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Effect of Alkaline Mineral Complex Buffer Supplementation on Milk Performance, Serum Variables, Rumen Fermentation and Rumen Microbiota of Transition Dairy Cows

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Abstract: The present study investigates the effect of 50 mL AMCB taken daily as a dietary supplement on the rumen fermentation, microbiota, and production performance of 40 Holstein dairy cows in the transition period with 2.76 ± 0.48 parity and 650 ± 25 kg body weight. AMCB supplementation stabilized rumen pH, improved rumen microbiota richness and partial probiotic colonization, and considerably increased dry matter intake, milk production, protein content, and yield. Moreover, after calving, AMCB supplementation considerably reduced the serum blood urea nitrogen, malondialdehyde, hydrogen peroxide, alanine aminotransferase, and aspartate transaminase levels and increased the serum immunoglobulin G and A levels. The results indicated that AMCB dietary supplementation improved postpartum dry matter intake, production performance, partial immune function, antioxidant capacity, and rumen microbiota richness in Holstein dairy cows in the transition period. AMC is an excellent candidate for use as a rumen buffer.

Keywords: alkaline mineral complex buffer; transition dairy cows; milk performance; rumen fermentation; rumen microbiota

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1. Introduction

The transition period (3 weeks before and after calving), the initial time when dairy cows create value, is also the most critical stage for dairy cows [1]. During this period, dairy cows experience substantial challenges to their physiology and metabolism, including childbirth stress, lactation stress, and changes in diet structure and environment, directly or indirectly affecting cow health [2,3]. Only when dairy cows transfer from pregnancy to lactation smoothly can there be a solid foundation for the high yield of the whole lactation cycle. During the first three weeks of cow lactation, milk production and protein, fat, and lactose levels rapidly increased [4]. Negative energy balance (NEB)-induced abnormal glucose and lipid metabolism primarily affects perinatal cows due to the lack of dry matter intake (DMI) and energy output intensification [5], with clinical symptoms primarily including increased body fat decomposition and decreased insulin sensitivity. However, these changes are a normal adaptation process for high-yielding cows. When cows fail to adapt to this metabolic challenge, it leads to disorders, including fatty liver and ketosis [6]. With the rapid development of the cattle breeding industry, the proportion of dairy cows suffering from nutritional and metabolic diseases has gradually increased, and the perinatal period has become the intensive period for dairy cow metabolic diseases [7,8]. Previous studies show that involuntary culling caused by abnormal glucose and lipid metabolism

in dairy cows during the perinatal period accounts for a 20.7% removal rate of adult cows in China, with an approximately $734 million annual loss [9]. Therefore, the health management of dairy cows during the transition period is particularly important.

The composition and abundance of rumen microbiota in dairy cows are relatively stable without external influence [10]. The change of feed for dairy cows during the transition period disturbs rumen microbiota homeostasis [11]. The altered dietary composition and nutrition, and the diversity and metabolism of digestive tract microbiota decrease their abundance in the digestive tract, particularly the starch-degrading bacteria [12]. Moreover, the change in the diet of postpartum cows shifts the rumen function and fermentation mode from prenatal acetic acid to propionic acid fermentation [13]. When a cow is fed a high-coarse diet before calving and changes to a high-concentrate diet after calving, the proportion of Bacteroides and Firmicutes in the rumen increases [14]. During the postpartum phase, a large amount of volatile fatty acid (VFA) and lactic acid accumulate in the rumen due to the sudden increase in concentrate content of the diet, which readily causes subacute rumen acidosis (SARA) [15,16]. SARA reduces milk performance and compromises the health of dairy cows [17]. SARA reduces the DMI, milk fat rate, and production, causes systemic inflammation, and induces lipopolysaccharide production in the rumen [17,18]. Lipopolysaccharide enter a cow's body through the hindgut, causing cow inflammation, increasing the triglyceride (TG) content in the cow liver through high-density lipoprotein and chyle particles and inhibiting the energy supply from fat in the liver [19,20]. Thus, enhancing rumen health in dairy cows is very important, and attention should be paid to feeding and managing dairy cows during the transition period.

Supplementing dairy cow diets with buffers or alkaline substances could stabilize the rumen pH and prevent the effects of SARA [21–23]. Sodium bicarbonate is the most commonly used rumen buffer [24]. Adding sodium bicarbonate to the cow diet stabilized the changes in rumen pH [25]. Additionally, a previous study on supplementing buffers in a high-concentrate dairy cow diet revealed that supplementing reduces the variability of DMI [26]. Supplementing dairy cow diets with buffers during the transition period is a common method for stabilizing rumen pH. The alkaline mineral complex buffer (AMCB) used in the present study is an alkaline solution (pH 9.1) containing zinc, sodium, potassium, and germanium, and its properties are based on its mineral composition [27]. The minerals in AMCB are essential for organ and physiological functions such as digestion and immune biosynthesis [28,29]. The alkaline mineral complex has biological benefits and therapeutic effects, including improving the quality of life of cancer patients, promoting antioxidation, promoting intestinal health, and treating intestinal inflammatory diseases and diarrhea [30–32]. The biological benefits and therapeutic effects of the alkaline mineral complex have been explored in monogastric animals in previous studies. For example, an alkaline mineral complex was proven to improve intestinal morphology and inflammatory reactions, promote intestinal health, and accelerate the growth performance of weaned piglets [33]. Moreover, the alkaline mineral complex improves the structure and function of intestinal microbiota, maintains intestinal epithelial regeneration, and improves the anti-diarrhea ability of piglets [27,34], confirming the biological benefits and therapeutic effects of the alkaline mineral complex. However, the importance of AMCB during the transition period for dairy cows has not been revealed. Although AMCB has superior biological benefits to traditional buffers, there are no studies on its application in dairy cows.

In the present study, we hypothesize that AMCB supplementation stabilizes rumen pH, promotes rumen microbiota richness, increases DMI, exerts biological benefits, and enhances the milk performance and health of dairy cows during the transition period. Therefore, the present study investigates the effect of AMCB supplementation to the dairy cow diet on rumen fermentation, microbiota, partial blood biochemical parameters, immune function, antioxidant capacity, and milk performance of transition dairy cows.

2. Materials and Methods

2.1. Experimental Animals and Treatments

All procedures for reducing animal pain were performed after ratification by the Experimental Animal Welfare and Animal Experiment Ethics Review Committee of China Agricultural University (Approval No. AW01103202-1-32). Animal studies were conducted following the approval of the State Council (Order No. 676) of the People's Republic of China, which regulates the administration of experimental animals.

The farm used in the present study is a commercial dairy farm in Yinchuan, Ningxia, China. According to the principle of similar parity, calving days, and milk yield of previous parity, 40 Holstein transition cows with 2.76 ± 0.48 parity and 650.00 ± 25.36 kg body weight were selected, and each cow was used as an experimental unit. The Holstein dairy cows were randomly divided into two experimental groups (A [AMCB supplemented] and B [without AMCB supplemented]) arranged in a completely randomized design. All cows were kept in free stalls, and a fence separated the two groups. The cows were fed twice daily at 7:30 and 15:30 with ad *libitum* drinking water. The cows were milked mechanically three times daily at 6:30, 14:30, and 20:30. The study period included 14 days of adaptation, followed by 42 days (the period between 3 weeks before and after calving) of experimentation. During the investigation, there was no manual intervention in the health of cows.

2.2. Experimental Feed

The experimental feed was prepared according to the Nutrient Requirements of Dairy Cattle [35] and mixed with feed ingredients available on the experimental dairy farm. Briefly, the feed of prenatal cows had high fiber and low energy, and the feed of postpartum cows had high feed concentrate and high energy. The ingredients and nutrient compositions of the prenatal and postpartum feeds are presented in Tables 1 and 2, respectively. AMCB is a liquid colloidal substance that is soluble in water. In order to ensure that the AMCB of cows could be evenly stirred, according to previous research, each cow was supplemented with 50 mL daily, diluted with water in a ratio of 1:4, and thoroughly stirred with the total mixed ration (TMR) until evenly mixed [36]. AMCB was supplemented in the TMR of the A group once daily at 7:30. Purified drinking water was used to avoid contamination from other mineral elements. The AMCB used in this experiment was obtained from the Nail Biotechnology Company, Beijing, China, and its composition is presented in Table 3.

Table 1. The formula and nutrient compositions of prenatal feed of dairy cows.

Dietary Composition (Fresh Weight)	Content	Nutrient Compositions (% of Dry Matter Unless Noted)	Content
Corn silage, kg/d	10.20	Dry matter (% of fresh weight), %	43.59 ± 0.19
Oat hay, kg/d	7.50	Total digestible nutrients, %	63.10
Corn starch, kg/d	0.52	NEL, Mcal/kg [1]	1.43
Cottonseed meal, kg/d	0.52	Crude protein, %	12.00 ± 0.27
Corn distillers dried grains with solubles, kg/d1	1.29	Neutral detergent fiber, %	46.85 ± 4.21
Rapeseed meal, kg/d	1.29	Acid detergent fiber, %	33.50 ± 0.17
Commercial Premix, kg/d [2]	0.41	Lignin, %	4.46 ± 0.32
Total, kg/d	21.73	Starch, %	12.12 ± 0.26
		Ether extract, %	4.16 ± 0.24
		Ash, %	10.48 ± 0.47

[1] NEL: net energy of milk production. [2] Commercial premix (Hua Sheng Mu Ge Feed Co., Ltd., Yinchuan, China).

Table 2. The formula and nutrient compositions of postpartum feed of dairy cows.

Dietary Composition (Fresh Weight)	Content	Nutrient Compositions (% of Dry Matter Unless Noted)	Content
Corn silage, kg/d	26.50	Dry matter (% of fresh weight), %	48.57 ± 2.21
Alfalfa hay, kg/d	3.40	NEL, Mcal/kg	1.63
Cotton seed, kg/d	1.35	Total digestible nutrients, %	72.15
steam-flaked corn, kg/d	0.80	Crude protein, %	16.29 ± 0.12
Bran, kg/d	1.15	Neutral detergent fiber, %	30.16 ± 1.75
Corn starch, kg/d	4.69	Acid detergent fiber, %	23.05 ± 1.25
Soybean meal, kg/d	2.84	Lignin, %	3.73 ± 0.36
Cottonseed meal, kg/d	0.86	Starch, %	26.21 ± 0.34
Corn distillers dried grains with solubles, kg/d	2.59	Ether extract, %	5.87 ± 0.23
Expanded soybean, kg/d	0.31	Ash, %	9.45 ± 0.32
Calcium bicarbonate, kg/d	0.25		
Sodium bicarbonate, kg/d	0.19		
Commercial premix [1], kg/d [1]	0.05		
Commercial premix [2], kg/d [2]	0.62		
Total, kg/d	45.60		

[1] Commercial premix1 (Hua Sheng Mu Ge Feed Co., Ltd., Yinchuan, China). [2] Commercial premix2 (Mengtai Dadi Biotechnology Development Co., Ltd., Hohhot, China).

Table 3. The composition of alkaline mineral complex buffer concentrate.

Ingredient	Content	Chemical Formula
Sodium metasilicate pentahydrate	200 g/L	$5H_2O{\cdot}Na_2SiO_3$
Potassium bicarbonate	100 g/L	$KHCO_3$
Zinc oxide	10 mg/L	ZnO
Bis-(carboxyethyl germanium) sesquioxide	1 mg/L	Ge-132

2.3. Feed and Milk Collection, Preservation, and Analysis and DMI Recording

The feed intake of each group was weighed and recorded daily and summarized weekly. The DMI of Holstein dairy cows in each experimental group was calculated considering the feed dry weight [37]. TMR samples were collected weekly and combined to determine the nutrient compositions [38]. All feed samples were dried at 65 °C for 48 h to prepare air-dried feed samples, and the dried feed samples were crushed and stored until use [39]. The crude protein and ether extract of dried feed samples were determined using the method of the Association of Official Agricultural Chemists [40]. Neutral and acid detergent fibers and lignin content of dried feed samples were determined via the method described by Van Soest [41], and the starch content of dried feed samples was determined using colorimetry [42].

Milk production of individual cows was recorded daily. Milk samples were collected from all dairy cows included in the experiment. Individual milk samples collected at 6:30, 14:30, and 20:30, 7, 14, and 21 days after calving were mixed at a ration of 4:4:3 according to [42] to determine milk compositions. The compositions of 4% fat corrected milk (FCM) and energy corrected milk (ECM) were calculated using a formula by Liu et al. as follows [43]:

$$4\%\ \text{FCM (kg)} = (0.4 + [15 \times \text{milk fat (\%)}] \times \text{milk production [kg]})$$

$$\text{ECM (kg)} = (0.327 \times \text{milk production (kg)} + (12.95 \times \text{milk fat yield (kg)}) + (7.2 \times \text{milk protein yield (kg)})$$

One milk aliquot was preserved using potassium dichromate. The milking routine (milk fat, lactose, protein contents, and somatic cell count) was determined using a milk composition analyzer (Milko Scipe Jule, Beijing Lanbosi Technology Co., Ltd., Beijing, China). The other milk aliquots were stored at −20 °C until the determination of milk urea nitrogen using the urea nitrogen kit (Nanjing Jiancheng Co., Ltd., Nanjing, China).

2.4. Collection, Preservation and Pretreatment of Cow Blood Samples

Blood samples were collected from the tail vein of 10 Holstein dairy cows randomly selected from each experimental group on the 14th day before the expected calving day, calving day, and 7, 14, and 21 days after calving using a vacuum tube without an anticoagulant 2 h after feeding. The blood samples were centrifuged at 3000× *g* at 4 °C for 15 min to collect the serum samples. The radioimmunoassay method was used to measure the insulin level using a BFM-96 instrument (Zhongcheng Jidian Technology Development Co., Ltd., Hefei, China). The TG, total cholesterol (TC), alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), creatinine (Cr), total bilirubin (T-BIL), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and hydrogen peroxide (H_2O_2) levels were determined using a commercial reagent kit (Nanjing Jiancheng Co., Ltd., Nanjing, China) on an automatic biochemical analyzer (CLS880, Zecen Biotechnology Co., Ltd., Taizhou, China) according to the manufacturer's instructions. The β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin 6 (IL-6), and granulocyte-macrophage colony-stimulating factor (GM-CSF) levels were analyzed using enzyme-linked immunosorbent assay kits (Abcam, Cambridge, UK) with a microplate reader (Thermo Multiskan Ascent, Thermo Fisher Scientific, Shanghai, China).

2.5. Collection, Preservation and Pretreatment of Rumen Fluid Samples

Rumen fluid samples were randomly collected from eight Holstein dairy cows from each experimental group using the random method 14 days before the expected delivery date and 7, 14, and 21 days after calving using a rumen fluid collection tube (A1141K, Anscitech Co., Ltd., Wuhan, China) 2 h before the afternoon feeding. The rumen fluid samples were analyzed to determine the proportion of rumen fermentation and microorganism diversity. First, rumen fluid was collected from eight Holstein dairy cows in each experimental group (A and B). Next, the rumen fluid collection tube was washed with fresh water, and the rumen fluid was collected after leaching through four layers of sterile gauze (Thermo Fisher Scientific). Next, a 50 mL rumen fluid sample was saved and frozen at −20 °C until the measurement of VFAs. Simultaneously, a pH meter (pHS-3E, INESA Scientific Instrument Co., Ltd., Shanghai, China) was used to determine the pH of rumen fluid samples. The remaining rumen liquid (approximately 1.8 mL) was frozen at −80 °C until the rumen microbiota diversity analysis. Rumen ammonia nitrogen concentration was determined spectrophotometrically as described by Broderick and Kang [44], and VFA concentration was determined using a gas chromatography-mass spectrometer (6890 N; Agilent Technologies, Avondale, PA, USA), as described by Zhang and Yang [45], respectively.

A soil DNA Kit (M5635-02 [OMEGA Bio-Tek, Norcross, GA, USA]) was used for extracting DNA from rumen fluid samples according to the manufacturer's manual. NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis were used to measure the quantity and quality of extracted DNA, respectively. DNA amplification using polymerase chain reaction was performed on the V3–V4 region of the 16S rRNA gene in bacteria, according to Claesson et al. for primer design for the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer 806R (5′-GACTACHVGGGTWTCTAAT-3′) [46]. The DNA extracted from the rumen microbiota samples were sent to Shanghai Bioprofile Technology Company Ltd., Shanghai, China, for sequencing on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

2.6. Data Analysis

The data displayed in the tables and figures are indicated as the mean ± standard error of the mean (SEM). The effect of AMCB dietary supplementation on milk performance, DMI, serum variables, and rumen fermentation of Holstein dairy cows in the transition period was

statistically analyzed using the mixed procedure of SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). The following model indicates the interaction effects between treatment and group:

$$Y = \mu + T_i + G_j + TG_{ij} + E_{ij}k_l$$

where Y is the dependent variable, μ is the overall mean, T_i is the time effect, G_j is the group effect, TG_{ij} is the interaction effect between T and G, and $E_{ij}k_l$ is the random residual error. Means were considered significantly different at $p < 0.05$. Significant differences in the means of the effect of AMCB supplementation on serum variables and rumen fermentation were determined using a one-way analysis of variance in SAS 9.4 (SAS Institute Inc.). The mean differences were considered statistically significant at $p < 0.05$. The difference was considered to have the following significant trend: $0.05 < p < 0.10$.

Sequence data analyses were mainly performed using QIIME2 [47] (https://library.qiime2.org) (accessed on 13 September 2022) and R3.2.0 (R Core Team, Vienna, Austria). The abundance curve of amplicon sequence variant (ASV) horizontal sorting was generated to compare the abundance and evenness of ASV between samples. The β diversity analysis used Jaccard [48], Bray–Curtis [49], and UniFrac distance metrics [50] to identify the structural changes of the rumen microbiota. The sequencing data were visualized using the principal coordinate analysis (PCoA) method with arithmetic means hierarchical clustering [51].

3. Results

3.1. Milk Performance and DMI

The results of the effects of AMCB dietary supplementation on milk performance and DMI are presented in Table 4 and Figure 1. AMCB dietary supplementation significantly increased DMI, average milk production, protein content, and yield of postpartum Holstein dairy cows ($p < 0.05$), whereas it did not significantly affect prenatal DMI. AMCB dietary supplementation significantly affected DMI, milk production, FCM, ECM, protein, urea nitrogen, lactose yield, fat yield, and protein yield ($p < 0.05$). AMCB dietary supplementation significantly decreased milk fat and urea nitrogen levels ($p < 0.05$). AMCB dietary supplementation increased ECM and milk lactose yield, although these changes were not statistically significant ($0.05 < p < 0.10$). These results demonstrated the essential role of AMCB dietary supplementation in the enhancement of the production performance of Holstein dairy cows.

Table 4. Effects of alkaline mineral complex buffer water dietary supplementation on milk performance and dry matter intake of Holstein dairy cows in the transition period.

Item	Group		SEM [5]	*p*-Value		
	A [1]	B [2]		Group	Time	Group × Time
Dry matter intake (before calving), kg	13.69	13.79	0.14	0.47	0.12	0.72
Dry matter intake (after calving), kg	20.16	18.29	0.70	0.01	0.01	0.34
Milk production, kg/d	38.25	36.10	1.03	0.03	<0.01	0.01
FCM yield, kg [3]	35.02	34.14	0.95	0.36	<0.01	0.02
ECM yield, kg [4]	39.14	37.32	1.05	0.09	<0.01	0.01
Milk lactose, %	5.03	5.10	0.06	0.06	<0.01	<0.01
Milk fat, %	3.43	3.64	0.09	0.03	0.29	0.73
Milk protein, %	3.45	3.22	<0.01	<0.01	<0.01	<0.01
Milk somatic cell count, ×1000/mL	58.65	58.83	0.19	0.90	<0.01	0.90
Milk urea nitrogen, mg/dL	12.30	13.64	0.26	<0.01	<0.01	0.01
Milk lactose yield, kg/d	1.92	1.83	0.05	0.08	<0.01	0.01
Milk fat yield, kg/d	1.31	1.31	<0.01	0.95	<0.01	0.02
Milk protein yield, kg/d	1.33	1.18	0.04	<0.01	<0.01	<0.01

[1] A, the group supplemented with an alkaline mineral complex buffer during the experiment. [2] B, control group; [3] FCM, fat-corrected milk; [4] ECM, energy-corrected milk. [5] SEM, standard error of means. Data are presented as the means ± standard error of means (*n* = 20 per experimental group).

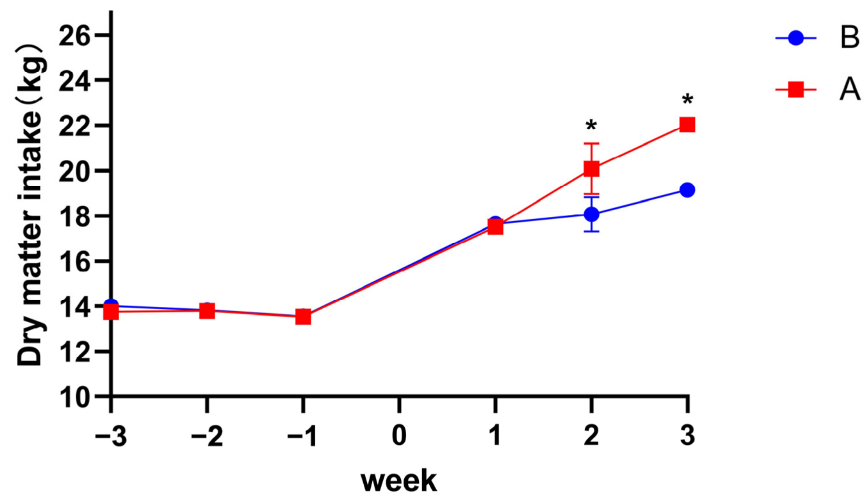

Figure 1. Effects of alkaline mineral complex buffer dietary supplementation on dry matter intake (DMI) of transition dairy cows. A, the group supplemented with AMCB; B, control group. *, significant difference between groups A and B. Data are presented as the means ± standard error of the mean (SEM [n = 20 per experimental group]).

3.2. Serum Variables

The serum variables before and after calving were analyzed considering the different physiological stages and feed intake before and after calving. The effects of AMCB dietary supplementation on serum variables are presented in Table 5 and Table S1. Before calving, AMCB dietary supplementation significantly decreased the MDA level ($p < 0.05$) but had no significant effect on other serum variables. After calving, AMCB dietary supplementation significantly decreased the ALT, AST, BUN, MDA, and hydrogen peroxide levels and increased the TC level ($p < 0.05$). AMCB dietary supplementation increased IgG and IgA ($0.05 < p < 0.10$), indicating that AMCB dietary supplementation could reduce liver damage and enhance the partial antioxidant capacity and immunity of transition dairy cows.

Table 5. Effects of alkaline mineral complex buffer supplementation on serum variables of dairy cows 7, 14, and 21 days after calving [3].

Item	Group		SEM	p-Value		
	A [1]	B [2]		Group	Time	Group × Time
INS, mIU/mL	16.31	16.58	1.07	0.80	0.47	0.63
GLU, mmol/L	3.62	3.64	0.02	0.84	0.15	0.38
TC, mmol/L	3.24	2.73	0.13	<0.01	<0.01	0.57
TG, mmol/L	0.19	0.20	<0.01	0.31	0.12	0.10
ALT, U/L	22.76	25.79	1.04	<0.01	0.47	0.38
AST, U/L	95.14	120.05	8.24	<0.01	<0.01	0.06
TP, g/L	69.92	70.38	0.46	0.70	<0.01	0.23
ALB, g/L	31.82	32.43	0.61	0.33	0.02	0.27
GLB, g/L	38.10	37.95	1.20	0.90	<0.01	0.42
T-BIL, umol/L	4.45	4.46	0.44	0.99	<0.01	0.70
ALP, U/L	42.39	38.12	4.77	0.37	0.90	0.29
BUN, mmol/L	5.27	5.74	0.21	0.03	<0.01	0.09
Cr, umol/L	84.79	86.64	2.02	0.36	<0.01	0.09
SOD, U/mL	44.17	43.17	0.94	0.29	0.45	0.12
MDA, nmol/mL	1.47	1.52	0.03	0.04	0.29	0.11
H_2O_2, mmol/L	25.13	49.46	5.34	<0.01	<0.01	<0.01
CAT, U/ML	17.33	18.28	0.58	0.11	0.23	0.10

Table 5. *Cont.*

Item	Group		SEM	*p*-Value		
	A [1]	B [2]		Group	Time	Group × Time
BHBA, mmol/L	0.46	0.45	0.01	0.48	0.74	0.48
NEFA, umol/L	38.03	37.91	1.37	0.93	<0.01	0.52
IgG, mg/mL	17.57	16.90	0.39	0.09	0.06	0.17
IgA, ug/mL	588.52	561.34	14.53	0.07	0.54	0.60
IgM, mg/mL	4.14	3.86	0.17	0.10	0.44	0.29
IL-6, ng/L	401.35	398.48	12.71	0.82	0.59	0.27
GM-CSF, pg/mL	54.90	55.13	1.98	0.91	<0.01	0.80

[1] A, the group supplemented with an alkaline mineral complex buffer. [2] B, control group. [3] Abbreviations in the table: TG is triglyceride; TC is total cholesterol; ALT is alanine aminotransferase; AST is aspartate transaminase; BUN is blood urea nitrogen; ALP is alkaline phosphatase; TP is total protein; ALB is albumin; Cr is creatinine; T-BIL is total bilirubin; MDA is malondialdehyde; SOD is superoxide dismutase; CAT is catalase; H_2O_2 is hydrogen peroxide; BHBA is β-hydroxybutyric acid; NEFA is non-esterified fatty acid; IgA is immunoglobulin A; IgG is immunoglobulin G; IgM is immunoglobulin M; IL-6 is interleukin 6; GM-CSF is granulocyte-macrophage colony-stimulating factor. Data are presented as the means ± standard error of the mean (SEM [n = 10 per experimental group]).

3.3. Rumen Fermentation

The effects of AMCB dietary supplementation on rumen fermentation are presented in Table 6. AMCB dietary supplementation decreased the butyric acid of postpartum dairy cows ($0.05 < p < 0.10$). AMCB dietary supplementation significantly affected the rumen pH of postpartum dairy cows but not rumen fermentation in dairy cows in the transition period ($p < 0.05$), indicating that AMCB supplementation significantly regulated the rumen pH of transition Holstein dairy cows.

Table 6. Effects of alkaline mineral complex buffer dietary supplementation on rumen fermentation of Holstein dairy cows after calving.

Item	Physiological Stage	Group		SEM	*p*-Value		
		A [1]	B [2]		Group	Time	Group × Time
pH	Prenatal [4]	6.79	7.30	0.25	0.10	-	-
	Postpartum [5]	6.75	6.38	0.12	0.01	0.36	0.04
Ammonia nitrogen, mg/dL	Prenatal	1.61	1.49	0.10	0.24	-	-
	Postpartum	1.29	1.31	0.02	0.77	<0.01	0.40
Acetic acid, mmol/L	Prenatal	53.90	46.76	7.04	0.34	-	-
	Postpartum	53.07	57.59	4.10	0.28	0.28	0.67
Propionic acid, mmol/L	Prenatal	17.16	15.45	1.85	0.38	-	-
	Postpartum	23.34	25.94	2.44	0.30	0.20	0.31
Butyric acid, mmol/L	Prenatal	10.21	10.44	1.53	0.89	-	-
	Postpartum	9.18	11.32	1.09	0.06	0.77	0.39
Acetic acid/propionic acid	Prenatal	3.12	3.03	0.15	0.60	-	-
	Postpartum	2.32	2.29	0.13	0.86	0.77	0.27
Total VFA, mmol/L [3]	Prenatal	81.27	72.65	10.00	0.41	-	-
	Postpartum	85.59	94.85	6.48	0.17	0.21	0.51

[1] A, the group supplemented with an alkaline mineral complex buffer. [2] B, control group. [3] VFA, volatile fatty acid. [4] Prenatal, the collection time of rumen fluid samples was 14 days before calving. [5] Postpartum, the collection time of rumen fluid samples were 7 days, 14 days, and 21 days after calving. Data are presented as the means ± standard error of the mean (SEM [n = 8 per experimental group]).

3.4. Rumen Microbiota Analysis

The present study further investigated the effects of AMCB dietary supplementation on rumen microbiota. The species accumulation curve proved that the sequencing data in the present study was sufficient to reflect the microbial information in the rumen microbiota samples (Figure S1). Alpha diversity analysis indicated that AMCB dietary supplementation affects the abundance and diversity of the rumen microbiota (Figure 2). AMCB dietary

supplementation significantly increased the Chao1, Simpson, and Shannon indices of rumen microbiota 14 days before the expected calving day. The Chao1 and Simpson indices of rumen microbiota of group A cows significantly increased and decreased, respectively, 21 days after calving.

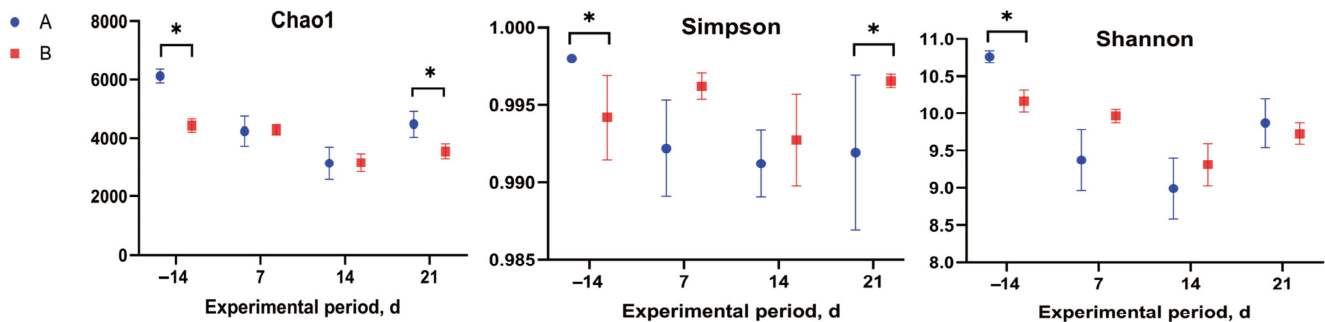

Figure 2. Effects of alkaline mineral complex buffer dietary supplementation on alpha diversity of rumen bacteria of transition dairy cows. Data are presented as the means ± standard error of the mean (SEM [n = 8 per experimental group]). A, the group supplemented with AMCB; B, control group. * $p < 0.05$.

We further analyzed the effects of AMCB dietary supplementation on rumen microbiota (Figure 3 and Tables 7 and 8). At the phylum level, the Firmicutes, Bacteroidetes, and Proteobacteria were predominant in the rumen microbiota (Figure 3A and Table 7). At the genus level, AMCB dietary supplementation markedly influenced rumen microbiota composition (Figure 3B,D and Table 8). PCoA results indicated that the rumen microbiota structure was not different between the two groups, whereas it differed before and after delivery (Figure 3C). The heatmap reveals that AMCB dietary supplementation increased the abundance of *Fibrobacter* during the study period (Figure 3D).

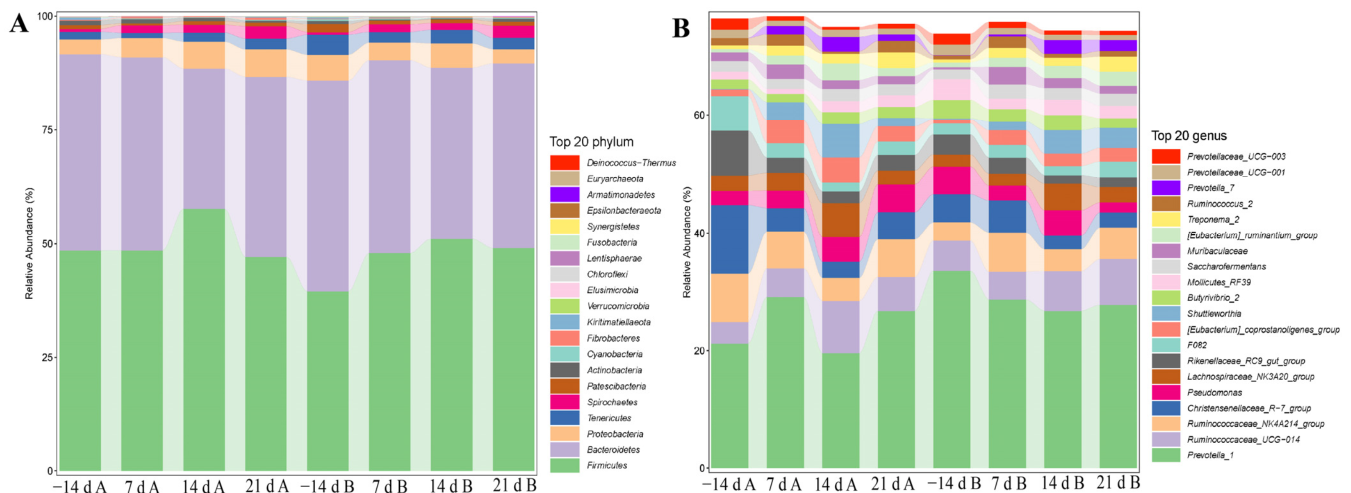

Figure 3. *Cont.*

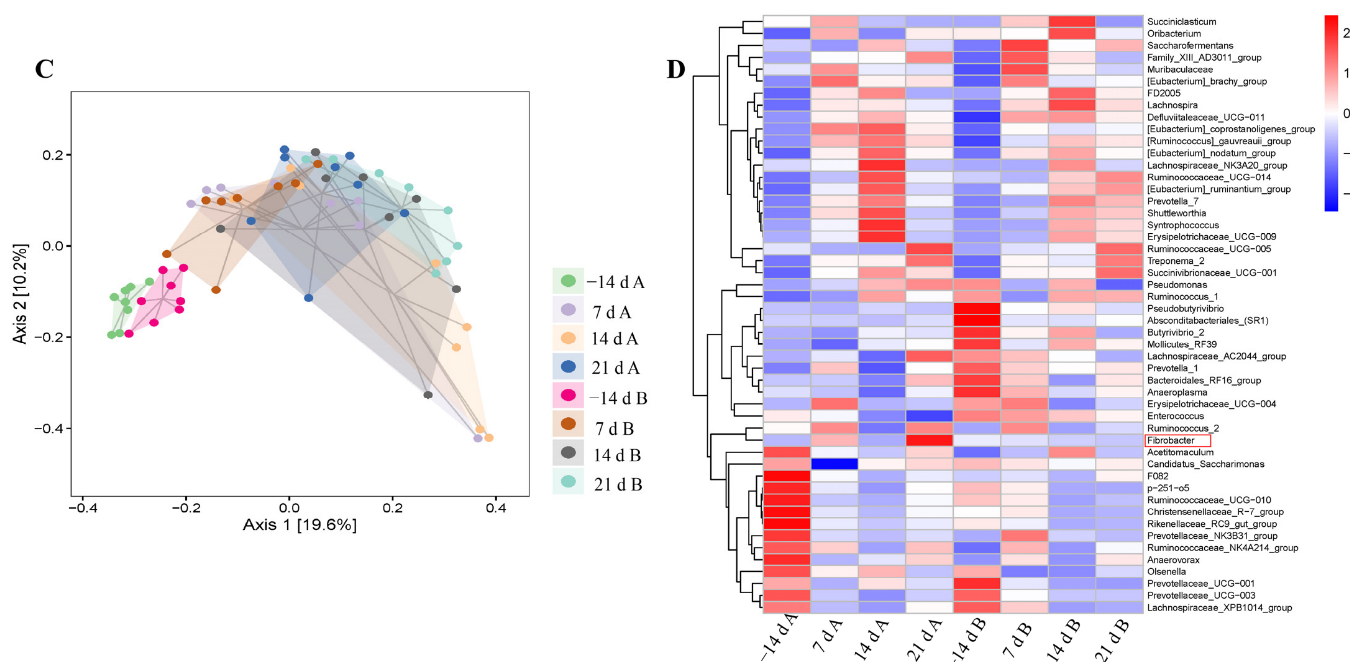

Figure 3. Effects of alkaline mineral complex buffer dietary supplementation on rumen microbiota of transition dairy cows. Effects of AMCB supplementation on rumen bacteria at the (**A**) phylum level (top 20 phylum) and (**B**) genus level (top 20 genus). (**C**) Principal coordinate analysis (PCoA) of rumen microbiota samples. (**D**) genus level species composition heatmap for clustering of rumen microbial samples (select the ASV drawing with the top 50 reads). The red box contains bacteria that have significantly changed during the experiment period. n = 8 per experimental group.

Table 7. Effects of alkaline mineral complex buffer dietary supplementation on the abundance of rumen bacteria at the phylum level (Top 10 phylum).

Plylum, %	−14d A	7d A	14d A	21d A	−14d B	7d B	14d B	21d B
Firmicutes	48.53	48.51	57.67	47.08	39.45	47.93	51.09	49.02
Bacteroidetes	43.15	42.54	30.83	39.61	46.39	42.46	37.58	40.61
Proteobacteria	3.23	4.20	5.95	6.06	5.73	3.84	5.39	3.17
Tenericutes	1.69	1.08	1.95	2.35	4.38	2.31	2.95	2.50
Spirochaetes	0.62	1.68	1.72	2.67	0.56	1.66	1.46	2.61
Patescibacteria	0.84	0.41	0.78	0.82	1.74	0.82	0.78	0.90
Actinobacteria	0.98	0.92	0.70	0.45	0.73	0.22	0.26	0.67
Cyanobacteria	0.03	0.20	0.15	0.18	0.34	0.29	0.20	0.25
Fibrobacteres	0.13	0.28	0.11	0.46	0.18	0.17	0.15	0.14
Kiritimatiellaeota	0.50	0.07	0.04	0.19	0.29	0.15	0.03	0.03

We conducted LEfSe analysis on rumen microbiota samples (Figures 4 and S2) to analyze the significant differences in rumen microbiota. AMCB dietary supplementation measurably influenced the rumen microbiota. Multiple rumen microbial communities were considerably affected during the study period (Figure 4). Firmicutes and Actinobacteria abundance increased in group A cows 14 days before the calving and 7 days after calving (Figure 4A,B). Contrastingly, AMCB dietary supplementation markedly influenced the rumen microbiota 14 days after calving, and the abundance of total bacteria markedly increased (Figure 4C). Moreover, AMCB dietary supplementation markedly increased the abundance of Kiritimatiellaeota, Verrucomicrobia, and Deltaproteobacteria 21 days after calving. Additionally, AMCB dietary supplementation considerably increased the abundance of *Eggerthellaceae* during the study period (Figure 4), indicating that AMCB dietary supplementation plays an important role in the structure of the rumen microbiota of Holstein dairy cows in the transition period.

Table 8. Effects of alkaline mineral complex buffer dietary supplementation on the abundance of rumen bacteria at the genus level (Top 20 genus).

Genus, %	−14d A	7d A	14d A	21d A	−14d B	7d B	14d B	21d B
Prevotella_1	21.25	29.07	19.54	26.75	33.58	28.66	26.74	27.75
Ruminococcaceae_UCG-014	3.61	4.92	8.90	5.75	5.14	4.76	6.76	7.84
Ruminococcaceae_NK4A214_group	8.23	6.28	3.92	6.46	3.10	6.68	3.76	5.35
Christensenellaceae_R-7_group	11.66	3.98	2.75	4.60	4.78	5.41	2.32	2.56
Pseudomonas	2.39	2.95	4.20	4.71	4.67	2.52	4.29	1.67
Lachnospiraceae_NK3A20_group	2.64	3.02	5.75	2.35	2.05	2.07	4.52	2.60
Rikenellaceae_RC9_gut_group	7.59	2.57	1.97	2.62	3.40	2.70	1.43	1.62
F082	5.88	2.49	1.51	2.30	1.91	2.17	1.57	2.74
[Eubacterium]_coprostanoligenes_group	1.12	3.84	4.28	2.60	0.62	2.46	2.08	2.32
Shuttleworthia	0.09	3.07	5.69	1.38	0.15	1.47	3.96	3.43
Butyrivibrio_2	1.61	1.44	1.96	1.86	3.18	2.10	2.53	1.58
Mollicutes_RF39	1.34	0.85	1.85	2.03	3.56	1.82	2.66	2.12
Saccharofermentans	1.83	1.71	2.11	1.87	1.64	2.41	1.96	2.13
Muribaculaceae	1.45	2.49	1.50	1.41	0.36	2.99	1.74	1.31
[Eubacterium]_ruminantium_group	0.60	1.54	2.81	1.35	0.90	1.59	2.06	2.37
Treponema_2	0.61	1.67	1.71	2.67	0.52	1.66	1.46	2.60
unclassified_Lachnospiraceae	0.83	1.83	2.57	1.18	1.23	0.97	2.12	1.94
Ruminococcus_2	1.23	1.89	0.38	1.94	0.67	1.91	0.63	0.96
Prevotella_7	0.01	1.40	2.44	1.10	0.01	0.40	2.34	1.86
Prevotellaceae_UCG-001	1.41	0.89	1.24	1.03	1.81	1.06	0.85	0.83

Figure 4. *Cont.*

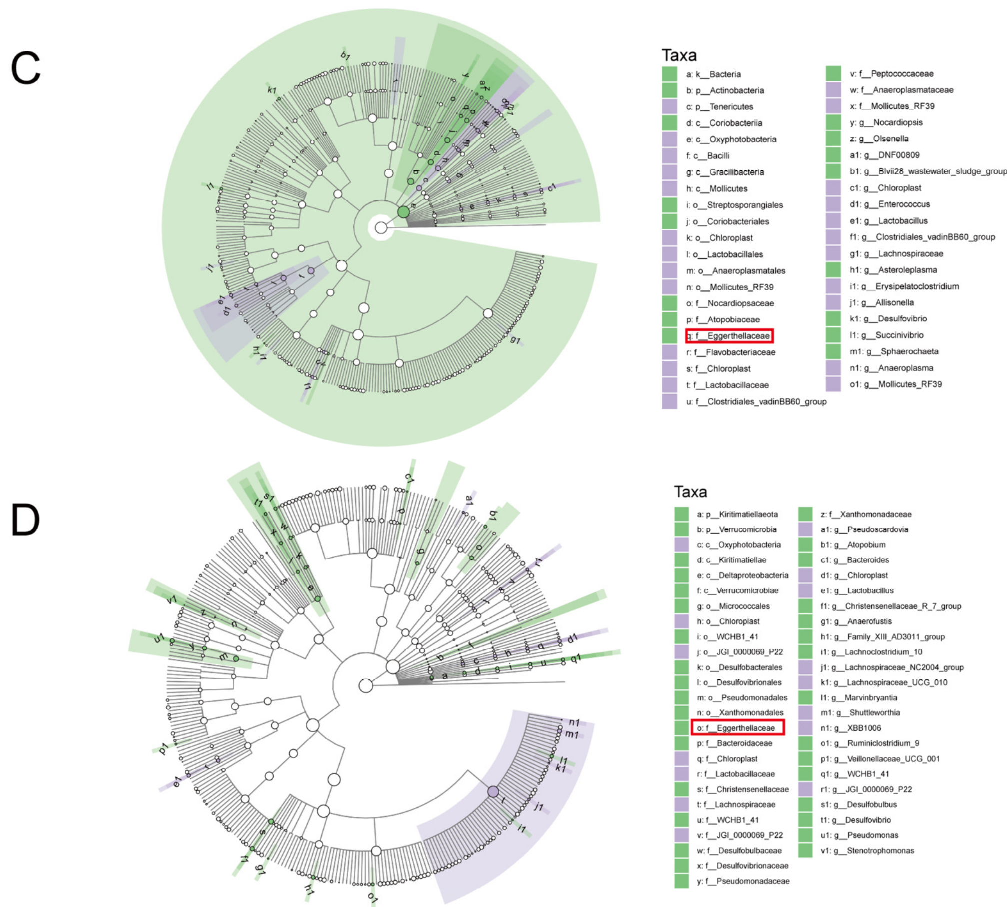

Figure 4. LEfSe analysis on rumen microbiota. (**A**) 14 days before the calving day (expected), (**B**) 7 days after calving, (**C**) 14 days after calving, and (**D**) 21 days after calving. Taxonomic dendrogram shows the hierarchical relationship of the main taxa from phylum to genus (from inner circle to outer circle) in the sample community. The node size corresponds to the average relative abundance of the classification unit; Hollow nodes represent classification units with insignificant inter group differences, while nodes with other colors indicate that these classification units exhibit a significant difference between groups and are more abundant in the samples represented by that color. The letters indicate the names of taxonomic units with significant differences between groups. A, the group supplemented with AMCB; B, control group. The red box contains bacteria that have significantly changed during the experiment. $n = 8$ per experimental group.

4. Discussion

Transition dairy cows have drastic changes in their endocrine and metabolic processes to meet the nutritional needs of early lactation [52]. However, various animal health problems during the transition period are the main risk factors affecting milk production and reproductive ability [53]. The DMI of cows during the transition period decreases by approximately 30% [54]. During the first three weeks of lactation, the rate of increase in milk production, milk protein, milk fat, and lactose substantially exceeds the rate of increase in DMI, developing NEB during the transition period [4]. Therefore, increasing the DMI of postpartum cows alleviates the NEB in cows, enhancing the production performance. In the present study, AMCB dietary supplementation considerably increased DMI during the postpartum phase. DMI is correlated with rumen pH and the diversity and quantity of rumen microorganisms [55,56]. The decreased acidity during rumen fermentation can negatively impact the fermentation efficiency of rumen microbiota, leading to toxin production [57]. The optimal pH range for rumen bacterial activity is from 5.5 to 7.3, and rumen bacteria have the best activity at pH 6.7 [58,59]. AMCB dietary supplementation stabilizes

the rumen pH and improves rumen microbiota richness, thus increasing DMI. A previous study has shown that DMI and milk yield in dairy cows are positively correlated [60], which may increase milk production. In the present study, the milk yield of Holstein dairy cows markedly improved owing to the considerable increase in DMI in the postpartum phase. Additionally, the FCM and ECM yields increased after ACMB dietary supplementation, although these changes were not statistically significant. Increased milk production could have considerable economic benefits.

Generally, milk fat and yield are negatively correlated [61]. AMCB dietary supplementation did not considerably affect acetic acid production; its deficiency causes classical diet-induced milk fat reduction [62]. In the present study, the acetic acid in the rumen did not increase with increasing milk production, resulting in an inadequate supply of acetic acid for milk fat synthesis and a low milk fat yield. Milk protein is an important indicator of milk quality and one of its main nutrients [63]. AMCB dietary supplementation markedly increased milk protein yield during the study period. Milk protein synthesis is directly or indirectly correlated with diet [64]. Milk urea nitrogen and BUN levels are positively correlated [65]. Urea nitrogen is a byproduct of protein metabolism, which can indirectly reflect the level of protein synthesis in the body [66,67]. A previous study revealed that animals with low urea nitrogen concentrations had normal amino acid metabolism and high protein synthesis rates [68]. In the present study, AMCB dietary supplementation considerably reduced blood and milk urea nitrogen levels in the postpartum phase, indicating that AMCB supplementation improves dietary protein utilization rate during the postpartum phase and may increase milk protein. Thus, AMCB supplementation improves the production performance of dairy cows during the transition period.

The metabolism and endocrine system of cows undergo drastic changes and adapt to early lactation during the transition period to ensure fetal nutrition [52]. During the transition period, cows are prone to NEB, thus increasing body fat degeneration [69]. AMCB dietary supplementation did not considerably affect VFA, NEFA, and BHBA levels during the study period because supplementation does not alter the dietary types and composition or affect the energy level of the feed. Interestingly, the TC level markedly increased in the postpartum phase in the present study, indicating that AMCB dietary supplementation actively synthesized lipoprotein and improved nutritional metabolism. Cholesterol synthesis is very active in the intestine, which helps absorb dietary lipids; therefore, high serum cholesterol may reflect the high DMI of dairy cows [1]. Dairy cows in the transition period are generally in the NEB state, which intensifies proper fat mobilization and causes excessive liver operational load, which impairs liver function [70,71]. AST and ALT are common indicators for monitoring liver injury, and their increased activity is often caused by the increase in cell membrane permeability caused by liver cell damage and necrosis, which releases these two enzymes into the bloodstream [72]. AST and ALT levels were higher during the postpartum phase than their respective reference levels, indicating a high liver burden in postpartum cows [73]. In the present study, there were considerable decreases in AST and ALT levels in the postpartum phase, indicating that AMCB dietary supplementation ameliorated the effects of liver damage and that the liver responded to the minerals in AMCB, which have an ameliorative effect [74]. Furthermore, the high DMI could have improved the NEB and reduced the liver burden of Holstein dairy cows during the transition period.

AMCB is an excellent source of silicon and zinc, which are correlated with the immune system and have antioxidant effects [29,75,76]. MDA is a lipid hydroperoxide decomposition product used to indicate cellular and tissue oxidative damage [77]. Hydrogen peroxide increases MDA production, decreasing the antioxidant ability [78]. In the present study, hydrogen peroxide levels considerably decreased in the postpartum phase, indicating that AMCB dietary supplementation improved partial antioxidant capacity. IgA and IgG indicate the strength and weakness of the immune system, respectively, and affect the survival and well-being of cows [79,80]. In the present study, AMCB dietary supplementation increased IgG and IgA levels in the postpartum phase, although these changes were not

statistically significant. Thus, AMCB dietary supplementation improved the health of dairy cows during the transition period.

Rumen microbiota has an essential role in ruminants. Cellulose is fermented and degraded into VFA, which ruminants absorb as nutrients owing to the unique physiological structure of the rumen and in synergy with rumen microorganisms [81]. The rumen microbial ecosystem is highly responsive to changes in diet and composition, which is a key factor affecting rumen microbial activity and function [82]. In the present study, rumen microbiota structures were different before and after calving owing to the considerable changes in diet composition and nutrition level of transition dairy cows. AMCB dietary supplementation increased the overall number of rumen microbiota during the study period and promoted the growth of rumen microorganisms by regulating the rumen pH. Rumen microbiota is correlated with DMI in dairy cows. Bacteroidetes and Firmicutes are essential in the rumen micro-ecosystem of ruminants, accounting for >90% bacterial abundance in the rumen [83]. A previous study found that the Bacteroidetes to Firmicutes ratio in dairy cows with a high DMI was lower than that in cows with a low DMI [84], consistent with the results of the present study.

Notably, AMCB dietary supplementation changed the composition and structure of rumen microorganisms during the study period. AMCB dietary supplementation considerably increased the abundance of Firmicutes 14 days before the expected delivery date. The increase in Firmicutes to Bacteroidetes ratio may reflect an increased ability to ferment dietary polysaccharides [85], consistent with LEfSe analysis results. The abundance of *Ruminococcaceae* and *Christensenellaceae* considerably increased 14 days before the expected delivery date. *Ruminococcaceae* mainly ferment cellulose disaccharides and cellulose [86], whereas *Christensenellaceae* ferment glucose to acetate and butyrate under anaerobic conditions [87], indicating that AMCB dietary supplementation improved the dietary polysaccharide degradation rate 14 days before the calving. The significant regulation of rumen microbiota through AMCB dietary supplementation was mainly observed 14 days postpartum, as the abundance of *Bacteria* considerably increased 14 days after calving. The high abundance of total bacteria may be due to high DMI and conducive rumen pH, which promote the growth of rumen microorganisms and digestion. AMCB dietary supplementation considerably increased the abundance of *Eggerthellaceae* and *Fibrobacter* during the study period. *Eggerthellaceae* belong to the phylum Actinobacteria [88]. Bach et al. demonstrated that the abundance of *Eggerthellaceae* is positively correlated with feed efficiency [11]. A previous study revealed that *Eggerthellaceae* are particularly involved in metabolizing daidzein and genistein and converting them to food polyphenols [89]. The main observed positive effects of polyphenols were their antioxidant activity in blood and milk and blood urea reduction [90]. AMCB dietary supplementation improved partial antioxidant capacity and decreased milk and serum urea nitrogen, which may be owing to the increased abundance of *Eggerthellaceae* after calving. *Fibrobacter* lives in the rumen and produces cellulase, which can break down lignocellulose for ruminant absorption [91,92]. The rumen microbial analysis results indicated that AMCB dietary supplementation improves rumen microorganism richness and partial probiotic colonization.

5. Conclusions

The present study investigates the effect of 50 mL AMCB taken daily as a dietary supplement on the rumen fermentation, microbiota, and production performance of 40 Holstein dairy cows in the transition period with a 2.76 $\pm$ 0.48 parity and 650 $\pm$ 25 kg body weight. AMCB dietary supplementation stabilized the rumen pH, improved rumen microbiota richness and partial probiotic colonization, and considerably increased DMI. Furthermore, AMCB dietary supplementation considerably increased milk performance owing to the high DMI. Notably, AMCB dietary supplementation considerably decreased serum ALT and AST levels, which indicates reduced liver damage in transition dairy cows. Additionally, AMCB dietary supplementation considerably reduced the BUN, MDA, and hydrogen peroxide levels and increased IgG and IgA after calving, indicating that AMCB supplemen-

tation considerably enhanced the partial antioxidant capacity and immunity of transition period dairy cows. Overall, AMCB dietary supplementation improved production performance, partial immune function, and antioxidant capacity and promoted the richness of rumen microorganisms of transition Holstein dairy cows. Therefore, AMC is an excellent candidate for use as a rumen buffer.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9090792/s1, Table S1: Effects of alkaline mineral complex buffer dietary supplementation on serum variables of dairy cows before calving; Figure S1: Species accumulation curve of rumen microbiota samples; Figure S2: LEfSe analysis of rumen microbiota in the experimental period.

Author Contributions: C.G.: Conceptualization, methodology, data analysis, investigation and writing—original draft; F.K.: Reviewing and editing; S.L.: Reviewing, editing, supervision, resources and project administration; X.W.: Methodology and validation; X.S.: Data analysis; W.D.: Methodology; D.D.: Investigations; S.W.: Writing, reviewing and editing; B.X.: Writing, reviewing and editing; X.X.: Methodology, resources and project administration. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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