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Research Article

Shifts in Gut Microbe Population in Periparturient Goats

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Abstract

Many factors influence the gut microbiome. The gastrointestinal tract of goats is inhabited by diverse and complex microbial communities including bacteria, protozoa, fungi, archaea, and viruses. This study investigated the shifts in the bacterial community during the periparturient period. Fecal samples were collected from Five BoerXSpanish goats at 14 days and 7 days before and after parturition. Fecal DNA was isolated using the QIAamp (R) DNA isolation stool mini kit. The Nanodrop spectrophotometer was used to determine the concentration and purity of microbial DNA. Fecal samples were amplified using RT-PCR to determine the presence of total microbial DNA and relative abundance of *Bifidobacteria spp* and *Lactobacillus spp.* The housekeeping genes GAPDH and β-actin were used to normalize the data. Relative abundance was calculated using the Livak method were samples taken from 2 weeks before kidding served as the control group. *Bifidobacteria, Lactobacillus,* and 16S primers detected microbial DNA in fecal samples. There was an increase in *Bifidobacteria,* and *Lactobacillus* 7 days before kidding. Gut microbial diversity changes in periparturient goats.

Introduction

Shifts in ruminal bacteria are considered to be beneficial to feed efficiency during the peripartal period [1]. The periparturient period is defined as the period from 3 weeks prepartum to 3 weeks postpartum [2,3]. Immunomodulatory properties of Bifidobacterium and the mechanisms and molecular players underlying these processes have implications for animal health [4,5]. The gastrointestinal tract of goats is colonized by a complex microbial community. Diverse microbiota such as bacteria, archaea, protozoa, and fungi play a role in the host's nutrient uptake and energy metabolism in ruminants [6]. *Lactobacillus* is a gram-positive bacterial species inhabiting the gastrointestinal tract of vertebrates [7]. Although greatly outnumbered by anaerobic bacterial species in the intestinal tract, *lactobacilli* are often detected in fecal samples [8]. *Bifidobacterium* is among the first microbes to colonize the gastrointestinal tract and are believed to exert positive health benefits on their host [9]. Previous studies have shown their use as probiotics especially in dairy products [10,11].

Lactobacillus and *Bifidobacterium,* are commonly used as probiotics in functional foods and animal feed [12,13]. These species have been shown to protect against enteric infection [4]. The objective of this study was to evaluate the shifts in the bacterial community in goat feces during the periparturient period.

Materials and Methods

Animals and Housing

Five female BoerXSpanish goats were used from North Carolina Agricultural and Technical State University Farm according to the guiding principles for the Institutional Animal Care and Use Committee (IACUC ID: 15-006.0).

Collection of Samples

Fecal samples were collected and evaluated once a week throughout the experiment.

Isolation of Microbial DNA

The QIAamp (R) DNA isolation stool mini kit (QIAGEN Sciences, Maryland) was used to isolate DNA from fecal samples as recommended by the manufacturer. The concentration (260nm) and quality or purity (260/280nm) of the isolated DNA samples were determined using the Nanodrop Spectrophotometer 1000 3.7.1 (Thermo Scientific Inc., MA).

Amplification of Microbial DNA Using PCR

DNA isolated from the fecal samples was amplified using PCR to determine the presence of total microbial DNA and relative abundance of *Bifidobacteria spp* and *Lactobacillus spp.* The GAPDH gene was used as a housekeeping gene and for normalizing data. Specific primers for the amplification of variable regions of 16S rRNA gene for *Bifidobacteria spp* and *Lactobacillus spp* were used (Table 1).

PCR was done using the CFX Connect Real-time system (Bio-Rad Laboratories, Inc., USA) [14]. Amplification consisted of one cycle of 95°C (10 minutes), 40 cycles of denaturation at 95°C (15s) and annealing/extension at 60°C (1minute) [15,16] in the CFX Connect Real-time system (BIO-RAD Laboratories, Hercules, CA)

Table 1: *The sequence of bacteria primers used.*

Gene	Primers	Sequence 5'>3'	Source
Bifidobacterium spp	Forward Reverse	CGCGTCYGGTGTGAAAG CCCCACATCCAGCATCCA	[17]
Lactobacillus spp	Forward Reverse	GAGGCAGCAGTAGGGAATCTTC GGCCAGTTACTACCTCTATCCTTCTTC	[17]
Bacterial 16S rRNA	Forward Reverse	ACTCCTACGGGAGGCAGCA GGACTACHVGGTWTCTAAT	[18]
GAPDH	Forward Reverse	GTCTTCACCACCATGGAG CTCCATGGTGGTGAAGAC	[19]
β-actin	Forward Reverse	CCAGATCATGTTCGAGACTTTCAA TCCCCAGAGTCCATGACAATG	[20]

Results

DNA Concentration and Purity

The lowest concentration of DNA in goat fecal samples was isolated during the periods one week before and one week after kidding. The concentration of isolated DNA ranged from 9.8ng/µl to 51ng/µl. The highest concentration was observed 2-weeks before kidding. The lowest concentration was observed 1-week after kidding.

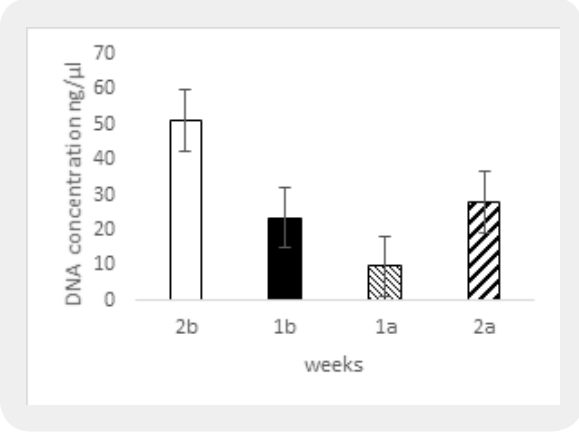

Figure 1: *DNA concentration during the periparturient period in goats. A – After, B – Before*

Microbial DNA

The relative abundance of total microbe (16S), *Bifidobacterium spp* and *Lactobacillus spp* changed during the periparturient period. The relative abundance was high for *Bifidobacterium spp* and *Lactobacillus spp* 7 days before kidding.

Table 2: *Relative abundance of* ***Bifidobacterium spp*** *and* ***Lactobacillus spp*** *over six (6) Weeks around the periparturient period*

	2B	1B	1A	2A
Bifidobacteria spp	1.0	3.00	1.87	2.11
Lactobacillus spp	1.0	55.14	46.69	0.41
16S	1.0	15.73	7.39	0.58

A - After, B - Before

Discussion

Understanding the shifts in bacterial communities in goats during the periparturient period is very important. The gastrointestinal tract is colonized by a diverse array of microflora which may be detrimental or beneficial to the host. *Bifidobacteria* and *lactobacillus* bacteria have been found in ruminant fecal samples and used as probiotics for improved production and have also been used to monitor food safety for mutton and other products [17]. In our study, both *Bifidobacteria* and *Lactobacillus* were present in goat feces, and their abundance varied during the periparturient period. Previous studies conducted by [14] reported the expression of both *Bifidobacteria* and *Lactobacillus* in sheep during the periparturient period. This result corroborates with the result from our study.

Our results also show an increased population of *Bifidobacteria* and *Lactobacillus* 7 days before kidding. It has been shown that oral administration of *Lactobacillus casei* activated immune cells of the innate immune response and increased the expression of innate immune receptor, TLR2 [21]. In ruminants, the probiotic *Lactobacillus rhamnosus* have been shown to amend *E. coli* induced inflammation in primary bovine mammary epithelial cells. Results from this study may suggest the need to study the role of shifts in *Bifidobacteria* and *lactobacillus* on host health and well-being during the peripartal period.

Conclusion

Both *Bifidobacteria* and *Lactobacillus* were present in goat feces, and their abundance varied during the periparturient period. Further studies are needed to determine the association to innate immunity during this period. Detection of fecal microbes in goats may be affected by the period of sampling.

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