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Saccharomyces cerevisiae Fermentation Products Promote the Growth of Pre-Weaning Calves by Increasing Apparent Nutrient Digestibility, and Modulating the Gut Microbiota

Qian Lei [1], Zhiqiang Cheng [1], Yuncheng Huang [1], Xue Li [1], Osmond Datsomo [1], Maocheng Jiang [1], Kexin Wang [1], Qianbo Ma [1], Xiaoxiao Gong [1], Yongjiu Huo [1,2]. and Miao Lin [1,2,*]

[1] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China;
[2] Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, 225009, China; lq92779277@163.com (Q.L.); zqcheng1998@163.com (Z.C.); 1074458154@qq.com (Y.C.); LX929492@163.com (X.L.); datsomorosmond@gmail.com (O.D.); Jmcheng1993@163.com (J.C.); awkx5527@163.comB (K.W.); mqb1601555436@163.com (Q.M.); 18762329160@163.com (X.G.); huoyj@126.com (Y.H.).
* Correspondence: linmiao@yzu.edu.cn; Tel.: 18252725959

Simple Summary: *Saccharomyces cerevisiae* fermentation products (SCFP) are a type of green feed additive with nutritional and health benefits, making them an attractive alternative to antibiotics with promising potential for development and application. This study found that supplementation with SCFP improved ADG and serum hormones, enhanced immunity, and regulated gut microbiota, thereby jointly promoting the growth of pre-weaning calves. These findings will provide valuable information for promoting the early development of calves, increasing daily weight gain, improving immunity, raising healthy calves, and improving the economic efficiency of livestock farms.

Abstract: *Saccharomyces cerevisiae* fermentation products (SCFP) can potentially promote gastrointestinal growth and immunity in young livestock. However, reports within the existing literature on the effects of SCFP supplements on calves have been inconsistent; therefore, we perform the following experiments to resolve the inconsistencies. A total of 22 Holstein calves [10 d after birth, BW = 48.93 ± 3.99 kg (mean ± SD)] were assigned randomly into two groups, namely the control group (CON) and SCFP group, each having 11 replicas. The calves in the CON were fed a basal diet, while the SCFP group was fed the basal diet supplemented with 5g/head/d SCFP (NutriTek, Diamond V, Cedar Rapids, IA52404, United States) incorporated into feed. All the calves were regularly fed thrice daily at 08:00, 14:30, and 21:00 and had free access to water. A 5-day adaptation phase was followed by a 45-day experimental period. The results showed that compared to the CON, at the end of the d 45 trial, the body weight was significantly greater in the SCFP group ($p < 0.05$), and during the 1-45 days, the ADG was higher ($p < 0.05$). The FCR in the 30-45 days SCFP group was higher ($p < 0.05$). Furthermore, the apparent digestibility of DM, CP, EE, ADF, Ca, and P were significantly increased in the SCFP group, except for NDF ($p < 0.05$). The concentration of GH and IGF-1 in serum showed a tremendous increase ($p < 0.05$) with SCFP supplementation on d 15 and d 45. On d 15, SCFP supplementation significantly increased the serum IgA contents ($p < 0.05$). Notably, on d 15 and d 45, the serum concentrations of IL-1β, IL-6, and TNF-α reduced ($p < 0.001$). Moreover, the *Actinobacteriota* in the SCFP group were significantly lower than those in the CON group ($p = 0.034$). SCFP significantly increased the abundance of *Butyricimonas*, *Parabacteroides*, and *Ruminococcus*. The differences among sob, Chao1, and PD-tree groups were statistically significant ($p < 0.05$). In conclusion, these results demonstrate that SCFP supplementation improved ADG, apparent digestibility and serum hormone, enhanced immunity, and regulated gut microbiota, thereby jointly promoting the growth of pre-weaning calves.

Keywords: Holstein calf; Growth; Gut microbiota; *Saccharomyces cerevisiae* fermentation products

1. Introduction

According to a study published by the US National Animal Health Surveillance System in 2018, 39% of calf deaths in the first 3 weeks of life are caused by diarrhea [1]. Chuang et al. conducted a study that revealed significant differences in microbiota structure between healthy and diarrheal calves and biases in bacterial community prediction of metagenomic function. The study also found a close association with immune-related markers, which provided a new idea for the relationship between paroxysmal calf diarrhea and gastrointestinal microbiota [2].

Calf gut microbes are a complex ecosystem, harboring various microbial communities that rely on each other and cooperate to maintain the stability of the intestinal environment [3–5]. During the growth of calves, gut microbes play an essential role in feed decomposition, nutrient absorption, and energy production [6,7]. By producing enzymes and other substances, these microorganisms help calves break down nutrients such as cellulose, protein, and fat in their feed, thereby improving the absorption and utilization of nutrients by calves and promoting growth [8–10].

In recent decades, the use of green and safe feed additives in livestock production has received attention due to the need for antibiotic alternatives, as antibiotic overuse has led to bacterial resistance [11,12]. Calves are important economic animals in agriculture, and their health is essential for sustainable animal husbandry [13,14]. Yeast-beneficial microorganisms show more advantages than other microorganisms in the animal production and feed industries. Yeast, the most commonly used feed additive in animal feeding, has been shown to effectively regulate the gut microbial balance, especially during periods of stress [15,16]. Therefore, yeast and yeast products play a vital role in the development of animal husbandry.

In this context, it is imperative to study the effects and potential of *saccharomyces cerevisiae* on the gut microbes of calves. SCFP is produced through the anaerobic fermentation of yeast, which has beneficial metabolites, such as B vitamins, amino acids, nucleotides, lipids, and organic acids [17]. For example, Alugongo et al. demonstrated that supplementation with SCFP could improve the intestinal health of pre-weaning dairy calves [18]. Centeno-Martinez et al. showed that supplementation of yeast fermentation products had no significant effect on fecal microbial community structure but tended to increase the uniformity of the microbial community [19]. Therefore, exploring its application in neonatal ruminants is of profound significance.

This study aimed to determine whether the addition of SCFP could improve the gut microbiota of pre-weaning calves and promote their growth. The product is postulated to affect growth-related growth hormones, immunoglobulin, and immune factors in the blood.

2. Materials and Methods

2.1. Animal, Experimental Design, diet, and Management

All animals were cared for according to protocols approved by Yangzhou University Laboratory Animal Care and Use Committee.

The study was conducted at the Gaoyou Experimental Ranch of Yangzhou University (Gaoyou, Jiangsu, China). A total of 22 Holstein calves [10 d after birth, BW = 48.93 ± 3.99 kg (mean ± SD)] were used. All calves were randomly allocated into 2 treatment groups with 11 replicates each, and each replicate had a calf. The calves in the CON group were only fed a basal diet, while the SCFP group was fed the basal diet supplemented with 5g/head/d SCFP products (NutriTek, Diamond V). The level of SCFP was based on the manufacturer's (Cedar Rapids, IA 52404, United States) recommendation for ruminants.

All the calves were fed thrice daily at 08:00, 14:30, and 21:00. Milk was offered in plastic tubs in equal amounts three times a day, and calves had free access to water. A 5-day adaptation phase was followed by a 45-day experimental period. The whole milk was pasteurized by heating up to 70 °C

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for 30 minutes and then cooled to 39-42 °C using cold-water circulation around the container before feeding to calves' liquid feed according to the pasture feeding system. The starters were fed at 7-d age, and the refusal of the previous day was recorded at 09:00 per day. Oat hay was included in the diet on day 30 of age. The chemical compositions of starters, oat hay, and SCFP are shown in Table 1.

Table 1. Chemical compositions of the calf starters, oat hay, and SCFP (% of DM).

Items	Starter	Oat hay	SCFP
DM	94.00	89.00	91.60
CP	21.07	8.52	18.73
EE	3.25	5.27	1.25
Ash	9.94	9.30	11.92
NDF	22.99	47.33	31.47
ADF	14.85	32.06	22.20
Ca	1.12	0.47	0.67
P	0.62	0.28	0.76
NaCl	0.60	—	0.15
MOS	—	—	2.00

Abbreviations: DM = Dry matter; CP = Crude Protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; Ca = Calcium; P = Phosphorus; NaCl = Sodium chloride; MOS = Mannosan; SCFP = *Saccharomyces cerevisiae* fermentation products (NutriTek, Diamond V; Cedar Rapids, IA52404, United States).

2.2. Body weight and feed intake

The body weight (BW) of all calves was measured during the period 1-15 days, 15-30 days, and 30-45 days, and the average daily gain (ADG) was calculated according to initial and final BW. The accurate starters and oat hay consumption of each calf were recorded and converted into average daily dry matter intake (ADMI). The feed conversion ratio was calculated by dividing ADG by ADMI.

2.3. Blood collection and analyses

Blood samples were collected via jugular venipuncture into 10-ml vacutainer tubes without additive anticoagulant on days 15 and 45 before morning feeding. Then, the samples were centrifuged at 3500 × g for 15 min, and then serum samples were collected and stored at −80 °C until further analysis. The detection of growth hormone (GH), insulin-like growth factor 1 (IGF-1), glucagon-like peptide-1 (GLP-1) and -2, (GLP-2), immunoglobulin A (IgA), and immunoglobulin M (IgM), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) were performed by a biotechnology testing company (Hengyi Biotechnology Co., Ltd., Jiangsu, China).

2.4. Collection and measurements of fecal samples

Fecal samples were collected aseptically from calves at the end of the experiment. Diluted sulfuric acid (10%) was mixed with fecal samples (2 ml per 100 g sample) for nitrogen fixation. All feed and fecal samples were oven-dried at 65 ℃ and grounded through a 0.35-mm screen for later determinations. The fecal dry matter (DM), ether extract (EE), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were analyzed and determined following the method described by Ji [20]. The kit method (China Nanjing Construction Co., LTD) was used to detect calcium and phosphorus concentrations. In addition, acid insoluble ash (AIA) was used as an internal marker to determine the nutrient digestibility, which was calculated using the equation:

> Nutrient digestibility (%) = [1 - (A1 × F2) / (A2 × F1)] × 100 according to Álvarez-Rodríguez [21].

Where A1 and A2 are the nutrient content in feces and diet (%), F1 and F2 are the AIA content in feces and diet (%), respectively.

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Fresh fecal samples (2 g) were collected directly from the rectum by rectal palpation. Samples were stored in 5 ml Cryovial Plastic Frozen Test Tubes on ice during sampling and then frozen at −20°C immediately until further analysis. The fresh fecal sample was collected and measured by high-throughput sequencing by Genedenovo Biological Technology Co., Ltd (Guangzhou, China). Microbial DNA was extracted using the HiPure Soil DNA Kits (or HiPure Stool DNA Kits) (Magen, Guangzhou, China) according to the manufacturer's protocol. The hypervariable V3-V4 region of the 16S-rDNA gene was amplified by PCR using the primers 341F: CCTACGGGNGGCWGCAG; 806R: GGACTACHVGGGTATCTAAT, where the barcode was an eight-base sequence unique to each sample. After raw reads were obtained by sequencing, DADA2 software [22] was used for data filtering and quality control. ASVs (Amplicon Sequence Variants) with single-base precision were clustered, which was equivalent to OTU clustered with 100% similarity. Species composition analysis, Indicator species analysis (observed OTUs and LEfSe analysis), and Alpha-diversity (α-diversity index) were performed using QIIME2.

2.5. Statistical analysis

A completely randomized test design was used in the study. All data were analyzed using the independent sample t-test of the IBM SPSS Statistics software (version 25.0). Data are shown as means and standard error of the mean (SEM). Differences with $p < 0.05$ and $p < 0.001$ were considered significant and highly significant, respectively. The DNA sequencing data were analyzed on a free online platform of Omicsmart tools (https://www.omicsmart.com /tools).

3. Results

3.1. Growth performance

The effects of SCFP on the growth performance in pre-weaning dairy calves are presented in Table 2. The initial BW didn't show a difference ($p > 0.05$) between the CON and SCFP, but at the end of the trial, there was an increase in BW in the SCFP group ($p < 0.05$). The ADMI was similar ($p > 0.05$) between the two groups. From 1 to 45 days, the ADG gain in the SCFP group was increased ($p < 0.05$). The FCR in the 30 to 45-day SCFP group was higher ($p < 0.05$) than the CON group.

Table 2. Effects of SCFP supplementation on growth performance in pre-weaning dairy calves.

Items	CON[1]	SCFP[2]	SEM	*p*
BW (kg)				
d 1	52.36	53.18	2.23	0.717
d 15	60.27	63.55	1.87	0.096
d 30	70.68	74.00	2.76	0.244
d 45	86.41	92.36	2.83	0.048
ADG (g/d)				
d 1-15	527.27	690.91	77.58	0.048
d 15-30	693.94	696.97	69.67	0.966
d 30-45	1048.49	1224.24	78.55	0.037
d 1-45	756.57	870.71	34.09	0.003
ADMI (g/d)				
d 1-15	121.42	142.86	27.25	0.461
d 15-30	916.04	919.81	126.11	0.977
d 30-45	2472.73	2455.74	153.59	0.916
d 1-45	1330.34	1340.95	111.32	0.927
FCR (g/g)				
d 1-15	4.41	5.18	1.09	0.512
d 15-30	0.74	0.79	0.18	0.791
d 30-45	0.43	0.65	0.04	0.027

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d 1-45	0.57	0.65	0.04	0.103

Abbreviations: CON = Control group; SCFP = *Saccharomyces cerevisiae* fermentation products (NutriTek, Diamond V; Cedar Rapids, IA52404, United States); SEM = Standard error of the mean; BW = Body weight; ADG = Average daily gain; ADMI = Average daily dry matter intake; FCR = Feed conversion ratio=ADG/ADMI. [1]CON, control with no SCFP and fed basal ration. [2]SCFP, fed basal ration and 5 g/d SCFP (NutriTek, Diamond V, Cedar Rapids, IA52404, United States) per calf.

3.2. Apparent nutrient digestibility

The effects of SCFP on the apparent nutrient digestibility of pre-weaned dairy calves are presented in Table 3. Except for NDF, the apparent digestibility of DM, CP, EE, ADF, Ca, and P were 76.21%, 62.33%, 64.85%, 49.09%, 66.99%, and 65.45%, which were significantly higher than CON ($p < 0.05$).

Table 3. Effects of SCFP on apparent nutrient digestibility of pre-weaning calves (%).

Digestibility	CON	SCFP	SEM	*p*
DM	71.03	76.21	0.93	＜0.001
CP	60.00	62.33	1.10	0.047
Fat	59.63	64.85	2.11	0.022
DNF	50.32	52.72	2.55	0.357
ADF	43.28	49.09	2.69	0.043
Ca	61.83	66.99	1.65	0.005
P	54.91	65.45	3.89	0.014

Abbreviations: CON = Control group; SCFP = *Saccharomyces cerevisiae* fermentation products (NutriTek, Diamond V; Cedar Rapids, IA52404, United States); SEM = Standard error of the mean; DM = Dry matter; CP = Crude Protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; Ca = Calcium; P = Phosphorus.

3.3. Growth-related hormones

The concentrations of GH, IGF-1, GLP-1, and GLP-2 in serum are presented in Table 4. The concentration of GH and IGF-1 in serum did increase by adding SCFP on d 15 and d 45 ($p < 0.05$). Furthermore, on d 45, SCFP supplementation significantly increased ($p < 0.05$) the GLP-1 content in the serum of pre-weaning dairy calves. Additionally, the SCFP group exhibited a slightly higher GLP-2 than the CON group.

Table 4. Effects of SCFP supplementation on plasma growth-related hormones in pre-weaning dairy calves.

Items	CON	SCFP	SEM	*p*
GH (ng/mL)				
d 15	9.66	12.75	0.90	0.003
d 45	11.61	16.45	1.57	0.007
IGF-1 (ng/mL)				
d 15	171.55	226.65	10.29	＜0.001
d 45	210.87	257.12	5.82	＜0.001
GLP-1 (pmol/L)				
d 15	26.45	29.40	1.99	0.157
d 45	25.07	33.53	1.56	＜0.001
GLP-2 (pmol/L)				
d 15	417.07	439.15	30.24	0.475
d 45	411.89	487.35	39.01	0.070

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Abbreviations: CON = Control group; SCFP = *Saccharomyces cerevisiae* fermentation products (NutriTek, Diamond V; Cedar Rapids, IA52404, United States); SEM = standard error of the mean; GH = Growth hormone; IGF-1 = insulin-like growth factor 1; GLP-1 = glucagon-like peptide-1; GLP-2 = glucagon-like peptide-2.

3.4. Serum immunoglobulin and cytokine

The IgA, IgM, TNF-α, IL-1β, and IL-6 concentrations in serum are presented in Table 5. No obvious difference ($p > 0.05$) in IgM contents was observed between the CON and SCFP groups on d 15 and d 45. However, on d 15 and d 45, SCFP supplementation significantly increased the IgA contents in the serum of pre-weaning dairy calves ($p < 0.05$). Notably, on d 15 and d 45, by feeding SCFP, the concentrations of TNF-α, IL-1β, and IL-6 in the serum were reduced ($p < 0.001$).

Table 5. Effects of SCFP supplementation on plasma serum immunoglobulin and cytokine in pre-weaning dairy calves.

Items	CON	SCFP	SEM	*p*
IgA (g/L)				
d 15	0.033	0.047	0.006	0.029
d 45	0.045	0.048	0.002	0.138
IgM (g/L)				
d 15	0.018	0.019	0.004	0.884
d 45	0.027	0.026	0.002	0.643
TNF-α (pg/mL)				
d 15	64.107	48.309	2.217	＜0.001
d 45	54.473	40.962	2.109	＜0.001
IL-1β (pg/mL)				
d 15	26.764	20.067	0.950	＜0.001
d 45	23.449	17.396	1.036	＜0.001
IL-6 (pg/mL)				
d 15	135.530	108.411	4.079	＜0.001
d 45	118.935	94.387	2.549	＜0.001

Abbreviations: CON = Control group; SCFP = *Saccharomyces cerevisiae* fermentation products (NutriTek, Diamond V; Cedar Rapids, IA52404, United States); SEM = Standard error of the mean; IgA = Immunoglobulin A; IgM = Immunoglobulin M; TNF-α = Tumor necrosis factor-alpha; IL - 1β = Interleukin-1β; IL-6 = Interleukin-6.

3.5. Gut microbiota community

3.5.1. OTU Statistics

Figure 1 shows the results of OTU. There were 8695 and 9636 OTUs in the CON and SCFP groups, respectively. The two groups shared 1876 OTUs.

As shown in Figure 2, the SCFP group had significantly high values in sob, chao1, and PD-whole tree ($p < 0.05$) (Figure 2 A, B, D), while Shannon had no significant difference (Figure 2 C).

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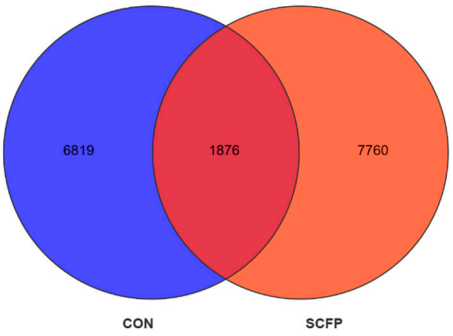

Figure 1. Venn diagram of OTU in CON and SCFP groups.

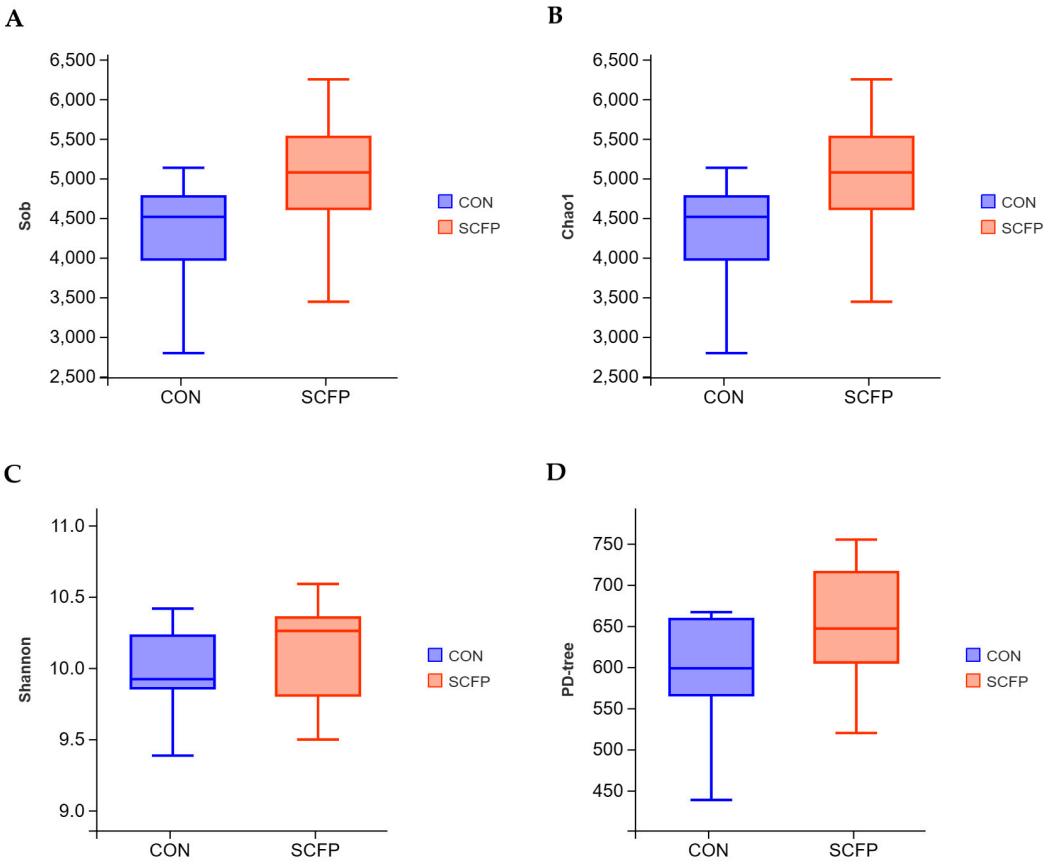

Figure 2. The observed_species (Sob) addresses the quantity of OTU identified by sequencing; The Chao1 index is mainly concerned with the species richness information of samples; The Shannon comprehensively reflects the richness and evenness of species; The PD-whole tree index is based on phylogenetic characteristics of OTU sequence evolutionary tree to assess the degree of diversity. (A) Observed_species (Sob) in α-variety; (B) Chao1 in α-variety; (C) Shannon in α-variety; (D) PD whole tree in α-variety.

3.5.2. Species composition analysis

In this study, at the phylum level, the top 10 most abundant species were Firmicutes, Bacteroidota, Spirochaetota, Proteobacteria, Verrucomicrobiota, Actinobacteriota, Cyanobacteriota, Desulfobacterota, Fusobacteriota, and Fibrobacterota. The relative abundance of gut microbiota community species distribution for each group is presented in Figure 3A. Additionally, the relative abundance of the Actinobacteriota CON group was significantly higher than the SCFP group ($p = 0.034$). (Figure 3B)

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At the genus level, the top 10 most abundant species were Ruminococcaceae_UCG-005, Prevotella, Bacteroides, Treponema, Prevotellaceae_UCG-003, Rikenellaceae_RC9_gut_group, Alloprevotella, and Christensenellaceae_R-7_group, Erysipelotrichaceae_UCG-002, and Ruminococcus. The relative abundance of gut microbiota community species distribution for each group is presented in Figure 3C. Additionally, the relative abundance of Erysipelotrichaceae_UCG-002, Lachnospiraceae_UCG-010, Oscillibacter, Negativibacillus, Olsenella, Anaeroplasma, and Ruminococcus_gauvreauii_group was significantly higher in the CON group ($p < 0.05$). However, Ruminococcus, Lachnospiraceae_AC2044_group, Parabacteroides, and Butyricimonas were significantly more abundant in the SCFP group ($p < 0.05$) (Figure 3D).

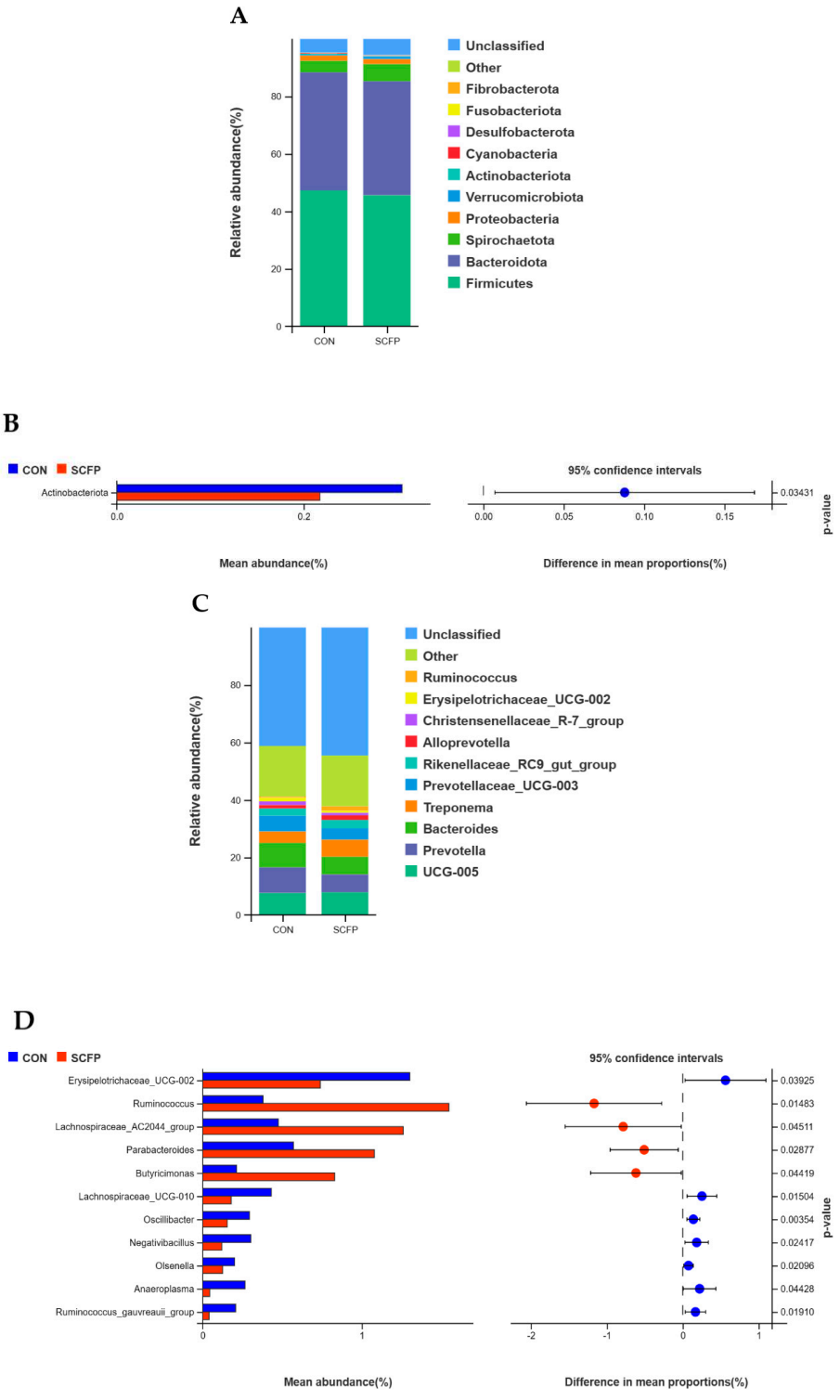

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Figure 3. Comparison of the relative abundance of gut microbiota community species distribution for each group in preweaning calves (Only the top 10 abundant phyla are presented). (A) Phylum level; (C) Genus level. Species differences between the two groups (phylum to species level, filtered for species whose sum abundance was less than 0.1% in all samples) were analyzed by Welch's t-test, and the results were presented as P-value <0.05 (or 0.01) was used, and a smaller P-value indicated a more significant difference. (B) Bar graph of difference by t-test at the phylum level; (D) Bar graph of difference by t-test at the genus level.

3.5.3. LEfSe analysis of gut microbiota

LEfSe analysis was used to distinguish the modified bacterial aggregates (LDA score > 3.0). In Figure 4A, from outside to inside, each circle successively addresses species at the class, order, family, genus, and species level. Based on this, compared with the CON group, there were three different microbiotas in the SCFP supplemented group, which were *Butyricimonas*, *Marinifilaceae* (a, b), *Parabacteroides_sp*, *Parabacteroides* (d, e), and *Ruminococcus* (o). Meanwhile, in the CON group, *Erysipelotrichaceae_UCG_002*, *Erysipelatoclostridiaceae*, *Erysipelotrichaceae*, *Erysipelotrichales*, and *Bacilli* (h, i, k, l, m) were significant.

The specific differences between the SCFP group and CON group were as follows: the abundance of *Ruminococcus*, *Marinifilaceae*, *Butyricimonas*, *Parabacteroides*, and P*arabacteroides_sp* in the SCFP group was higher than that in the CON group. On the other hand, in the CON group, the abundance of *Lachnospiraceae_UCG_010*, *Anaerovoracaceae*, *Acholeplasmataceae*, *Acholeplasmatales*, *Faecalitalea*, *Erysipelotrichaceae_UCG_002*, E*rysipelotrichaceae*, *Prevotella_sp_DJF_CP65*, *Erysipelatoclostridiaceae*, *Erysipelotrichales*, and *Bacilli* was significantly higher. (Figure 4B).

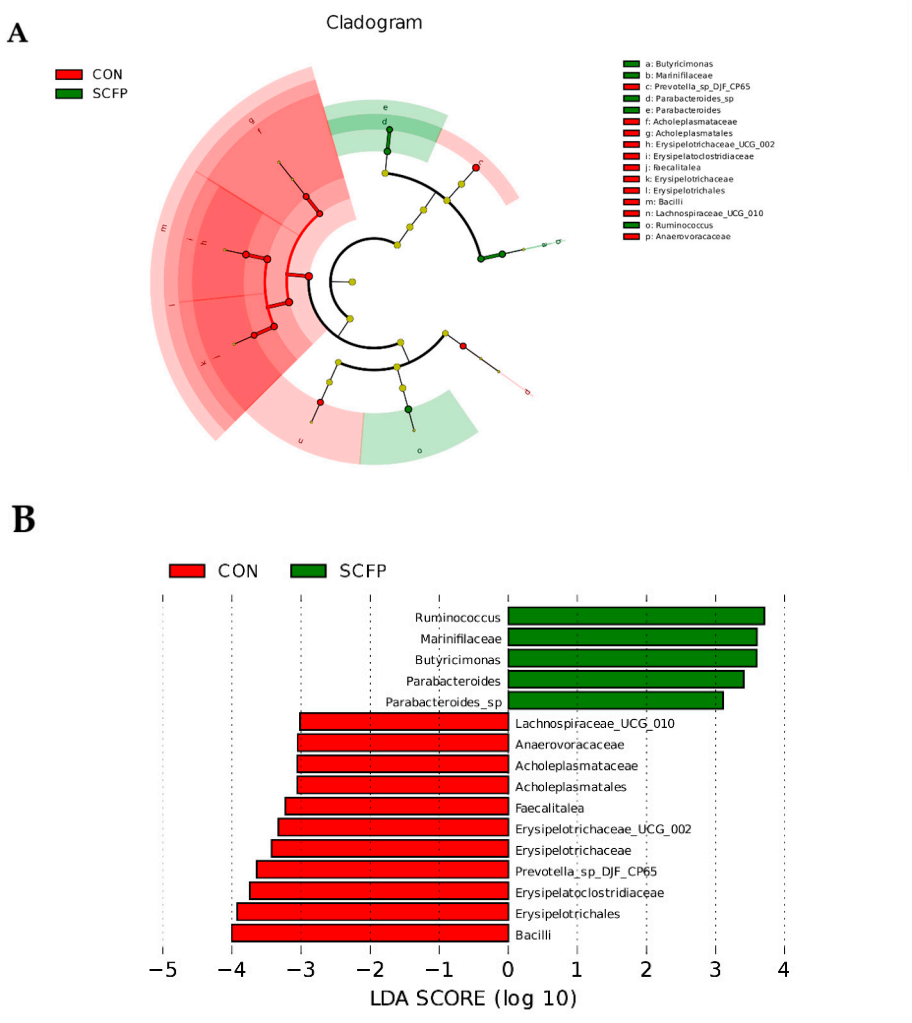

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Figure 4. LEfSe analysis was used to further evaluate the effect of SCFP on the composition of the gut microbiota in pre-weaned dairy calves. (A) Evolutionary branching of gut microbiota of pre-weaned dairy calves in CON and SCFP. The red nodes address the microbiome that assumes a significant part in the CON, and the green nodes address the microbiome that assumes a significant part in the SCFP. Species with no tremendous distinction are yellow nodes; (B) Correlation of direct discriminant examination (LDA) of the stomach microbiota of pre-weaned dairy calves in CON and SCFP, shows species with contrasts in LDA scores over the set point (3.0), i.e., statistically significant.

4. Discussion

Numerous studies have proven that feed additives can improve gut microbiota and thus promote animal growth. There are five most common feed additives: antibiotics, ionic carriers, probiotics, prebiotics, and plant extracts [23]. Probiotics, including s*accharomyces cerevisiae* supplements, can improve DMI and nutrient utilization efficiency [24].

This study researched the impact of SCFP on the development execution of pre-weaning calves. The outcomes showed that the admission of ADMI was practically similar in the two groups of calves, with an expansion in mean day-to-day weight gain from 1-45 days and an increase in feed transformation in the SCFP from 30-45 days. Similar results were reported by Mitchell and Heinrichs [25], who observed yeast supplementation did not affect calves' intake from 7 to 16 weeks fed different forages. However, improving the average daily weight gain and feed conversion ratio is beneficial in reducing the effects of weaning stress on calves. As a rule, better growth performance of ruminants is associated with improved gut microbiota and immunity.

Improving feed digestibility is essential for promoting healthy growth in calves. Zhang showed that supplementation with SCFP increased the apparent nutrient digestibility of DM, CP, NDF, and ADF in calves [26]. The results of this experiment are similar in that except for NDF, compared with the CON group, the SCFP group's apparent digestibility of DM, CP, EE, ADF, Ca, and P increased significantly. The higher apparent digestibility of nutrients, including DM, CP, NDF, and ADF, indicates that the higher feed conversion efficiency and utilization ultimately promote animal growth and development [27]. The apparent digestibility of NDF and ADF can reflect the ability of the animal to utilize dietary fiber [28]. These results suggest that adding SCFP to calves' diets before weaning can improve calves' gastrointestinal environment, improve gastrointestinal digestion and absorption of nutrients in feed, and increase the digestibility of nutrients.

Supplementation with SCFP was shown to promote growth in pre-weaning calves by testing serum growth-related developmental hormones. In most tissues, GH stimulates the synthesis of IGF-1 [29,30], which plays a vital role in regulating somatic cell growth and development [31]. IGF-1 concentration is related to hormonal and nutritional levels in the animal organism [32]. A study conducted by Graham et al. revealed that low birth weight and reduced serum IGF-1 concentrations could contribute to poor growth in Holstein dairy calves during their early stages of life [33]. However, the study found that the SCFP group had significantly increased levels of IGF-1 in their blood serum. Additionally, microbial dysbiosis and immaturity can result in decreased GH/IGF-1 signaling [34].

Multiple studies have demonstrated that gut bacteria in animals have an impact on their growth, partially through the GH/IGF-1 pathway. However, there has been no study to date that specifically investigates the role of GH in the gut microbiome. A study conducted by Jensen found that GH gene-disrupted mice (a model of GH deficiency) at 6 months of age had significantly reduced abundance of *Proteobacteria*, *Campylobacterota*, and *Actinobacteriota* in their gut. Conversely, bovine GH transgenic mice (a model of chronic, excess GH action) showed a trending increase in these phyla compared to the respective control group [35].

Both GLP-1 and GLP-2 are released by intestinal L cells in response to various stimuli such as nutrition, hormones, and neurological signals [36]. Previous studies have shown that GLP-1 and GLP-2 are simultaneously secreted in sheep [37]. However, in calves, the secretion of GLP-1 and GLP-2 may not always occur in parallel depending on the initial intake [38]. Rises in plasma levels of GLP-1 and GLP-2 indicating the possibility of co-secretion of intestinal peptide. According to the results

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of this experiment, within 45 days, GLP-1 and GLP-2 concentrations in the SCFP group had a rising trend. Additionally, Cani et al. discovered that specific changes in the gut microbiota can lead to increased GLP-2 concentrations [39]. This suggests that SCFP may promote the growth of intestinal microorganisms.

Numerous studies have demonstrated that incorporating SCFP into animal feed can enhance the animal's immune response [40,41]. B lymphocytes in the blood produce three types of immunoglobulins, namely IgA, IgG, and IgM, which play a crucial role in humoral immunity. The concentration of these immunoglobulins in the serum reflects the strength of the body's immune defense. IgA serves to prevent pathogenic microbes from adhering to mucous membranes and protects against pathogenic bacteria [42]. In this particular study, calves treated with SCFP showed higher levels of blood IgA, indicating an improvement in the immunological function of pre-weaning calves. Weaning stress often leads to gastrointestinal issues in calves and triggers a pro-inflammatory response [43]. Inflammatory mediators such as IL-1β, IL-6, and TNF-α are important indicators of inflammation in the body, with elevated levels suggesting the presence of inflammation [44]. The results of this study showed that compared with the CON group, the serum concentrations of IL-1β, IL-6, and TNF-α in the SCFP group were significantly lower. This demonstrates how adding SCFP to a diet can strengthen pre-weaning calves' immune systems while also reducing the inflammatory response in weaned calves.

Previous research has found that the SCFP nutritious metabolite, mannan oligosaccharide, and β-glucans can benefit various bacteria [45]. To better understand the influence of SCFP on gut microbiota diversity, 16 s rDNA sequencing and bioinformatics analysis were performed. Gut microbiota can participate in various physiological pathways and influence animal growth and metabolism [46]. The sequencing data analysis showed that the number of OTU in group CON and SCFP was 8695 and 9636, respectively. Cao's results showed that among the 88 samples, an average of 1582, 1563, and 1588 OTUs were detected for CON, SCFP1, and SCFP2, respectively [47]. However, unlike in this trial, Cao studied low numbers of OTUs, which different SCFP products could cause.

The structural composition and variety of the gut microbial flora are critical in sustaining the intestinal microecological environment [48]. Studies have shown that there are *Firmicutes*, *Bacteroides*, *Spirochaetota*, *Proteobacteria*, *Verrucomicrobiota*, *Actinobacteriota*, and *Fusobacteriota* in the intestinal tract, among which *Firmicutes* and *Bacteroides* are the proportion can be as high as 90% in total [49]. The results of this experiment show that *Firmicutes*, *Bacteroidota*, *Spirochaetota*, *Proteobacteria*, *Verrucomicrobiota*, and *Actinobacteriota* were the dominant intestinal flora in pre-weaning calves. *Firmicutes* play a major role in the processes of protein utilization and fermentation of carbohydrates [50,51]. Calves with a gut microbiome populated by *Firmicutes* can consume more energy from their diet and maintain superior physical health [52]. *Proteobacteria* make up most of the bacteria in the intestinal flora, according to Shin et al. [53]. These bacteria are directly connected to enteritis, immunological dysregulation, and bacterial flora imbalance. *Proteobacteria* abundance is an essential measure of gut health since it correlates with how healthy the gut [53]. It has been known that pathogenic *E. coli* manifests its effects by promoting the relative abundance of *Proteobacteria* and decreasing the relative abundance of *Firmicutes* at the phylum level [54]. According to the consequences of this experiment, *Proteobacteria* was once slightly decreased in the group supplemented with SCFP. In this way, it was assumed that the supplementation of SCFP could further develop gut microbiota and advance gastrointestinal wellbeing, advancing calves' development and improvement. In addition, the *Actinobacteriota* in the SCFP group were significantly lower than those in the CON group. Li et al found that fecal *Actinobacteriota* abundance increased by 2.5-fold after chemotherapy compared with before chemotherapy and that intestinal *Actinobacteriota* may have positive clinical outcomes in patients with colorectal cancer [55]. However, the results of this experiment were the opposite. The number of *Actinobacteriota* decreased in the group supplemented with SCFP, which may alleviate intestinal inflammation, so the number of *Actinobacteriota* decreased.

Past investigations have shown that the advantageous impacts of SCFP on the ruminal microbiome were interceded by expanding the overall overflow of a few individuals from the

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Bacteroidetes, like *Prevotella*, as well as certain individuals from the *Rumenococcaceae* and *Lachnospiraceae* families, including the sort *Dorea*, *Blautia*, and *Roseburia* [56]. This experiment's dominant bacterial groups at the genus level are *UCG-005*, *Prevotella*, *Bacteroides*, and *Treponema*. Nonetheless, the results of this test showed that *Erysipelotrichaceae_UCG-002* was higher than the SCFP group, and *Ruminococcus* was higher than the CON group. Hao et al. indicated that *Erysipelotrichaceae_UCG_002*, *Syntrophococcus*, and *Shuttleworthia* have been negatively correlated with rumen pH and speculated that these genera are involved in fiber digestion [57]. *Ruminococcus* is dominant in the cellulose-decomposing bacteria population [58]. Previous research has shown that many of the microbes in gastroenterology are common fibro-degradant bacteria, most of which can degrade proteins [59,60]. The results of this trial exhibit that *Erysipelotrichaceae_UCG-002* is higher than SCFP and *Ruminococcus* higher than CON. As a result, it is speculated that supplementation with SCFP will increase the ability of intestinal degradation of fibrin, promote dietary absorption, and improve the apparent digestibility of CP, DNF, and ADF, thereby increasing the ADG.

LefSe analysis was performed to find species with significant differences in abundance between groups. *Ruminococcus*, *Butyricimonas*, and *Parabacteroides* were significantly enriched in the SCFP group. *Butyricimonas* is a Gram-negative anaerobic bacterial genus of the family *Odoribacteraceae*. They are present in the gut of several mammals, including rats and humans [61,62]. Lee et al. showed that *Butyricimonas* is associated with glucose regulation in obese mice, and in a mouse model of obesity induced by a high-fat diet, Treatment with live and heat-killed *Butyricimonas* improved body weight [63]. In previous animal studies, two *Parabacteroides* species, including *Parabacteroides distasonis* and *Parabacteroides goldsteinii*, played roles in anti-obesity, hyperglycemia, and insulin resistance [64,65]. Altogether, we speculate that SCFP supplementation enhances *Butyricimonas* and *Parabacteroides* and regulates intestinal function, thereby increasing the body weight of pre-weaning calves.

5. Conclusions

The experiment showed that supplementation SCFP positively affected pre-weaning calves, including the increase of ADG, FCR, apparent nutrient digestibility and immunity. More to the point, supplement SCFP increased GH, IGF-1, GLP-1 and GLP-2 concentrations. In addition, SCFP reduced the abundance of *Actinobacteriota* and increased the abundance of *Butyricimonas*, *Parabacteroides*, and *Ruminococcus*. Therefore, supplementing SCFP regulates gut microbiota and promotes animal growth.

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Conflicts of Interest: The authors declare that there are no conflicts of interest.

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