Effect of Weaning Age on Rumen Microbial Diversity in Hu Sheep Lambs (Postprint)

Authors: Ma Wanhao, Zhang Ning, Li Fei, Li Fadi

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effect of weaning age on rumen microbial diversity in Hu sheep lambs. Sixty-six Hu sheep male lambs were selected; six lambs were slaughtered at 1, 14, and 28 days of age, respectively. The remaining 48 lambs were randomly divided into two groups and weaned at 28 and 56 days of age, respectively. At 42, 56, 70, and 84 days of age, six lambs from each group were slaughtered to collect rumen content samples. All lambs were fed starter diet starting at 7 days of age. The experimental period was from 1 to 84 days of age. The results showed that weaning age had no significant effect on the relative abundances of *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Streptococcus bovis*, *Fibrobacter succinogenes*, and *Treponema bryantii* ($P>0.05$). With increasing age, rumen microbial diversity in lambs continuously increased, and lambs weaned at 28 days of age exhibited higher rumen microbial diversity compared with those weaned at 56 days of age. These results suggest that rumen microbial diversity is influenced by age and weaning age, and early weaning can accelerate the establishment of rumen microbiota.

Full Text

Effects of Weaning Age on Rumen Microbial Diversity in Hu Lambs

MA Wanhao[1], ZHANG Ning[1],[2], LI Fei[1]*, LI Fadi[1],[3]

[1]Key Laboratory of Grassland Farming Systems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China
[2]New Hope Liuhe Group Co., Ltd., Beijing 100102, China
[3]Biotechnology Engineering Laboratory of Gansu Meat Sheep Breeding, Minqin 733300, China

Abstract

This study investigated the effects of weaning age on rumen microbial diversity in Hu lambs. Sixty-six male Hu lambs with similar birth weights were selected. Six lambs were slaughtered at 1, 14, and 28 days of age, respectively. The remaining 48 lambs were randomly divided into two groups weaned at 28 or 56 days of age, with six lambs from each group slaughtered at 42, 56, 70, and 84 days of age to collect rumen content samples. All lambs were fed starter from 7 days of age. The experimental period spanned from 1 to 84 days of age. The results showed that weaning age had no significant effect on the relative abundance of *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Streptococcus bovis*, *Fibrobacter succinogenes*, and *Treponema bryantii* ($P>0.05$). Rumen microbial diversity increased continuously with age, and lambs weaned at 28 days of age exhibited higher rumen microbial diversity compared to those weaned at 56 days of age. These findings indicate that rumen microbial diversity is influenced by both age and weaning age, and that early weaning can accelerate the establishment of rumen microflora.

Keywords: Hu lambs; weaning age; microbial diversity

Introduction

The rumen of ruminants is a complex ecosystem harboring a vast population of microorganisms. Based on their distribution patterns, these microbes can be categorized into liquid-associated, solid-associated, and mixed-phase communities. Among them, bacteria play the primary role in digestion and degradation, and can be classified into functional groups including cellulolytic bacteria, amylolytic bacteria, hemicellulolytic bacteria, lipolytic bacteria, lactate-utilizing bacteria, and lactate-producing bacteria [1].

Rumen microbial communities are influenced by dietary intake [2-3]. Different weaning and supplementation times can alter nutrient intake in ruminants, affecting rumen microbial community structure and rumen tissue development, which subsequently impacts feed utilization efficiency [4-5]. Additionally, as lambs grow older, their feed intake changes, and numerous external microbes colonize the rumen, gradually enriching the rumen microbial community. The ability to degrade and utilize cellulose marks the establishment of rumination function in lambs, which is associated with the colonization of cellulolytic bacteria in the rumen. Therefore, this study selected several major cellulolytic bacteria including *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens*, along with one hemicellulolytic bacterium *Treponema bryantii*, as target organisms to investigate dynamic changes in rumen microbes. Given the importance of dietary starch for lamb growth and development, one major amylolytic bacterium *Streptococcus bovis* was also included in this study. Denaturing gradient gel electrophoresis (DGGE) technology offers advantages including high reliability, reproducibility, and convenience

[6], and has been widely applied in microbial community diversity analysis. Using real-time quantitative PCR and DGGE techniques, this study examined the effects of weaning age on rumen microbial diversity in lambs while simultaneously monitoring microbial dynamics from birth to 84 days of age, providing fundamental data for clarifying the impact of weaning age on rumen microbial community establishment.

Materials and Methods

Experimental Animals and Design

Sixty-six male Hu lambs with similar birth weights [(3.51±0.57) kg] and good health status were selected. Six lambs were slaughtered at each of 1, 14, and 28 days of age. The remaining 48 lambs were randomly assigned to two groups (28-day weaning group and 56-day weaning group) according to similar body weight principles. All experimental animals were fed starter from 7 days of age and transitioned to grower pellets at 59 days of age over a 10-day adaptation period. Six lambs from each weaning group were slaughtered at 42, 56, 70, and 84 days of age. All experimental lambs were purchased from Jinchuan Zhongtian Sheep Industry Co., Ltd. in Gansu Province. The experimental period spanned from 1 to 84 days of age.

Sample Collection

After slaughter, rumen contents were removed and homogenized using a blender. All contents were filtered through four layers of cheesecloth to obtain rumen fluid, which was then aliquoted into cryovials and preserved in a liquid nitrogen tank. Samples were stored at -80°C in the laboratory until analysis.

PCR Analysis

Total microbial DNA was extracted using a Stool DNA Kit (Stool DNA Kit 50, OMEGA, USA) following the manufacturer' s instructions. Primers were synthesized by Shanghai Sangon Biotech Co., Ltd. based on references by Firkins et al. [7], Khafipour et al. [8], and Ley et al. [9], with primer sequences listed in Table 1 .

Real-time quantitative PCR was performed using a Bio-Rad CFX 96 Real-Time PCR Detection System (Bio-Rad, USA). A 20 µL reaction system was established using SYBR Premix Ex TaqTM (Beijing TransGen Biotech Co., Ltd.), containing 10 µL SYBR Green I fluorescent dye, 0.4 µL each of forward and reverse primers, 2 µL total DNA template, and 7.2 µL double-distilled deionized water. Reaction conditions were: pre-denaturation at 95°C for 10 min; 40 cycles of denaturation at 95°C for 15 s, annealing at respective temperatures for 30 s, and extension at 72°C for 30 s with fluorescence signal acquisition; followed by melting curve analysis according to instrument instructions.

PCR amplification of the rumen microbial 16S rDNA V3 region was performed in a 50 µL system containing: 2 µL total DNA template (approximately 100 ng), 0.6 µL each of GC-338f and 533r primers, 5 µL buffer, 4 µL dNTPs, 0.8 µL Taq polymerase (TIANGEN Taq Polymerase), and 37 µL double-distilled deionized water. The GC-338f primer sequence was 5 -CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACGGGAGGCAGCAG-3 (GC clamp underlined), and the 533r primer sequence was 5 -TTACCGCGGCTGCTGGCAC-3 . The PCR program was: 94°C for 5 min; 10 cycles of 94°C for 30 s, 60°C for 20 s (decreasing 0.5°C per cycle), and 72°C for 30 s; 25 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s; final extension at 72°C for 30 s.

The relative abundance of target microorganisms was calculated using the following formula:
Microbial relative abundance (%) = 2^-(Ct_target - Ct_total bacteria), where Ct_target is the cycle threshold value obtained with target bacteria primers and Ct_total bacteria is the cycle threshold value obtained with general bacterial primers.

DGGE Analysis

DGGE analysis was performed according to the method described by Zhang Ning [10].

Cluster Analysis

UPGAMA cluster analysis was conducted according to the method described by Zhou Yiyi [11].

Statistical Analysis

DGGE profiles were processed using Quantity-One software (Bio-Rad) to compare similarities among samples in each lane and perform UPGAMA cluster analysis [11]. The software was also used to calculate Shannon-Wiener diversity indices from DGGE profiles. Data were analyzed using SPSS 23.0 software for one-way ANOVA, with significance level set at P<0.05.

Results and Analysis

Total DNA extraction was verified using 2% agarose gel electrophoresis, and gel imaging analysis confirmed successful extraction of microbial total DNA. Using the extracted microbial total DNA as template, the 16S rDNA V3 region fragment was successfully amplified (Figure 1 [Figure 1: see original paper]).

The effects of weaning age on the relative abundance of *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Streptococcus bovis*, *Fibrobacter succinogenes*, and *Treponema bryantii* are shown in Figure 2 [Figure 2: see original paper]. Weaning age had no significant effect on the relative abundance of these bacteria (P>0.05). The relative abundance of *Butyrivibrio fibrisolvens* in the 28-day weaning group showed a pattern of being low at 28 days, peaking at 42 days, and then gradually declining. In the 56-day weaning group, the relative abundance of *Butyrivibrio fibrisolvens* was low at 28 days, peaked at 42 days, then declined to its lowest point at 70 days, with a slight increase at 84 days; the value at 42 days was significantly higher than at 70 and 84 days (P<0.05), with no significant differences among other ages (P>0.05). At 70 days of age, the relative abundance of *Butyrivibrio fibrisolvens* in the 28-day weaning group was significantly higher than in the 56-day weaning group (P<0.05).

Data points within the same group marked with different lowercase letters indicate significant differences (P<0.05); data points at the same time point marked with * indicate significant differences between groups (P<0.05).

The DGGE profiles (Figures 3 [Figure 3: see original paper] and 4 [Figure 4: see original paper]) and cluster analysis (Figure 5 [Figure 5: see original paper]) are presented for representative samples only. DGGE profiles of rumen microbes in lambs weaned at 28 and 56 days of age are shown in Figure 3. DGGE profiles of rumen microbes in lambs from 1 to 84 days of age are shown in Figure 4, with cluster analysis results presented in Figure 5. The results indicate that similarity among different lambs at the same time point gradually increased with age, with the greatest fluctuations occurring during 1-14 days and 56-70 days of age.

Shannon-Wiener diversity indices calculated from DGGE profiles are presented in Table 2 . The results showed that at 42 days of age, the diversity index of the 28-day weaning group was significantly higher than that of the 56-day weaning group (P<0.05), while at 70 days of age, no significant difference was observed between the two weaning groups (P>0.05). From the perspective of overall rumen microbial community development, diversity indices at 70 and 84 days of age were significantly higher than at all other ages (P<0.05), while the index at 1 day of age was significantly lower than at all other ages (P<0.05), with no significant differences among other ages (P>0.05).

Values in the same row with different lowercase letter superscripts indicate significant differences (P<0.05).

Discussion

In this study, the relative abundance of *Butyrivibrio fibrisolvens* in the 28-day weaning group was significantly higher than in the 56-day weaning group at 70 days of age, while age-related changes caused differences in the relative abundance of *Butyrivibrio fibrisolvens* in the 56-day weaning group. Jami et al. [12] used real-time quantitative PCR to examine changes in bacterial populations in the rumen of dairy calves from birth to adulthood, finding that the number of *Butyrivibrio fibrisolvens* decreased after weaning. Another study from our research group showed that feed intake increased after weaning in the 56-day weaning group (569.03 g/d vs. 333.83 g/d) [13]. The difference in *Butyrivibrio fibrisolvens* abundance between the 28-day and 56-day weaning groups at

70 days of age may be attributed to increased feed intake leading to decreased rumen pH, which is unfavorable for *Butyrivibrio fibrisolvens* growth.

Chai Jianmin [14] investigated three early weaning treatments at 10, 20, and 30 days of age in lambs and found that, compared with 60-day weaning, early weaning significantly affected Shannon and Simpson indices of rumen microbes, indicating that weaning has a substantial impact on rumen microbial communities. Han Xufeng [15] fed Shaanbei white cashmere goats with three diets differing in concentrate-to-forage ratios (30:70, 50:50, and 70:30) and found that as the dietary concentrate ratio increased, several functional groups of rumen bacteria changed, with cellulolytic bacteria showing a significant decreasing trend. Jami et al. [12] examined rumen microbes in Israeli Holstein cattle at five different age stages from birth to 24 months, revealing significant changes in both diversity and quantity with age.

At 42 days of age, differences in band number and intensity between the 28-day and 56-day weaning groups were apparent, likely because the 28-day weaning group increased starter intake after weaning. Since microbial community abundance on different feed particles is generally similar [16], starter consumption accelerated microbial colonization in the rumen. At 70 days of age, the 56-day weaning group showed higher band numbers and intensity than the 28-day weaning group, as starter intake increased after weaning in the 56-day group, leading to accelerated microbial colonization. Chen [17] found that when calf diets were switched to high-grain diets with relatively high starch levels, rumen microbial community diversity decreased. Additionally, according to studies by Khafipour et al. [8], Tajima et al. [18], and Zhang Hongtao [19], increased dietary neutral detergent fiber (NDF) levels enhanced both species richness and diversity of rumen microbial communities. In this study, when switching from starter to grower pellets, starch level decreased from 38.81% to 28.93% while NDF level increased from 18% to 22%, which may explain the greater microbial species and abundance in the 56-day weaning group.

As lambs aged, solid feed intake increased, causing changes in rumen microbial communities [20]. DGGE profiles at 14 days of age showed more bands and greater intensity than at 7 days of age, likely because supplementation with solid starter at 7 days of age, although consumed in small amounts by young lambs, allowed microbial colonization through feed licking [21]. DGGE profiles at 70 days of age showed more bands than at 56 days of age. Given that total volatile fatty acid concentration increased significantly from 56 to 70 days of age (102.31 mmol/L vs. 96.72 mmol/L) while rumen pH also tended to increase [22], this suggests that after switching from starter to grower pellets, rumen microbial fermentation of feed proceeded rapidly. Increased fermentation substrates favored microbial proliferation, and relatively higher pH was also beneficial for cellulolytic bacteria growth. Therefore, this phenomenon may be caused by the combined effects of increased starter intake after weaning and changes in solid feed composition.

Bomba et al. [23] compared rumen microbial development in calves weaned at

7 weeks versus 9 weeks of age and found that early starter supplementation resulted in similar rumen microbial development between 7-week and 9-week-old calves. Our study also implemented early supplementation when weaning at 28 days of age, and our results are consistent with those of Bomba et al. [23]. Yáñez-Ruiz et al. [24] reported that newborn ruminants harbor minimal or no microbes in the rumen, with microbial colonization occurring gradually through environmental contact. In this study, within-group similarity increased with age, consistent with the findings of Rey et al. [25]. As ruminants age, rumen microbial communities gradually stabilize and reach a dynamic equilibrium, indicating that the influence of diet-introduced microbes on rumen microbial communities diminishes over time. Among the age periods showing increasing within-group similarity from 1 to 84 days of age, the greatest fluctuations occurred during 1-14 days and 56-70 days of age. In this study, starter supplementation at 7 days of age and weaning at 56 days of age altered nutrient intake composition, thereby causing changes in rumen microbial communities.

Bomba et al. [23] found that rumen microbial diversity indices increased significantly after weaning in calves. In this study, the 28-day weaning group had already been weaned at 42 days of age, while the 56-day weaning group had not, resulting in a significantly higher diversity index in the 28-day weaning group at 42 days of age. These results indicate that starter supplementation at 7 days of age and weaning at 56 days of age substantially affected rumen microbial communities. Jami et al. [12] found that altering dietary nutrient composition in ruminants could directly affect rumen microbial community structure. The supplementation and weaning treatments in this study indirectly altered dietary nutrient composition.

Conclusions

1. Under conditions of starter feeding from 7 days of age, lambs weaned at 28 days of age exhibited greater rumen microbial diversity at 42 days of age compared to lambs weaned at 56 days of age.
2. Rumen microbial diversity in lambs gradually increased with age, showing a significant increase at 14 days of age (after starter supplementation from 7 days) compared to 1 day of age, and a significant increase at 70 days of age compared to pre-weaning (56 days of age).

References

[1] Feng Yanglian. Ruminant Nutrition [M]. Beijing: Science Press, 2006.
[2] Dan Ruifang, Zhang Haitao, Long Ruijun, et al. Review of research methods for rumen microbial ecology [J]. Pratacultural Science, 2007, 24(7): 77-82.
[3] De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa [J]. Proceedings of the National Academy of Sciences of the United States of America, 2010, 107(33): 14691-14696.

[4] Chen Hao, Liu Ting, Wu Jianping, et al. Effects of oregano essential oil on growth and blood immunity of newborn calves [J]. Pratacultural Science, 2017, 34(10): 2141-2148.
[5] Luo Dian, Chai Linrong, Chang Shenghua, et al. Management and optimization of yak grazing systems on the Qinghai-Tibet Plateau [J]. Pratacultural Science, 2017, 34(4): 881-891.
[6] Gong Manli, Ren Nanqi, Xing Defeng. DGGE/TGGE technology and its application in microbial molecular ecology [J]. Acta Microbiologica Sinica, 2004, 44(6): 845-848.
[7] Firkins J L, Yu Z. Ruminant nutrition symposium: how to use data on the rumen microbiome to improve our understanding of ruminant nutrition [J]. Journal of Animal Science, 2015, 93(6): 1450-1470.
[8] Khafipour E, Li S C, Plaizier J C, et al. Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis [J]. Applied and Environmental Microbiology, 2009, 75(22): 7115-7124.
[9] Ley R E, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes [J]. Science, 2008, 320(5883): 1647-1649.
[10] Zhang Ning. Effects of weaning and supplementation time on muscle fatty acids and rumen microbial diversity in Hu lambs [D]. Master' s Thesis. Lanzhou: Lanzhou University, 2016.
[11] Zhou Yiyi. Study on the microbiological mechanism of tea saponin inhibiting methane production in Hu sheep [D]. Master' s Thesis. Hangzhou: Zhejiang University, 2009.
[12] Jami E, Israel A, Kotser A, et al. Exploring the bovine rumen bacterial community from birth to adulthood [J]. The ISME Journal, 2013, 7(6): 1069-1079.
[13] Ma Zhiyuan. Effects of early weaning on performance and gastrointestinal development in Hu lambs [D]. Master' s Thesis. Lanzhou: Lanzhou University, 2015.
[14] Chai Jianmin. Effects of weaning age on growth performance and gastrointestinal development in lambs [D]. Master' s Thesis. Beijing: Chinese Academy of Agricultural Sciences, 2015.
[15] Han Xufeng. Study on effects of age and dietary concentrate-to-forage ratio on rumen microbial community in Shaanbei white cashmere goats [D]. Doctoral Thesis. Yangling: Northwest A&F University, 2015.
[16] Martin C, Michalet-Doreau B. Variations in mass and enzyme activity of rumen microorganisms: effect of barley and buffer supplements [J]. Journal of the Science of Food and Agriculture, 1995, 67(3): 407-413.
[17] Chen Y H. Changes in ruminal bacterial communities of beef cattle during high concentrated transition experimental induced subacute acidosis [D]. Master' s Thesis. Edmonton: University of Alberta, 2011.
[18] Tajima K, Aminov R I, Nagamine T, et al. Diet-dependent shifts in the bacterial population of rumen revealed with real-time PCR [J]. Applied and Environmental Microbiology, 2001, 67(6): 2766-2774.
[19] Zhang Hongtao. Effects of different corn silage levels on rumen microbiome and metabolome in Holstein heifers [D]. Doctoral Thesis. Beijing: China Agri-

cultural University, 2017.
[20] Khan M A, Bach A, Weary D M, et al. Invited review: transitioning from milk to solid feed in dairy heifers [J]. Journal of Dairy Science, 2016, 99(2): 885-902.
[21] El-Shazly K, Hungate R E. Fermentation capacity as a measure of net growth of rumen microorganisms [J]. Applied Microbiology, 1965, 13: 62-93.
[22] Liu T, Li F D, Wang W M, et al. Effects of lamb early starter feeding on the expression of genes involved in volatile fatty acid transport and pH regulation in rumen tissue [J]. Animal Feed Science and Technology, 2016, 217: 27-35.
[23] Bomba A, Kmet V, Koniarová I, et al. Production of volatile fatty acids in the rumen calves during dietary-microbial stimulation early weaning [J]. Veterinární Medicina, 1989, 34(3): 141-148.
[24] Yáñez-Ruiz D R, Abecia L, Newbold C J. Manipulating rumen microbiome and fermentation through interventions during early life: a review [J]. Frontiers in Microbiology, 2015, 6: 1133.
[25] Rey M, Enjalbert F, Combes S, et al. Establishment of ruminal bacterial community in dairy calves from birth to weaning: sequential microbial development [J]. Journal of Applied Microbiology, 2014, 116(2): 245-257.