated whether the extract influenced blood derivatives of reactive oxygen metabolites (d-ROM), serum antioxidant capacity, fecal microbiota, growth rates, and severity of BP and NCD-related clinical parameters. In this randomized clinical trial, 80 healthy female dairy calves were allocated into 2 groups: a control group (CTRL, n = 40) and an HS-supplemented group (HS, n = 40), which received a milk replacer enriched with HS extract (5 g/d) from d 3 to d 18 of life. Daily clinical examinations were conducted for all calves until they reached 21 d of age. Additionally, thoracic ultrasonography (TUS) was performed at 21, 40, and 60 d of age, and weights were recorded at enrollment (d 3), 21 d, and 60 d. These repeated outcomes were analyzed using generalized estimating equations. Blood samples were collected from each calf at the beginning of the study and after HS (or control) administration for the evaluation of serum antioxidant capacity using the oxygen adsorbent test (OXY-Adsorbent) and for determining d-ROM. Additionally, rectal swabs were taken from all calves at 2, 10, 18, and 24 d of life for the analysis of fecal microbiota. The duration of diarrhea in the CTRL group was 22% higher than in HS (incidence rate ratio = 1.22; 95% CI: 1.03–1.45). Calves in the CTRL group had higher odds of elevated fecal scores (odds ratio = 1.23; 95% CI: 1.02–1.52). At 21 d of life, median OXY-Adsorbent values were 397.29 µmol (25th percentile [25°P] = 360.51; 75th percentile [75°P] = 426.30) in CTRL and 440.40 µmol (25°P: 400.97; 75°P: 479.14) in HS, with a significant difference between groups The ADG, fecal microbiota, d-ROM, BP, disease incidence, and all clinical parameters evaluated were not affected by HS administration. In conclusion, these findings suggest that the administration of HS resulted in a reduction in the duration of watery feces during NCD episodes and an improvement in serum antioxidant capacity in treated calves. However, HS supplementation did not demonstrate any effects on the overall clinical health issues associated with NCD, lung lesions identified via TUS, or weight gain. Future research on HS supplementation in calves should be conducted over a more extended administration period and include comprehensive cost-benefit analyses.

Key words: calves, diarrhea, bronchopneumonia, phytotherapy, hazelnut

INTRODUCTION

Neonatal calf diarrhea (**NCD**) and bronchopneumonia (**BP**) significantly affect morbidity and mortality in preweaning calves, raising concerns for animal welfare (Martin et al., 2022; AHAW et al., 2023) and increasing antimicrobial use in the dairy sector (Baptiste and Kyvsgaard, 2017; Redding et al., 2019). The overuse or misuse of antimicrobials for these diseases (Ollivett, 2020; Eibl et al., 2021) has led to a decline in bacterial susceptibility over the years (Stanford et al., 2020; Formenti et al., 2021; Zhang et al., 2022). As concerns regarding antimicrobial resistance grow from a one-health perspective, it is imperative to restrict the use of these compounds in veterinary medicine to instances where they are truly necessary. Considering this, there

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has been a marked increase in interest in unconventional molecules to enhance the health status of farm animals. One promising strategy is phytotherapy, which uses plants or extracts recognized for their pharmacological properties or beneficial chemical components, such as polyphenols. These compounds have been shown to possess antioxidant and anti-inflammatory effects, regulate immune responses, support intestinal microbiota, and exhibit both antimicrobial and antihelminthic activities (Lavecchia et al., 2013; Kodithuwakku et al., 2021; Turini et al., 2022; Nabi et al., 2023). Additionally, these products are often derived from agricultural waste, thereby promoting a circular economy by transforming waste into valuable resources while minimizing environmental impact. In this context, many studies have explored the effects of phytotherapeutic solutions, both in vivo and in vitro, with a focus on their potential health benefits for food animals (Ayrle et al., 2016; Olagaray and Bradford, 2019). However, the outcomes of clinical trials involving phytotherapeutic supplementation in dairy calves have led to variable results. For instance, in several studies, the phytotherapeutic compounds used demonstrated a significant reduction in NCD episodes in calves (Weyl-Feinstein et al., 2014; Mendonça et al., 2021; Serri et al., 2022; Hu et al., 2023; Dell'Anno et al., 2024). In contrast, other studies reported only uncertain health or average daily gain improvements in calves, revealing no significant differences in NCD episodes or BP-related clinical signs between treated and control groups (Maciej et al., 2016; De Paris et al., 2020; Ayrle et al., 2021; Bonelli et al., 2021; Kodithuwakku et al., 2021; Turini et al., 2022; McNeil et al., 2023; Nora et al., 2024; Velázquez-Cruz et al., 2025;).

Hazelnuts (*Corylus avellana*) offer intriguing possibilities in this context. As the second most consumed tree nut worldwide, they find widespread application in the confectionery industry, which generates a notable by-product: hazelnut skin (**HS**). This by-product can be processed to yield an extract that is rich in phenolic compounds, known for their potent antioxidant and antimicrobial properties (Capaldi et al., 2025). Recent research suggests that supplementing the diet of farm animals with HS improves the nutritional quality of animal products and contributes to improved animal health (Candellone et al., 2019; Renna et al., 2020; Menci et al., 2023; Ciliberti et al., 2024).

Research has not yet investigated the effects of HS extract supplementation on the health of newborn dairy calves. Therefore, our primary objective in this randomized controlled study was to evaluate the effect of administering HS extract for 15 d to Holstein heifers aged 3 to 18 d of life. We focused on the incidence and duration of NCD, BP-related lung lesions identified through thoracic ultrasonography (**TUS**), and weight gain. Additionally, the study aimed to estimate blood derivatives of reactive oxygen metabolites (**d-ROM**), serum antioxidant capacity, and fecal microbiota, as well as therapeutic and mortality rates. We hypothesized that HS extract supplementation would clinically influence NCD and BP while promoting growth rates, enhancing antioxidant defenses, and positively affecting fecal microbiota by promoting beneficial bacteria and inhibiting harmful pathogens.

MATERIALS AND METHODS

Study Design and Ethics Statement

We conducted a parallel, randomized clinical trial with longitudinal follow-up, following the Consolidated Standards of Reporting Trials (CONSORT) guidelines, using a sample of dairy calves collected from a commercial dairy farm in January and July 2024. This study received ethical approval from the Institutional Animal Welfare Organization of the University of Milan (approval number 142_2023).

Setting

This study was conducted on a dairy farm selected for its convenience, following the farm practitioner and with the owner's consent to collaborate with the Ruminant and Swine Clinic of the Department of Veterinary Medicine and Animal Sciences at the University of Milan (Lodi, Italy). The farm was located in Codogno, Lodi, Italy (45°09′40″ N, 9°40′06″ E) and consisted of 600 lactating cows. On this farm, calves were separated from their dams immediately after birth and fed 4 L of high-quality colostrum (Brix ≥22%) using a nipple bottle or an esophageal tube. At 48 h of age, jugular blood samples were collected in anticoagulant-free tubes, and serum total protein (**STP**) levels were assessed using an optical refractometer. Calves were housed in individual straw-bedded hutches in an indoor barn, allowing visual and tactile contact with neighboring calves. Their daily feeding regimen consisted of 2.5 L of milk replacer (Emme Erre Flash 22.5, Tredì), administered twice daily at 38°C, with free access to water and grain. From d 8 to 14, an oral rehydration solution (Reidral Record, Tecnozoo) was provided for all calves. At 20 d of age, calves were moved to multiple pens, each housing 10 to 15 calves, and fed using multibucket systems. Weaning was completed between 75 and 80 d of age. Dams were vaccinated against coronavirus, rotavirus, and *E. coli* K99 (Kolibin RC Neo, Bio98) during the late stages of gestation. Calves received intranasal vaccination with a complex that included live-lyophilized bovine parainfluenza 3 and bovine respiratory syncytial virus (Rispoval RS+PI-3 Intranasal, Zoetis) between 3 and 10 d of age.

From 15 to 30 d of age, calves were given subcutaneous vaccinations with inactivated BRSV, PI3-V, and *Mannheimia haemolytica* antigens (Bovilis Bovipast RSP, MSD Animal Health) in 2 doses, with the second dose administered 21 to 28 d later. In addition, all calves received oral treatment with halofuginone (100 µg/kg; Halofusol, Alivira) from 2 to 8 d of age. The treatment protocols for the primary pathological conditions in calves were based on the prescriptions from the farm practitioner. Throughout this study, the farm management practices remained unchanged, and the study group did not intervene in the treatments provided by the farm veterinarian. In summary, calves exhibiting cough, fever, or respiratory distress were administered a treatment regimen that included florfenicol (Effe 70, Fatro; 40 mg/kg, given subcutaneously once) and meloxicam (Metacam, Boehringer Ingelheim; 0.5 mg/kg, given subcutaneously once). Calves affected by NCD were treated according to their clinical signs. Dehydrated calves with diminished sucking reflexes received intravenous infusion therapy with a saline solution and sodium bicarbonate, along with gentamicin (Aagent, Fatro; 4 mg/kg, intramuscularly for 3 d), flunixin meglumine (Alivios, Fatro) at a dose of 2.2 mg/kg intravenously, and vitamin supplements. The farm practitioner was responsible for determining the appropriate dosages and the number of interventions based on the severity of the clinical signs. Additionally, in instances of bloody or watery feces, gentamicin or flunixin meglumine, or both, could be administered without the need for intravenous fluid therapy.

Sample Size Calculation

To ensure external validity and to ground our calculations in realistic conditions, we selected the farm enrolled in the trial based on the prevalence of NCD and BP reported by the farmer. According to reported treatments in 2023, the prevalence was approximately 15% for NCD and 10% for BP. For the a priori calculation, we concentrated on outcomes that were measured repeatedly throughout the study. Specifically, we chose TUS, which was assessed 3 times during the trial, and BW (measured 3 times) as the reference variables for our computations. We opted for these data because other clinical parameters, such as fecal score, were collected more frequently (at 18 different time points), which would have resulted in impractically small estimates for the required sample size. The a priori sample size was determined using G*Power (Version 3.1, Heinrich-Heine-Universität, Düsseldorf, Germany), with a focus on longitudinal clinical scores as primary outcomes. We employed ANOVA (repeated measures, within-between interaction), targeting an effect size of 0.20, an α level of 0.05, and a power $(1 - \beta)$ of 0.95, with 2 groups and 3 repeated assessments. This planning aimed to detect treatment effects on repeated clinical scores over time. Given the limited data regarding the expected effect of HS in calves, we referred to previous studies assessing the effects of polyphenol-rich substances on BW (Serri et al., 2022; Nowroozinia et al., 2022), which indicated a small-to-moderate effect size consistent with 0.20 in G*Power. The analysis showed that 72 animals were necessary. To account for potential data loss due to neonatal mortality, the minimum number of animals was increased by 10%. Consequently, the final number of animals included in the study was rounded to 80.

Eligibility Criteria

This study focused on dairy calves on their third day of life that met the following criteria:

- Only female Holstein heifers were included in the study.
- Calves delivered through both eutocic and nontwin births were considered eligible.
- Calves must have exhibited no signs of illness 2 d before participating in the trial.
- Calves must have ingested all the colostrum provided according to the farm's management protocols.
- They must have consumed all the milk offered to them within 2 d before participating in the trial.
- Two-day-old calves were required to have an STP concentration ≥58 g/L to avoid health potential bias due to failure of transfer of passive immunity (**FTPI**).

Randomization Method, Grouping, and Protocol

Following the determination of a minimum required sample size of 80 calves, we developed an a priori randomization list using an Android application (Randomizer, Darshan Institute of Engineering and Technology, Rajkot, India). This list enabled us to consecutively assign each eligible calf born on the farm to either a control (**CTRL**, n = 40) group or an HS-supplemented group (n = 40). The CTRL group received a milk replacer following the farm's guidelines, and the HS group was supplemented with HS extract (5 g/d) during the 6 a.m. milk feeding for 15 d, from d 3 to d 18 of life. The HS extract was stored on-site in a dry location in premeasured 5-g samples. A trained farm employee, who identified HS group calves using pen tags, mixed the extract with the milk using a whisk. If the supplemented milk was not entirely consumed, it was stored in the farm refrigerator and offered to the calf later in the day.

HS Extract Characteristics

The extract derived from hazelnut skin administered to the HS group was obtained using green extraction methods, as detailed by Capaldi et al. (2025). Specifically, the extract used in this study was referenced as batch 4 extract, originating from a batch provided by a feed company that sources various HS from multiple suppliers (Capaldi et al., 2025). The extraction was carried out using microwave-assisted subcritical water extraction, achieving an extraction yield of approximately 25%. Quantitative analysis of the HS extract revealed a total phenolic content of 778.4 ± 16.0 mg gallic acid equivalent/g of dry extract. Specifically, the breakdown of phenolic compounds included 1.36 ± 0.031 mg/g of phenolic acids, 14.84 ± 0.06 mg/g of flavan-3-ols, and 0.51 ± 0.003 mg/g of flavonols. Consequently, the HS group in this study received a total administered dose of 3,892 mg of polyphenols per calf per day. Additionally, the antioxidant activity measured using the 1,1-diphenyl-2-picrylhydrazyl method yielded a value of 4.51 mmol Trolox equivalent/g extract, and the oxygen radical absorbance capacity test indicated a value of 6.86 mmol Trolox equivalent/g for the batch 4 extract.

Measurements and Clinical Status Evaluations

We measured and recorded milk wastage for both groups during each feeding session. All enrolled calves were weighed using an electronic scale (EOS 300K100XL, Kern & Sohn GmbH, Balingen, Germany) available on the farm at enrollment (d 3 of life), after individual housing (d 21 of life), and again at d 60 of life. Each calf underwent a clinical evaluation from the day of enrollment until it reached 21 d of age, resulting in a total of 18 clinical evaluations per individual. The clinical signs assessed during these evaluations are detailed in the medical record outlined in Table 1. The calves were examined following their afternoon feeding. One of the primary authors (EG) conducted all clinical assessments. Before the onset of the study, AB provided EG with initial training, which consisted of 5 sessions at dairy farms, each lasting approximately 2 h. Additionally, AB continuously monitored the scoring on the des-

Table 1. Scoring system and categories used to examine the longitudinal effect of oral administration of HS extract on 80 newborn Holstein heifers from the day of enrollment until they reached 21 d of age, divided into HS and CTRL groups

Score	Clinical sign
Suckle reflex	
0	Strong
1	Weak
2	Absent or chewing movements
Vigor	
0	Standing securely without assistance, curious, alert
1	Standing up after encouragement, weak, "sad calf"
2	Sternal recumbency, standing after lifting, "drunken gait," insecure posture
3	Permanent sternal/costal recumbency
4	Lateral recumbency, sometimes comatose
Fecal score	
0	Normal (firm but not hard)
1	Soft (does not hold form, piles but spreads slightly)
2	Runny (spreads readily)
3	Watery (liquid consistency, splatters)
Umbilicus	
0	Normal, pencil size, dry, and painless
1	Bigger than normal, but dry and painless
2	Bigger than normal, wet, or painful
3	Bigger than normal, with pus draining and evidence of pain (any presence of internal umbilical swelling ranks as a 3 and should be described as involving $_{\text{arteries}}$/urachus or vein)
Hydration status	Enophthalmos in mm
Rectal temperature	
Spontaneous cough	
Yes	
No	
Joint swelling	
Yes	
No	
Neurological signs	
Yes	
No	

ignated days for TUS to ensure accuracy and consistency in the recorded clinical signs. Calves exhibiting watery feces (fecal score ≥2; Renaud et al., 2020) were diagnosed with NCD. The duration of an NCD episode was defined as the number of days from the initial onset of diarrhea (fecal score ≥ 2) until the fecal score dropped below 2. Furthermore, each enrolled calf had been submitted to systematic TUS conducted by the first author using a portable unit (Draminski Blue, Draminski Ultrasound Scanners, Ząbruk, Poland) with a 7.5 MHz rectal transducer set to a depth of 8 cm. The thorax was not shaved, and 70% isopropyl alcohol was applied to the hair. Bilateral TUS was performed at 21, 40, and 60 d of age in intercostal spaces 10–1 on the right and 10–2 on the left, using a 0-to-5-point scoring scale (Ollivett and Buczinski, 2016). Scores indicated lung condition, with 0 indicating normal aeration and higher scores reflecting an increasing severity of pneumonia, up to a score of 5 for lobar pneumonia affecting 3 or more lobes. Cranial and caudal aspects of the cranial lobe were individually evaluated.

Blood Oxidative Stress Assay

Each enrolled calf from both groups underwent venous blood sampling via jugular venipuncture with a 20-gauge hypodermic needle at enrollment and the end of HS (or CTRL) administration (d 18 of life). The collected blood was stored in a sterile Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ) without an anticoagulant. Samples were transported to the clinic in a portable cooler at 5°C and centrifuged at 20°C for 10 min at 900 × *g* within 4 h of collection. The obtained serum was transferred into 2-mL Eppendorf tubes and stored at −80°C until the blood antioxidant status of all calves was evaluated at the study's conclusion. Serum antioxidant capacity was assessed using the OXY-Adsorbent test (Diacron International, Grosseto, Italy). Briefly, serum samples were diluted 1:100 in distilled water and mixed with a solution containing hypochlorous acid (HClO). After incubation at 37°C for 10 min, a chromogenic solution was added, and absorbance was measured at 505 nm. Results were expressed as μmol of HClO per mL of sample (Pirro et al., 2015). The d-ROM determination was evaluated using the d-ROM test (Diacron International Srl, Grosseto, Italy) according to the manufacturer's instructions and as described by Sauerwein et al. (2020). Briefly, a mixture of acetate buffer and chromogenic solution was added to each sample, and sera were incubated at 37°C for 30 min. Absorbance was measured by photometric reading at 505 nm, and the results were expressed in UCarr (1 UCarr corresponds to 0.08 mg H_2O_2/dL), using a human standard serum as a calibrator after subtracting the blank.

Fecal Microbiota Sampling and Analysis

Starting from the second month of the trial, rectal swabs were collected from all enrolled calves at 4 time points: d 2 (**T0**), 10 (**T1**), 18 (**T2**), and 24 (**T3**) of life. Fecal samples were stored at −80°C until DNA extraction was performed. For the microbiota analysis, calves were retrospectively excluded if they died within the first 24 d of life, received antibiotic treatment during this period, or had missing samples at any of the 4 time points. Bacterial DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. The DNA quality and quantity were assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The isolated DNA was then stored at −20°C until it was used. Bacterial DNA amplification and library preparation were performed following the procedure previously described in Penati et al. (2021).

Statistical Analysis of Clinical and Antioxidant Status

All statistical analyses were performed using SPSS software (version 29 for Mac; IBM Corp., Armonk, NY). Descriptive statistics were reported as mean ± SD or median with 25th and 75th percentiles, depending on the data distribution. Normality was assessed using the Shapiro–Wilk test. Categorical variables were expressed as frequencies and percentages. Comparisons between the 2 groups for single, nonrepeated continuous variables were performed using the independent samples *t*-test or the Mann–Whitney U test, depending on the distribution of the data. Single, nonrepeated categorical variables were compared using the chi-squared test. To assess the effect of HS supplementation on various clinical outcomes, we conducted a series of generalized estimating equations (**GEE**), creating a distinct model for each outcome of interest. In each model, the clinical variable under investigation was designated as the dependent variable, and the treatment group (CTRL vs. HS) was included as a fixed effect. Calf ID was specified as the subject variable to account for repeated measurements, and time points were incorporated as within-subject factors. Neonatal diarrhea duration, measured in days, was analyzed as a count variable, with calves that did not develop NCD included in the analysis and coded as having 0 d of NCD. A Poisson distribution with a log link function was applied for this analysis. All other clinical outcomes—fecal score, TUS score, suckle reflex, vigor score, umbilicus condition, dehydration status, cough, joint swelling, neurological signs, and therapy—were treated as ordinal categorical variables and analyzed separately using GEE with ordi-

nal logistic regression and cumulative logit link. Model fit was assessed using the Quasi-likelihood under the independence model criterion. Results were presented as odds ratios (**OR**) or incidence rate ratio (**IRR**) with 95% CI, and significance was established at $P < 0.05$.

Fecal Microbiota Evaluation

Paired-end reads obtained from fecal samples were imported and processed using a dedicated pipeline for performing microbial community analysis (QIIME2, version 2024.10; Bolyen et al., 2019). Primer sequences were trimmed using the cutadapt plugin (Callahan et al., 2016). Subsequent denoising and chimera removal were performed with a dedicated software package (DADA2; https://benjjneb.github.io/dada2/) applying truncation lengths of 220 base pairs for forward reads and 200 base pairs for reverse reads (Callahan et al., 2016). Overall sequencing quality was high, with Phred scores above Q30 (corresponding to a 99.9% base-calling accuracy, or an error rate of 1 in 1,000 bases) across most read lengths (median forward: 36.7; median reverse: 35.0). After the denoising step, approximately 18.5% of input reads per sample remained as nonchimeric, resulting in an average of 25,989 high-quality reads per sample available for downstream analyses. Sequences classified as mitochondria or chloroplasts were filtered out before taxonomic assignment. To normalize sample depths, different rarefaction curves were evaluated at 11,000; 5,000; 1,000; and 500 sequences per sample. A depth of 5,000 was selected because it retained 95.8% of the samples, providing a good balance between sequencing depth and sample retention. Taxonomic classification was then performed using the pretrained SILVA 138 reference database (https://www.arb-silva.de/) adapted to the V3-V4 region. The alignment of representative sequences with MAFFT (Katoh and Stanley, 2013) and the construction of a rooted tree using FastTree (Price et al., 2010) were performed to infer phylogenetic relationships. Diversity analysis was computed using the core-metrics-phylogenetic pipeline. Alpha diversity (Faith's phylogenic diversity index) and β diversity (unweighted UniFrac) were evaluated, and statistical comparisons were conducted using Kruskal–Wallis tests and permutational multivariate ANOVA (**PERMANOVA**), respectively. In β diversity (PERMANOVA) and differential abundance testing (Wilcoxon rank-sum test), *P*-values were adjusted using the false discovery rate (**FDR**) method for multiple comparisons, and results are reported as q-values. Bar plots were generated in R (https://www.r-project.org) to visualize taxonomic composition at both phylum and class levels. Raw sequencing data have been submitted to the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena) under the accession number PRJEB96918. The script used for the analyses has been deposited in Figshare (https://doi.org/10.6084/m9.figshare.30074860.v1).

RESULTS

Clinical Data

During the study period, a total of 235 calves were born on the farm, from which a final study sample of 80 calves was derived, resulting in the exclusion of 155 calves. Specifically, 107 of those were mixed breeds, 12 were male Holstein-Friesians, and 8 were stillborn. Among the female Holstein-Friesian calves, 12 were excluded because they had been sold and relocated to another farm, and 3 were excluded due to complications arising from dystocia. Additionally, 13 calves were excluded because their STP levels, measured 36 to 48 h after birth, fell below 58 g/L.

Concerning STP levels, no statistical difference was found between groups ($P = 0.304$) in enrolled calves. In the CTRL group, STP was 66 g/L (25th percentile [**25°P**]: 6.2; 75th percentile [**75°P**]: 7.15), while in HS, it was 64 g/L (25°P: 6.00; 75°P: 7.00). During the study period, 4 enrolled calves died; the mortality rate was 2.5% (n = 1/40) in the CTRL group and 7.5% (n = 3/40) in the HS group. The difference in mortality was not significant ($P = 0.305$). Body weight was measured at d 21, 24, and 60 of life. At d 24, the mean ± SD weight was 42.1 ± 4.2 kg in the CTRL group and 41.8 ± 3.8 kg in the HS group; at d 21, it was 45.4 ± 4.7 kg in CTRL and 46.2 ± 3.8 kg in HS, and at d 60 it was 66.8 ± 7.3 kg in CTRL and 67.2 ± 8.2 kg in HS. The differences were not significant ($P = 0.280$).

During the study period, 20 calves were treated for BP, 19 with florfenicol and meloxicam and one with meloxicam alone. Fourteen calves were treated for NCD; among these, 6 required intravenous fluids, gentamicin, and flunixin meglumine; 2 received gentamicin and flunixin meglumine; and 6 were treated with flunixin meglumine only. Three calves were treated with antibiotics for umbilical infections, and 7 were treated for recurrent fever with antibiotics and anti-inflammatory drugs (6 calves) and one with anti-inflammatory drugs alone. Overall, 7 calves underwent more than one antibiotic course due to either recurrence or occurrence of different conditions.

The frequency of NCD (number of episodes per calf) and its duration (in days) were analyzed in the study. The percentage and number of calves experiencing at least one NCD event were 83.7% (n = 33/40) in the CTRL group and 72.5% (n = 29/40) in the HS group. The incidence of NCD did not differ significantly between the 2 groups ($P = 0.648$). When considering repeated measurements using GEE models, HS supplementation was associated with a shorter duration of NCD. The average duration

was 7.23 ± 2.53 d for CTRL calves compared with 5.63 ± 2.57 d for HS calves. According to the regression model, the expected duration of NCD was approximately 22% longer in CTRL group calves compared with those in the HS group (IRR = 1.22; 95% CI: 1.03–1.45; P = 0.024). Hazelnut skin supplementation also had a significant effect on mean fecal scores. Higher fecal scores were observed more frequently in CTRL calves, and the GEE analysis confirmed that CTRL animals had higher odds of presenting increased fecal scores compared with HS calves (OR = 1.23; 95% CI: 1.02–1.52; P = 0.049). No significant differences were noted for respiratory clinical signs or TUS findings. Lung consolidation greater than 1 cm was recorded in 65% of CTRL calves (26/40) and 47.5% of HS calves (19/40), but this difference was not significant (P = 0.115, chi-squared test). Other clinical variables, including suckle reflex, vigor score, umbilical scores, dehydration, cough, joint swelling, neurological signs, and therapy, did not show significant differences between the groups. Comprehensive results are presented in Table 2.

Antioxidant Status

At d 2 of life, OXY-Adsorbent value levels showed a median of 365.99 µmol HClO/mL (25°P: 326.02; 75°P: 388.63) in CTRL and 382.51 µmol HClO/mL (25°P: 332.70; 75°P: 417.96) in HS (P = 0.315). At d 21 of life, median OXY-Adsorbent values were 397.29 µmol HClO/mL (25°P: 360.51; 75°P: 426.30) in CTRL and 440.40 µmol HClO/mL (25°P: 400.97; 75°P: 479.14) in HS, with a significant difference between groups (P < 0.001). At d 2 of life, d-ROM values were 174.82 ± 29.74 mg H_2O_2/dL in CTRL and 174.62 ± 37.00 mg H_2O_2/dL in HS groups (P = 0.545). At the end of HS administration (d 18 of life), d-ROM values were 175.85 ± 46.84 mg H_2O_2/dL in CTRL and 177.05 ± 55.54 mg H_2O_2/dL in HS (P = 0.733), with no significant differences observed at either time point.

Fecal Microbiota Results

Out of the initial 80 calves enrolled in the study, 54 were selected for microbiota sampling. The first 26 calves enrolled in this study were excluded from fecal microbiota sampling because of the uncertainty regarding the effects of the polyphenol extract during the early phase of the trial. Among the 54 sampled calves, 2 were subsequently excluded due to mortality within the first 24 d of life, 21 were excluded due to antibiotic treatment during the same period, and 7 were excluded due to missing samples at one or more time points. The final data set consisted of 24 calves evenly distributed between the CTRL group and HS groups (n = 12 per group). The composition of the microbial community was evaluated to assess both temporal dynamics (2–24 d of life) and potential differences between the HS group versus the CTRL group using 16S rRNA gene sequencing data. For the analysis, 92 samples were retained after quality filtering and rarefaction, with a minimum of 5,000 sequences per sample. Alpha diversity, measured using Faith's phylogenetic diversity index, showed no significant differences between HS groups (P = 0.134) or among time points (P = 0.135), indicating similar within-sample diversity across the study, as reported in Table 3. Similarly, no significant differences were observed in β diversity when comparing the CTRL and HS groups. Permutational multivariate ANOVA, based on unweighted UniFrac distances, yielded a pseudo-F value of 0.98 with q = 0.499 (Table 4), indicating no significant differences in the overall microbial community structure between the HS and CTRL groups. In contrast, β diversity assessed between different times (T0 to T3) revealed differences in microbial community structure over time (Table 4). Permutational multivariate ANOVA tests confirmed that microbial communities differed significantly across time points (q <0.005 for all pairwise comparisons). From Table 4, it is evident that β diversity distances increased progressively from T0 to T3, indicating a temporal divergence in the fecal microbial community structure during calf development. This pattern is illustrated in the principal coordinates analysis (**PCoA**) shown in Figure 1a, where samples cluster by time point with limited overlap between ellipses, consistent with the temporal divergence observed in Table 4. In contrast, Figure 1b shows the PCoA plot by the HS group, where the HS group and CTRL samples display overlapping distributions, confirming the nonsignificant PERMANOVA result (q > 0.2). In addition to the observed differences in community structure, we further examined the taxonomic composition of the fecal microbiota. From Figure 2a, it is possible to observe that the fecal microbiota at the phylum level is composed principally of *Firmicutes* and *Actinobacteriota*, which together represent the majority of reads across all samples. Phyla less represented include *Proteobacteria* and *Verrucomicrobiota*. At the class level, as shown in Figure 2b, *Clostridia* (*Firmicutes*) and *Actinobacteria* (*Actinobacteriota*) appear as the most abundant taxa throughout the different times of collection. In contrast, *Fusobacteria* (*Fusobacteriota*) and *Campylobacteria* (*Campylobacterota*) exhibit greater variability between individuals and across time points, potentially reflecting individual responses to environmental changes or microbial succession during early development. To investigate potential differences in the relative abundance of these taxa between groups, differential abundance analysis was performed at the phylum and class levels. This analysis was conducted

Table 2. Frequency and percentage of clinical scores recorded from 3 to 21 d of age in 80 newborn Holstein heifers, categorized into 2 experimental groups: a control group (CTRL; n = 40 calves) and a group supplemented with HS extract (n = 40 calves) at a dosage of 5 g/d, administered for 15 d from d 3 to 18 of age[1]

Clinical sign/score	CTRL	HS	*P*-value
Suckle reflex[2]			0.753
0	636 (83.7)	599 (82.2)	
1	120 (15.8)	129 (17.7)	
2	4 (0.5)	1 (0.1)	
Vigor score[3]			0.783
0	742 (97.6)	709 (97.3)	
1	16 (2.1)	18 (2.5)	
2	2 (0.3)	2 (0.3)	
Fecal score[4]			0.049
0	231 (30.4)	238 (32.7)	
1	240 (31.6)	265 (36.4)	
2	153 (20.1)	120 (16.5)	
3	136 (17.9)	105 (14.4)	
Umbilicus[5]			0.189
0	500 (65.8)	449 (61.6)	
1	251 (33.0)	224 (30.7)	
2	9 (1.2)	30 (4.1)	
3	—	26 (3.6)	
Dehydration[6] (mm)			0.610
0	713 (93.8)	676 (92.7)	
2	45 (5.9)	51 (7.0)	
4	2 (0.3)	2 (0.3)	
Cough[7]			0.645
0	730 (96.1)	694 (95.2)	
1	30 (3.9)	35 (4.8)	
Joint[8]			0.117
0	753 (99.2)	710 (97.4)	
1	6 (0.8)	19 (2.6)	
Neurological signs[9]			0.384
0	754 (99.2)	727 (99.7)	
1	6 (0.8)	2 (0.3)	
Therapy[10]			0.940
0	699 (92.0)	672 (92.2)	
1	61 (8.0)	57 (7.8)	
TUS[11]			0.539
2	17 (15.0)	13 (11.1)	
3	16 (14.2)	9 (7.7)	
4	3 (2.7)	8 (6.8)	
5	—	9 (7.7)	

[1]Each score category is detailed as frequency and percentage (%, in parentheses) within each group. The *P*-value indicates the significance derived from the generalized estimating equation model, where the variable displayed in the table was treated as the dependent variable and the HS group was included as a predictor, while accounting for repeated measures over time. Missing scores in the table were not recorded in enrolled calves during the study period.

[2]Suckle reflex: 0 = strong; 1 = weak, 2 = absent or chewing movements.

[3]Vigor score: 0 = standing securely without assistance, curious, alert; 1 = standing up after encouragement, weak, "sad calf;" 2 = sternal recumbency, standing after lifting, "drunken gait," insecure posture; 3 = permanent sternal/costal recumbency; 4 = lateral recumbency, sometimes comatose.

[4]Fecal score: 0 = normal (firm but not hard); 1 = soft (does not hold form, piles but spreads slightly); 2 = runny (spreads readily); 3 = watery (liquid consistency, splatters).

[5]Umbilicus: 0 = normal, pencil size, dry, and painless; 1 = bigger than normal, but dry and painless; 2 = bigger than normal, wet, or painful; 3 = bigger than normal, with pus draining and evidence of pain (any presence of internal umbilical swelling ranks as a 3 and should be described as involving $_{\text{arteries/}}$urachus or vein).

[6]Dehydration: expressed in mm.

[7]Spontaneous cough: 0 = no; 1 = yes.

[8]Joint: 0 = normal joints; 1 = swollen joints.

[9]Neurological signs: nystagmus or opisthotonus or seizures, 0 = no; 1 = yes.

[10]Therapy, the treatment protocols for the primary pathological conditions in calves were based on the prescriptions from the farm practitioner. Dehydrated calves with NCD received intravenous fluid therapy along with flunixin meglumine, vitamin complex supplementation, and antibiotics if they experienced severe critical illness or bloody feces. Those exhibiting cough, fever, or labored breathing were treated with antimicrobial therapy and meloxicam.

[11]Thoracic ultrasonography (TUS): 0 = no lesions; 1 = diffuse comet tails; 2 = patchy lesions; consolidation of ≥ 1 cm but < 3 cm, between a normal aired lung parenchyma; 3 = lobar pneumonia affecting only 1 lobe; 4 = lobar pneumonia affecting 2 lobes; 5 = indicates lobar pneumonia affecting 3 or more lobes.

Table 3. Results from the Kruskal–Wallis test assessing fecal microbiota α diversity, measured by Faith's Phylogenetic Diversity index, obtained from 24 calves divided into 2 experimental groups: a CTRL group (n = 12 calves) and a group supplemented with HS extract (n = 12) at a dosage of 5 g/d from d 3 and continuing until d 18 of age[1]

Item	Chi-squared	df	*P*-value
Group (HS, CTRL)	2.248	1	0.134
Time (d 2, 10, 18, 24)	5.564	3	0.135

[1]Fecal microbiota evaluations were conducted on d 2, 10, 18, and 24 of life. The accompanying table presents the chi-squared statistic, degrees of freedom (df), which indicates the number of independent categories minus 1, and the corresponding *P*-value that reflects the level of significance. The term "Group" pertains to the treatment effect (CTRL vs. HS), and "Time" refers to the effect of the sampling day (d 2, 10, 18, and 24 of life).

to compare the HS group and CTRL group within each time point using the Wilcoxon rank-sum test, with FDR correction for multiple testing. No taxa were found to differ significantly in relative abundance between groups at any time point (q >0.05).

DISCUSSION

The present study aimed to assess whether administering HS extract to dairy heifers during their early life stages could alleviate the morbidity and severity associated with NCD and BP while influencing growth rates, fecal microbiota, and oxidative status. The findings indicated that the duration of diarrhea was shorter in the HS group. Moreover, the HS group exhibited a higher serum antioxidant capacity than their CTRL counterparts. However, the results showed no significant differences between the groups in terms of weight gain, fecal microbiota, d-ROM, mortality rate, disease incidence, and other parameters evaluated for general health and clinical conditions.

Two noteworthy findings merit reporting. First, the lack of differences in milk consumption between the groups is noteworthy, indicating that the HS extract did not compromise feed palatability—an issue often seen with tannin compounds, which can lead to unpleasant sensations of dryness and roughness (Oliveira et al., 2010; Renna et al., 2020). However, these results should be interpreted with caution, given the low quantity of milk provided to calves during the initial days of life on the farm where this trial was conducted. This may have prompted the calves to consume the milk despite any potential unpleasant taste. The second finding pertains to the homogeneity of both study groups in terms of BW and STP at the time of enrollment, which facilitated a more objective assessment of the results related to HS supplementation.

Comparing the effects of these compounds on the incidence of NCD in the literature can be challenging due to the diverse assessment methods and the subjective nature of fecal scoring. However, other studies that have applied an established visual method to accurately assess fecal dry matter decline (Renaud et al., 2020) have revealed that calves supplemented with polyphenols experience a reduction in diarrhea duration and an overall improvement in fecal score (Bonelli et al., 2021; Turini et al., 2022). The observed decrease in fecal scores is promising because it may correlate with reduced fluid and electrolyte loss during episodes of NCD, ultimately leading to enhanced health outcomes. The protective effects of polyphenols against intestinal disorders are primarily attributed to their phenolic hydroxyl groups, which facilitate various activities that can influence the intestinal bacterial population. These activities promote the growth of beneficial bacteria that are crucial for maintaining the integrity of the intestinal barrier (refer to the review by Makarewicz et al., 2021). However, our study did not reveal a significant effect on the composition and diversity of fecal microbiota in neonatal calves supplemented with HS. Analyses of both α and β diversity showed no differences between the study groups ($P > 0.1$), suggesting that HS supplementation resulted in no detectable

Table 4. Results from PERMANOVA were obtained to compare β diversity, measured by unweighted UniFrac distances, among 24 calves assigned to 2 experimental groups: a CTRL group (n = 12 calves) and a group receiving HS extract (n = calves) at a dosage of 5 g/d from d 3 to d 18 of age[1]

Item	Group 1	Group 2	Sample size	Permutations	Pseudo-F	q-Value
Groups	HS	CTRL	92	999	0.984	0.499
Time points	d 2	d 10	48	999	2.095	0.001
	d 2	d 18	46	999	2.720	0.001
	d 2	d 24	46	999	2.796	0.001
	d 10	d 18	46	999	1.427	0.002
	d 10	d 24	46	999	1.944	0.001
	d 18	d 24	44	999	1.587	0.001

[1]The fecal microbiota was assessed on d 2 (T0), 10 (T1), 18 (T2), and 24 (T3) of life. The columns labeled "Group 1" and "Group 2" represent the 2 conditions being compared, either treatment groups (HS vs. CTRL) or sampling time points (T0, T1, T2, T3). The sample size indicates the total number of samples included in each comparison. The pseudo-F value denotes the test statistic, calculated as the ratio of between-group to within-group variance. The q-value reflects the FDR-adjusted *P*-value, which accounts for multiple comparisons.

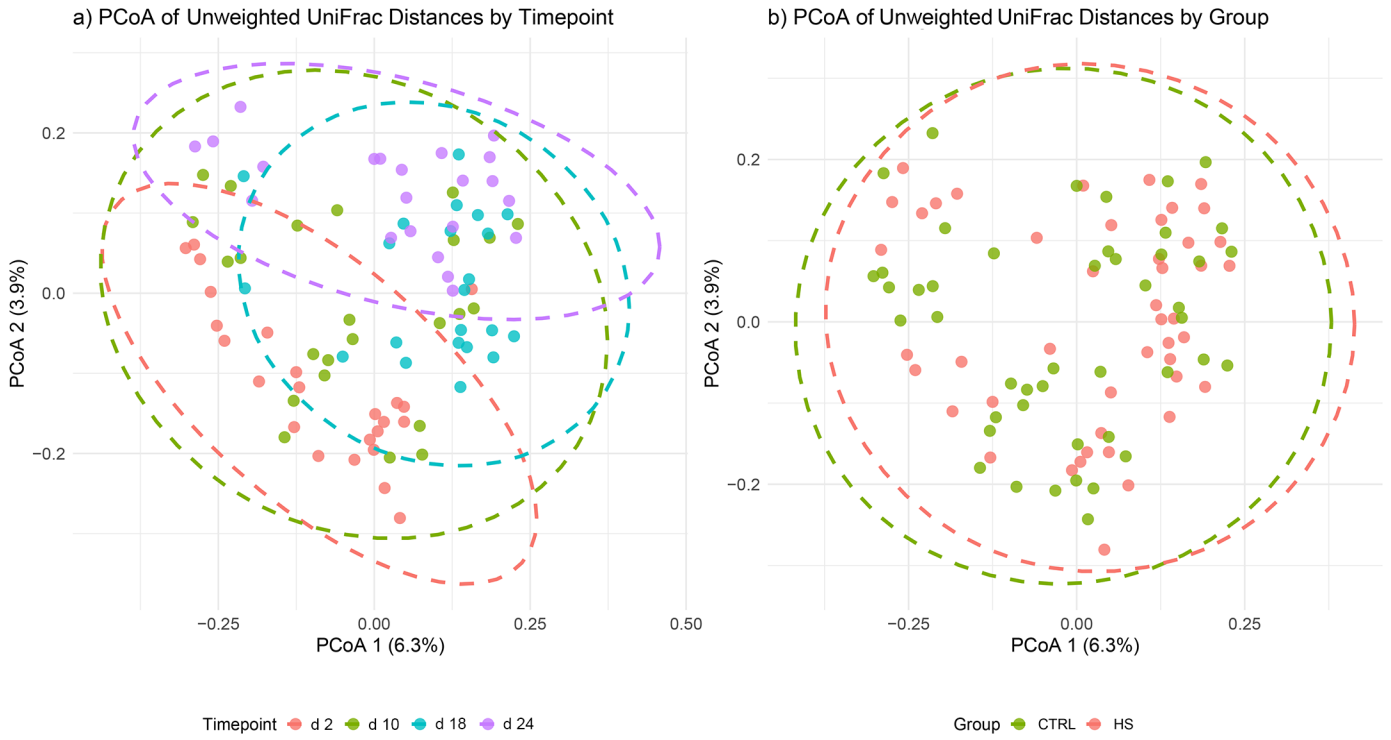

Figure 1. Principal coordinates analysis (PCoA) plots based on unweighted UniFrac distances of fecal microbiota from 24 calves divided into 2 experimental groups: a CTRL group (n = 12 calves) and a group administered HS extract (n = 12 calves) at a dosage of 5 g/d from d 3 to d 18 of age. The assessment of fecal microbiota occurred on d 2, 10, 18, and 24 of their lives. (a) Samples are color-coded by time point; (b) samples are color-coded by HS group (HS group vs. CTRL group). Ellipses indicate the 95% CI.

modulation of the fecal microbiome. Notably, significant changes were observed over time, particularly between d 2 of life and d 24 of life, reflecting the natural maturation process of the bacterial populations (Malmuthuge and Guan, 2017; Alipour et al., 2018). Similar findings have been documented by others (Nora et al., 2024). These results underscore the challenges associated with assessing changes in intestinal bacterial populations following the administration of these compounds. Such challenges can arise from the varying interactions based on the type, length, and quantity of polyphenol administered, as well as the distinct effects of these molecules that may vary depending on the specific phenolic compounds and the individual bacterial strains involved (Makarewicz et al., 2021; Nora et al., 2024). Interestingly, at d 10 of life and d 18 of life, the high abundance of *Actinobacteriota* phyla in both groups contrasts with previous microbiome studies on neonatal dairy calves, which identified *Firmicutes* as the predominant phylum (Gomez et al., 2017; Alipour et al., 2018). Several factors can affect the proportion of *Actinobacteriota*, including host-related characteristics and methodological considerations. Preweaning diet and colostrum significantly influence the microbial colonization of the gut in neonatal dairy calves, particularly promoting the growth of *Bifidobacterium* species (Carroll et al., 2025). Furthermore, methodological techniques, such as DNA extraction protocols and sequencing methods, can affect the detection and relative abundance of microbial taxa (Knight et al., 2018; Cremonesi et al., 2021). Although 16S rRNA sequencing is a cost-effective method for characterizing microbial diversity, more advanced methods can provide higher taxonomic resolution, offering a more nuanced understanding of shifts within microbial communities (Matchado et al., 2024).

One possible explanation for the observed reduction in mean fecal scores and the duration of NCD episodes in calves within the HS group may be linked to the anti-inflammatory properties of polyphenolic compounds. These substances have demonstrated the ability to regulate critical inflammatory pathways, particularly in the gastrointestinal tract (Gessner et al., 2012; Vendrame and Klimis-Zacas, 2015; Ismail et al., 2025). This regulation may correspond to a diminished release of pathogen-related inflammatory mediators, which play a role in promoting water and electrolyte secretion by affecting the enteric nervous system during NCD episodes (Foster and Smith, 2009). The reduction in inflammatory bowel processes may also be associated with the effects of polyphenols on oxidative stress (Singh et al., 2020). In this study, dietary supplementation of calves with HS

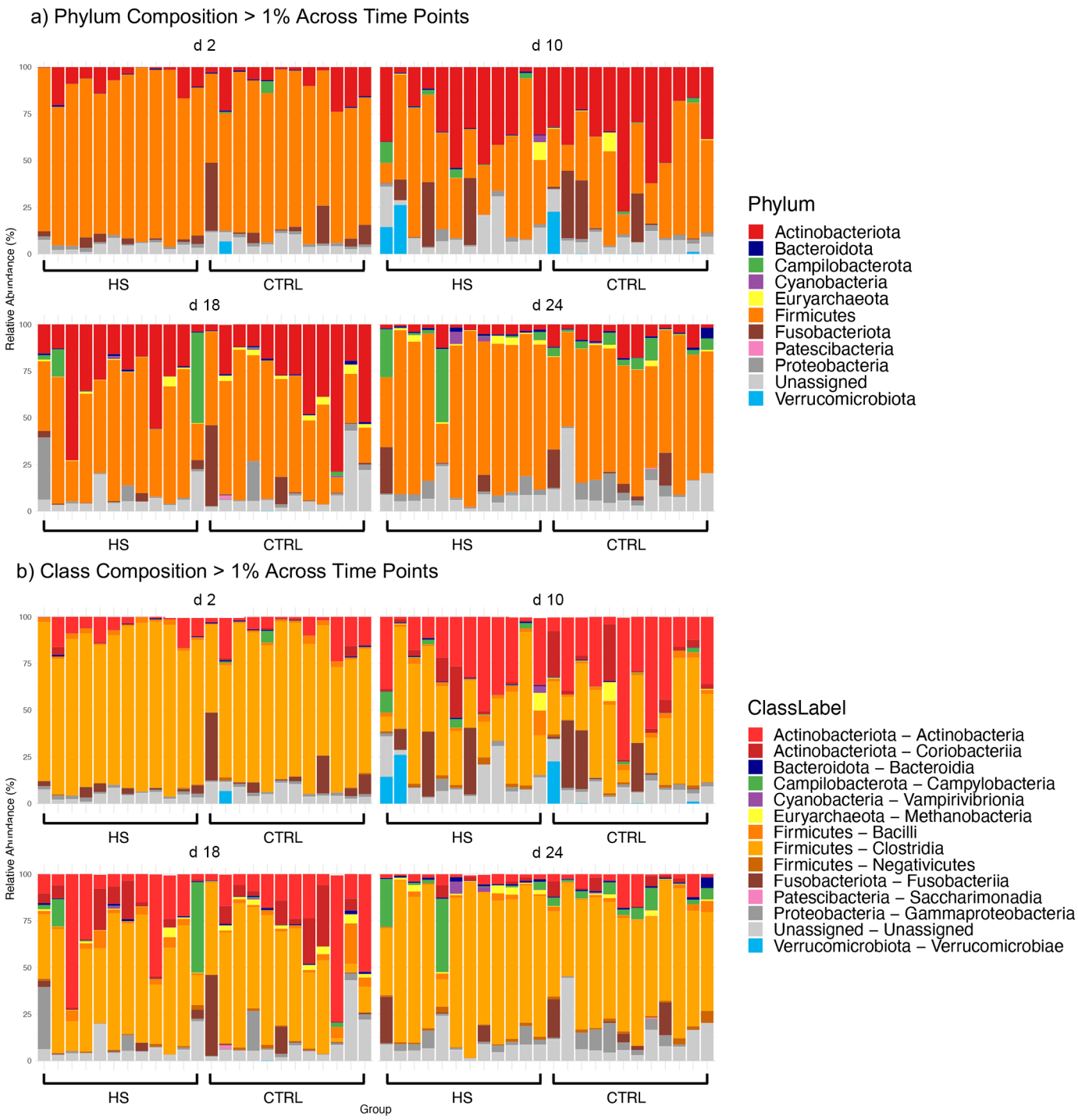

Figure 2. Fecal microbiota composition at the phylum and class levels in 24 calves, divided into 2 experimental groups: a CTRL group (n = 12 calves) and a group treated with HS extract (n = 12 calves), administered at a dosage of 5 g/d from d 3 to 18 of age. Assessments were conducted on d 2, 10, 18, and 24. The stacked bar plots display the relative abundance of taxa exceeding 1% by sample, categorized by group (HS vs. CTRL) and time point at both the phylum (a) and class (b) levels.

extract led to a significant improvement in serum antioxidant capacity at d 21 of life, as evidenced by elevated OXY-Adsorbent values in the HS group compared with the CTRL group ($P < 0.001$). This improvement is likely attributed to the high concentration of bioactive compounds in HS, particularly polyphenols and vitamin E,

which bolster endogenous antioxidant defenses (Pfeil et al., 2024). Interestingly, no significant differences were found in d-ROM values between groups at any time point, indicating that systemic oxidative load—reflected by hydroperoxide accumulation—remained unchanged. This apparent dissociation between increased OXY-Adsorbent values and stable d-ROM can be attributed to the distinct physiological roles of the 2 markers; OXY-Adsorbent value reflects the antioxidant potential available to counteract oxidants, and d-ROM indicate the level of oxidative challenge the organism is currently facing (Kilk et al., 2014; Yuba et al., 2024). Previous research suggests that polyphenol supplementation raises antioxidant status without necessarily reducing oxidative damage, with variations influenced by species, age, dosage, and diet composition (Orzuna-Orzuna et al., 2021). Overall, the findings imply that polyphenol-rich supplementation aids endogenous antioxidant systems rather than directly reducing reactive oxygen species production or reversing oxidative damage.

Although this study and a variety of others in the literature highlight that the administration of polyphenols can reduce the incidence and duration of NCD in calves, the actual evaluation of the effectiveness of these substances in justifying their routine use on dairy farms remains a contentious topic. Other authors have reported no improvement in fecal scores of dairy calves following polyphenolic supplementation (Oliveira et al., 2010). Similarly, research assessing not only fecal consistency but also clinical signs associated with NCD and the necessity for diarrhea-related treatments generally supports the finding that polyphenol supplementation has minimal effect on these outcomes (Bonelli et al., 2021; Kodithuwakku et al., 2021; Turini et al., 2022; McNeil et al., 2023). Our results align with this trend; the lack of significant differences between the CTRL and HS groups regarding mortality, morbidity, or therapeutic interventions associated with NCD underscores the limited clinical effect of HS extract supplementation within our study sample. These results, therefore, need to be interpreted with caution. In calculating the sample size, we considered repeated measurements of TUS and changes in calf BW at 3 observation points. To accurately evaluate the effect of HS supplementation on individual outcome measures, such as mortality or disease treatment, a considerably larger number of calves would have been necessary. Moreover, the absence of significant differences between the 2 study groups in terms of TUS-detected lung lesions further indicates that HS supplementation had a poor clinical effect on our calves. These results are consistent with other studies, which indicated that respiratory patterns were not affected by polyphenol supplementation in calves (Serri et al., 2022; McNeil et al., 2023; Velázquez-Cruz et al., 2025). Importantly, this study is the first to investigate the effects of polyphenol administration on BP incidence, using longitudinal TUS as a diagnostic tool. The lack of significant differences—despite employing a more advanced diagnostic method rather than relying solely on BP-related clinical signs—highlights the ineffectiveness of these substances in producing any substantial effects on BP outcomes. Similarly, the absence of significant differences in weight gain between the 2 groups suggests that HS extract supplementation does not influence growth, which was consistent with the findings of Bonelli et al. (2021) and Turini et al. (2022). This contrasts with Serri et al. (2022), who showed that 4 g/d of hydrolyzable tannin extract significantly improved ADG and final BW compared with lower or no supplementation, and with Nowroozinia et al. (2022), who found that calves receiving fennel seed powder (1.5 or 3 g/d) had better growth than the control group. Two factors may clarify this observation. First, our study involved administering an HS-based extract over a practical duration of 15 d, which was designed to effectively manage costs and facilitate ease of administration before housing calves in multiple pens. The lack of a significant increase in BW in the HS group may be attributed to the shorter supplementation period; Oliveira et al. (2010) reported that pomegranate extract improved performance only after a longer supplementation duration of 30 to 70 d. Second, BP and NCD can considerably affect ADG in dairy calves (Schinwald et al., 2022; Sáadatnia et al., 2023). The absence of significant differences in the severity and incidence of these diseases in our study groups may have limited the ability to detect a substantial difference in weight gain.

This study has several limitations. The investigation was performed on a commercial dairy farm chosen for convenience, which may have introduced selection bias. To mitigate this issue, we implemented a randomized clinical trial with a longitudinal evaluation of enrolled calves sourced from a single location, theoretically ensuring uniform exposure to BP- and NCD-related pathogens. However, our study design could have been enhanced by incorporating a blinded assessment procedure after enrollment in the HS or CTRL group. Although we initially intended to evaluate both groups blindly, the use of HS resulted in a reddish coloration of feces, rendering blind assessment practically unfeasible. Additionally, we did not perform virological and parasitological fecal tests or etiological assessments related to BP, which limited our ability to link the study results to specific identified pathogens. Caution should be exercised when interpreting microbiota results because the small sample size may have restricted the detection of subtle effects and may not have accurately reflected the entire study population. Furthermore, we restricted HS administration to 15 d, leaving the possibility that longer-term administration

could have yielded more favorable clinical effects. Although mortality was numerically higher in the HS group (7.5% vs. 2.5%), this difference was not significant (P = 0.305) and may represent incidental findings rather than effects attributable to HS supplementation; however, the lack of demonstrated causality warrants cautious interpretation.

The exclusion of calves with FTPI and the potential impact on the external validity of the results should be noted. However, this pilot study aimed to minimize sources of bias and to evaluate the effects of the hazelnut extract in a population of calves as homogeneous as possible even in terms of immune status. For this reason, calves with FTPI were excluded to avoid potential confounding effects on the outcomes. Consequently, the efficacy of the hazelnut extract in calves affected by FTPI remains unknown and should be addressed in future studies. Additionally, the lack of an evaluation of agreement during clinical assessment training between the first and second authors of this paper could be a source of assessment bias.

As previously mentioned, a further limitation of this study is that, for single-incidence outcomes such as the number of NCD and BP cases, mortality, or disease treatment, the sample size was limited. The post hoc power analysis showed that the achieved power was only 50% for NCD and 63% for BP, confirming that the study was underpowered to detect differences in disease incidence. Based on the observed effect sizes, the required sample size to reach adequate power would have been substantially larger, ranging from approximately 125 to over 450 calves per group, depending on the outcome and the desired power level (80%–95%). This limited statistical power may have contributed to the lack of statistical significance in these outcomes. Therefore, future studies with larger populations are warranted to properly evaluate the effect of HS supplementation on disease incidence and severity.

CONCLUSIONS

In conclusion, our study provides intriguing evidence supporting the hypothesis that HS supplementation could be beneficial in certain aspects. It enhances fecal scores and decreases the duration of watery feces during episodes of NCD while also boosting the antioxidant capacity. We recommend that future research on HS supplementation in calves be carried out over extended administration periods, supplemented by comprehensive cost-benefit analyses. This would enable a more in-depth examination of the potential benefits of such supplementation for dairy farming, particularly in terms of its impact on mortality rates, therapeutic interventions, and ADG. Furthermore, enrolling a significantly larger number of calves will be crucial for a thorough evaluation of these single-incidence outcomes. Subsequent trials should also investigate longer supplementation durations, such as 30 to 60 d, to more effectively influence microbiota composition and growth trajectories.

NOTES

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Nonstandard abbreviations used: BP = bronchopneumonia; CTRL = control group; df = degrees of freedom; d-ROM = blood derivatives of reactive oxygen metabolites; FDR = false discovery rate; FTPI = failure of transfer of passive immunity; GEE = generalized estimating equation; HS = hazelnut skin; HS group = HS-supplemented group; IRR = incidence rate ratio; NCD = neonatal calf diarrhea; OR = odds ratio; 25°P = 25th percentile; 75°P = 75th percentile; PCoA = principal coordinates analysis; PERMANOVA = permutational multivariate ANOVA; STP = serum total protein; TUS = thoracic ultrasonography.

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