Effects of antibiotic residues in milk on growth, ruminal fermentation, and microbial community of preweaning dairy calves

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ABSTRACT

The aim of this study was to evaluate the effects of antibiotic residues in milk on growth, ruminal fermentation, and microbial community of dairy calves in their first 35 d of age. Twenty newborn Holstein bull calves were assigned to 1 of 2 treatments equally: milk replacer without antibiotics (control) and milk replacer plus 4 antibiotics: 0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur (ANT). Starter intake and fecal consistency scores of each calf were recorded on a daily basis. Body weight, withers height, body length, and heart girth were measured on d 1, 7, 14, 21, 28, and 35 before feeding in the morning. Rumen fluid was collected on d 15, 25, and 35 to determine ruminal pH, volatile fatty acids (VFA), and NH_3-N concentrations. A total of 10 (5 per treatment) samples of rumen fluid taken on d 35 were analyzed for microbial community. Rumen tissues from the cranial ventral sac and cranial dorsal sac were collected from 8 calves of each group for morphology analysis on d 35 after being harvested. The results showed that calves in 2 treatments had similar starter intake, body weight, withers height, body length, heart girth, and average daily gain. The ANT group showed a lower diarrhea frequency in wk 4, and no differences were found for other weeks. Calves in the ANT group exhibited a greater concentration of acetic acid in the rumen and no differences for other VFA, total VFA, rumen pH, or NH_3-N. As for rumen morphology, the length of papillae from cranial ventral sac of the ANT group was longer than that of the control group. The results of ruminal microbial community showed that antibiotic residues had minor effects on bacteria phyla and bacteria diversity. At the genus level, calves in the ANT group showed lower richness of *Prevotella* and higher richness of *Acetitomaculum*. In conclusion, antibiotic residues stimulated the development of ruminal papillae and increased the production of acetic acid in rumen, which might be caused by the influence of antibiotics on the ruminal microbial community.

Key words: calf, antibiotics, ruminal fermentation, microbial community

INTRODUCTION

Antibiotics have been an effective medical treatment since the 20th century (Zaffiri et al., 2012). However, the overuse of antibiotics causes many problems such as the disturbance of intestinal microbes and antibiotic resistance (Phillips et al., 2004). Studies have shown that the use of antibiotics during infancy increases the risk of developing allergies and obesity when babies grow up, which has a close relation to the disturbance of gut microbes due to antibiotics (Johnson et al., 2005; Ajslev et al., 2011). These side effects of antibiotics have raised a concern about using antibiotics on husbandry animals.

Antibiotics are commonly used to treat mastitis, reproductive diseases, and hoof diseases in dairy farms. Antibiotics are injected into either vein or muscle, then end up in milk at very low concentrations. Penicillin G residue can be detected from the milk 9 d after treatment (Seymour et al., 1988) and 20% of milk samples showed positive results of gentamicin 6 d after treatment for mammary inflammation (Martins et al., 2014). The milk containing antibiotics along with transitional milk from fresh cows and milk containing high SCC from cows with mastitis make up waste milk, which cannot be sold commercially. Waste milk is usually used to feed calves for economic reasons. Milk is the major feedstuff for calves and plays an important role on health and growth. Many studies report consistent results that waste milk causes antibiotic resistance in gut bacteria (Wray et al., 1990; Aust et al., 2013; Maynou et al., 2017). As for weight gain, some reported that calves fed with waste milk had higher weight gain than those fed with milk replacer (Brunton et al., 2014) or bulk milk (Zou et al., 2017), whereas others did not observe any

differences on weight gain due to milk type (Wray et al., 1990; Aust et al., 2013). The growth-promoting effect of waste milk is explained by higher nutrient density (Zou et al., 2017) or antibiotics (Aust et al., 2013) in waste milk. However, the nutrient density and antibiotics in waste milk vary in each study, which makes it difficult to determine which factor contributes to the differences in weight gain, therefore quantified antibiotics and milk replacer were used in our study to avoid the instability of waste milk. Although milk bypasses the rumen into the abomasum due to esophageal groove reflex, the disturbances of milk containing antibiotics on the rumen microbes of calves are still reported (Li et al., 2017), which is reasonable because the gastrointestinal ecosystem is open and integrated (Savage, 1977). Rumen development is essential for young ruminants, so this study focused on how antibiotic residues affected growth performances, ruminal fermentation, and rumen microbial community of calves. Our hypothesis was that antibiotic residues would not significantly change the growth performances of calves, but would change the rumen microbe compositions, and thereby would affect the ruminal fermentation.

MATERIALS AND METHODS

Antibiotic Residues Testing

This study was conducted in Dingzhou, Hebei Province, China. The experimental design and procedures were executed according to the protocols approved by the Ethical Committee of the College of Animal Science and Technology, China Agricultural University (no. 2016DR07). Waste milk samples were collected for 3 wk (3 times per week, 9 samples in total) from the dairy farm before the animal trial to determine the types and dosages of antibiotics used in this study. All samples were stored at −20°C and thawed at 4°C for 24 h before testing. According to the treatment protocols, penicillin, streptomycin, tetracycline, and ceftiofur were 4 mostly used antibiotics on the farm; therefore, the concentrations of these antibiotics were tested using a Penicillin ELISA kit (Wdwk Bio Co., Ltd., Beijing, China), Streptomycin ELISA kit (Wdwk Bio Co., Ltd.), Tetracyclines ELISA kit (Wdwk Bio Co., Ltd.), and Ceftiofur ELISA kit (Wdwk Bio Co., Ltd.) separately. The results were used to determine the amounts of antibiotics added in milk for the animal trial.

Animals, Treatments, and Management

The animal trial was conducted from October to December in 2016, using a randomized complete block design according to birth date. Twenty Holstein neona-

Table 1. Nutrient composition of milk replacer and starter

Nutrient composition (%)	Milk replacer	Starter
DM	96.0	89.1
CP, DM basis	22.0	22.9
Ether extract, DM basis	20.0	3.5
Ash, DM basis	9.0	7.0
Ca, DM basis	0.6	1.7
P, DM basis	0.6	0.5
Lactose, DM basis	39.7	—
NDF, DM basis	—	21.8
ADF, DM basis	—	7.5

tal male calves (40.6 ± 3.3 kg of BW) were equally assigned to 1 of 2 treatments: control group (milk replacer with no antibiotics; **CON**) and antibiotic group (milk replacer plus 0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur; **ANT**). All the calves received 4 L of colostrum within 1 h after birth and were moved into individual hutches with free access to starter and water from d 2. Milk replacer was fed twice a day at 0730 and 1600 h from d 2 to 5 in the amount of 2 L/meal, from d 6 to 14 in the amount of 3 L/meal and from d 15 to 35 in the amount of 4 L/meal. The study ended when all calves reached d 35 of age, and 16 of them (8 per treatment) were slaughtered 2 h after the morning feeding. The milk replacer (FrieslandCampina Co., Ltd., Amersfoort, the Netherlands) used in this study contained no antibiotics. One kg of powder formed 8 L of liquid milk under 45 to 55°C; then the milk was cooled down to 38 to 40°C and antibiotics were added before feeding. Starter was offered once daily after milk feeding in the morning. The nutrient compositions of milk replacer and starter are shown in Table 1.

Sample Collection

Starter Intake and Growth Performance Measurements. Newly fed and refused starter was recorded daily to calculate individual starter intake. Body weight, withers height, body length, and heart girth of each calf were measured on d 1, 7, 14, 28, and 35 before the morning milk feeding.

Fecal Scoring. Fecal consistency scores for all calves were determined daily based on a 1 to 4 system according to the guidelines suggested by Larson (Larson et al., 1977). A fecal score of 3 and above was considered a diarrhea day. Diarrhea frequency was calculated with the following equation weekly:

$$\text{diarrhea frequency} = [(\text{number of diarrhea calves}$$
$$\times \text{ days of diarrhea})/(\text{total number of calves}$$
$$\times \text{ days of trial})] \times 100\%.$$

Rumen Fluid. Rumen fluid was collected on d 15, 25, and 35 of age by a flexible esophageal tube (2 mm of wall thickness and 6 mm of internal diameter; Anscitech Co., Ltd., Wuhan, Hubei, China) from all calves 4 h after the morning milk feeding. The pH of rumen fluid was tested immediately after sample collection. Rumen liquid fraction was obtained by filtering rumen fluid through 4 layers of cheesecloth and 30 mL of the liquid was stored at −20°C for later analysis of VFA (Erwin et al., 1961) and NH$_3$-N (Broderick and Kang, 1980). Ten milliliters of the liquid collected on d 35 from 10 calves (5 per treatment) was stored at −80°C for later analysis of microbial composition and population.

Rumen Tissues. On d 35 of age, 16 calves (8 per treatment were randomly selected) were harvested. After removing the digesta, tissues from the rumen were rinsed in saline. Two 1-cm^2 rumen tissue samples were removed from the center of each area of cranial ventral sac and cranial dorsal sac (Lesmeister et al., 2004). The tissue samples were then fixed in 4% phosphate-buffered paraformaldehyde solution. After rinsing with water, the samples were dehydrated in a graded series of ethanol (50, 70, 80, 90, and 100%), cleared with xylene twice, and separately embedded in paraffin blocks in a vertical direction to keep the orientation of the rumen papillae the same as the cutting direction when isolating tissue slices. For each block, 5 cuts of 3- to 4-µm-thick sections were isolated and stained with hematoxylin/eosin, resulting in 10 regions of each rumen site for histology analysis. As shown in Figure 1, the 5 longest papillae were selected from each rumen tissue slice for papillae length (**PL**) and papillae width (**PW**) measurements through a computerized micrometer according to Malhi et al. (2013) under a microscope (Olympus CKX53, Tokyo, Japan) at 100× magnification.

Bacteria Richness. Ten rumen fluid samples (5 per treatment were randomly selected) on d 35 were collected for microbial analysis. The DNA from samples was extracted using the E.Z.N.A. Bacterial DNA Kit (Omega Bio-Tek, Norcross, GA) according to the manufacturer's instructions. Thirty-nanogram DNA samples were used for 50-µL PCR reaction mixtures. A pair of 2-µL primers with 10 µM forward primer (338F 5′-ACTCCTACGGGAGGCAGCAG-3′) and 10 µM reverse primer (806R 5′-GGACTACHVGGGTWTC-TAAT-3′) was used to amplify a region covering the V3–V4 region of bacterial 16S rRNA genes. The PCR conditions were as follows: an initial predenaturation at 95°C for 5 min, denaturation by 28 cycles of 95°C for 30 s, annealing at 56°C for 30 s, elongation at 72°C for 40 s, and then a final extension at 72°C for 10 min and holding at 4°C. Sequencing was done on the Illumina

MiSeq platform at Beijing Allwegene Technology Co., Ltd. (Beijing, China). After trimming the adaptor and primer sequences from Illumina reads, the raw sequences were assembled for each sample according to the unique barcode using QIIME (V1.8, http://qiime.org/). Quality filtering was performed under specific filtering conditions to obtain the high-quality clean tags according to QIIME (V1.8, http://qiime.org/). High-quality sequences were clustered into operational taxonomic units (**OTU**), defined as comprising sequences with less than a 3% difference using UPARSE (V7.0, http://drive5.com/uparse/). Chimeric OTU were removed before further analysis by UCHIME (Edgar, 2010). Chao1, Shannon, and observed species were used to estimate α diversity. The OTU were denominated according to the Silva bacteria database (http://www.arb-silva.de).

Statistical Analysis

Continuous variables with repeated measurements including starter intake, BW, body measurements, rumen VFA, NH$_3$-N, and pH were analyzed using a mixed effect model with treatment, time, and the interaction between them as fixed effects, and animal within treatment as a random effect. Variables of rumen morphological parameters were also analyzed using a mixed effect model, but with treatment alone as a fixed effect, and animal within treatment as a random effect. Diarrhea frequency was compared using the χ^2 test. Variables of microbial parameters were analyzed with the Kruskal-Wallis test. MIXED procedure, FREQ pro-

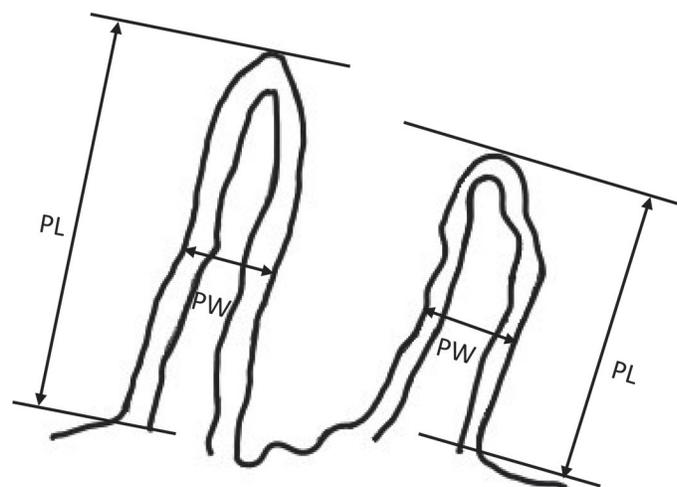**Figure 1.** Papillae length (PL) and papillae width (PW) measured for rumen papillae through a computerized micrometer at 100× magnification.

cedure, and NPAR1WAY procedure of SAS 9.2 (SAS Institute Inc., Cary, NC) were used to analyze the data in this study. Significance was indicated at $P < 0.05$. Data were reported as least squares means. Not all the calves were used for rumen tissue sampling and bacteria richness analysis out of the consideration of budget. The sample sizes of histology analysis and microbial analysis were estimated to obtain a power of 0.8 under a significance level of 0.05.

RESULTS

Antibiotic Concentrations in Waste Milk

The concentrations of penicillin, streptomycin, tetracycline, and ceftiofur residues in waste milk were 0.024 $\pm$ 0.034 mg/L, 0.019 $\pm$ 0.008 mg/L, 0.08 $\pm$ 0.05 mg/L, and 0.76 $\pm$ 0.43 mg/L (mean $\pm$ SD) separately. The large standard deviations indicated great variations of antibiotic residues; thus, medians (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.10 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) were used as the concentrations of antibiotics in the animal trial.

Starter Intake, Body Weight, Body Measurements, and Diarrhea Frequency

As shown in Table 2, calves fed with either treatment consumed similar amounts of starter ($P = 0.58$). Antibiotic residues did not affect the BW ($P = 0.32$), withers height ($P = 0.41$), body length ($P = 0.30$), heart girth ($P = 0.64$), or ADG ($P = 0.71$). Diarrhea frequency (Figure 2) for calves in the CON group was higher than the ANT group in wk 4 ($P = 0.03$), and no differences were found in other weeks ($P > 0.05$).

Ruminal Fermentation and Morphology

Rumen pH, VFA, and NH_3-N were similar between 2 treatments (Table 3), except that calves in the ANT group exhibited a greater concentration of acetic acid
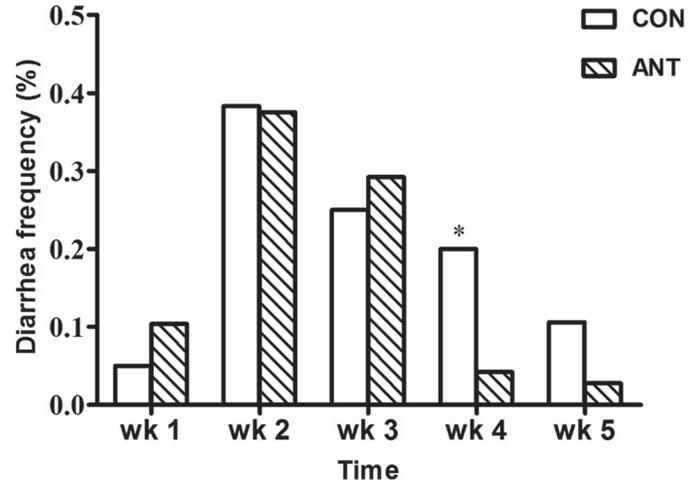

Figure 2. The effect of antibiotic residues on diarrhea frequency. CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer (n = 10). *$P < 0.05$: difference between CON and ANT.

than the CON group ($P = 0.008$). Rumen fermentation was affected by the age of calves ($P < 0.01$).

Effects of antibiotic residues on the PL and PW are presented in Table 4. Calves in the ANT group exhibited a longer PL at the cranial ventral sac than the CON group ($P = 0.04$). No differences were found for PL at the cranial dorsal sac or PW in both sacs ($P > 0.05$).

Diversity of Rumen Bacteria

Chao1 index, Shannon index, and observed species were used to evaluate the diversity of rumen bacteria (Figure 3). No differences were found for Chao1 index, Shannon index, or observed species ($P > 0.05$).

Bacteria Richness

Bacteroidetes, *Firmicutes*, *Proteobacteria*, *Synergistetes*, and *Actinobacteria* were 5 major bacterial

Table 2. The effect of antibiotic residues on starter intake and growth of dairy calves (n = 10)

Item	Treatment[1]			P-value		
	CON	ANT	SEM	Treatment	Time	Treatment × time
Starter intake (g/d)	75.76	92.74	22.67	0.58	<0.001	0.84
BW (kg)	48.66	47.24	1.02	0.32	<0.001	0.93
Withers height (cm)	79.19	78.43	0.67	0.41	<0.001	0.02
Body length (cm)	72.64	71.74	0.65	0.30	<0.001	0.60
Heart girth (cm)	86.46	86.10	0.57	0.64	<0.001	0.99
ADG (g)	487.14	503.57	33.22	0.71	—	—

[1]CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer.

Table 3. The effect of antibiotic residues on rumen pH, VFA, and NH$_3$-N (n = 10)

Item	Treatment[1]			P-value		
	CON	ANT	SEM	Treatment	Time	Treatment × time
pH	6.29	6.32	0.15	0.86	<0.001	0.81
Acetic acid (mmol/L)	24.86	33.73	1.82	0.008	<0.001	0.09
Propionic acid (mmol/L)	18.59	15.26	2.35	0.32	<0.001	0.28
Butyric acid (mmol/L)	5.64	3.76	0.91	0.17	<0.001	0.03
Isovaleric acid (mmol/L)	0.82	0.65	0.15	0.47	0.005	0.38
Valeric acid (mmol/L)	1.34	0.93	0.31	0.35	<0.001	0.41
Total VFA (mmol/L)	52.17	53.96	4.22	0.77	<0.001	0.09
NH$_3$-N (mg/dL)	5.74	5.28	0.92	0.74	<0.001	0.52

[1]CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer.

phyla in rumen fluid of calves for both groups, accounting for more than 98% of the total rumen bacterial community (Figure 4). No differences were found in phyla relative abundance ($P > 0.05$) with addition of antibiotics.

The rumen bacteria richness at genus level was shown in Figure 5. The 10 most common rumen bacteria at genus level for the CON group were *Prevotella*, *Selenomonas*, *Succinivibrio*, unidentified, U29-B03, *Ruminobacter*, *Ruminococcaceae* UCG-014, *Prevotellaceae* NK3B31 group, *Lachnospiraceae* NK3A20 group, and *Succiniclasticum*. The 10 most common rumen bacteria at genus level for the ANT group were *Succinivibrio*, *Prevotella*, *Lachnospiraceae* NK3A20 group, unidentified, *Olsenella*, *Succiniclasticum*, *Atopobium*, *Megasphaera*, *Acetitomaculum*, and *Bacteroides*. Antibiotic residues decreased the relative abundance of *Prevotella* ($P = 0.036$) and increased the relative abundance of *Acetitomaculum* ($P = 0.046$) in the rumen. No differences were observed for other species ($P > 0.05$).

DISCUSSION

Our study investigated the effects of antibiotic residues on the growth, rumen fermentation, rumen histology, and rumen bacteria, and tried to find the connections between each aspect.

Growth and Health

The growth-promoting effect of antibiotics was explained by the modification of intestinal microbiota for a healthy intestinal environment (Gaskins et al., 2002), or the inhibition of immune functions to save energy for growth purposes (Roura et al., 1992; Bhandari et al., 2008). However, this effect is more likely to occur when animals are poorly managed and antibiotics are fed at high concentrations (Langford et al., 2003). No sig-

nificant differences were observed on growth or starter intake in our study, which was consistent with previous studies reporting that milk containing subtherapeutic antibiotics does not influence the growth of animals (Langford et al., 2003; Thames et al., 2012).

The effect of antibiotic residues on diarrhea was minor according to previous studies (Langford et al., 2003; Thames et al., 2012). Brunton et al. (2014) reported a lower diarrhea incidence for calves fed with waste milk containing antibiotic residues compared with those fed with milk replacer, but they attributed this effect to individual differences and group housing. There is not enough evidence to support the diarrhea-reducing effect of antibiotic residues, thus the lower diarrhea frequency observed for the ANT group in wk 4 was probably due to individual differences as well.

Rumen Fermentation and Rumen Histology

Rumen pH is affected by several factors, including the concentration of VFA and NH$_3$-N, the secretion of saliva, and outflow of rumen fluid. Lower pH can pro-

Table 4. The effect of antibiotic residues on rumen papillae length and width (n = 8)

Item[1]	Treatment[2]			
	CON	ANT	SEM	P-value
PL (μm)				
CVS	346.54	451.27	32.77	0.04
CDS	375.66	389.58	36.99	0.79
PW (μm)				
CVS	112.26	126.48	7.44	0.20
CDS	105.43	120.79	7.20	0.15

[1]PL = papillae length; PW = papillae width; CVS = cranial ventral sac; CDS = cranial dorsal sac.

[2]CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer.
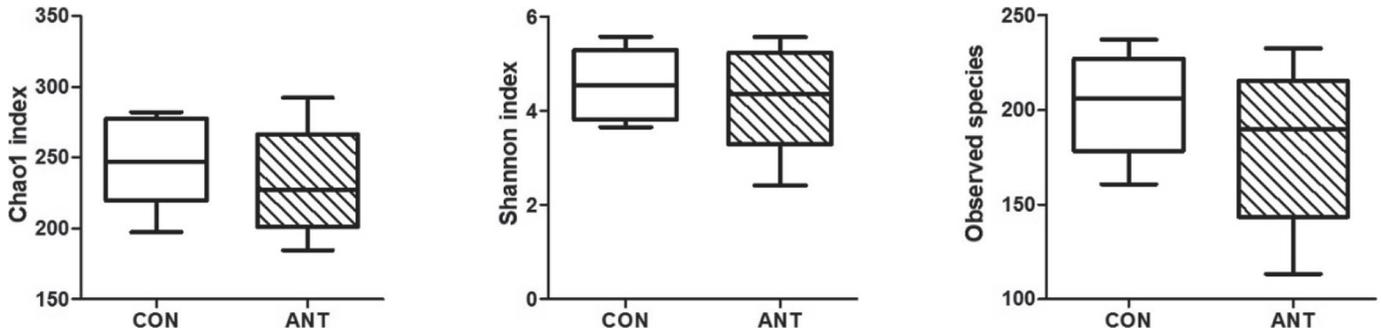
Figure 3. The effect of antibiotic residues on the α diversity of rumen bacteria on d 35. CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer (n = 5). The central rectangle spans the first quartile to the third quartile. The segment inside the rectangle shows the median, and whiskers above and below the box show the locations of the minimum and maximum.

mote rumen epithelium to absorb VFA and accelerate rumen development (Baldwin and McLeod, 2000). In this study, the rumen pH of newborn calves was high because of low feed intake and inactivity of rumen, and

Figure 4. The effect of antibiotic residues on the relative abundance (%) of the most abundant rumen bacterial phyla. CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer (n = 5).

it decreased with age, which was relative to the increase of starter intake (Robinson et al., 1986).

Antibiotics are believed to be able to inhibit rumen fermentation through suppressing rumen microbes (Owens and Basalan, 2016). However, we observed a greater concentration of acetic acid for calves fed with antibiotics. Wasserman et al. (1952) suggested that penicillin and streptomycin suppressed fiber degradation at high concentrations while promoting fiber degradation and increasing acetic acid concentration at low concentrations in vitro. Low concentrations of penicillin could also increase the proportion of acetic acid without significantly changing total VFA in vitro (De Jong, 1989). Xiong et al. (2017) reported that tetracycline increased acetic acid production under anaerobic conditions. Our results along with previous studies (Wasserman et al., 1952; De Jong, 1989; Xiong et al., 2017) showed that a low concentration of several antibiotics increased the production of acetic acid in the rumen.

Ruminal environment has a great influence on the development of rumen papillae, therefore daily ration changes affect the development of the rumen significantly (Lesmeister et al., 2004). Volatile fatty acids stimulate the development of rumen papillae and epithelia and thus are considered one of the most important factors affecting rumen development (Tamate et al., 1962). Acetate, propionate, and butyrate are all able to stimulate rumen development (Sander et al., 1959; Sakata and Tamate, 1979). Although ANT group had higher acetic acid concentration, the total VFA concentration was not different from the CON group, and thus the longer PL at the cranial ventral sac of calves in ANT group might be due to other reasons. Niwińska and Strzetelski (2005) and Górka et al. (2011) did not find direct connections between the concentration of rumen VFA and the development of rumen papillae as
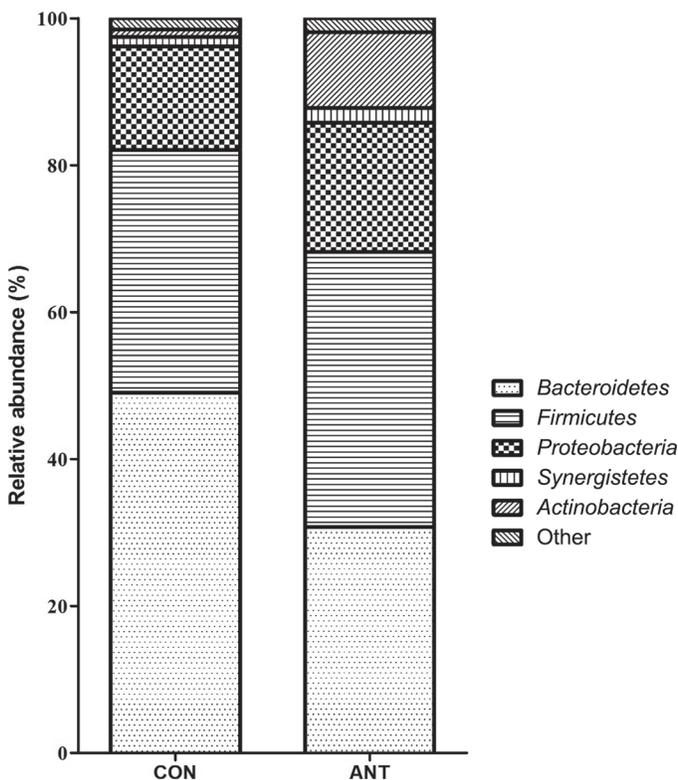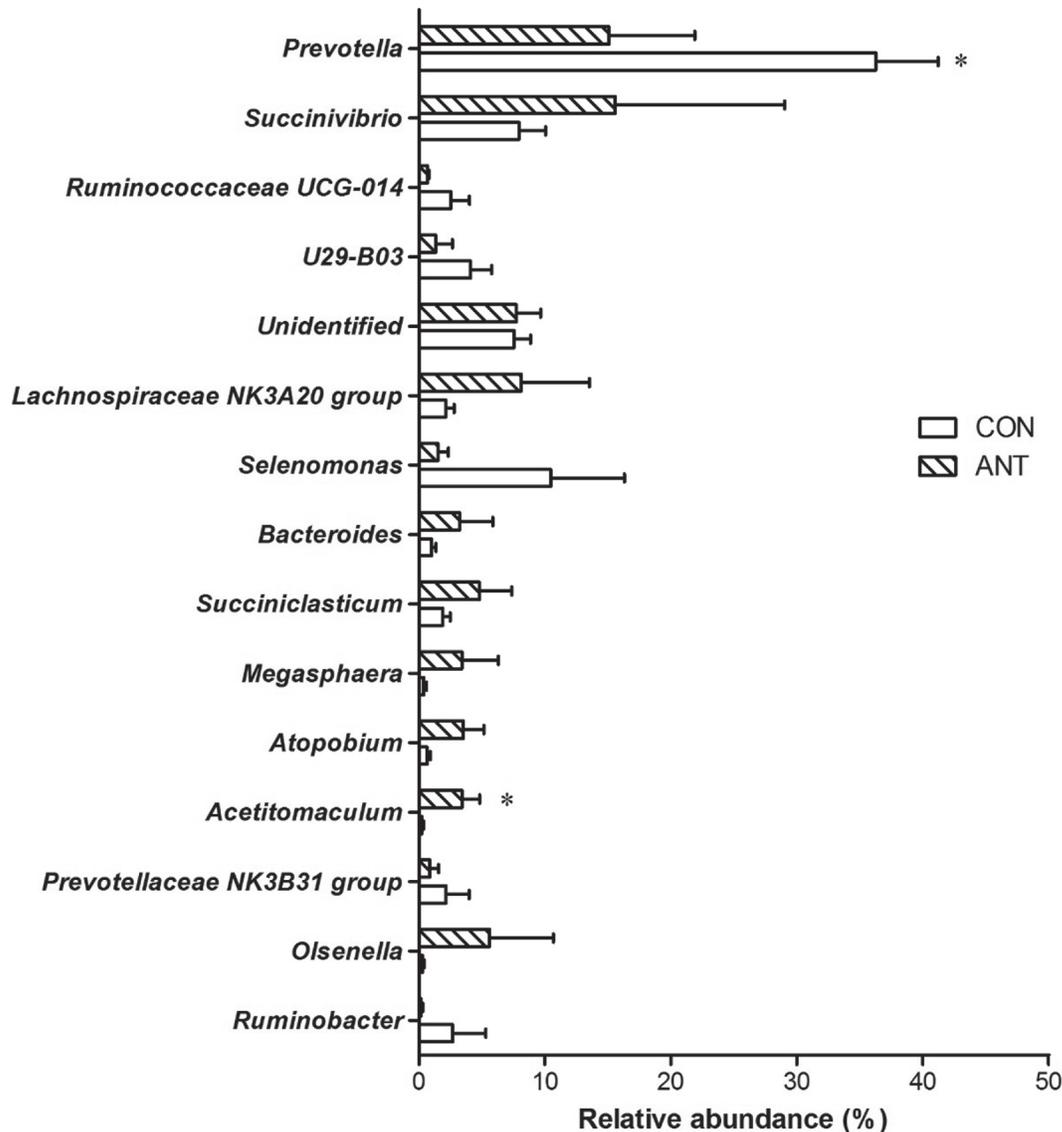
Figure 5. The effect of antibiotic residues on the relative abundance (%) of the most abundant rumen bacterial genera. CON = no antibiotics; ANT = antibiotics (0.024 mg/L of penicillin, 0.025 mg/L of streptomycin, 0.1 mg/L of tetracycline, and 0.33 mg/L of ceftiofur) in milk replacer. *$P < 0.05$ (n = 5). The error bars represent the SD.

well. Insulin appears to affect the absorptive surface of the ruminal wall (Hugi et al., 1997), so the analysis of blood insulin level could possibly help explain the longer PL caused by antibiotic residues in the future.

Diversity of Rumen Bacteria

We did not observe any differences on Chao1 index, Shannon index, or observed species between the 2 treatments. Similar results were reported by Van Vleck Pereira et al. (2016), who fed calves with a different combination of antibiotics but at similar dosage to our

study and found no difference in the diversity of feces microbiota. The inhibition of antibiotics on diversity of intestinal microbiota was reported in several studies (Keesing et al., 2010; Livanos et al., 2016), but the dosages used in this study might not be enough to cause a difference on the diversity of rumen bacteria.

Bacteria Richness

The 3 most common bacteria phyla, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, were observed for both groups in this study. The same rumen bacteria for

dairy calves were reported as the dominant phyla by previous studies (Li et al., 2012; Rey et al., 2014), and *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are also taxonomic groups represented within the cattle gastrointestinal tract (Mao et al., 2015). Overall, a limited influence of antibiotic residues on rumen bacteria was observed at the phylum level in our study. Additionally, Van Vleck Pereira et al. (2016) reported that fecal bacteria phyla are not significantly changed by antibiotic residues.

The compositions of rumen bacteria at the genus level were altered by antibiotic residues. As one of core bacterial microbiome at the genus level (Henderson et al., 2015), *Prevotella* was the only bacteria genus whose relative abundance was decreased by antibiotic residues, which might be due to the susceptibility of *Prevotella* to penicillin (Falagas and Siakavellas, 2000) and tetracycline (Niederau et al., 1980). As the most abundant bacteria genus, the greatest exposure of *Prevotella* to the antibiotics might contribute to its lower abundance. The decreased relative abundance of *Prevotella* was also observed in fecal bacteria for elderly people after antibiotic treatment (Bartosch et al., 2004).

Limited studies have investigated the effect of antibiotics on *Acetitomaculum*. Hill et al. (2010) reported a decreased relative abundance of *Acetitomaculum* in the colon after antibiotic treatment for mice, which is contrary to our results, probably because they used a different combination of antibiotics and a higher dosage. Carey el al. (2016) added triclosan to anaerobic digesters and observed very close results to ours in terms of microbial community. They reported that high concentration of triclosan selected for the phyla *Actinobacteria* and *Firmicutes*, with *Firmicutes* as the most abundant phylum in triclosan-containing digesters. *Firmicutes* was also the most abundant phylum for calves treated with antibiotics in our study. In addition, they observed that *Succinivibrio*, *Atopobium*, *Olsenella*, and *Acetitomaculum* were enriched in the digesters treated with triclosan. In our study, *Atopobium* and *Olsenella* were among the 10 most abundant genera for the ANT group but not for the CON group, and *Acetitomaculum* was enriched for calves fed with antibiotic residues. As a synthetic antimicrobial agent, triclosan is not an antibiotic but has antibiotic properties (Dhillon et al., 2015). The consistency between the 2 studies indicated a similar regulation of triclosan and the antibiotic residues in this study on the microbial community. The selectiveness of the antibiotic residues on rumen bacteria needs further study.

The increase of the relative abundance of *Acetitomaculum* for the ANT group might thereby contribute to the higher concentration of acetic acid because

Acetitomaculum is a member of acetogenic bacteria (Le Van et al., 1998). On the other hand, *Prevotella* is hydrogen-consuming bacterium and can produce propionate through the fermentation of sugars or lactate (Li et al., 2013). The decrease of the abundance of *Prevotella* did not significantly decrease the propionic acid concentration for the ANT group, but could possibly eliminate the difference on total VFA concentration, given that the ANT group had a higher acetic acid concentration but a similar total VFA concentration as the CON group.

CONCLUSIONS

The antibiotic residues had no significant effects on the growth of calves. This might suggest that antibiotic residue in waste milk is not a factor affecting the growth or health of calves. The antibiotic residues increased PL in the rumen cranial ventral sac, the reason for which is unclear. The antibiotic residues had limited effects on rumen bacteria, but increased the relative abundance of *Acetitomaculum*, which might contribute to the increased concentration of acetic acid in the rumen.

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