Postprint: Developmental Changes in Ileal Microbiota Colonization and Digestive Function in Goat Kids

Authors: Yan Qiongxian, Tan Zhiliang, Li Xiaopeng, Jiao Jinzhen

Date: 2017-10-10T00:00:00+00:00

Abstract

This study aimed to investigate the developmental changes of ileal bacterial colonization and digestive function in goat lambs. Forty-four Xiangdong black goat lambs were used, with 4 randomly selected lambs slaughtered for sampling at 1, 7, and 14 days of age (non-ruminant stage), 28 and 42 days (transition stage), and 56 and 70 days of age (ruminant stage). Ileal digesta were collected to determine acetic acid content, amylase, cellulase, and xylanase activities, total bacterial count, and the percentages of selected functional bacteria (Prevotella, methanogens, fiber-degrading bacteria, and starch-degrading bacteria) relative to total bacteria. The results showed that acetic acid was detected in ileal digesta starting at 14 days of age, and its content increased significantly with age (P 0.05), with the fastest increase observed at 56-70 days of age; age significantly or very significantly increased cellulase and xylanase activities ($P<0.05$ or $P<0.01$); age very significantly increased total bacterial count and the percentages of methanogens (first detected at 7 days of age), Prevotella, and Ruminococcus relative to total bacteria ($P<0.01$); fiber-degrading bacteria (Fibrobacter succinogenes and Ruminococcus flavefaciens) and starch-degrading bacteria (Ruminobacter amylophilus) were both first detected at 28 days of age. In conclusion, the ileal fiber-degrading digestive capacity in goat lambs was established after day 28 and gradually improved thereafter, while large numbers of methanogens could be detected from day 7 and gradually stabilized.

Full Text

Developmental Changes in Bacterial Colonization and Digestive Function in the Ileum of Goat Kids

LI Xiaopeng[1],[2], JIAO Jinzhen[1],[2], YAN Qiaoxian[1], TAN Zhiliang[1]*

[1]Key Laboratory for Agro-Ecological Processes in Subtropical Regions, Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production, South-Central Experimental Station of Animal Nutrition and Feed Science, Ministry of Agriculture, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China

Abstract: This study investigated the developmental changes in bacterial colonization and digestive function in the ileum of goat kids. Forty-four Xiangdong black goat kids were randomly selected for sampling at 1, 7, and 14 days of age (non-ruminant stage), 28 and 42 days of age (transition stage), and 56 and 70 days of age (ruminant stage). Ileal chyme was collected to determine acetate content, activities of amylase, cellulase, and xylanase, total bacterial numbers, and the percentages of selected functional bacteria (Prevotella, methanogens, cellulolytic bacteria, and amylolytic bacteria) relative to total bacteria. The results showed that acetate was first detected in ileal chyme at 14 days of age, and its content increased significantly with age ($P < 0.05$), with the fastest growth observed between 56 and 70 days. Age significantly or highly significantly increased cellulase and xylanase activities ($P < 0.05$ or $P < 0.01$). Age also highly significantly increased total bacterial numbers and the percentages of Prevotella, *Prevotella ruminicola*, and methanogens (first detected at 7 days of age) relative to total bacteria ($P < 0.01$). Both cellulolytic bacteria (*Fibrobacter succinogenes* and *Ruminococcus flavefaciens*) and amylolytic bacteria (*Ruminobacter amylophilus*) were first detected at 28 days of age. In conclusion, the fiber-degrading capacity in the ileum of goat kids is established after 28 days of age and gradually improves thereafter. Substantial methanogen colonization can be detected as early as 7 days of age, after which it gradually stabilizes.

Keywords: bacterial colonization; digestive function establishment; ileum

Introduction

Ruminants possess unique physiological characteristics distinct from monogastric animals or other non-herbivores, harboring large microbial communities in their gastrointestinal tract that can digest cellulose and other substances indigestible to most animals. Through microbial biotransformation, these compounds are converted into animal protein (meat and milk) for human utilization [1]. The gastrointestinal microbiota of ruminants is not present at birth but is gradually established through colonization, survival, and reproduction as the animal contacts its mother and environment, with microbial communities adapting to and being selected by the digestive tract environment [2]. Typically, ruminants are in a non-ruminant stage from 1 to 20 days of age, a transition stage from 21 to 56 days, and enter the ruminant stage after 57 days. During this functional development, both gastrointestinal physiology and microbial communities undergo substantial transformations. Throughout this process, gut microorganisms secrete various functional enzymes to degrade and digest nutrients, progressively establishing and improving digestive, absorptive,

and metabolic functions [3-4]. Hungate [5] pioneered systematic research on the rumen microbial ecosystem in 1966, and over the subsequent half-century, increasing numbers of researchers have focused on rumen microbial digestive and metabolic functions. In contrast, the nutritional digestion, absorption, and metabolic functions of the abundant post-ruminal gut microorganisms in ruminants have not received adequate attention.

Based on this analysis, the present study selected Xiangdong black goats as experimental animals to reveal the developmental patterns of small intestinal bacterial community succession and nutritional digestive function establishment in ruminants by measuring key functional enzyme activities (amylase, xylanase, and cellulase), acetate content, and major functional bacterial populations in the ileum.

Materials and Methods

Experimental Animals and Design

Sixty multiparous Xiangdong black goat does with good body condition, similar age [(2.0 ± 0.3) years], and similar body weight [(25.0 ± 1.0) kg] were selected. Following previously established protocols [6], estrus synchronization was performed, and ultrasonic examination was conducted on day 60 of gestation. Forty-four pregnant does carrying single fetuses were selected as experimental subjects and housed individually in well-ventilated pens with suitable temperature [(24 ± 1) °C] and natural lighting. Kids were numbered and weighed at birth.

Feeding and Management

All kids (n = 44) were fed exclusively with doe milk for the first 20 days postpartum, receiving 1 L of goat milk per day divided into two feedings (08:00 and 17:00). Four kids were randomly selected for slaughter at each of 1, 7, and 14 days of age. The remaining kids were gradually weaned between 21 and 40 days of age and supplemented with starter feed and forage, receiving 0.5 L of goat milk, 0.12 kg of starter feed, and 0.04 kg of fresh forage per day. From 41 to 70 days of age, each kid received 0.17 kg of starter feed and 0.06 kg of forage daily. Four kids were randomly selected for slaughter at 28, 42, 56, and 70 days of age.

Milk and Starter Feed Composition

Every 100 g of goat milk contained 12.9 g total solids, 3.7 g crude fat, 3.5 g crude protein, 2.9 g casein, 4.5 g lactose, and 0.8 g crude ash, with calcium content of 134 mg/L and gross energy of 2.72 kJ/L. The starter feed composition is shown in Table 1 .

Table 1 Starter feed composition (DM basis), %

Items	Content
Corn flour	
Fat powder	
Whey powder	
Milk powder	
Soybean meal	
Fish meal	
$CaHPO_4$	
$CaCO_3$	
NaCl	
Premix	
Total	

One kilogram of premix contained: $MgSO_4 \cdot H_2O$ 119.0 g, $FeSO_4 \cdot 7H_2O$ 2.5 g, $CuSO_4 \cdot 5H_2O$ 0.8 g, $MnSO_4 \cdot H_2O$ 3.0 g, $ZnSO_4 \cdot H_2O$ 5.0 g, Na_2SeO_3 10.0 mg, KI 40.0 mg, $CoCl_2 \cdot 6H_2O$ 30.0 mg, VA 95,000 IU, VD 17,500 IU, VE 18,000 IU.

Sample Collection

After slaughter, the entire intestinal tract was immediately placed in CO_2-filled plastic bags and transferred to the laboratory within 30 minutes under anaerobic conditions. All ileal chyme was weighed (approximately 5 g per kid at 1, 7, and 14 days of age; approximately 15 g per kid at 28, 42, 56, and 70 days of age). Two grams of chyme were stored at -80 °C for DNA extraction and bacterial quantification. Three grams of chyme were mixed with 1 mL of 25% metaphosphoric acid and 6 mL of water, centrifuged (17,000 × g, 4 °C, 10 min), and the supernatant stored at -20 °C for acetate determination [7]. Four grams of chyme from kids at 28, 42, 56, and 70 days of age were pre-warmed at 39 °C, diluted 1:3 with sterile 0.1 mol/L citrate-phosphate buffer (pH 6.6) under anaerobic conditions, and stored at -20 °C for enzyme activity analysis.

Determination of Digestive Enzyme Activities and Acetate Content

Ileal chyme acetate content was determined by gas chromatography (7890A, Agilent) according to the method of García-González et al. [8]. For enzyme activity assays, samples were thawed and bacterial cells were disrupted by ultrasonication under anaerobic conditions, followed by centrifugation (15,000 × g, 4 °C, 20 min). The supernatant was used as the enzyme solution. For amylase, cellulase (CMCase), and xylanase, 0.5 mL of enzyme solution, 0.5 mL of 0.1 mol/L citrate-phosphate buffer (pH = 6.6), and 1.0 g of corresponding substrate (starch, carboxymethyl cellulose, and xylan, all from Sigma) dissolved in 100 mL of water were mixed and incubated at 39 °C for 15, 30, and 15 min, respectively. Two blank controls (substrate only or enzyme solution only) were

included to correct results. One unit of enzyme activity was defined as the amount of enzyme producing 1 mol/L of reducing sugar per minute.

Quantification of Functional Bacteria

Selected bacteria included: total bacteria (general bacteria, GB); Prevotella genus, including *Prevotella* (Pre) and *Prevotella ruminicola* (Pr), which are typical protein-degrading bacteria and also starch-degrading bacteria; two cellulolytic bacteria (Cb), *Fibrobacter succinogenes* (Fs) and *Ruminococcus flavefaciens* (Rf); two amylolytic bacteria (Ab), *Selenomonas ruminantium* (SELE) and *Ruminobacter amylophilus* (RAMY); and methanogens (Met), which are evolutionarily distinct microorganisms (archaea) different from bacteria. Known rumen archaea are strictly anaerobic methanogens, most of which are hydrogenotrophic and can utilize hydrogen and CO_2 released during carbohydrate degradation to produce methane [9]. These bacteria were selected because they play important roles in nutrient digestion. Bacteria-specific primers for real-time quantitative PCR (qPCR) are listed in Table 2 .

Table 2 Primers of bacteria used for real-time quantitative PCR

Primer names	Primer sequences (5 -3)	Product size/bp	Amplification efficiency/%	Reference
Met-F	GGATTAGATACCCGGTAGT			Hook et al. [10]
Met-R	GTTGATCCAATTAAACCGCA			
GB-F	CGGCAACGAGCGCAACCC			Denman et al. [11]
GB-R	CCATTGTAGCACGTGTGTAGCC			
Pre-F	GGTTCTGAGAGGAAGGTCCCC			Stevenson et al. [12]
Pre-R	TCCTGCACGCTACTTGGCTG			
Pr-F	GAAAGTCGGATTAATGCTCTATGTTG			Stevenson et al. [12]
Pr-R	CATCCTATAGCGGTAAACCTTTGG			
Fs-F	GTTCGGAATTACTGGGCGTAAA			Denman et al. [11]
Fs-R	CGCCTGCCCCTGAACTATC			
Rf-F	CGAACGGAGATAATTTGAGTTTACTTAGG			Stevenson et al. [12]
Rf-R	CGGTCTCTGTATGTTATGAGGTATTACC			

Primer names	Primer sequences (5 -3)	Product size/bp	Amplification efficiency/%	Reference
SELE-F	CAATAAGCATTCCGCCTGGG			Stevenson et al. [12]
SELE-R	TTCACTCAATGTCAAGCCCTGG			
RAMY-F	CTGGGGAGCTGCCTGAATG			Stevenson et al. [12]
RAMY-R	CTGGGGAGCTGCCTGAATG			

Total DNA Extraction

Total bacterial DNA from ileal chyme was extracted using the QIAamp DNA Stool Mini Kit (Qiagen). Approximately 0.2 g of chyme sample was processed according to the manufacturer' s instructions, with one modification: after adding ASL buffer, samples were incubated at 95 °C for 10 min to ensure lysis of both Gram-negative and Gram-positive bacterial cell walls. Extracted DNA was dissolved in 50 L of sterile nuclease-free water, and nucleic acid concentration (ng/ L) and purity (OD_{260} nm/OD_{280} nm) were measured using a NanoDrop ND-100 spectrophotometer. DNA was stored at -20 °C until use.

Real-Time Quantitative PCR Analysis

Target Fragment Amplification PCR was performed using total ileal bacterial DNA as template. Reaction systems and conditions followed those described by Jiao et al. [9]. After PCR, 20 L of product was analyzed by 2% agarose gel electrophoresis.

Cloning of Amplification Products PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System according to the manufacturer' s protocol. Purified products were ligated into pMD®18-T Vector and transformed into 50 L of *E. coli* competent cells. Transformed cells were incubated in 950 L of pre-warmed SOC medium at 37 °C and 180 r/min for 1.5 h. Then, 50 L of culture was plated onto LB agar plates containing ampicillin (Amp), isopropyl β-D-1-thiogalactopyranoside (IPTG), and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), and incubated at 37 °C for 12–16 h until colonies appeared. Single white colonies were selected for culture and colony PCR identification. Positive clones were sequenced by Sangon Biotech (Shanghai) Co., Ltd. Correct plasmid DNA was extracted from verified clones using the TaKaRa MiniBEST Plasmid Purification Kit Ver. 3.0.

Standard Curve Construction and Sample Detection Positive plasmid clones for each bacterial group were quantified using a NanoDrop ND-100 spectrophotometer. Copy numbers were calculated based on molecular mass and

concentration to prepare standards. Known copy number plasmid standards were serially diluted 10-fold, and 5–8 dilution gradients were used as templates for PCR.

Real-time qPCR conditions were identical for standard plasmids and samples, using a 10 L reaction system: 5 L SYBR Green I premix, 0.2 L ROX, 0.2 L each of forward and reverse primers (10 mol/mL), 1 L plasmid DNA template or sample DNA template (diluted to ~10 ng/ L), 0.05 L bovine serum albumin (BSA), and 3.35 L sterile deionized water. Cycling conditions: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 60 °C for 30 s (with fluorescence acquisition); and a melting curve from 60 °C to 95 °C.

Bacterial Quantity Calculation Cycle threshold (Ct) values from qPCR were converted to log values using corresponding standard curves. Bacterial numbers [log (cells/g)] were calculated according to the formula in reference [13], and functional bacterial percentages relative to total bacteria were computed.

Statistical Analysis

Data were analyzed using SPSS 21.0 software for one-way ANOVA and bivariate correlations. Results are expressed as means ± standard deviation, with $P < 0.05$ considered statistically significant.

Results

Acetate Content

Since acetate was not detected in ileal chyme at 1 and 7 days of age, Table 3 only shows acetate content at 14, 28, 42, 56, and 70 days of age. Age had a significant effect on ileal acetate content ($P = 0.045$), with values at 28, 42, and 56 days significantly higher than at 14 days ($P < 0.05$) but significantly lower than at 70 days ($P < 0.05$).

Table 3 Acetate concentration in ileal chymus of lambs at different days of age, %

Item	Days of age	P-value
Acetate	1.97 ± 0.13 , 3.8 ± 3.17 , 3.97 ± 2.77 , 3.50 ± 1.95 , 7.61 ± 2.38	0.045

Values in the same row with different letter superscripts differ significantly ($P < 0.05$), while those with the same or no superscripts do not differ significantly ($P > 0.05$). The same applies below.

Digestive Enzyme Activities

Due to insufficient ileal chyme samples at 1, 7, and 14 days of age, enzyme activities could not be measured. Therefore, Table 4 only shows enzyme activities at 28, 42, 56, and 70 days of age. Amylase activity showed fluctuating increases with age but did not change significantly ($P = 0.118$). Age significantly or highly significantly affected cellulase ($P = 0.030$) and xylanase activities ($P = 0.001$), with values at 42, 56, and 70 days significantly higher than at 28 days ($P < 0.05$).

Table 4 Digestive enzyme activities in ileal chymus of lambs at different days of age, U/g

Items	Days of age	P-value
Amylase	0.73 ± 0.05, 1.17 ± 0.45, 0.75 ± 0.14, 0.78 ± 0.25	0.118
CMCase	0.028 ± 0.007 , 0.076 ± 0.036 , 0.080 ± 0.010 , 0.075 ± 0.028	0.030
Xylanase	0.089 ± 0.007 , 0.240 ± 0.093 , 0.240 ± 0.036 , 0.330 ± 0.066	0.001

Bacterial Numbers

As shown in Table 5 , age had a highly significant effect on total bacterial numbers in ileal chyme ($P < 0.01$). Total bacteria at 7, 14, 28, and 42 days were significantly higher than at 1 day ($P < 0.05$) but significantly lower than at 56 and 70 days ($P < 0.05$). Age also highly significantly affected the percentages of *Prevotella* ($P < 0.01$), *Prevotella ruminicola* ($P < 0.01$), methanogens ($P < 0.01$), *Fibrobacter succinogenes* ($P = 0.006$), *Ruminococcus flavefaciens* ($P = 0.002$), and *Ruminobacter amylophilus* ($P = 0.001$) relative to total bacteria, but did not significantly affect *Selenomonas ruminantium* percentage ($P = 0.714$). With increasing age, the percentages of *Prevotella*, *P. ruminicola*, methanogens, and *F. succinogenes* showed gradual increasing trends, while *R. flavefaciens*, *R. amylophilus*, and *S. ruminantium* did not follow similar patterns. Peak percentages of *R. flavefaciens* and *R. amylophilus* occurred at 42 and 28 days, respectively. The dominant bacteria in goat kid ileal chyme were *Prevotella*, *P. ruminicola*, methanogens, and *R. amylophilus*, while *F. succinogenes* and *R. flavefaciens* were relatively less abundant, and *S. ruminantium* was the least abundant.

Table 5 Total bacterial numbers and functional bacterial percentages in ileal chymus of lambs at different days of age

Items	Days of age	P-value
GB/[lg(cells/g)]	5.59 ± 0.28 , 8.22 ± 0.34 , 8.30 ± 0.25 , 7.96 ± 0.62 , 8.23 ± 0.44 , 10.01 ± 0.93 , 9.73 ± 0.38	< 0.001
Pre/%	0.27, 4.92 ± 2.94 , 19.91 ± 10.95 , 0.36 ± 0.15, 1.85 ± 0.87 , 5.26 ± 0.064 , 9.75 ± 6.94 , 1.01 ± 0.56	< 0.001
Pre	5.74 ± 0.22 , 11.24 ± 10.05 , 0.82 ± 1.09 , 5.96 ± 0.48 , 8.55 ± 3.04 , 1.86 ± 1.35 , 6.55 ± 0.19 , 44.49 ± 19.37	< 0.001
Pr	55.28 ± 16.13 , 14.64 ± 8.43 , 7.14 ± 0.41 , 2.51 ± 1.76 , 7.22 ± 0.30 , 4.02	< 0.001
Met/%	0.16, < 0.001	
Cb/%	0.30 ± 0.32 , 0.87 ± 0.81 , 0.20 ± 0.25 , 14.11 ± 8.30 , 1.51 ± 0.46 , 1.52 ± 1.12	0.006
Fs	1.13 ± 0.76 , 0.62 ± 0.77 , 0.70 ± 0.63, 0.53 ± 0.37, 0.91 ± 0.49, 53.89 ± 26.50	0.002
Rf	0.67 ± 0.41, 4.95 ± 3.48 , 0.43 ± 0.43, 22.70 ± 12.04 , 0.53 ± 0.28, 3.42 ± 3.64	0.001
Ab/%		
SELE		
RAMY		

Correlation Analysis

Table 6 shows correlation coefficients between bacterial composition and digestive function indices, with absolute values > 0.40 considered significant. Acetate content was significantly positively correlated with percentages of *Prevotella*, *P. ruminicola*, and methanogens ($P < 0.05$). Amylase activity was significantly positively correlated only with *R. flavefaciens* percentage ($P < 0.05$). Cellulase activity was highly significantly positively correlated with methanogen percentage ($P < 0.01$) and significantly negatively correlated with *R. amylophilus* percentage ($P < 0.05$). Xylanase activity was significantly positively correlated with percentages of *Prevotella*, *P. ruminicola*, methanogens, and *F. succinogenes* ($P < 0.05$), but highly significantly negatively correlated with *R. amylophilus* percentage ($P < 0.01$).

Table 6 Correlation coefficients between bacterial community and digestive functional indexes in ileal chymus of lambs

Items	Acetate	Amylase	Cellulase	Xylanase
Pre	0.547*	0.620*	0.456*	0.594*
Pr	0.625**	-0.576*	0.601*	0.595*
SELE	0.750*	0.546*	-0.704**	
Met				
Fs				
Rf				
RAMY				

** meant extremely significant difference ($P < 0.01$), and * meant significant difference ($P < 0.05$).

Discussion

Development of Ileal Digestive Function After Birth in Goat Kids

Direct studies have confirmed that besides the rumen' s powerful capacity to degrade fibrous materials, the small intestine (including jejunum and ileum) of ruminants also possesses strong compensatory digestive ability for dietary fiber [14-15]. This suggests that the establishment and perfection of post-ruminal digestive capacity are directly related to the quantity and types of bacteria colonizing the hindgut, with the most direct indicators being volatile fatty acid (VFA) production capacity and enzyme activity. As ruminants age, various digestive enzymes (especially fiber-degrading enzymes) secreted by small intestinal bacteria enable feed materials (particularly fiber) entering the hindgut to be converted into VFAs, representing a gradual establishment and improvement of carbohydrate degradation capacity [3-4]. Studies have shown that strong fiber digestion also exists in the cecum and colon of ruminants [16-17]. Our results demonstrate that acetate content in goat kid ileal chyme increased gradually with age, accompanied by synchronous increases in cellulase and xylanase activities. Based on these data, we infer that small intestinal (ileal) bacteria began developing the capacity to degrade and digest fibrous materials from 14 days of age. Which bacteria play dominant roles will be discussed further below.

Zhang et al. [18] reported that ileal amylase activity increased gradually with lamb age, stabilizing at 3 months, likely due to increasing feed intake, changing food sources, and progressive gut development and bacterial evolution. However, under our experimental conditions, ileal amylase activity did not change significantly between 28 and 70 days of age, while cellulase and xylanase activities increased markedly. The possible reason is that the ileum is not the primary site for amylase secretion, while gradual weaning increased hindgut bacterial colonization, enhancing the response to fibrous materials (cellulose, hemicellulose, and xylan) entering the hindgut and consequently increasing cellulase and xylanase secretion capacity.

Bacterial Colonization in the Ileum After Birth

Although ileal bacterial types and numbers cannot compare with those in the rumen, our results show that ileal bacterial numbers reached 10^8–10^9 cells/g. Bacterial numbers in goat kid ileum increased by three orders of magnitude within the first week after birth, likely originating from maternal bacteria, milk, and the surrounding environment [19-20]. Generally, lambs lack rumination function and have immature rumen development during the first 3 weeks postpartum, making doe milk the primary nutrient source for hindgut bacterial colonization and survival [21]. In this study, Prevotella and amylolytic bacteria were among the earliest colonizers in the ileum after birth, and measurable acetate was detected in ileal chyme at 14 days of age because these bacteria participate in converting lactic acid, lactose, and other oligosaccharides entering the hindgut into VFAs [21]. Total bacterial numbers in goat kid ileal chyme peaked and remained relatively stable at 56 and 70 days of age, suggesting that hindgut bacterial colonization is essentially completed by approximately 2 months of age. Regarding functional bacteria, the percentages of *Prevotella*, *P. ruminicola*, and *F. succinogenes* increased significantly with age, coinciding with increased acetate content and cellulase and xylanase activities. Considering that *Prevotella* and *P. ruminicola* were dominant groups in ileal chyme, ileal fiber digestion capacity appears to depend on increased numbers of these two bacteria. Correlation analysis also confirmed that their percentages were significantly positively correlated with acetate content.

The peak numbers of *R. flavefaciens* and *R. amylophilus* at 42 and 28 days of age, respectively, may be related to dietary changes during gradual weaning and warrant further investigation. Additionally, at 42 days of age when complete weaning and diet change occurred, bacterial numbers indicated that weaning particularly caused dramatic changes in *R. flavefaciens* and *R. amylophilus* numbers compared with 28 and 56 days. Similar results were observed in the cecum and colon by Jiao et al. [9]. Although methanogens were not the most abundant bacteria in the ileum, they were second only to Prevotella and remained relatively stable from the first detection at 7 days of age, indicating that methanogen colonization in the ruminant hindgut may be essentially completed within the first week.

Conclusions

1. The capacity for fiber degradation and digestion in the goat ileum is not present at birth but is established gradually after 28 days of age along with colonization of functional bacteria such as *Prevotella* and *P. ruminicola*.
2. The earliest colonizing bacteria in the ileum are Prevotella, *Selenomonas ruminantium*, and methanogens, while cellulolytic bacteria including *F. succinogenes* and *R. flavefaciens* begin colonizing at 28 days of age.
3. Methanogens are abundant not only in the rumen but also in the ileum, where their numbers remain stable regardless of dietary changes.

References

[1] LIU Minxiong. Ruminant Digestive Physiology [M]. Beijing: Beijing Agricultural University Press, 1991: 12.
[2] FENG Yanglian. Ruminant Nutrition [M]. Beijing: Science Press, 2004.
[3] KRAUSE D O, NAGARAJA T G, WRIGHT A D G, et al. Board-invited review: rumen microbiology: leading in microbial ecology [J]. Journal of Animal Science, 2013, 91(1): 331–341.
[4] REY M, ENJALBERT F, MONTEILS V. Establishment of ruminal enzyme activities and fermentation capacity in dairy calves from birth through weaning [J]. Journal of Dairy Science, 2012, 95(3): 1500–1512.
[5] HUNGATE R E. The Rumen and Its Microbes [M]. New York: Academic Press, 1966.
[6] HE Z X, WU D Q, SUN Z H, et al. Protein or energy restriction during late gestation alters fetal growth and visceral organ mass: an evidence of intrauterine programming in goats [J]. Animal Reproduction Science, 2013, 137(3/4): 177–182.
[7] MAO S Y, HUO W J, ZHU W Y. Use of pyrosequencing to characterize the microbiota in the ileum of goats fed with increasing proportion dietary grain [J]. Current Microbiology, 2013, 67(3): 341–350.
[8] GARCÍA-GONZÁLEZ R, LÓPEZ S, FERNÁNDEZ M, et al. Screening the activity of plants and spices for decreasing ruminal methane production in vitro [J]. Animal Feed Science and Technology, 2008, 147(1/2/3): 36–52.
[9] JIAO Jinzhen, WANG Pengpeng, TANG Shaoxun, et al. Study on the quantitative distribution characteristics of important functional microorganisms in different gastrointestinal tract segments of Liuyang black goats [J]. Acta Veterinaria et Zootechnica Sinica, 2013, 44(10): 1590–1599.
[10] HOOK S E, NORTHWOOD K S, WRIGHT A D G, et al. Long-term monensin supplementation does not significantly affect the quantity or diversity of methanogens in the rumen of lactating dairy cows [J]. Applied and Environmental Microbiology, 2009, 75(2): 374–380.
[11] DENMAN S E, MCSWEENEY C S. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen [J]. FEMS Microbiology Ecology, 2006, 58(3): 572–582.
[12] STEVENSON D M, WEIMER P J. Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR [J]. Applied Microbiology and Biotechnology, 2007, 75(1): 165–174.
[13] ZHOU M, HERNANDEZ-SANABRIA E, GUAN L L. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies [J]. Applied and Environmental Microbiology, 2009, 75(20): 6524–6533.
[14] TAN Z L, LU D X, HU M, et al. Effect of dietary structural to nonstructural carbohydrate ratio on rumen degradability and digestibility of fiber fractions of wheat straw in sheep [J]. Asian-Australasian Journal of Animal Sciences, 2002, 15(11): 1591–1598.
[15] TAN Z L, LU D X, HU M, et al. Effects of dietary nitrogen sources on

fiber digestion and ruminal fluid characteristics in sheep fed wheat straw [J]. Asian-Australasian Journal of Animal Sciences, 2001, 14(10): 1374-1382.
[16] CUMMINGS J H. Short chain fatty acids in the human colon [J]. Gut, 1981, 22(9): 763-779.
[17] JIAO J Z, WANG P P, HE Z X, et al. In vitro evaluation on neutral detergent fiber and cellulose digestion by post-ruminal microorganisms in goats [J]. Journal of the Science of Food and Agriculture, 2014, 94(9): 1745-1752.
[18] ZHANG Yingjie, LIU Yueqin, SUN Hongxin, et al. Study on developmental patterns of pH and major digestive enzymes in lamb small intestine [J]. Acta Veterinaria et Zootechnica Sinica, 2005, 36(2): 149-152.
[19] MÄNDAR R, MIKELSAAR M. Transmission of mother' s microflora to the newborn at birth [J]. Biology of the Neonate, 1996, 69(1): 30-35.
[20] HUNT K M, FOSTER J A, FORNEY L J, et al. Characterization of the diversity and temporal stability of bacterial communities in human milk [J]. PLoS One, 2011, 6(6): e21313.
[21] ØRSKOV E R, BENZIE D, KAY R N B. The effects of feeding procedure on closure of the oesophageal groove in young sheep [J]. British Journal of Nutrition, 1970, 24(3): 785-795.
[22] FAUBLADIER C, JULLIAND V, DANEL J, et al. Bacterial carbohydrate-degrading capacity in foal faeces: changes from birth to pre-weaning and the impact of maternal supplementation with fermented feed products [J]. British Journal of Nutrition, 2013, 110(6): 1040-1052.

Note: Figure translations are in progress. See original paper for figures.