Journal of Agricultural Science; Vol. 14, No. 4; 2022
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Molecular Detection of Age-Related Abundance and Diversity of *Bifidobacterium* spp. in Pastured Goats

Eunice N. Ndegwa[1] & Ako Agyemang[2]

[1] Agricultural Research Station, Virginia State University, Petersburg,VA, USA

[2] USDA Agricultural Research Services (ARS), Beltsville, MD, USA

Correspondence: Eunice N. Ndegwa, Agricultural Research Station, Virginia State University, 23806, Petersburg, VA, USA. Tel: 1-804-524-3264. E-mail: endegwa@vsu.edu

Received: August 24, 2021 Accepted: February 25, 2022 Online Published: March 15, 2022

doi:10.5539/jas.v14n4p51 URL: https://doi.org/10.5539/jas.v14n4p51

Abstract

Bifidobacterium spp. are among bacteria being developed as probiotics for farm animals. Understanding diversity of the normal gut inhabitants is a prerequisite to developing species-specific probiotics strains that are likely to establish in the host. For many farm animals including goats, the normal gut *Bifidobacterium* inhabitants have not been characterized including age based differences. The objective of this exploratory study was to gain preliminary understanding of abundance and diversity of *Bifidobacterium* species in goats of different ages. Molecular methods have given scientists fast methods for exploratory insights into previously uncharacterized microbiomes. Consequently, in this study we utilized molecular assays using genus and species specific primers to characterize *Bifidobacterium* in pastured goats. Although *Bifidobacterium* were detected in all ages, younger animals had higher counts than older goats. In goats below six months of age, *B. angulatum*, *B. dentium*, *B. gallicum*, *B. animalis* sub *animalis*, *B. longum* and *B. catenulatum* were detected. In goats six months and older, *B. dentium* and *B. gallicum* were predominant. In some goats however, the specific strains could not be identified with the currently available primers indicating there are goat specific unique strains that are yet to be characterized. Further research to characterize and isolate *Bifidobacterium* in goats are needed for future probiotic applications. In conclusion, *Bifidobacterium* spp. were common in all age groups of pastured goats but more abundant in pre-weaned goats compared to goats over six months. In addition age related differences in the diversity of *Bifidobacterium* spp. in goats were reported.

Keywords: abundance, *Bifidobacterium*, molecular detection, age-related, diversity, goats

1. Introduction

Among the complex microbial communities found in the mammalian gut, the significant role of *Bifidobacterium* species in host health is well described (Abatenh, Gizaw, Tsegay, Tefera, & Aynalem, 2018; Abdelhamid, El-Masry, & El-Dougdoug, 2019; Liu, Ren, Song, Wang, & Sun, 2015; Ross, Mills, Hill, Fitzgerald, & Stanton, 2010; Russell, Ross, Fitzgerald, & Stanton, 2011; Steele, Malmuthuge, & Guan, 2015; Zuo et al., 2016). *Bifidobacterium* species benefits are due to ability to produce several metabolites that are beneficial to the host. These benefits include, antitumor compounds, immune modulation, antimicrobial effects, ability to reduce cholesterol in humans and bioactive fatty acid (conjugated linoleic acid-CLA) production (Abatenh et al., 2018; Russell et al., 2011). This knowledge has led to development of human specific commercial probiotic supplements containing *Bifidobacterium* spp. (Guglielmetti, Mora, Gschwender, & Popp, 2011; Picard et al., 2005; Whorwell et al., 2006). However, little progress has been made on development of these species for livestock species. The abundance and diversity of *Bifidobacterium* species in human and a few animals has been described (Endo, Futagawa-Endo, & Dicks, 2010; Paliy et al., 2020; Ritchie, Burke, Garcia-Mazcorro, Steiner, & Suchodolski, 2010; Roger, Costabile, Holland, Hoyles, & McCartney, 2010; Vlková et al., 2010; Yang et al., 2019) and the results indicate inter and intra species differences. In one animal study, *Bifidobacterium* abundance and diversity differed comparing wild herbivores, carnivores and omnivores. *Bifidobacterium* were rare in herbivores while they were detected in all carnivores and most omnivores. In herbivores, *Bifidobacterium pseudolongum* was the only species detected (Endo et al., 2010). In another study in horses, *Bifidobacterium* were rarely detected while *Lactobacillus* species were common (Endo, Futagawa-Endo, & Dicks, 2009). One study in calves detected *Bifidobacterium animalis* ssp. *animalis*, *B. thermophilum* , *B. choerinum* and *B. longum*

ssp. s*uis* (Vlková et al., 2010). A few other studies have evaluated the *Bifodobacterium* in sheep and detected different strains in both young and adult sheep (Bunešová, Vlková, Killer, Rada, & Ročková, 2012; Lamendella, Santo Domingo, Kelty, & Oerther, 2008) while one study described detection of *Bifidobacterium* genus in jejunum of young goats (Zhang, Wu, Zhou, Tan, & Jiao, 2020). Very few studies have evaluated samples from goats and even then, these were single time-point studies (Lamendella et al., 2008) which yield minimal information and do not highlight age based differences which have been known to exist (Kato et al., 2017; Lim, Song, Kang, & Nam, 2019; Odamaki et al., 2016; Salazar et al., 2019). Development of *Bifidobacterium* species as probiotic for use in animals requires an initial understanding of the normal inhabitants in healthy animals of all age groups. In farm animals like goats, this information is lacking. We however hypothesize that as in other mammalian species, goats harbor *Bifidobacterium* species that are yet uncharacterized. Molecular based methods are ideal for preliminary studies to gain broad insight into diversity of microbiome (Endo et al., 2009) in understudied hosts like goats. Thus the objective of this study, was to evaluate the the presence, abundance and diversity of *Bifidobacterium* in fecal samples of different age groups of pastured goats using molecular assays applying *Bifidobacterium* genus and species-specific primers.

2. Materials and Methods

2.1 Experimental Animals and Husbandry

The experiment was carried out at Virginia State University, Petersburg, USA using animals reared under pasture at the institution's Randolph farm small ruminant paddocks. The goats age ranged from three days of age to over 4 years and all had been born and raised on the same farm. The goat herd is closed with breeding does and bucks that range between eighteen months and five years of age. Animals are born and raised on the farm until one year of age when replacement does and bucks are selected and the rest of animals disposed off through the local livestock market. Animals are typically raised on pasture that include naturally occurring annual grasses and shrubs and are rotated as needed. The goats kid on pasture in April, and are maintained on these pastures until around three months of age. Both does and nursing kids are reared together on pasture until June/July when the kids are routinely weaned at around three months of age. After weaning (three months), the goats (male and female separately) are moved to designated smaller paddocks for ease of health monitoring. While at these paddocks they are routinely supplemented with baled hay *ad lib* to meet their energy requirement. The goats are also supplemented with a corn soybean concentrate in shared feeders daily at 2% their weight. Afterwards at six months of age, the goats are moved to pasture and grazed alongside other adults in the herd in separate male and females paddocks.The fecal samples were collected from goats between April 2017 and April 2019. The study was approved by Virginia State University animal care and use committee and protocols were carried out with minimal discomfort to the animals.

2.2 Sample Collection

Fecal samples were collected rectally from healthy goats between April 2017 to April 2019 either with a wet cotton tipped swab in goat kids less than one month or lubricated glove from the rectum in older animals. Groups of goats sampled included goats between 3-7 days of age, one (1) month of age, two (2) months of age, six (6) months of age and goats older than one year. Pre-weaned goats (first week of life upto weaning day) were sampled alongside their nursing does (included as part of yearling and over). Other adults of breeding age were sampled on pasture before the breeding season (Oct/Nov). To evaluate if there are any changes around weaning at three months, goats were sampled on the day of weaning (0DPW), one day after weaning (1DPW), 2 days after weaning (2DPW) and then one week after weaning (7DPW). Goat kids with diarrhea between the age one month and seven days after weaning were sampled separately.

2.3 DNA Extraction

DNA extraction was carried out using a bead-beating column based protocol following the EZNA stool DNA extraction following the manufacturer's instructions (E.Z.N.A. ® Stool DNA Kit). Approximately 200mg of fecal samples or 200 mls of fecal solution (in young goat kids) was used for DNA extraction for all samples. DNA concentration and purity was determined by Nanodrop and samples were stored in -80 ^{o}C until further processing and analysis.

2.4 Detection of Bifidobacterium spp.

Two genus specific primers that have been widely applied for detection of *Bifidobacterium* were used in two different PCR protocols. One primer, the Lm26/lm3 (Kaufmann, Pfefferkorn, Teuber, & Meile, 1997) amplifies a fragment of approximately 1400 bp while the other primer, g-Bifid primers (Matsuki et al., 2002) amplifies a 550 bp fragment (Table 1). The amplitaq Gold 360 master mix was used following the manufacturers

recommendations in a 25 ul total volume and 200 ng of DNA. The PCR protocol for the Lm26/Lm3 primer included 35 cycles of initial denaturation 94 ^{o}C, 1minute, annealing 55 ^{o}C, 3minutes, extension 72 ^{o}C, 4minutes followed by 4 ^{o}C infinite hold while for the g-Bifid, the protocol included 35 cycles of denaturation 95 ^{o}C 30 sec, annealing 55 ^{o}C for 30 sec, extension 72 for 1 min and a final extension of 72 for 5 min. Amplified fragments were visualized on a 1% ethidium bromide stained gels on a Gel Imager (Applied Biosystems).

2.5 Bifidobacterium Quantification

Samples that were positive by either detection assay were further subjected to a SYBR green quantification QPCR protocol using primers targeting a conserved 16S region (241 base pair) in the *Bidodobacterium* spp. genus (Rinttilä, Kassinen, Malinen, Krogius, & Palva, 2004). Between 10-100 ng of DNA was used for the quantification assay following the Applied Bisosystems PowerUp™ SYBR™ Green Master Mix reaction set up recommendations in a 10ul total volume and primer specific annealing temperatures as shown in table 1. Melting curve analysis was used to confirm the specificity of the primers. A PCR product generated from DNA extracted from a mixture of *Bifidobacterium* strains (*B. longum*, *B. infantis*, *B. lactis*, and *B. breve*) from a commercial food grade supplement (Mood probiotics®) was used as standard curve for quantification. The minimum detection limit for the quantification assay was ten genome copies.

2.6 Evaluation of Diversity of Bifidobacterium Species

The diversity of *Bifidobacterium* spp. was determined by PCR using species specific primers listed in Table 1 (Matsuki, Watanabe, & Tanaka, 2003; Matsuki, Watanabe, Tanaka, Fukuda, & Oyaizu, 1999). All samples that were positive with the *Bifidobacterium* genus specific primers Lm26/lm3 and or g-Bifid were subjected to several PCR assays using previously described primers that target most *Bifidobacterium* that have been characterized. In absence of published primers for *Bifidobacterium* in other mammalian species except human, the primers used for this exploratory study are those recommended and used in human studies (Matsuki et al., 2003) and have also been used to study diversity in other animal studies (Hernández-Rodríguez, Vásquez-Aguilar, Serio-Silva, Rebollar, & Azaola-Espinosa, 2019; Lamendella et al., 2008) that included sheep, goats and cattle. Additionally, some *Bifidobacterium* strains are known to colonize the gut of diverse species of mammals. The annealing temperature for each of the primers are listed in Table 1. The PCR products were visualized in a 1.5% ethidium bromide stained gel depending on the size of the fragment.

Table 1. Primers used for detection and diversity evaluation of *Bifidobacterium* species in

Primer name	Sequence	Size	AT	Reference	Target gene
g-Bifid-F	CTCCTGGAAACGGGTGG	549-563	52	Matsuki et al., 2002; Matsuki et al., 2003	16s rRNA gene
g-Bifid-R	GGTGTTCTTCCCGATATCTACA				
Lm3	CGGGTGCT**Ic**CCCACTTTCATG	1400	57	Kaufmann et al., 1997; Matsuki et al., 2003	16s rRNA gene
Lm26	GATTCTGGCTCAGGATGAACG				
BiADO-1	CTCCAGTTGGATGCATGTC	279	55	Matsuki, Watanabe, Tanaka, & Oyaizu, 1998	16s rRNA gene
BiADO-2	CGAAGGCTTGCTCCCAGT				
BiBIF-1	CCACATGATCGCATGTGATTG	279	55	Matsuki et al., 1998	16s rRNA gene
BiBIF-2	CCGAAGGCTTGCTCCCAAA				
BiINF-1	TTCCAGTTGATCGCATGGTC	828	55	Ventura, Reniero, & Zink, 2001	16s rRNA gene
BiINF-2	GGAAACCCCATCTCTGGGAT				
Biani-F	GCTACAACTCAAAGCATTAC	550	47	Hong & Chen, 2007	16s-23s rRNA intergenic spacer
Biani-R	GTACTTCCGCCTCAGCGAT				
BiBRE-1	CCGGATGCTCCATCACAC	288	55	Matsuki et al., 1998	16s rRNA gene
BiBRE-2	ACAAAGTGCCTTGCTCCCT				
BiANG-1	CAGTCCATCGCATGGTGGT	275	55	Matsuki et al., 1998	16s rRNA gene
BiANG-2	GAAGGCTTGCTCCCCAAC				
BiCATg-1	CGGATGCTCCGACTCCT	285	55	Matsuki et al., 1998	16s rRNA gene
BiCATg-2	CGAAGGCTTGCTCCCGAT				
BiLON-1	TTCCAGTTGATCGCATGGTC	277	55	Matsuki et al., 1998	16s rRNA gene
BiLON-2	TCGCGCTTGCTCCCCGAT				
Bflac2	GTGGAGACACGGTTTCCC	680	50	Matsuki et al., 1999	16s rRNA gene
Bflac5	CACACCACACAATCCAATAC				
BiDEN-1	ATCCCGGGGGTTCGCCT	387	55	Matsuki et al., 1999	16s rRNA gene
BiDEN-2	GAAGGGCTTGCTCCCGA				
BiGAL-1	TAATACCGGATGTTCCGCTC	303	55	Matsuki et al., 1999	16s rRNA gene
BiGAL-2	ACATCCCCGAAAGGACGC				
Bifi F	TCGCGTC(C/T)GGTGTGAAAG	241		Rinttilä et al., 2004	16s rRNA gene
Bifi R	CCACATCCAGC(A/G)TCCAC				

2.7 Statistical Analysis

Abundance of *Bifidobacterium* spp. in log genomes/100 ng of DNA in all age groups were calculated. Pairwise comparison of mean log genomes between age groups was done using Students t test calculator, https://www.socscistatistics.com/tests/studentttest/default.aspx, 2021. A P value of < 0.05 was considered significant for all comparisons.

3. Results

3.1 Detection of Bifidobacterium spp. Using Genus Specific Primers in Pastured Goats

In total 236 individual animal fecal samples from different age groups and health status were evaluated over the two years for the presence, diversity and abundance of *Bifidobacterium* species using the universal primers LM26/lm3 and g-Bifid. *Bifidobacterium* were detected in all ages ranging from a few days after birth to adult healthy goats over one year (Table 2). *Bifidobacterium* were also detected in diarrheaic young animals below three months as well as all animals during the peri-weaning period. The two primers (Lm26/Lm3 and g-Bifid) performed similarly except in rare cases where one animal would not give a product with one of the primers. In the youngest age group (3-7 days), ten of the samples did not yield a product with either of the two primers. Interestingly the Lm26/lm3 primer gave multiple bands (data not shown) in addition to the 1400 bp expected fragment in goats (3-7 dys old) in contrast with other age groups where this primer gave a single PCR product approximately 1400 base pairs. This may indicate the presence of other *Bifidobacterium* like bacteria in young animals during the first one week.

Table 2. Detection of *Bifidobacterium* species using genus specific primers detected in feces of different age groups of pastured goats

Age/Animal group	Lm26/lm3	g-Bifid
3-7 days old (n = 46)	36	36
One month old (n = 8)	8	8
Two months (n = 20)	20	19
Weaning ODPW(n = 11)	11	11
1DPW (n = 11)	11	11
2DPW (n = 11)	10	11
7DPW (n = 11)	11	11
Diarrhea (1-3 months (n = 23))	22	23
6 Months (n = 29)	28	28
Yearling and over (n = 66)	66	64

3.2 Abundance of Bifidobacterium in Different Age Groups and Health Status

We compared the mean *Bifidobacterium* counts in goats of different age groups to evaluate if any differences existed. Figure 1 shows the relative abundance of *Bifidobacterium* species in different age groups of pastured goats. In general, the abundance of Bifidobacterium spp. was higher in animals less than three months compared to older animals (six months and above). There was a clear pattern where the highest count were detected in goats between 3-7 days of age (mean log = 2.73/100 ng DNA) while the lowest counts were detected in goat kids seven days after weaning (mean log = 0.17/100 ng DNA). Comparing the *Bifidobacterium* counts in pre-weaned goats, the decrease in counts from the first week of life ($M = 2.73$, $SD = 2.23$) to one month old goat kids ($M = 1.96$, $SD = 1.33$) was not significant ($t(51) = 0.95$, $p = 0.18$) while at two months old ($M = 1.58$, $SD = 1.97$) the decrease in counts was significant ($t(51) = 1.95$, $p = 0.03$). The mean counts detected on the day of weaning (ODPW) ($M = 1.55$, $SD = 3.24$) although lower than the first week of life ($M = 2.73$, $SD = 2.23$) were not significant ($t(54) = 1.41$, $p = 0.08$). However comparing the *Bifidobacterium* mean counts one day after weaning ($M = 1.08$, $SD = 1.46$) and one week after weaning ($M = 0.17$, $SD = 0.33$), the decrease in the counts was significant ($t(20) = 1.92$, $p = 0.03$). There was a slight increase in the counts between one week after weaning and at six month of age but this was not significant. In comparison, *Bifidobacterium* counts detected at first week of life and those detected at six months and over one year of age were also significantly different ($p < 0.05$). No significant change in counts was detected in goats older than one year compared to six months. We also compared the mean *Bifidobacterium* spp. counts between healthy and diarrhearic goat kids three months old and below. Although the combined mean of *Bifidobacterium* counts detected in healthy goats aged between one month-three months (data not shown) was higher ($M = 1.26$, $SD = 1.96$) than goats within the same age group but with diarrhea ($M = 0.93$, $SD = 0.99$), the difference was not significant ($t(83) = 0.56$, $p = 0.29$).

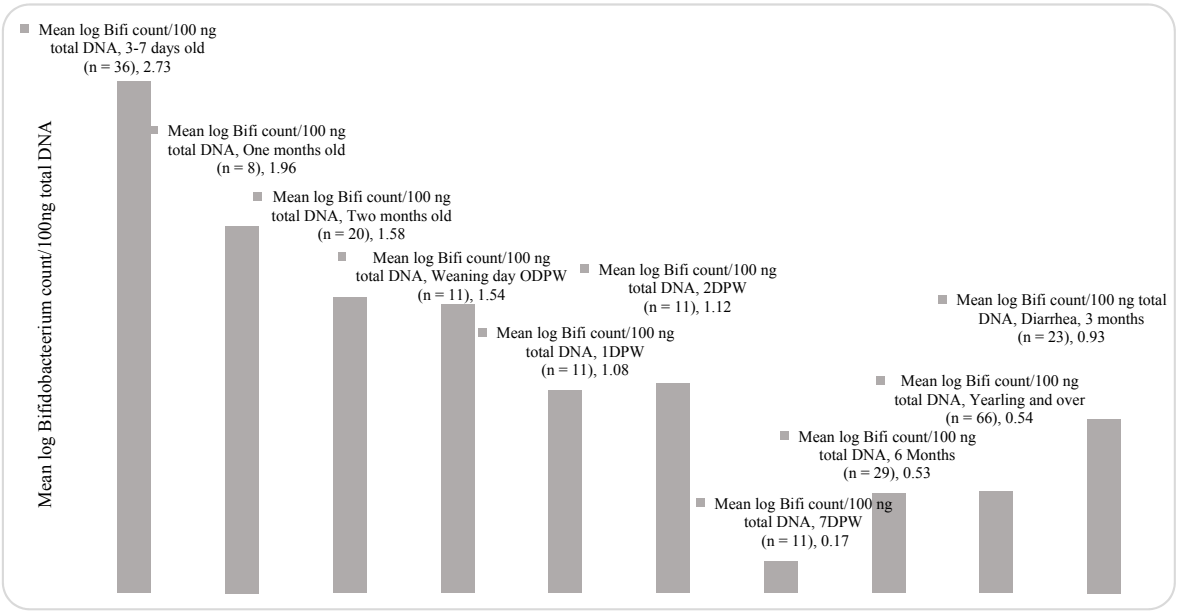

Figure 1. Relative abundance of *Bifidobacterium* spp. in fecal samples of pastured goats of different age groups. DPW = days post weaning

3.3 Bifidobacterium Species Diversity in Pastured Goats

Samples that produced a PCR product with either of the universal primers were subjected to multiple PCR assays with the species-specific primers listed in table 1. Overall, in goats six months and those over one year, 86% and 93% of the samples respectively had at least one species of *Bifidobacterium* amplified with the set of primers. However in goats three months and below, over 40% of the samples did not yield any product with the primers used in the study indicating the presence of unique yet to be characterized goat specific gut *Bifidobacterium* spp. (Table 3). Of the 11 primers used to evaluate the *Bifidobacterium* species diversity, seven primers gave the expected size PCR product for the following species (*B. angulatum*, *B. animalis*, *B. bifid*, *B. catenulatum*, *B. dentium*, *B. gallicum* and *B.longum*) in at least one sample. Two (*B. adolescentis* and *B. infantis*) did not yield any positive sample (Table 3) and two amplified products of a different size than expected (*B. lactis* and *B. breve*). The latter indicates that goats may be hosts to *B. lactis* and *B. breve* like species that are yet to be identified and sequenced that share some sequence similarities to those found in humans. While *B. angulatum*, *B. catenulatum,* and *B. biden* were detected in most age groups of goats, *B. gallicum* and *B. dentium* were predominantly detected in goats six months and older. On the other hand, *B. bifid* was only detected in goats during the first week of life; *B. animalis* in goats two months and below, while *B. longum* was detected in goats three months and below. In goats six months of age and those over one year, *B. gallicum* was detected in 52% of all the fecal samples in each respectively. *Bifidobacterium dentium w*as the most abundant in goats at six months of (68%) but was also frequently detected in goats over one year of age (38%).

Table 3. Results from species-specific *Bifidobacterium* PCR assays using fecal DNA from different age groups of goats

Total evaluated in each age group	Total positive	B.ado	B.ang	B.ani	B.bre	B.cat	B.den	B.bif	B.gal	B.inf	B.lac	B.lon
3-7 days old (n = 36)	20(47)	-	6	-	-	8	-	13	-	-	-	7
One month old (n = 7)	3(43)	-	2	1	-	-	-	-	-	-	-	-
Two month old (n = 20)	11(55)	-	-	6	-	1	4	-	3	-	-	1
Weaning day ODPW(n = 11)	6(55)	-	2	-	-	-	2	-	-	-	-	-
1DPW (n = 11)	4(36)	-	-	-	-	-	1	-	-	-	-	1
2DPW (n = 11)	6(55)	-	3	-	-	-	4	-	-	-	-	1
7DPW (n = 11)	6(55)	-	-	-	-	1	-	-	-	-	-	1
Diarrhea (3months (n = 13)	4(31)	-	3	-	-	-	1	-	-	-	-	-
6 Months (n = 28)	24(86)	-	-	-	-	-	20	-	15	-	-	-
Yearling and over (n = 56)	52(93)	-	6	-	-	8	25	-	34	-	-	-

Note. B. = *Bifidobacterium*, ado = adolescentis, ang = angulatum, ani = animalis subsp animalis, bre = breve, cat = catenulatum, den = dentium, bif = bifidum, gal = gallicum, inf = infantis, lac = lactis, lon = longum.

5. Discussion

Our results indicate that *Bifidobacterium* species are present in all age groups of pastured goats beginning from few days of age to goats over four years. We also report differences in abundance and diversity of *Bifodobacterium* between different age groups of pastured goats. To our knowledge, this is the first study that have looked at *Bifidobacterium* presence, abundance and diversity in goats of different age groups reared under similar husbandry practices. These findings agree with previous studies in human which have also reported the presence of *Bifidobacterium* species in all age groups beginning with infants to people over one hundred years (Kato et al., 2017). In other animal species, studies have evaluated the presence of *Bifidobacterium* at specific time points (Simpson, Stanton, Fitzgerald, & Ross, 2003). In agreement with our findings, one study evaluated the presence and diversity of *Bifidobacterium* in wild animals found in animal houses in South Africa representing carnivores, omnivores and herbivores (wallaby, springbok, giraffe, eland, zebra and buffalo) using culture and molecular methods (Endo, Futagawa-Endo, & Dicks, 2010). They detected *Bifidobacterium* in only about 20% of the twenty-six different animal fecal samples evaluated using both methods. Both techniques detected *Bifidobacterium* but only in less than half of the herbivores (wallabies and springboks) but authors did not specify the ages. The differences between our study findings and these findings may be due to the primers used in the study to detect *Bifidobacterium* spp. In agreement with our study, two studies in US and Europe also evaluated the presence of *Bifidobacterium* in fecal samples from different animal species including ruminants (Gavini et al., 2006; Lamendella et al., 2008). They both reported *Bifidobacterium* species in most of the animals evaluated including goats, sheep and cattle but again the age groups were not indicated. While a few studies have specifically evaluated and detected *Bifidobacterium* species in lambs (Bunešová et al., 2012; Vlková et al., 2010), both studied young lambs during the first forty days of life. Some studies have utilized 16s metagenomics to examine microbial diversity and abundance in sheep and goats at six and twelve months of age and detected relatively low levels *Bifidobacterium* in both (Shabana, Albakri, & Bouqellah, 2021) and in goats from birth to weaning (Zhuang et al., 2020). However the specific species in each of these age groups are not highlighted. These findings may also be explained by the different molecular techniques applied in the studies. Overall, the the findings in this study and others indicates that *Bifidobacterium* species may play important role in the gut homeostasis in the mammalian species including goats.

We also detected differences in abundance of *Bifidobacterium* spp. in different age groups of goats with the highest abundance being detected in young goat kids during the first week of life. The counts generally declined with age with the lowest counts being detected one week after weaning. Although no other studies in ruminants were found comparing sequential age based *Bifidobacterium* spp. abundance in different age groups of ruminants, higher *Bifidobacterium* counts were also reported in younger pigs compared to older pigs in one study (Lim et al., 2019) and counts were generally higher in younger compared to older people in human studies (Arboleya, Watkins, Stanton, & Ross, 2016; Salazar et al., 2019). This may indicate that growth of *Bifidobacterium* may be favored by nutrients available in the gut of younger animals compared to older animals. In this study, the young animals were on combined milk diet (suckling) and browsing until weaning. It is interesting to note that the

lowest *Bifidobacterium* spp. counts were reported one week after weaning in this study, which may point to a role of a milk diet in the abundance of *Bifidobacterium* spp. in goats.

Seven different species of *Bifidobacterium* were detected in pastured goats in this study (*B. angulatum*, *B. animalis*, *B. bifid*, *B. catenulatum*, *B. dentium*, *B. gallicum* and *B. longum*). Several different species/subspecies are also currently known to inhabit the human gut (Kato et al., 2017). In one study using molecular methods that examined fecal samples from ruminants, *B. dentium* and *B. angulatum* were detected in goats similar to our study while in sheep in addition to these species *B. adolescentis*, *B.gallicum*, and *B. longum* were also detected (Lamendella et al., 2008). Another culture based study detected *B. pseudolongum* in both sheep and goats while *B. bifidum* was only detected in goats (Gavini et al., 2006). In a study in young lambs utilizing culture based methods, *B. animalis sub animalis*, *B. choerinum*, *B. pseudolongum* and *B. pseudocatenulatum* were detected (Bunešová et al., 2012). The differences in the findings of our study and other studies involving goats may be due to the ages of the animals involved. While our study evalauated fecal samples from several age groups most of the other studies are single point experiments or do not indicate the ages from which samples were collected from (Gavini et al., 2006; Lamendella et al., 2008). It is also possible that husbandry practices including the diet and geographical location may influence the diversity of *Bifidobacterium* spp. as is reported for many other bacteria. It is worth noting also that in this study however, over 44% of fecal samples that had a positive amplification of *Bifidobacterium* using genus specific PCR assay did not yield any product with the species specific assays applied here especially in goats less than six months of age. This means that further research is needed to characterize and isolate these goat specific *Bifidobacterium* species.

Bifidobacterium species diversity was detected based on age indicating that specific species are more adapted to specific conditions present in the gut of the different age groups while others are not age specific. Notably, the *B. angulatum*, *B. catelanum*, *B. dentium* and *B. gallicum* were found in goats few days of age to goats over one year. On the other hand*, B.bifid* was only detected during the first week of life, *B.animalis sub animalis* and *B. longum* were only detected in young animals less than three months. However, *B. dentium* and *B.gallicum* species were found in high proportion of goats six months and older, while they were rare in younger animals. In previous studies reporting *Bifidobacterium* species in other animals including goats, no age data on the sample sources were reported making it difficult to compare age based differences detected in this study with other studies. In humans however, age related differences in diversity of *Bifidobacterium* species have been reported (Arboleya et al., 2016; Kato et al., 2017). Age based differences could be explained by the difference in dietary components and therefore nutrients available for each age group that may favor growth of specicific species of *Bifidobacterium* in the gut of the animals.Thus, these aged based differences in diversity of *Bifidobacterium* species is new information that has not been studied or reported in goats before and may also exist in other mammalian species that have not been studied. This information is significant as it may be utilized in selecting and developing age specific probiotic *Bifidobacterium* species that are likely to establish in goats of different age groups.

6. Conclusion

In this study we have documented preliminary insight into *Bifidobacterium* species in pastured goats highlighting prevalence and age based differences in abundance and diversity. This study detected higher abundance of *Bifidobacterium* spp. in preweaned goats compared with goats six months and over, we also report differences in *Bifidobacterium* spp. diversity based on age. This information is critical as research for identification and development of host specific beneficial gut microbes continues to gain interest. However, more research is needed to isolate and characterize these bacteria species including understanding their growth, biochemical and potential beneficial characteristics in goats.

Acknowledgements

Funding for the work was supported USDA NIFA Evans Allen project 1013065 at Agricultural Research Station (ARS), Virginia State University, USA.

Authors wish to acknowledge the VSU small ruminant herd manager, Ms. Amanda Miller and her team for the overall maintenance of the herd and assistance in handling animals during sample collection.

Eunice Ndegwa designed study and carried out gene detection, data analysis and manuscript preparation. Ako Agyemang contributed in sample collection and DNA extraction.

References

Abatenh, E., Gizaw, B., Tsegay, Z., Tefera, G., & Aynalem, E. (2018). Health benefits of probiotics. *J Bacteriol Infec Dis, 2*(1). Retrieved from http://www.alliedacademies.org/journal-bacteriology-infectious-diseases

Abdelhamid, A. G., El-Masry, S. S., & El-Dougdoug, N. K. (2019). Probiotic *Lactobacillus* and *Bifidobacterium* strains possess safety characteristics, antiviral activities and host adherence factors revealed by genome mining. *EPMA Journal, 10*(4), 337-350. https://doi.org/10.1007/s13167-019-00184-z

Arboleya, S., Watkins, C., Stanton, C., & Ross, R. P. (2016). Gut *Bifidobacteria* populations in human health and aging. *Frontiers in Microbiology, 7*, 1204. https://doi.org/10.3389/fmicb.2016.01204

Bunešová, V., Vlková, E., Killer, J., Rada, V., & Ročková, Š. (2012). Identification of *Bifidobacterium* strains from faeces of lambs. *Small Ruminant Research, 105*(1), 355-360. https://doi.org/10.1016/j.smallrumres.2011.12.004

Endo, A., Futagawa-Endo, Y., & Dicks, L. M. T. (2009). *Lactobacillus* and *Bifidobacterium* diversity in horse feces, revealed by PCR-DGGE. *Current Microbiology, 59*(6), 651-655. https://doi.org/10.1007/s00284-009-9498-4

Endo, A., Futagawa-Endo, Y., & Dicks, L. M. T. (2010). Diversity of *Lactobacillus* and *Bifidobacterium* in feces of herbivores, omnivores and carnivores. *Anaerobe, 16*(6), 590-596. https://doi.org/10.1016/j.anaerobe.2010.10.005

Gavini, F., Delcenserie, V., Kopeinig, K., Pollinger, S., Beerens, H., Bonaparte, C., & Upmann, M. (2006). *Bifidobacterium* species isolated from animal feces and from beef and pork meat. *Journal of Food Protection, 69*(4), 871-877. https://doi.org/10.4315/0362-028X-69.4.871

Guglielmetti, S., Mora, D., Gschwender, M., & Popp, K. (2011). Randomised clinical trial: *Bifidobacterium bifidum* MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life—A double-blind, placebo-controlled study. *Alimentary Pharmacology & Therapeutics, 33*(10), 1123-1132. https://doi.org/10.1111/j.1365-2036.2011.04633.x

Hernández-Rodríguez, D., Vásquez-Aguilar, A. A., Serio-Silva, J. C., Rebollar, E. A., & Azaola-Espinosa, A. (2019). Molecular detection of *Bifidobacterium* spp. in faeces of black howler monkeys (*Alouatta pigra*). *Journal of Medical Primatology, 48*(2), 99-105. https://doi.org/10.1111/jmp.12395

Hong, W.-S., & Chen, M.-J. (2007). Rapid identification of *Bifidobacteria* in dairy products by gene-targeted species-specific PCR technique and DGGE. *Asian-Australasian Journal of Animal Sciences, 20*(12), 1887-1894. https://doi.org/10.5713/ajas.2007.1887

Kato, K., Odamaki, T., Mitsuyama, E., Sugahara, H., Xiao, J.-Z., & Osawa, R. (2017). Age-related changes in the composition of gut *Bifidobacterium* species. *Current Microbiology, 74*(8), 987-995. https://doi.org/10.1007/s00284-017-1272-4

Kaufmann, P., Pfefferkorn, A., Teuber, M., & Meile, L. (1997). Identification and quantification of *Bifidobacterium* species isolated from food with genus-specific 16S rRNA-targeted probes by colony hybridization and PCR. *Applied and Environmental Microbiology, 63*(4), 1268-1273. https://doi.org/10.1128/aem.63.4.1268-1273.1997

Lamendella, R., Santo Domingo, J. W., Kelty, C., & Oerther, D. B. (2008). *Bifidobacteria* in feces and environmental waters. *Applied and Environmental Microbiology, 74*(3), 575-584. https://doi.org/10.1128/AEM.01221-07

Lim, M. Y., Song, E.-J., Kang, K. S., & Nam, Y.-D. (2019). Age-related compositional and functional changes in micro-pig gut microbiome. *Geroscience, 41*(6), 935-944. https://doi.org/10.1007/s11357-019-00121-y

Liu, G., Ren, L., Song, Z., Wang, C., & Sun, B. (2015). Purification and characteristics of bifidocin A, a novel bacteriocin produced by *Bifidobacterium animals* BB04 from centenarians' intestine. *Food Control, 50*, 889-895. https://doi.org/10.1016/j.foodcont.2014.10.049

Matsuki, T., Watanabe, K., & Tanaka, R. (2003). Genus-and species-specific PCR primers for the detection and identification of bifidobacteria. *Current Issues in Intestinal Microbiology, 4*(2), 61-69.

Matsuki, T., Watanabe, K., Fujimoto, J., Miyamoto, Y., Takada, T., Matsumoto, K., ... Tanaka, R. (2002). Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Applied and Environmental Microbiology, 68*(11), 5445-5451. https://doi.org/10.1128/AEM.68.11.5445-5451.2002

Matsuki, T., Watanabe, K., Tanaka, R., & Oyaizu, H. (1998). Rapid identification of human intestinal Bifidobacteria by 16S rRNA-targeted species-and group-specific primers. *FEMS Microbiology Letters, 167*(2), 113-121. https://doi.org/10.1111/j.1574-6968.1998.tb13216.x

Matsuki, T., Watanabe, K., Tanaka, R., Fukuda, M., & Oyaizu, H. (1999). Distribution of Bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specific primers. *Applied and Environmental Microbiology, 65*(10), 4506-4512. https://doi.org/10.1128/AEM.65.10.4506-4512.1999

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J.-Z., ... Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiology, 16*(1), 1-12. https://doi.org/10.1186/s12866-016-0708-5

Paliy, A., Gujvinska, S., Livoshchenko, L., Nalivayko, L., Livoshchenko, Y. M., Risovaniy, V., ... Petrov, R. (2020). Specific composition of indigenous microflora (*Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp.) in farm animals. *Ukrainian Journal of Ecology, 10*(1). https://doi.org/10.15421/2020_7

Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F., & Matuchansky, C. (2005). Bifidobacteria as probiotic agents-physiological effects and clinical benefits. *Alimentary Pharmacology & Therapeutics, 22*(6), 495-512. https://doi.org/10.1111/j.1365-2036.2005.02615.x

Rinttilä, T., Kassinen, A., Malinen, E., Krogius, L., & Palva, A. (2004). Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology, 97*(6), 1166-1177. https://doi.org/10.1111/j.1365-2672.2004.02409.x

Ritchie, L. E., Burke, K. F., Garcia-Mazcorro, J. F., Steiner, J. M., & Suchodolski, J. S. (2010). Characterization of fecal microbiota in cats using universal 16S rRNA gene and group-specific primers for *Lactobacillus* and *Bifidobacterium* spp. *Veterinary Microbiology, 144*(1-2), 140-146. https://doi.org/10.1016/j.vetmic.2009.12.045

Roger, L. C., Costabile, A., Holland, D. T., Hoyles, L., & McCartney, A. L. (2010). Examination of faecal *Bifidobacterium* populations in breast-and formula-fed infants during the first 18 months of life. *Microbiology, 156*(11), 3329-3341. https://doi.org/10.1099/mic.0.043224-0

Ross, R. P., Mills, S., Hill, C., Fitzgerald, G. F., & Stanton, C. (2010). Specific metabolite production by gut microbiota as a basis for probiotic function. *International Dairy Journal, 20*(4), 269-276. https://doi.org/10.1016/j.idairyj.2009.12.003

Russell, D., Ross, R., Fitzgerald, G., & Stanton, C. (2011). Metabolic activities and probiotic potential of Bifidobacteria. *International Journal of Food Microbiology, 149*(1), 88-105. https://doi.org/10.1016/j.ijfoodmicro.2011.06.003

Salazar, N., Arboleya, S., Fernández-Navarro, T., de Los Reyes-Gavilán, C. G., Gonzalez, S., & Gueimonde, M. (2019). Age-associated changes in gut microbiota and dietary components related with the immune system in adulthood and old age: A cross-sectional study. *Nutrients, 11*(8), 1765. https://doi.org/10.3390/nu11081765

Shabana, I., Albakri, N., & Bouqellah, N. (2021). Metagenomic investigation of faecal microbiota in sheep and goats of the same ages. *Journal of Taibah University for Science, 15*(1), 1-9. https://doi.org/10.1080/16583655.2020.1864930

Simpson, P., Stanton, C., Fitzgerald, G., & Ross, R. (2003). Genomic diversity and relatedness of Bifidobacteria isolated from a porcine cecum. *Journal of Bacteriology, 185*(8), 2571-2581. https://doi.org/10.1128/JB.185.8.2571-2581.2003

Steele, M., Malmuthuge, N., & Guan, L. (2015). *Opportunities to improve gut health in ruminant production systems*. eCommons, Cornell University Library. Retrieved from https://ecommons.cornell.edu/bitstream/handle/1813/41221/CNC2015_3-Steele_manu.pdf;sequence=2

Ventura, M., Reniero, R., & Zink, R. (2001). Specific identification and targeted characterization of *Bifidobacterium lactis* from different environmental isolates by a combined multiplex-PCR approach. *Applied and Environmental Microbiology, 67*(6), 2760-2765. https://doi.org/10.1128/AEM.67.6.2760-2765.2001

Vlková, E., Grmanová, M., Killer, J., Mrázek, J., Kopečný, J., Bunešová, V., & Rada, V. (2010). Survival of Bifidobacteria administered to calves. *Folia Microbiologica, 55*(4), 390-392. https://doi.org/10.1007/s12223-010-0066-x

Whorwell, P. J., Altringer, L., Morel, J., Bond, Y., Charbonneau, D., O'mahony, L., ... Quigley, E. M. (2006). Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel

syndrome. *Official journal of the American College of Gastroenterology, 101*(7), 1581-1590. https://doi.org/10.1111/j.1572-0241.2006.00734.x

Yang, B., Chen, Y., Stanton, C., Ross, R. P., Lee, Y.-K., Zhao, J., ... Chen, W. (2019). *Bifidobacterium* and *Lactobacillus* composition at species level and gut microbiota diversity in infants before 6 weeks. *International Journal of Molecular Sciences, 20*(13), 3306. https://doi.org/10.3390/ijms20133306

Zhang, X., Wu, J., Zhou, C., Tan, Z., & Jiao, J. (2020). Spatial and temporal organization of jejunal microbiota in goats during animal development process. *Journal of Applied Microbiology, 131*(1), 68-79. https://doi.org/10.1111/jam.14961

Zhuang, Y., Chai, J., Cui, K., Bi, Y., Diao, Q., Huang, W., ... Zhang, N. (2020). Longitudinal investigation of the gut microbiota in goat kids from birth to postweaning. *Microorganisms, 8*(8), 1111. https://doi.org/10.3390/microorganisms8081111

Zuo, F., Yu, R., Feng, X., Chen, L., Zeng, Z., Khaskheli, G. B., ... Chen, S. (2016). Characterization and in vitro properties of potential probiotic *Bifidobacterium* strains isolated from breast-fed infant feces. *Annals of Microbiology, 66*(3), 1027-1037. https://doi.org/10.1007/s13213-015-1187-x