Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.205

RESEARCH ARTICLE

Psyllium and Inulin Supplementation Alleviates Diarrhea and Supports Gut Flora in Pre-Weaning Calves

Xia Yi[13], Wentao Lu[1], Dengke Liu[2], Haohua Zhuang[1], Chengcheng Peng[1], Yuhan Ge[1], Xinyue Zhang[1], Kaidi Shen[1], Jie Cao[1*] and Chong Ma[1*]

[1]College of Veterinary Medicine, China Agricultural University, Beijing 100193, China
[2]Hebei Shounong Modern Agricultural Technology Co., LTD, Hebei 073000, China
[3]School of Chemistry, University of Bristol, Bristol, BS8 1TS, United Kingdom
*Corresponding author: mach_cau@cau.edu.cn; caojie010@163.com

ARTICLE HISTORY (25-331)

Received: April 15, 2025
Revised: June 20, 2025
Accepted: June 21, 2025
Published online: July 21, 2025

Key words:
Calf diarrhea
Dietary fiber
Fecal flora
Intestinal health

ABSTRACT

Pre-weaning diets are critical to the intestinal health of calves, and dietary fiber is considered to regulate gut microbiota and enhance gastrointestinal conditions. However, the effects of different types of dietary fibers vary significantly, and the specific impacts on the intestinal microbiota of neonatal calves remain largely unexplored. This study investigates the effects of supplemental psyllium husk powder and inulin on growth performance, diarrhea prevention, and fecal microbiota composition in calves. A total of 136 healthy newborn Holstein calves were randomly assigned to one of three groups: control, psyllium husk, and inulin groups, with the experimental groups receiving a fiber supplementation of 0.2 g/kg body weight from birth until weaning. The daily mixed feed was added to milk and evenly divided into two to three feedings. Key parameters assessed included average daily gain (ADG), starter intake post-56 days, and diarrhea status. To better evaluate the effects of dietary fiber, calves that remained diarrhea-free throughout the study period were selected, with fecal samples collected from the same calves at age 0, 7, 14, 28, and 56 days. Results showed that psyllium husk and inulin supplementation significantly reduced the incidence and duration of diarrhea, promoted the growth of beneficial flora, especially acid-producing bacteria, and decreased the relative abundance of *Dorea* during the peak diarrhea period. By the end of the pre-weaning phase, both fibers increased the relative abundance of *Prevotella*, contributing to improved gastrointestinal health in calves. This study suggests that psyllium husk and inulin supplementation may offer a viable nutritional intervention strategy to enhance gut microbiota composition and health outcomes in pre-weaning calves.

To Cite This Article: Yi X, Lu W, Liu D, Zhuang H, Peng C, Ge Y, Zhang X, Shen K, Cao J and Ma C, xxxx. Psyllium and inulin supplementation alleviates diarrhea and supports gut flora in pre-weaning calves. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.205

INTRODUCTION

Diarrhea represents a substantial challenge in the dairy industry, particularly in pre-weaning calves, impacting growth performance, mortality and economic losses (Cho and Yoon 2014). Managing and preventing diarrhea in calves during the pre-weaning period is essential for ensuring optimal health and productivity in dairy herds. Because of the crucial role of diet in modulating gut microbiota composition and structure, the early establishment of gastrointestinal microbiota is crucial to healthy calf growth, a few imbalances can affect digestion, nutrient absorption, and overall gastrointestinal function (Xu *et al.* 2021) , the feeding choice during the pre-weaning period is of significant importance (Castro *et al.* 2016).

In recent years, there has been a growing interest in exploring dietary interventions (Cronin *et al.* 2021, Jha *et al.* 2019), including the supplementation of dietary fibers such as psyllium husk powder (Kodithuwakku *et al.* 2021) and inulin (Gao *et al.* 2024, Zhu *et al.* 2020). These strategies are considered a potential means to mitigate the incidence and severity of diarrhea in calves while promoting the establishment of a healthy gut microbiota. Psyllium husk powder, derived from Plantago ovata, is abundant in soluble and viscous fibers, including

carbohydrates such as xylose arabinose (Zhang *et al.* 2025). This property promotes nutrient absorption through efficient transit, regulating bowel movements and alleviating constipation or diarrhea (McRorie *et al.* 2021). Fermentation of psyllium in rumen produces short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate (Jalanka *et al.* 2019), which further supports the growth of beneficial bacteria and contributes to mucosal health. A study investigated the effect of timothy hay and psyllium husk fiber supplementation on the growth performance and fecal microbiota of pre-weaning calves within the first week after birth and observed that an early introduction of dietary fiber improved calves' ADG from birth to 21 days and facilitated the colonization of beneficial bacteria such as *Lactobacillus* and *Prevotella* in the intestine after the first 21 days (Kodithuwakku *et al.* 2021). These findings underscore the potential of dietary fiber supplementation, specifically psyllium husk, in promoting the growth and establishment of beneficial gut bacteria in calves.

Inulin, another fermentable fiber extracted from chicory roots, is formed through the polymerization of fructose with varying chain lengths, exhibiting prebiotic properties through gut fermentation (Shoaib *et al.* 2016). Inulin, primarily derived from chicory, offers various physiological benefits, including regulating gastrointestinal function, promoting mineral absorption, improving lipid metabolism, aiding in weight control, and stimulating vitamin synthesis (Gupta *et al.* 2019). It selectively promotes the growth of specific bacterial species, such as *Bifidobacterium* and *Lactobacillus* (Zhu *et al.* 2020). A daily supplementation of 6 g of inulin over an 8-week period in 5-week-old calves has been associated with a significant increase in ADG, indicating its potential to support early-stage growth and development (Jonova *et al.* 2018). Although links between dietary fiber intake and shifts in intestinal microbiota have been established, the mechanisms underlying these effects remain unclear.

Previous studies have primarily focused on the effects of dietary fiber supplements in promoting productivity and health (Bradford and Mullins 2012, Belorio and Gómez 2021). However, the comparative efficacy of different fermentable fibers, such as inulin and psyllium husk, in managing diarrhea remains underexplored. Additionally, comprehensive microbial compositional analyses are needed to fully understand the mechanisms underlying the effects of these fibers on gut health and diarrhea incidence. Therefore, further research is necessary to compare the effects of various fermentable fibers on diarrhea prevention and management, as well as to elucidate their impact on the composition and function of the gut microbiota.

Recent advancements in high-throughput sequencing technologies have facilitated a deeper understanding of the complex interactions between diet, gut microbiota and host health. Leveraging these tools, this study aims to evaluate the efficacy of psyllium husk powder and inulin supplementation in ameliorating diarrhea incidence and severity in pre-weaning calves, as well as their influence on the dynamic establishment of intestinal flora during the early postnatal period. Additionally, changes in fecal microbiota composition and clinical parameters will be examined to elucidate the mechanisms underlying the observed effects and provide valuable insights into the use of dietary fiber supplementation. This study aims to enhance our understanding of the role of dietary fiber supplementation in promoting gastrointestinal health and performance in pre-weaning calves. By investigating the mechanisms underlying the observed effects of psyllium husk powder and inulin supplementation on diarrhea management and early gut microbiota dynamics, we aim to inform evidence-based management strategies, enhancing the welfare and productivity of dairy herds.

MATERIALS AND METHODS

Animals and management: The research was conducted at a major dairy farm in northern China, which had approximately 19,000 cows. An individual hutch is used for the calves, and fine sand bedding is provided, allowing nose-to-nose contact between neighboring calves. Each calf received 4 liters of colostrum at the initial feeding, followed by an additional 2 liters in the second feeding within the first 24 hours after birth. After colostrum intake, all calves were given 2.5 liters of pasteurized whole milk as transitional milk from 2 to 7 days of age, followed by pasteurized raw milk from 8 to 28 days of age. The calves were fed milk replacer from day 29 until weaning at 65 days of age. The milk replacer used in the study contained no growth-promoting antibiotics, prebiotics, or probiotics. Starting from one week of age, the calves were provided with a commercial calf starter ration (Table S1). Clean and sufficient water was available to the calves *ad-libitum* consumption throughout the experiment.

Table S1: Composition of feeds

Item	Milk replacers	Starter
CP, %	≥ 20.0	≥ 20.0
Crude fat, %	≥ 16.5	≥ 2.0
Crude Ash, %	≤ 10.0	≤ 15
Crude fiber, %	≤ 1.5	≤ 8
Lysine, %	≥ 1.5	≥ 0.8
Methionine, %	≥ 0.4	-
Ca, %	≥ 0.5	0.4 - 1.5
P, %	≥0.5	0.4 - 1.0
NaCl, %	1.5 - 3.5	0.5 - 1.5

Enrollment and treatment: A total of 136 healthy newborn Holstein female calves were selected as participants for this study. The selection of healthy calves was based on the University of Wisconsin Calf Health Scoring Criteria, which apply scores for mental status, eye discharge, nasal discharge, and cough to assess the health status of the newborns (University of Wisconsin Calf Health Scoring Criteria, available at https://fyi.extension.wisc.edu/heifermgmt/files/2015/02/calf_health_scoring_chart.pdf). Each index was scored from 0 (indicating average) to 3 (indicating severe), and the overall health status of the calves was determined by a total score of 0. The newborn calves were randomly assigned to one of three groups: the control group (group C), the psyllium husk powder group (group P), and the inulin group (group I). The trial followed a randomized controlled design and was based on an expected diarrhea incidence of approximately 60% across the calves on the farm, a dietary fiber intervention was estimated to improve this by

30% at a significance level of α = 5% and statistical power of β = 20%. Using Evan's Awesome A/B Tools (available at https://www.evanmiller.org/ab-testing/sample-size.html), the minimum sample size required for each group was calculated to be 41 calves. Considering any potential dropouts, the estimated number of calves per group was set at 110% of the minimum, *i.e.,* 45 calves.

The psyllium husk powder used in the study was directly sourced from a supplier (Psyllium husk powder, Xi'an Xihai bio-technique Co., Ltd, Xi'an, China), with a purity of 99%. Inulin was commercially obtained (Inulin, Qingdao Kangda Shengwu Keji Co., Ltd., Qingdao, China), with a purity of 98%. The dietary fiber was added to the treatment groups at a dose of 0.2 g/kg body weight per day. From 2 to 28 days of age, the experimental groups received 10 g of dietary fiber daily, which was increased to 20 g from 29 to 56 days of age. Group C did not receive any dietary fiber supplementation. The dietary fiber was mixed into the liquid feed guided by the farm's feeding program, ensuring equal distribution among the feeds (Table S2**)**.

Table S2: Feeding scheme of pre-weaning calves

Age (d)	Feeding volume (Liter)	Number of Feeding	Type of liquated feed
1 - 7	3	3	Pasteurized milk
8 - 15	3.5	3	Pasteurized milk
16 - 28	2.5	3	Pasteurized milk
29 - 49	2.5	3	Milk replacer
50 - 52	2.5	2	Milk replacer
53 - 56	2.5	2	Milk replacer

Data and sample collection: The weight of calves was measured and recorded at 56 days of age. The ADG was calculated by dividing the difference in body weight from birth to 56 days of age by the number of days within that period. Two weeks after reaching 56 days of age (57–70 days of age), the daily intake of starter feed was measured and recorded for each calf. This was accomplished by weighing the starter feed provided and measuring the leftovers at 7 a.m. the following day. The daily starter intake was calculated as the total amount of starter feed if day minus the number of leftovers the next day.

Fecal consistency scores were recorded daily for each calf throughout the trial. The scores ranged from 0 to 3 and were applied by observing the freshest feces visible on the bedding. A fecal consistency score of 2 or higher indicated "diarrhea" in a calf, while a score below 2 indicated a "healthy" calf. The duration of diarrhea was recorded when a calf exhibited signs of diarrhea, with the onset marked as the age in days when a diarrhea score of 2 was first recorded. The end of a diarrhea episode was identified as the day when the score was below 2. The duration of diarrhea was calculated as the lapse of time between the two dates, representing the occurrence of diarrhea in the calf. The weekly diarrhea incidence was determined as the average of the 7-day diarrhea incident within that week. Diarrhea treatment and any other morbidities were documented according to the treatment measures and protocols followed on the farm.

To investigate the impact of dietary fiber supplementation on fecal microbiota in pre-weaning calves, fresh fecal samples were collected by utilizing rectal stimulation at 0, 7, 14, 28, and 56 days of age. Sterile gloves were used to collect the fecal samples, which were then immediately placed on ice, packed into 5mL sterile tubes, stored in liquid nitrogen, and transferred to a –80 °C refrigerator for preservation. Fecal samples were differentiated between healthy calves and those affected by diarrhea in each group throughout the experiment, The selected samples consisted of nine calves from group P at each of the five-time points (Pd0, Pd7, Pd14, Pd28, Pd56), eight calves from group I at each of the five-time points (Id0, Id7, Id14, Id28, Id56), and nine calves from the negative group C at each of the five points in time (Cd0, Cd7, Cd14, Cd28, Cd56). Discrepancies in the final numbers of calves from each subgroup across all detections were due to calf mortality or removal, insufficient feces for laboratory tests, or sample damage during the research. Once the samples were picked out according to the requirements, 2 g of fecal samples were kept in dry ice and sent to a company (Tiangen Biochemical Technology Ltd., China) for macro genome sequencing.

Metagenomic sequencing: The complete microbial DNA from the fecal samples was extracted by using the Magnetic Soil and Stool DNA Kit (DP210831, Tiangen Biochemical Technology Ltd., China) in preparation for metagenome sequencing. The sequencing activity was performed on an Illumina NovaSeq 6000 platform with 150 bp paired end reads, conducted by TIANGEN Biotech (Beijing) Co., Ltd. A total of 122 samples were subjected to sequencing.

Assembly and functional annotation from fecal metagenomes: To summarize the methodology, the quality filtering of the obtained data was performed using Trimmomatic (v0.39, available at https://github.com/usadellab/Trimmomatic/). Subsequently, each sample underwent de novo assembly using MEGAHIT (v1.2.9, available at https://github.com/voutcn/megahit) (Li *et al.* 2015) with the threshold to be a minimum contig size of 500 bp. Open reading frames (ORFs) were predicted from the assembled contigs using MetaGeneMark (v3.25, available at http://exon.gatech.edu/meta_gmhmmp.cgi). The assembled contigs were then pooled to generate nonredundant sequences by identifying and merging identical contigs using CD-HIT (v4.8.1, available at https://github.com/weizhongli/cdhit) (Fu *et al.* 2012). The original sequences were aligned to the predicted genes using BWA (Li and Durbin 2009)(v0.7.17, available at https://github.com/lh3/bwa) to estimate their abundances. Additionally, contigs were annotated against the CAZy database (dbCAN2 version, 31 July 2020) using HMMER (v3.3, available at http://hmmer.org/) and against the KEGG database (Release 95, 1 July 2020) using DIAMOND (v0.9.35, available at https://github.com/bbuchfink/diamond), with a significance cutoff of e-value $< 1 \times 10^{-5}$.

Statistical analysis: Data preprocessing for calf growth performance analysis was conducted using Excel 2019 software. Statistical analysis was conducted using SPSS 25.0 software, GraphPad Prism version 9, and R for generating visual presentations. Measurement data were presented as mean±standard deviation (X±SD). One-way

ANOVA and Bonferroni's method for multiple comparisons were used when significant differences were observed. Count data was expressed as frequency/ percentage and analyzed using the chi-square test (χ2 test). To analyze the differences between groups, PLS-DA was applied using R software. Differences in species composition and functional composition between samples were evaluated using LEfSe analysis. Kruskal-Wallis and Wilcoxon rank sum tests were employed to assess the differences in species abundance between treatments. A linear discriminant analysis threshold value of > 2 and a P-value of <0.05 were considered significant for determining group differences.

RESULTS

Growth, starter intake, and diarrhea occurrences: A total of 136 healthy Holstein newborn calves were enrolled in the study, with Group C and Group 1 consisting of 45 calves, and Group P including 46 calves. No significant differences were observed in birth parity or birth weight among the groups. Throughout the pre-weaning period, the ADG of Group C, Group P, and Group I is 810.56±93.54g (n = 45), 818.04±87.95 g (n = 46), 796.44±91.73 g (n = 45) (Table 1). There are no significant differences among groups (F = 0.644, P=0.527), although a slight reduction in ADG was noted in group I compared to the control. Starter feed intake in all groups gradually increased with age. At 57 days of age, the starter feed intake was 1.09±0.44 kg (n=13) in the blank control group, 1.23±0.48 kg (n=11) in the psyllium husk group, and 0.90±0.54 kg (n=11) in the inulin group. The trend in starter feed intake over the two weeks was generally consistent between the psyllium husk group and the blank control group. However, the inulin group had consistently a lower starter feed intake compared to both the blank control and psyllium husk groups over the two weeks. There were no significant differences in feed intake among the groups during this period (Table S3).

The incidence of diarrhea in the preweaning period was 80.0% (36/45) in Group C, 54.3% (25/46) in Group P, and 57.8% (26/45) in Group I. The incidence of diarrhea differed significantly among groups (χ^2 = 7.62, P<0.05), with lower incidences observed in both psyllium husk powder and inulin groups compared to the control. Furthermore, the duration and frequency of diarrhea were significantly lower in the intervention groups compared to the control (F = 10.24, P<0.0001), with no significant difference observed between psyllium husk powder and inulin groups (F = 3.55, P<0.05). By the second week, group C had 7.94% incidence, while it was 3.11% in group P and 6.21% in group I, with a significant difference observed (F = 3.709, P=0.045), particularly between group P and group C (P=0.045). The peak incidence occurred in the fourth week, with rates of 11.75% in Group C, 3.73% in Group P, and 6.83% in Group I, displaying a significant difference (F = 7.059, P=0.006) (Fig. 1, Table S4).

Analysis of the relative abundance of phylum, genus in calf fecal Microbiota: In this study, 122 fecal samples from the three experimental groups were analyzed, revealing *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Clostridium* as the predominant bacterial taxa at the phylum level. While *Firmicutes* and *Proteobacteria* dominated in the initial days, *Bacteroidetes* gradually increased with age, while *Actinobacteria* decreased (Fig. 2A). At the genus level,

Table 1: Information about pre-weaning calves in three groups

	Group					
Item	Control	Psyllium husk powder	Inulin	F value	*P* value	χ^2
N	45	46	45			
Birth weight, kg	37.36±4.39	36.89±3.29	37.29±5.15	0.153	0.858	
Average daily gain, g	859.44±102.34	876.48±95.28	848.53±90.07	0.644	0.527	
Diarrhea incidence, %	80.0[a]	54.3[b]	57.8[b]		P<0.05	7.62
Diarrhea duration, day	2.69±2.29[a]	0.96±1.38[b]	1.60±1.76[a]	10.24	P<0.0001	
Duration time	1.27±1.03	0.76±0.8	0.91±0.90	3.55	P<0.05	

[a–b]Mean values in the same row with different superscripts differ (P<0.01).

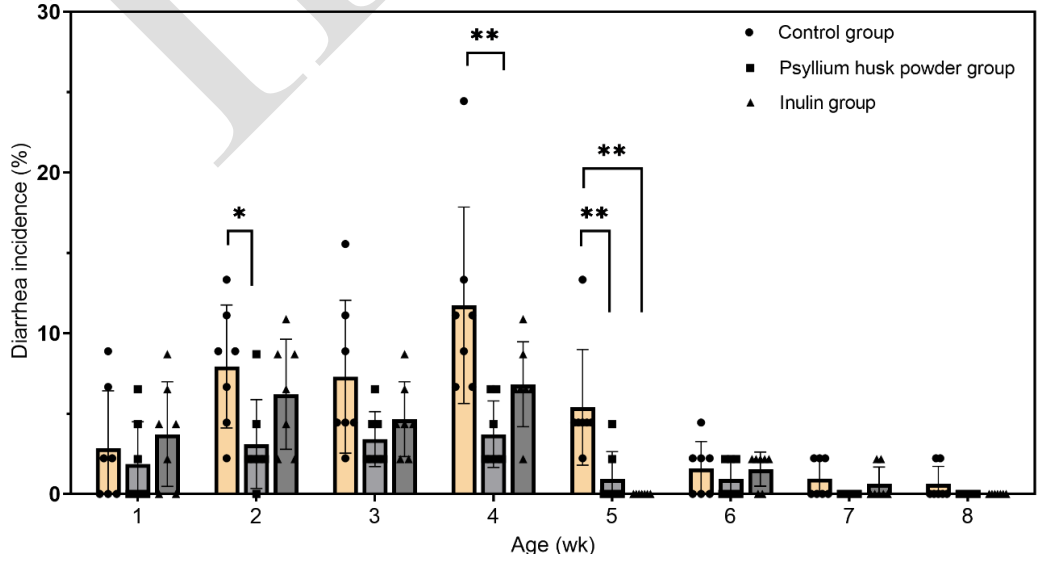

Fig. 1: Effects of dietary fiber supplementation weekly diarrhea. * indicates a significance level of P<0.05, and ** indicates a significance level of P<0.01.

Table S3: Starter intake of calves in each group after 56 days of age (kg)

		D57	D58	D59	D60	D61	D62	D63	D64	D65	D66	D67	D68	D69	D70
Control Group (n = 13)	C1	1.85	1.65	1.20	1.10	1.20	1.45	2.00	1.85	1.60	1.65	1.75	2.00	2.00	1.80
	C2	0.80	1.55	1.50	1.35	1.85	1.55	1.60	1.65	1.85	1.65	2.60	2.00	1.65	1.80
	C3	0.95	1.25	0.75	0.75	1.20	1.45	1.50	1.35	1.75	1.65	2.00	1.65	1.80	1.00
	C4	1.10	1.25	1.05	1.40	0.65	1.75	1.80	2.00	2.70	2.00	2.00	2.00	1.75	2.00
	C6	0.90	1.30	1.65	1.10	0.85	1.30	0.75	1.15	1.70	1.40	1.75	1.25	1.60	2.00
	C7	1.45	1.65	1.85	1.45	1.45	1.70	0.60	1.25	1.80	1.40	1.60	1.60	1.90	2.20
	C8	1.75	1.55	1.75	1.80	1.85	1.95	1.70	2.20	1.95	2.15	2.00	2.45	2.00	3.00
	C9	1.10	1.15	1.25	1.25	1.25	1.60	1.55	1.60	1.65	1.70	1.55	1.70	1.80	1.95
	C10	0.10	0.70	0.85	0.90	1.05	1.95	0.95	1.20	1.40	1.25	1.70	1.45	1.70	1.95
	C11	1.15	1.35	1.65	1.70	1.70	0.60	0.85	1.65	1.90	2.00	2.70	2.50	2.00	2.95
	C12	1.10	1.00	1.20	1.55	1.60	2.00	2.00	1.90	1.75	1.85	2.00	2.00	1.90	2.00
	C13	0.95	0.25	1.30	0.55	1.15	0.40	0.95	1.10	1.30	1.20	1.15	1.05	1.45	0.55
	C14	1.00	0.65	1.70	2.00	1.20	1.55	0.90	1.00	1.45	1.30	1.55	1.50	1.85	2.00
Psyllium husk powder (n = 11)	P1	0.65	0.90	1.40	0.95	0.90	1.25	1.25	1.60	1.67	1.70	1.75	1.45	2.00	1.30
	P2	1.80	1.65	1.05	1.70	1.60	1.85	1.60	1.85	1.70	1.50	1.50	2.45	2.00	1.55
	P3	1.55	1.60	1.00	1.05	1.00	1.25	1.25	1.45	1.50	1.15	1.80	1.90	2.00	1.45
	P4	1.00	1.75	1.00	1.30	1.50	1.50	1.30	1.60	1.60	1.25	1.75	1.55	1.85	1.30
	P5	2.00	1.45	1.50	1.05	1.15	1.30	1.35	1.80	1.85	1.55	2.00	2.45	1.90	1.90
	P6	0.90	0.70	0.85	0.70	1.00	0.90	1.05	1.10	1.30	1.05	1.45	1.50	1.75	1.55
	P7	1.10	0.80	1.40	1.40	1.20	1.50	1.60	1.55	1.95	1.55	1.95	1.50	1.85	1.80
	P8	0.85	1.15	1.95	1.30	1.35	1.50	1.40	2.00	1.75	1.95	2.00	1.75	2.00	1.65
	P9	1.85	1.60	1.75	1.50	2.00	1.95	1.55	2.00	2.95	2.00	3.00	2.00	2.00	2.00
	P11	0.85	1.10	1.00	1.90	1.10	1.45	0.95	1.15	1.35	0.80	1.30	1.10	1.10	1.55
	P12	0.95	1.45	1.65	1.40	0.80	0.65	0.55	0.60	1.05	0.75	0.75	0.75	0.85	1.65
Inulin group (n = 11)	I1	1.00	0.85	0.60	0.85	1.05	1.10	1.05	1.10	0.95	1.10	1.05	1.40	1.25	1.40
	I2	1.55	0.70	0.55	0.65	1.10	1.05	1.10	1.00	1.15	1.10	1.05	1.40	1.80	1.65
	I3	2.00	1.40	1.25	0.85	1.55	1.70	1.75	2.00	1.85	1.50	2.00	1.85	2.00	2.00
	I4	0.70	0.70	1.40	1.15	1.30	1.50	1.30	1.55	1.70	1.10	1.60	1.40	1.90	1.95
	I5	0.40	1.25	1.45	1.10	0.80	1.25	1.30	1.50	1.30	1.40	1.55	1.60	1.85	1.85
	I6	0.50	0.45	0.80	0.60	0.70	0.80	0.70	0.75	1.10	0.80	1.00	1.20	1.50	1.50
	I7	0.50	0.75	1.05	0.75	0.95	0.90	1.20	1.05	0.95	1.10	1.25	1.30	1.60	1.50
	I8	0.35	0.55	0.55	0.55	1.05	0.85	1.05	0.90	1.05	0.85	0.60	1.05	1.50	1.35
	I9	1.45	0.95	0.95	1.05	1.30	1.45	1.65	1.45	1.65	1.90	1.95	2.00	1.85	1.25

Table S4: Average daily diarrhea rate of calves in each group

	Age	wk1	wk2	wk3	wk4	wk5	wk6	wk7
Control Group (n = 45)	1 wk	2.22%	2.22%	0	0	0	6.67%	8.89%
	2 wks	13.33%	8.89%	8.89%	6.67%	2.22%	4.44%	11.11%
	3 wks	11.11%	15.56%	8.89%	2.22%	4.44%	4.44%	4.44%
	4 wks	8.89%	11.11%	13.33%	6.67%	6.67%	11.11%	24.44%
	5 wks	13.33%	4.44%	2.22%	4.44%	4.44%	4.44%	4.44%
	6 wks	0	4.44%	2.22%	2.22%	0	0	2.22%
	7 wks	0	0	0	2.22%	2.22%	2.22%	0
	8 wks	2.22%	0	2.22%	0	0	0	0
Psyllium husk powder (n = 46)	1 wk	2.17%	0	0	0	0	4.35%	6.52%
	2 wks	2.17%	2.17%	2.17%	8.70%	0	2.17%	4.35%
	3 wks	2.17%	6.52%	2.17%	4.35%	2.17%	2.17%	4.35%
	4 wks	4.35%	2.17%	6.52%	2.17%	2.17%	2.17%	6.52%
	5 wks	0	2.17%	0	4.35%	0	0	0
	6 wks	0	0	0	0	2.17%	2.17%	2.17%
	7 wks	0	0	0	0	0	0	0
	8 wks	0	0	0	0	0	0	0
Inulin group (n = 45)	1 wk	6.52%	0	0	2.17%	4.35%	4.35%	8.70%
	2 wks	6.52%	8.70%	10.87%	2.17%	4.35%	2.17%	8.70%
	3 wks	4.35%	4.35%	2.17%	4.35%	8.70%	6.52%	2.17%
	4 wks	2.17%	6.52%	8.70%	6.52%	6.52%	6.52%	10.87%
	5 wks	0	0	0	0	0	0	0
	6 wks	2.17%	2.17%	2.17%	2.17%	0	0	2.17%
	7 wks	2.17%	0	2.17%	0	0	0	0
	8 wks	0	0	0	0	0	0	0

significant differences were noted in the relative abundance of *Butyricoccus*, *Enterococcus*, and *Megamonas* among the groups, with variations observed at different ages (Fig. 2B). During different growth stages of calves, significant changes in the microbial community are observed. At day 0, calves exhibit a diverse yet relatively balanced distribution of microbial taxa, including *Parabacteroides*, *Mycoplasma*, and *Sharpea*. By day 7 and day 14, there is a noticeable increase in the relative abundance of *Dorea* and *Prevotella*, particularly in the psyllium husk powder group, suggesting that dietary fiber supplementation may stimulate the growth of specific taxa during early postnatal development.

The control group maintains a stable microbial composition over time, characterized by moderate levels of taxa such as *Lactobacillus* and *Faecalibacterium*. In contrast, Psyllium husk powder group shows significant fluctuations in microbial populations, with increased

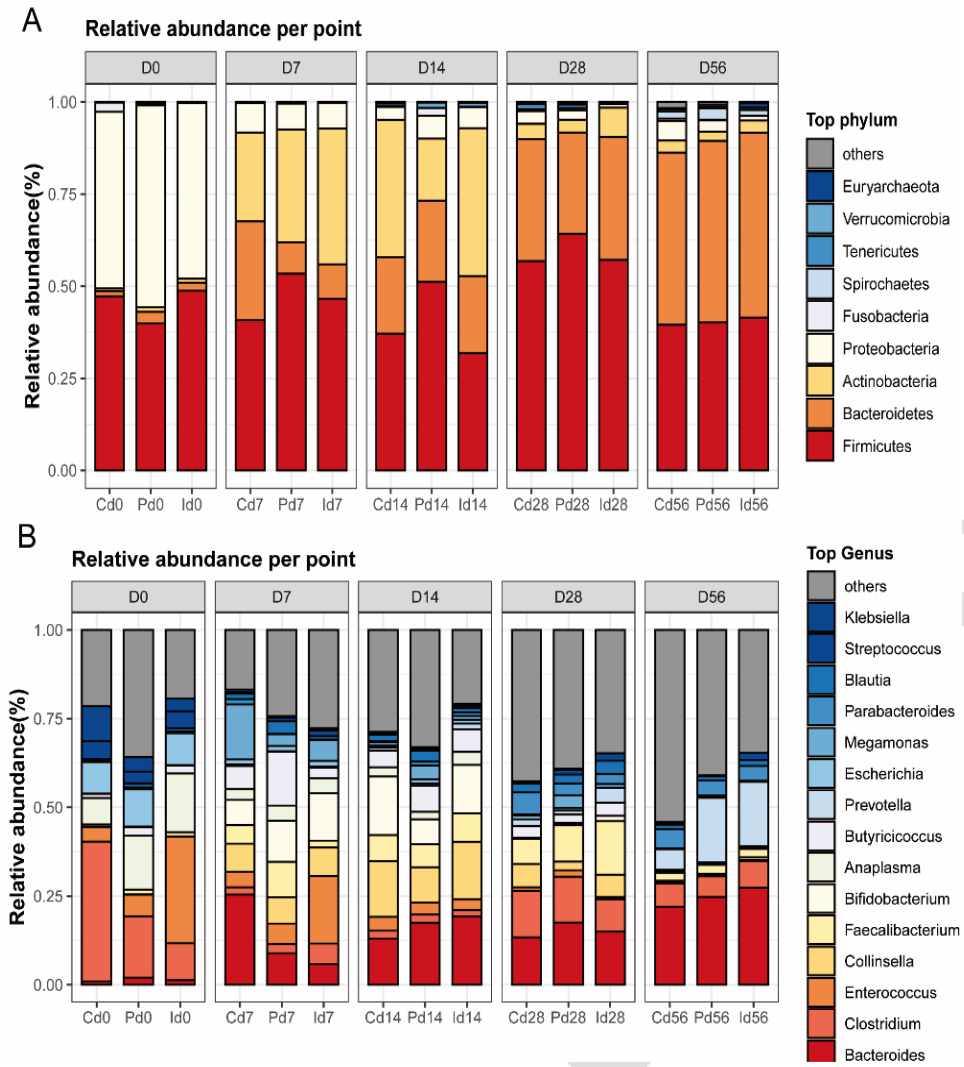

Fig. 2: Relative abundance of fecal microbiota at D0, D7, D14, D28, and D56 in each group. (A) Taxon bar plots at the phylum level depict the top 9 species with the highest abundance. (B) Taxon bar plots at the genus level display the top 15 species with the highest abundance. C represents the control group, P represents the psyllium husk powder group, and I represents the inulin group.

relative abundance of beneficial bacteria like *Bifidobacterium* and *Lactobacillus* at day 14 and day 28. The inulin group exhibits a distinct microbial profile with elevated levels of *Ruminococcus* at later time points, suggesting potential long-term effects on gut microbiota composition. Both psyllium husk powder and inulin groups demonstrate a marked decrease in the relative abundance of potential pathogens such as *Escherichia* and *Staphylococcus* over time. Throughout the various growth stages of calves, there are obvious shifts in dominant bacterial strains. Following supplementation with psyllium husk powder and inulin, there is a significant decrease in the relative abundance of harmful bacteria like *Dorea*, along with reductions in *Sharpea* and *Parabacteroides.* (Fig. 3).

Analysis of microbial alpha in calf fecal microorganisms: Analysis of microbial alpha diversity in calf fecal microorganisms revealed dynamic changes over the study period. Initially, the total microbial count in calf feces exhibited a progressive increase with age. Specifically, from 7 to 56 days old, both groups supplemented with group P and group I demonstrated greater microbial species richness compared to group C. However, statistical analysis indicated no significant differences in species richness between the groups at any time point (P>0.05). Additionally, as the calves matured, fecal microbial diversity consistently increased, eventually reaching a plateau at a comparable level across all groups. Importantly, there were no statistically significant variations in microbial diversity among the groups (P>0.05). These findings suggest that while age influences the total microbial count and richness in calf feces, dietary fiber supplementation with psyllium husk powder and inulin did not significantly alter microbial alpha diversity during the pre-weaning stage (Fig. 4).

PLS-DA analysis of intra-group and between groups differences in calf fecal microorganisms: The clustering patterns diversified with each time point, indicating evolving fecal microbial compositions influenced possibly by short-term dietary fiber intake. As calves advanced in age, the spatial separation between groups increased, reflecting shifts in fecal microbial community structure. At 56 days of age, intergroup differences in fecal microorganisms emerged at the genus level compared to earlier time points (Fig. 5).

At seven days of age: group P exhibited a significantly higher abundance of *Comamonas*, *Ruminococcus*, *Clostridia*ceae, *Neisseriales*, *Atopobiaceae*, and Rhodobacter, *Roseburia*, compared to Group C. Conversely, *Soehngenia* and *Acinetobacter* were

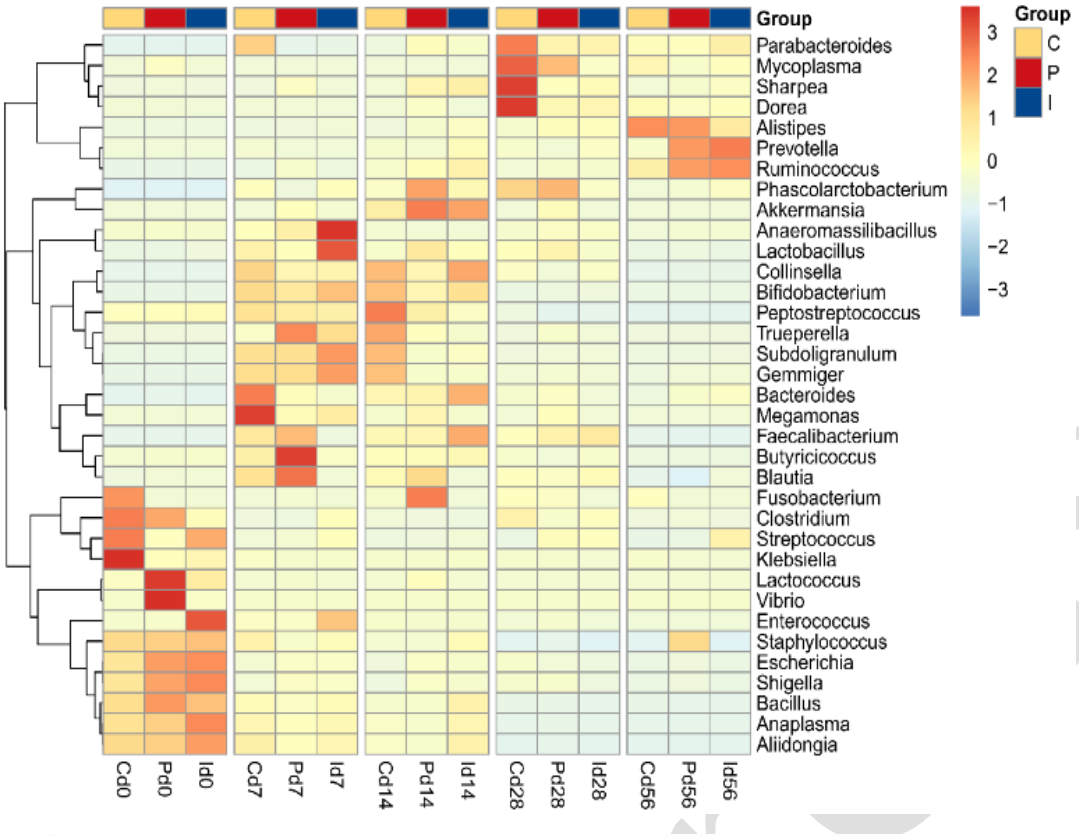

Fig. 3: Relative abundance heatmap at the genus level. It shows the temporal dynamics and relative abundance of various microbial taxa in fecal samples collected from pre-weaning calves subjected to different treatments: control (C), psyllium husk powder (P), and inulin (I). Samples were collected at specified time points (day 0, day 7, day 14, day 28, and day 56). The color scale of red indicates higher relative abundance and blue indicates lower relative abundance. The y-axis enumerates key microbial taxa, while the x-axis delineates the collection time points corresponding to their respective treatment groups.

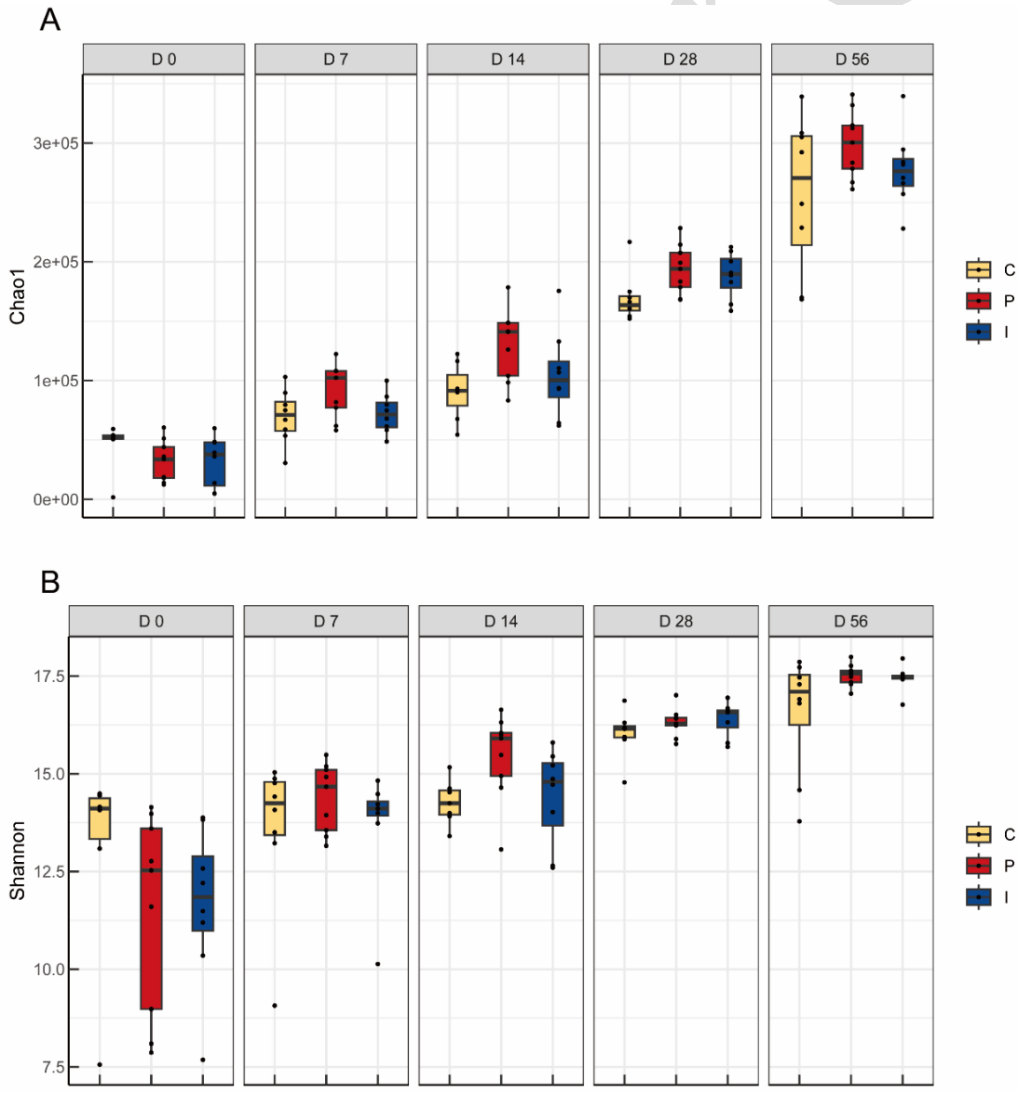

Fig. 4: Different dietary fiber supplements affect fecal bacterial α-diversity in dairy calves across different ages. (A) Bacterial Chao1 value. (B) Shannon index. C, control group. P, psyllium husk powder group. I, inulin group.

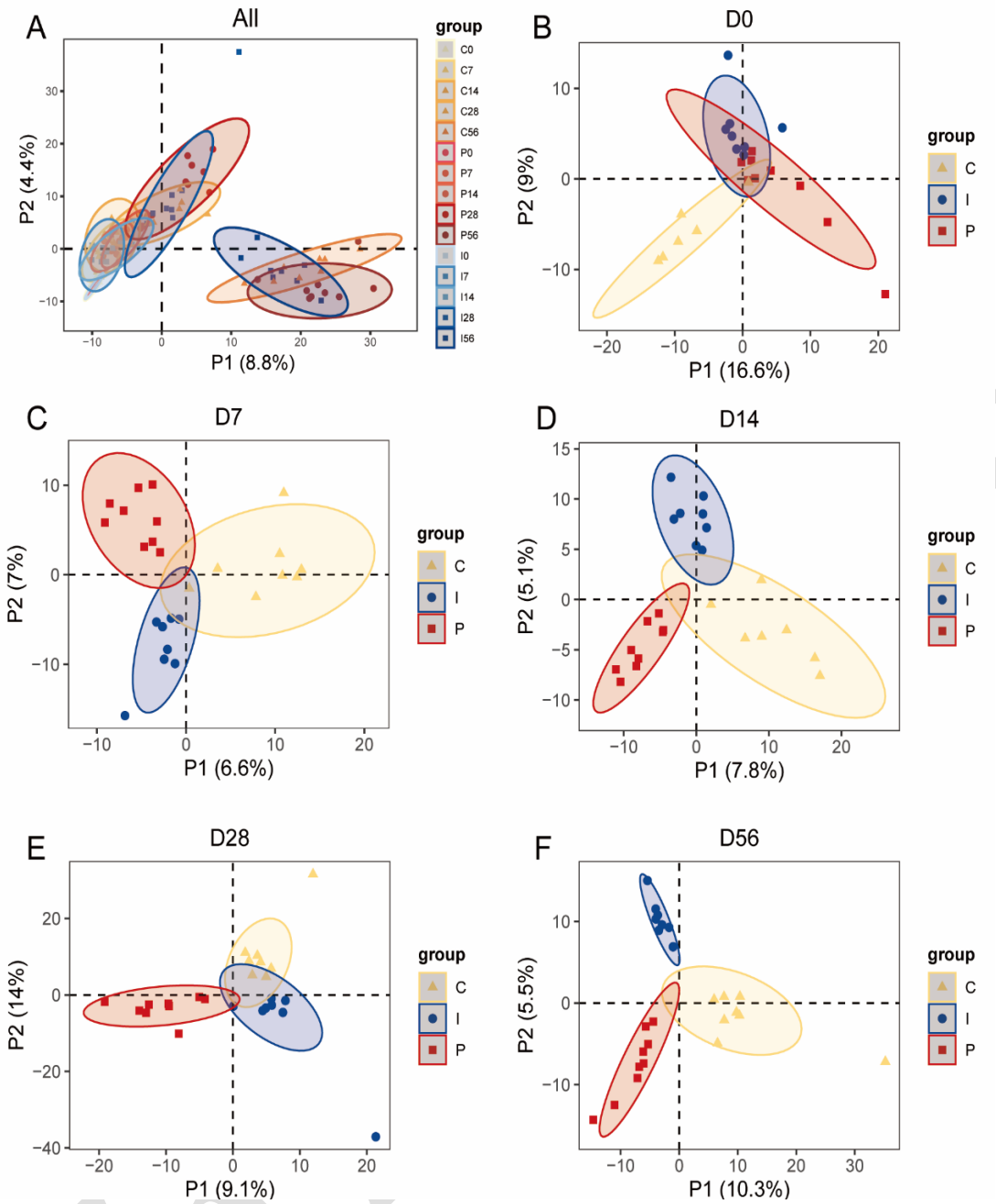

Fig. 5: Partial least squares discriminant Analysis (PLS-DA) for comparing groups at genus level. (A) Changes in beta diversity across all time points. (B) Changes in beta diversity at D0. (C) Changes in beta diversity at D7. (D) Changes in beta diversity at D14. (E) Changes in beta diversity at D28. (F) Changes in beta diversity at D56. C represents the control group, P represents the psyllium husk powder group, and I represents the inulin group.

significantly less abundant in group P than in group C. Group I showed elevated levels of *Streptococcus* and *Comamonas* compared to group C, while *Vibrio*, *Ruminococcus*, *Soehngenia*, and *Acinetobacter* were significantly lower. Additionally, *Erysipelotrichia*, *Lachnospiraceae*, *Ruminococcus*, *Dscillibacter*, and *Clostridioides* were significantly more abundant in group P than in group I**.** At 14 days of age, *Subdoligranulum* and *Ruthenibacterium* from the *Ruminococcaceae* family were significantly lower in group P compared to group C. Group I exhibited higher levels of *Methanobrevibacter*. Moreover, *Erysipelatodlostridium* and *Coprobacillus* were lower in group I than in group P, while *Sellimonas*, *Ensifer*, and *Bifidobacteriales* were significantly higher. At 28 days of age, group P showed an elevated abundance of *Pseudoflavonifractor*, *Roseburia*, *Intestinibacillus*, *Clostridia*, *Ruminococcus*, *Intestinimonas*, *Micrococcales*, and *Veillonella* compared to Group C. Conversely, *Dorea* was less abundant in group P than in group C (Fig. 6A). *Faecalibacterium*, *Prevotella*, *Streptococcus*, *Bifidobacteriaceae*, *Megamonas*, *Kibdelosporangium*, *Candidatus*, *Dichloromethanomonas*, and *Beijerinckiaceae* was significantly higher in group P compared to group C, while *Thermodesulfobacterium* and *Dorea* was significantly lower in group P compared to group C (Fig. 6B). Group P exhibited significantly lower abundance of *Coccidioides*, *Agathobaculum*, *Fusobacterium*, *Escherichia*, *Klebsiella*, *Paenibacillus*, *Clostridiaceae*, and *Enterobacteriaceae* compared to group I (Fig. 6C).

At 56 days of age, group P exhibited a higher abundance of *Prevotella*, *Lactobacillus*, and *Anaeromassilibacillus* compared to Group C, while *Eubacteriaceae* was lower in Group P than in Group C (Fig. 7A). *Prevotella*, *Sharpea*, *Streptococcus*, *Selenomonadaceae*, and *Lactobacillus* was higher in group P compared to group C. Additionally, *Proteobacteria*, including *Klebsiella*, *Pseudomonas*,

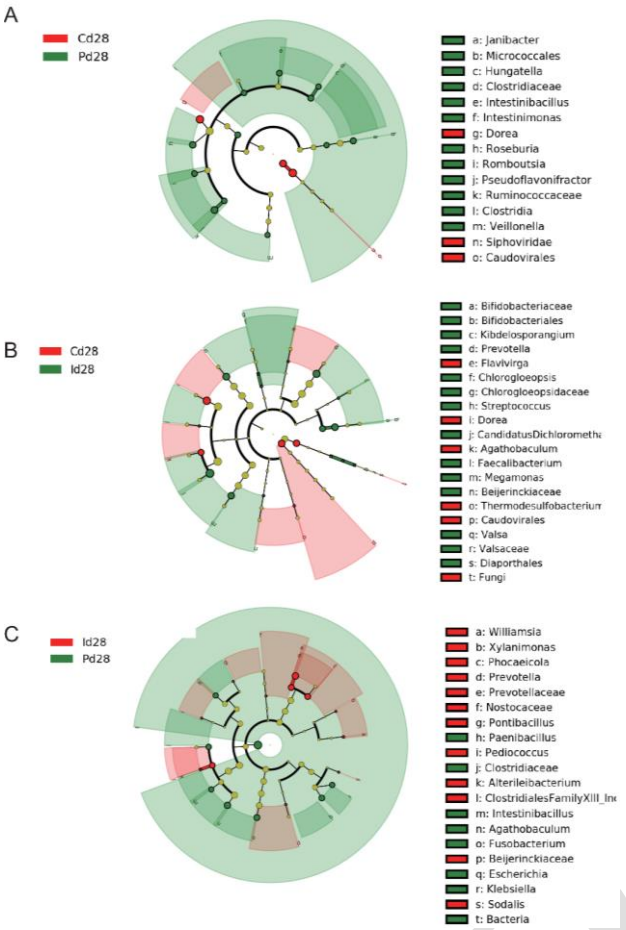

Fig. 6: LEfSe analysis of psyllium husk powder and inulin diets on fecal microbiota of dairy calves at 28 days of age. Differential taxonomic abundance between C and P groups (A), between C and I groups (B), and between P and I groups (C).

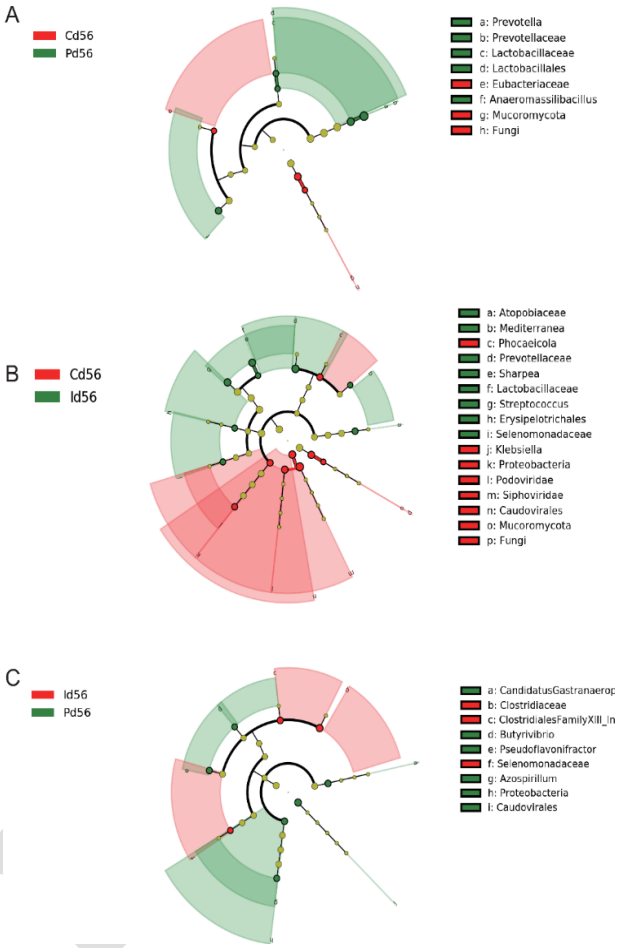

Fig. 7: LEfSe analysis of psyllium husk powder and inulin diets on fecal microbiota of dairy calves at 56 days of age. Differential taxonomic abundance between C and P groups (A), between C and I groups (B), and between P and I groups (C).

Butyrivibrio, and *Pseudoflavonifractor* were significantly higher in group P compared to group I (Fig. 7B). *Clostridiaceae* were lower in group P than in group I, while *Pseudomonas*, *Butyrivibrio*, and *Pseudoflavonifractor* were significantly higher. *Clostridia*ceae was also lower in group P compared to group I (Fig. 7C).

DISCUSSION

This study did not observe significant differences in ADG between the control and treatment groups during the pre-weaning period, which is consistent with previous research reporting mixed outcomes regarding the impact of dietary fiber on calf growth. For example, while some studies have demonstrated significant increases in ADG with inulin supplementation (Jonova *et al.* 2018, Jonova *et al.* 2021), others have reported no significant effects (Tóth *et al.* 2020). Similarly, supplementation with psyllium and timothy grass fiber has yielded inconsistent results in ADG (Kodithuwakku *et al.* 2021, Gleason *et al.* 2021). Moreover, while initial feed intake did not differ significantly between the control and treatment groups, post-weaning, calves supplemented with psyllium husk powder exhibited reduced feed intake compared to the control group. This finding suggests a potential impact of psyllium husk powder supplementation on post-weaning dietary transition and calf development**.** These discrepancies in the literature may derived from various factors, including differences in fiber types, dosage, feeding regimens, and calf management practices across studies (Bradford and Mullins 2012, Hou *et al.* 2023). Moreover, individual variations in calf physiology and genetics could also play a role in their response to dietary fiber supplementation (Castro *et al.* 2016, Gleason *et al.* 2021). Differences in gut microbiota composition may influence how calves utilize dietary fiber for growth and development (Xu *et al.* 2021, Du *et al.* 2023, Zhuang *et al.* 2024). Further research is suggested to elucidate the specific mechanisms underlying the effects of dietary fiber on calf growth and development. This may involve investigating the interaction between dietary fiber and gut microbiota composition, as well as exploring potential metabolic pathways involved in fiber fermentation and nutrient absorption in the calf intestine (Du *et al.* 2023, Suharoschi *et al.* 2019, Han *et al.* 2023, Qi *et al.* 2019). Additionally, studies evaluating the long-term effects of dietary fiber supplementation on calf growth and health outcomes are needed to provide a comprehensive understanding of its benefits and limitations in calf-rearing practices (Castells *et al.* 2015, Xiao *et al.* 2024).

In addition to growth performance, our study also found that both psyllium husk powder and inulin supplementation significantly reduced the duration and

incidence of diarrhea during peak periods, indicating their efficacy in managing calf diarrhea. In piglets, dietary fiber supplementation has similarly been associated with a reduction in diarrhea incidence (Uddin *et al.* 2023, Mu *et al.* 2017). Additionally, the role of dietary fiber in promoting gut health and reducing the risk of gastrointestinal diseases has been extensively documented in the literature (Cronin *et al.* 2021). Psyllium husk powder has been shown to improve intestinal barrier function and reduce inflammation (Garg *et al.* 2024). Similarly, inulin supplementation has been associated with increased production of SCFAs and modulation of gut microbiota composition, leading to improved gut health and reduced risk of diarrhea (Sheng *et al.* 2023, Ji *et al.* 2024). Psyllium husk powder and inulin represent two distinct types of dietary fiber, characterized by potentially significant differences in their chemical structure, solubility, and fermentative properties (Waleed *et al.* 2022, Verbeke 2022). These differences may influence their rates of degradation in the intestinal tract, the types and quantities of fermentation by-products produced, thereby impacting the composition and functionality of the intestinal microbiota.

Moreover, the efficacy of dietary fiber supplementation in managing calf diarrhea is supported by its ability to modulate gut microbiota composition. These results are consistent with previous research suggesting that dietary fiber supplementation can prevent gut flora dysbiosis and enhance the activity of beneficial gut microorganisms, particularly during periods of high disease incidence. Studies have shown that dietary fiber promotes the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* while inhibiting the proliferation of pathogenic species (Healey *et al.* 2018, Kiewiet *et al.* 2021). These changes in gut microbiota composition contribute to maintaining intestinal homeostasis and resilience against gastrointestinal infections (Qi *et al.* 2019, Ventura *et al.* 2016, Wang *et al.* 2022). Therefore, the observed reductions in diarrhea duration and incidence in calves supplemented with psyllium husk powder and inulin can be attributed, in part, to their modulatory effects on gut microbiota composition and function.

The alteration of fecal microflora composition in response to dietary fiber supplementation was another key finding of our study. Short-term dietary fiber intake significantly increased the abundance of fermentative strains such as *Roseburia*, *Clostridia*, *Ruminococcus*, *Intestinimonas*, and *Veillonella* in pre-weaning calves. Particularly, psyllium husk powder supplementation resulted in more extensive alterations in fecal microbial structure compared to inulin supplementation, with significant increases in beneficial strains such as *Coprobacillus*, *Paenibacillus*, and *Bacillus polymyxa*. These findings emphasize the differential effects of different types of dietary fiber on gut microbiota composition and function. The results revealed dynamic changes in the clustering patterns, indicating evolving fecal microbial compositions possibly influenced by short-term dietary fiber intake. As calves progressed in age, spatial separation between groups increased, suggesting significant shifts in fecal microbial community structure. These findings are similar with previous studies emphasizing the impact of age and dietary interventions on gut microbiota dynamics at a young age (Luo *et al.* 2022, Ronan *et al.* 2021). At 56 days of age, distinct intergroup differences in fecal microorganisms emerged at the genus level, indicating pronounced shifts in microbial taxa composition. This observation corroborates with a study highlighting the critical role of age-related transitions in shaping the gut microbiome of calves (Amin and Seifert 2021, Kim *et al.* 2021). Moreover, the differential responses observed in fecal microbial profiles between groups suggest a potential interaction between dietary fiber supplementation and gut microbiota composition. These findings demonstrate the modulatory effects of dietary fiber on the abundance and diversity of gut microbial communities in calves.

Psyllium husk powder and inulin increased the relative abundance of fermentative strains in the fecal microflora, although the specific responses varied. Inulin's highly fermentable nature may contribute to its efficacy in promoting the proliferation of beneficial bacteria like Bifidobacterium, while psyllium husk powder may induce broader alterations in microbial structure. Supplementation with both psyllium husk powder and inulin significantly reduced disease duration and diarrhea incidence, and the relative abundance of these fermentative strains in the microflora was enhanced. Specific microbial populations, such as *Roseburia*, *Prevotella*, *Lactobacillus*, and *Faecalibacterium*, exhibited significant increases with psyllium husk powder and inulin supplementation. These bacteria play crucial roles in fermentation, SCFA production, and intestinal health. Certain *Prevotella* species could potentially serve as probiotics for preventing early diarrhea and facilitating a smooth weaning transition period for calves (Chen *et al.* 2022, Wu *et al.* 2011). Conversely, the relative abundance of *Dorea* decreased, associated with abdominal discomfort and increased intestinal permeability in certain conditions, which is linked to increased intestinal permeability in IBS (Rangel *et al.* 2015, Gargari *et al.* 2024).

Conclusions: This study demonstrates the potential of psyllium husk powder and inulin supplementation in ameliorating diarrhea incidence and duration in pre-weaning calves. Dietary fiber supplementation increases gut microbial richness, enhancing gut microbial functionality and stability, indirectly reducing diarrhea risk.

These findings resonate with prior research emphasizing the pivotal role of dietary fiber in fostering the proliferation of beneficial bacteria and preserving gut homeostasis. Moreover, the divergent responses observed between psyllium husk powder and inulin supplementation suggest nuanced effects on microbial populations. Particularly, psyllium husk powder exhibited superior efficacy in mitigating diarrhea incidence, potentially attributed to its distinct influence on microbial communities, including significant increments in beneficial strains like *Coprobacillus* and *Paenibacillus*. This observation suggests that modulating the intestinal microbial environment through psyllium husk powder and inulin supplementation can effectively promote calf intestinal health, thereby mitigating the severity and

morbidity associated with diarrheal episodes in pre-weaning calves.

This study provides valuable insights into dietary fiber supplementation as a strategy to enhance calf health during the pre-weaning phase. Variations in dosage, duration of supplementation, and administration methods (such as direct feed addition or premix inclusion) can influence bioavailability and efficacy, leading to different physiological responses. Future research should explore the mechanisms behind microbiota alterations and the long-term effects of dietary fiber supplementation on calf growth and development.

Acknowledgments: We thank the Hebei Sunlon Livestock Development Co. for providing the experimental site.

Conflict of interest statement/Competing Interests: The authors declare that there are no conflicts of interest; we do not have any possible conflicts of interest.

Author contribution: CM, JC: Conceptualization, Methodology, Supervision, Writing - Review & Editing. XY: Methodology, Investigation, Software, Data curation, Visualization, Writing- Original draft preparation. DL: Resources, Project administration, Investigation HZ, CP, WL, YG, XZ, KS: Investigation.

REFERENCES

Amin N and Seifert J, 2021. Dynamic progression of the calf's microbiome and its influence on host health. Comp Struct Biotechnol J 19:989-1001.

Belorio M and Gómez M, 2021. Psyllium: A useful functional ingredient in food systems. Crit Rev Food Sci Nutr 62:527-538.

Bradford B J and Mullins C R, 2012. Invited review: Strategies for promoting productivity and health of dairy cattle by feeding nonforage fiber sources. J Dairy Sci 95:4735-4746.

Castells L, Bach A and Terré M, 2015. Short- and long-term effects of forage supplementation of calves during the preweaning period on performance, reproduction, and milk yield at first lactation. J Dairy Sci 98:4748-4753.

Castro J J, Gomez A, White B A, *et al.*, 2016. Changes in the intestinal bacterial community, short-chain fatty acid profile, and intestinal development of preweaned holstein calves. Effects of prebiotic supplementation depend on site and age. J Dairy Sci 99:9682-9702.

Chen H, Liu Y, Huang K, *et al.*, 2022. Fecal microbiota dynamics and its relationship to diarrhea and health in dairy calves. J Anim Sci Biotechnol 13:1-20.

Cho Y I and Yoon K J, 2014. An overview of calf diarrhea - infectious etiology, diagnosis, and intervention. J Vet Sci 15:1-17.

Cronin P, Joyce S A, O'toole P W, *et al.*, 2021. Dietary fibre modulates the gut microbiota. Nutrients 13:1-22.

Du Y, Gao Y, Hu M, *et al.*, 2023. Colonization and development of the gut microbiome in calves. J Anim Sci Biotechnol 14:1-15.

Fu L, Niu B, Zhu Z, *et al.*, 2012. Cd-hit: Accelerated for clustering the next-generation sequencing data. Bioinformatics 28:3150-3152.

Gargari G, Giacomo M, Cesare C, *et al.*, 2024. Collinsella aerofaciens as a predictive marker of response to probiotic treatment in non-constipated irritable bowel syndrome. Gut Microbes 16:1-16.

Garg P, Garg P K and Bhattacharya K, 2024. Psyllium husk positively alters gut microbiota, decreases inflammation, and has bowel-regulatory action, paving the way for physiologic management of irritable bowel syndrome. Gastroenterology 166:545-546.

Gao Y, Zhang W, Zhang T, *et al.*, 2024. Fructo-oligosaccharide supplementation enhances the growth of nursing dairy calves while stimulating the persistence of bifidobacterium and hindgut microbiome's maturation. J Dairy Sci 107:5626-5638.

Gleason C B, Settlage R E, Beckett L M, *et al.*, 2021. Characterizing effects of ingredients differing in ruminally degradable protein and fiber supplies on the ovine rumen microbiome using next-generation sequencing. Front Anim Sci 2:1-15.

Gupta N, Jangid A K, Pooja D, *et al.*, 2019. Inulin: A novel and stretchy polysaccharide tool for biomedical and nutritional applications. Int J Biol Macromol 132:852-863.

Healey G, Murphy R, Butts C, *et al.*, 2018. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: A randomised, double-blind, placebo-controlled, cross-over, human intervention study. Br J Nutr 119:176-189.

Han X, Ma Y, Ding S, *et al.*, 2023. Regulation of dietary fiber on intestinal microorganisms and its effects on animal health. Anim Nutr 14:356-369.

Hou P, Li B, Wang Y, *et al.*, 2023. The effect of dietary supplementation with zinc amino acids on immunity, antioxidant capacity, and gut microbiota composition in calves. Animals 13:1-15.

Jalanka J, Major G, Murray K, *et al.*, 2019. The effect of psyllium husk on intestinal microbiota in constipated patients and healthy controls. Int J Mol Sci 20:1-12.

Jha R, Fouhse J M, Tiwari U P, *et al.*, 2019. Dietary fiber and intestinal health of monogastric animals. Front Vet Sci 6:1-12.

Ji K, Zhang M, Du L, *et al.*, 2024. Exploring the role of inulin in targeting the gut microbiota: An innovative strategy for alleviating colonic fibrosis induced by irradiation. J Agric Food Chem 72:5710-5724.

Jonova S, Ilgaza A, Grinfelde I, *et al.*, 2018. Impact of the flour of jerusalem artichoke on the production of methane and carbon dioxide and growth performance in calves. Vet World 11:1532-1538.

Jonova S, Ilgaza A and Zolovs M, 2021. The impact of inulin and a novel synbiotic (yeast saccharomyces cerevisiae strain 1026 and inulin) on the development and functional state of the gastrointestinal canal of calves. Vet Med Int 2021:1-9.

Kiewiet M B G, Elderman M E, El Aidy S, *et al.*, 2021. Flexibility of gut microbiota in ageing individuals during dietary fiber long-chain inulin intake. Mol Nutr Food Res 65:1-10.

Kim E-T, Lee S-J, Kim T-Y, *et al.*, 2021. Dynamic changes in fecal microbial communities of neonatal dairy calves by aging and diarrhea. Animals 11:1-20.

Kodithuwakku K A H T, Owada H, Miura H, *et al.*, 2021. Effects of oral administration of timothy hay and psyllium on the growth performance and fecal microbiota of preweaning calves. J Dairy Sci 104:12472-12485.

Li D, Liu C M, Luo R, *et al.*, 2015. Megahit: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de bruijn graph. Bioinformatics 31:1674-1676.

Li H and Durbin R, 2009. Fast and accurate short read alignment with burrows–wheeler transform. Bioinformatics 25:1754-1760.

Luo C, Xia B, Zhong R, *et al.*, 2022. Early-life nutrition interventions improved growth performance and intestinal health via the gut microbiota in piglets. Front Nutr 8:1-15.

McRorie J W, Gibb R D, Sloan K J, *et al.*, 2021. Psyllium: The gel-forming nonfermented isolated fiber that delivers multiple fiber-related health benefits. Nutr Today 56:169-182.

Mu C, Zhang L, He X, *et al.*, 2017. Dietary fibres modulate the composition and activity of butyrate-producing bacteria in the large intestine of suckling piglets. Antonie van Leeuwenhoek 110:687-696.

Qi H, Li Y, Yun H, *et al.*, 2019. Lactobacillus maintains healthy gut mucosa by producing l-ornithine. Commun Biol 2:1-14.

Rangel I, Sundin J, Fuentes S, *et al.*, 2015. The relationship between faecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients and healthy subjects. Aliment Pharmacol Ther 42:1211-1221.

Ronan V, Yeasin R and Claud E C, 2021. Childhood development and the microbiome: The intestinal microbiota in maintenance of health and development of disease during childhood development. Gastroenterology 160:495-506.

Shoaib M, Shehzad A, Omar M, *et al.*, 2016. Inulin: Properties, health benefits and food applications. Carbohydr Polym 147:444-454.

Sheng W, Ji G and Zhang L, 2023. Immunomodulatory effects of inulin and its intestinal metabolites. Front Immunol 14:1-16.

Suharoschi R, Pop O L, Vlaic R A, *et al.* (2019). Dietary fiber and metabolism. Dietary fiber: Properties, recovery, and applications, Academic Press**:** 59-77.

Tóth S, Kovács M, Bóta B, *et al.*, 2020. Effect of mannanoligosaccharide (mos) and inulin supplementation on the performance and certain physiological parameters of calves reared on milk replacer. J Appl Anim Res 48:228-234.

Uddin M K, Mahmud M R, Hasan S, *et al.*, 2023. Dietary micro-fibrillated cellulose improves growth, reduces diarrhea, modulates gut microbiota, and increases butyrate production in post-weaning

piglets. Sci Rep 13:1-15.
Ventura M, Delgado S, Milani C, *et al.*, 2016. Bifidobacteria and their role as members of the human gut microbiota. Front Microbiol 1:1-23.
Verbeke K, 2022. Combining dietary fibres to reduce intestinal gas production in patients with ibs. Gut 71:848-849.
Waleed M, Saeed F, Afzaal M, *et al.*, 2022. Structural and nutritional properties of psyllium husk arabinoxylans with special reference to their antioxidant potential. Int J Food Prop 25:2505-2513.
Wang H, Huang X, Tan H, *et al.*, 2022. Interaction between dietary fiber and bifidobacteria in promoting intestinal health. Food Chem 393:1-11.
Wu G D, Chen J, Hoffmann C, *et al.*, 2011. Linking long-term dietary patterns with gut microbial enterotypes. Science 334:105-108.
Xiao J, Chen T, Peng R, *et al.*, 2024. How neonatal diet affects the long-term development of rumination behavior, rumen fermentation and feed digestion in dairy calves fed a high milk level? Anim Nutr 16:326-337.
Xu Q, Qiao Q, Gao Y, *et al.*, 2021. Gut microbiota and their role in health and metabolic disease of dairy cow. Front Nutr 8:1-13.
Zhang Y, Luo Y, Gao B, *et al.*, 2025. Psyllium: A nutraceutical and functional ingredient in foods. Annu Rev Food Sci Technol 16:355-377.
Zhuang Y, Liu S, Gao D, *et al.*, 2024. The bifidobacterium-dominated fecal microbiome in dairy calves shapes the characteristic growth phenotype of host. npj Biofilms Microbomes 10:1-18.
Zhu Y, Liu J, Lopez J M, *et al.*, 2020. Inulin fermentation by lactobacilli and bifidobacteria from dairy calves. Appl Environ Microbiol 87:1-12.