Animal Science Papers and Reports vol. 36 (2018), no. 1, 99-113
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Sex-associated effects of dietary tannic acid on the abundance and diversity of caecal microbes in Brandt's voles (*Lasiopodomys brandtii*)*

Manhong Ye[1,2], Mengting Xu[1], Mengmeng Ding[1], Jiayue Nie[1], Xiaoqian Ding[1], Jingjing Kuai[1], Wanhong Wei[1,2], Shengmei Yang[1,2], Bin Zhou[3]**

[1] College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, Jiangsu Province, China

[2] Joint International Research Laboratory of Agricultural & Agri-Product Safety, Yangzhou University, Yangzhou 225009, Jiangsu Province, China

[3] College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, Jiangsu Province, China

(Accepted November 9, 2018)

Tannic acid (TA) is one of the important plant secondary metabolites existed in the Brandt's vole's daily food. Its anti-nutritional and anti-oxidant effects have been previously reported. However, investigations about the microbial communities and alterations of gut microbiota in response to dietary TA have not been documented in Brandt's voles. To characterize the effects of dietary TA on the abundance and diversity of caecal microbes, 18 adult Brandt's voles were randomly assigned to 3 groups, where they were continuously fed with commercial rodent diet containing 0%, 3%, and 6% (w/w) TA for three weeks. The luminal contents of the caeca were collected for DNA extraction and high throughput sequencing was applied using an Illumina MiSeq. Significant differences in alpha diversity indices were detected between male and female voles. Female voles were significantly higher in Chao1, observed species, and the Shannon and Simpson indices than the males. The composition of bacterial community in the Brandt's voles was significantly affected by the administration of dietary TA. Gender, as well as the interaction between gender and TA played important roles in determining the bacterial structure. Female voles were observed to be more vulnerable than the males to the influence of dietary TA. This study was the first non-culture based microbiota analysis that revealed the caecal microbiota compositions in Brandt's voles. Our results

*This work was financially supported by the National Science Foundation of China (31370415).
**Corresponding author: smyangyzu@126.com

provided preliminary data about sex-associated differences in the response of the caecal microbiota to dietary TA in Brandt's voles.

KEYWORDS: Brandt's voles / caecum / gender effects / microbiota / tannic acid

The Brandt's vole (*Lasiopodomys brandtii*) is a dominant pest rodent in North China. It is also one of the smallest strictly herbivorous mammals mainly inhabiting the grasslands of Inner Mongolia of China, the Republic of Mongolia, and the region of Beigaer Lake in Russia [Li and Wang 2005]. Tannic acid (TA), one of the important plant secondary metabolites, is ubiquitously distributed in Brandt's vole's daily food, accounting for about 5% of the dry food [Li *et al*. 2007]. Negative effects of dietary TA on animals due to its interactions with protein [Torregrossa *et al*. 2014], carbohydrates [Mansoori and Modirsanei 2012], and minerals [Jaramillo *et al*. 2015] had been reported. As an anti-nutritional factor in food, TA reduced food efficiency and growth rate [Lee *et al*. 2010]. On the other side, dietary TA had favorable impacts on anti-oxidant and anti-inflammation abilities [Gülçin *et al*. 2010; Bouki *et al*. 2013]. Its influences on lipid metabolism [Ye *et al*. 2016, Masek and Starcevic 2017] and the composition of microbial flora [Yang *et al*. 2017] were also documented. We assumed that TA, one important component in the daily food of Brandt's vole, will have potential influences on the abundance and diversity of gut microbes. However, there has been relatively limited study about the microbial communities colonizing the wild or captive Brandt's voles. Neither is there investigation studying the alterations of gut microbiota in response to different concentrations of dietary TA in Brandt's voles.

As is known, intestinal microbial communities bear significant roles in promoting host development, nutrition and immunity [Jandhyala *et al*. 2015]. The large and diverse assemblage of bacteria in gut exhibits both temporal and spatial variations all along the individual's life span and can be influenced by various factors, such as diet [David *et al*. 2014, Espley *et al*. 2014], age [Yatsunenko *et al*. 2012], season [Davenport *et al*. 2014], genetics [Spor *et al*. 2011, Ussar *et al*. 2015], antimicrobial exposure [Zhang *et al*. 2016, Bessegatto *et al*. 2017], and concomitant disease [Zhao 2013]. Of all these factors, diet is one of the most important determinants in shaping the composition, diversity, and richness of the microbial communities. Up till now, the host and environmental factors that shape Brandt's voles' gut microbiota remain largely unexplored.

Using 16S rDNA sequencing technology, accumulating documents have provided a wealth of information on the composition and structure of the bacterial community in both domesticated and wild animals [Barker *et al*. 2013, Maurice *et al*. 2015, McLaughlin *et al*. 2015, Guass *et al*. 2016, Kohl *et al*. 2017, Li *et al*. 2017]. It has been proved to be a useful tool to understand the microbial ecology of organisms. Here, we provided an initial view of the profile of caecal bacteria in Brandt's voles using high-throughput sequencing and studied the alterations of their compositions in response to different concentrations of dietary TA. Our results revealed that the bacterial communities in Brandt's voles were significantly influenced by the

supplementation of dietary TA. Significant interactions between sex and dietary TA were also observed. Evidence for the impact of TA over the bacterial composition was observed at the phylum, class, order, family, and genus levels. Together, our results suggested that dietary TA has an important role in shaping the bacterial communities in Brandt's voles.

Material and methods

Ethical approval

The care and treatment of experimental animals conformed to the "Guide for the Care and Use of Laboratory Animals" of the Comparative Medical Centre of Yangzhou University (a registered animal facility for supervising experiments on laboratory animals). All procedures performed in this study involving animals were in accordance with the ethical standards of and approved by the Animal Ethical and Welfare Committee of Yangzhou University.

Animals and experimental design

Eighteen 90-day-old Brandt's voles, half male and half female, with an average weight of 44.07±4.09 g (47.71±1.78 g and 40.42±1.58 g for male and female voles, respectively) were obtained from a breeding colony maintained at the College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, Jiangsu Province, China. Founder animals in the colony were originally captured in the wild in Xilinguole League, Inner Mongolia Autonomous Region of China. The animals were housed individually in plastic cages and maintained under standard laboratory conditions (22 ± 2°C, 40-60% humidity, and a 12h/12h light/dark cycle). They were acclimated for one week with free access to water and commercial rodent diet (Xietong Bio-Pharm Co., Ltd., Nanjing, China) before being randomized into three groups. The control animals (group CT), fed with commercial diet containing no TA, were included in sub-groups CT_M and CT_F (for male and female voles, respectively). Animals treated with 3% (3% TE group) and 6% TA (6% TE group) were assigned into sub-groups of 3%TE_M (male), 3% TE_F (female), 6%TE_M (male), and 6% TE_F (female) groups, respectively. Each sub-group contained three animals. The nutrient contents of the commercial chow diet (containing no TA) were as follows: crude protein ≥15%, crude fat ≥4%, crude fiber 10-15%, ash ≤8%, calcium 1.0-1.8%, and phosphorus 0.6-1.2%.

TA with a molecular weight of 1701.2 was purchased as dry power from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. TA was added to the commercial diet at the expense of the whole diet. 3% (6%) TA-containing diets were prepared by adding 3 g (6 g) TA to 97 g (94 g) of the standard diet. The experiment lasted for three weeks and all animals had free access to tap water and diet during the whole experiment. Then all animals were sacrificed after being anesthetized by the intraperitoneal infusion of sodium pentobarbital (150 mg/kg).

Sampling

Caecal samples were collected immediately after the death of the animal. Briefly, the caeca were removed aseptically, clamped with forceps, and placed in sterile plastic bags on ice. Then the open ends of the caeca were cut with sterile scissors and the contents in the caeca were inverted into 2 mL centrifugal tubes. Approximately 0.5-1 g of contents was collected into the centrifuge tube and stored at -80℃ until DNA extraction, which was undertaken 3 days later. One caecal sample from a female vole in the 6% TE_F sub-group was accidently spilled. In order to prevent any potential contamination, only 17 caecal samples were used for further analysis.

DNA isolation and high-throughput sequencing

The following protocols were performed by staff at the Oebiotech Co., Ltd., Shanghai, China. Bacterial genomic DNA was extracted from the whole caecal contents containing solid and liquid fractions of each animal with the QIAamp DNA Stool mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The resulting metagenomic DNA samples were dissolved in 50 μL of elution buffer and then inspected on ethidium bromide-stained agarose gels. DNA concentrations were determined using the Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA). All DNA samples were stored at -20 °C until needed.

The bacterial 16S rRNA gene was amplified from the extracted DNA using primers 338F (5' -ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The primer pair contained the appropriate Illumina adapter sequence and an 8-bp barcode which allowed for post-sequencing identification. The resulting PCR products were confirmed on 2% agarose gel electrophoresis and purified using QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The purified amplicons were quantified using QuantiFluor™-ST (Promega, Madison, WI, USA), pooled in equimolar concentrations, and sequenced on Illumina MiSeq platform (Illimina, USA) to characterize total bacterial population.

Data availability and bioinformatics analysis

The raw sequences generated in this study were deposited into the NCBI Sequence Read Archive (SRA) database under the study PRJNA394059. Bioinformatics analysis was performed by staff at the Oebiotech Co., Ltd., Shanghai, China. Paired-end reads were truncated by cutting off the primer sequences using Trimmomatic software and merged using FLASH software (V1.2.7). Each sample was sorted according to the unique barcode. Quality filtering on the raw reads was performed by using the QIIME (V1.7.0) program. Chimeric sequences were removed by using Usearch software to obtain the clean sequences. The operational taxonomic units (OTUs) were mathematically defined as having a 3% sequence distance (e.g. 97% similarity). Diversity and richness were calculated using the Cluster Database at High Identity with Tolerance (CD-HIT). For each OTU, representative sequence was screened for

further annotation. The taxonomic information of each representative sequence was annotated using the GreenGene Database based on RDP classifier algorithm. Alpha-diversity indices including Chao1, Shannon-Wiener, and Simpson indices were also calculated from QIIME to estimate the community richness and diversity.

Statistical analysis

All data were presented as the mean ± standard error of the mean (SEM) for each group. Two-way analysis of variance (ANOVA) was used for analyzing the effects of TA, gender, and potential interaction between gender and TA on the composition of caecal bacteria, followed by the LSD method for post-hoc test using SPSS software (Chicago, IL, USA) (version 15.0 for windows). Values were considered significant when $p<0.05$.

Results and discussion

Summary of sequencing data

In the present study, 393,940 nucleotide sequences originating from 16S rRNA genes were retrieved from bacteria found in the caecal contents. Following quality control and removing of chimera, a total of 359,634 high quality sequences were obtained (Tab. 1) and the average number of sequences was about 21,154 per sample. Most of the sequences were distributed between 420-429 bp (76.26%) and 440-449 bp (22.56%) (Fig. 1). Based on the 97% species identity, the superior quality sequences were clustered into 3790 operational taxonomic units (OTUs).

Table 1 Raw and clean counts obtained and OTUs detected

Group	Sample name	Sex	Raw Counts	Clean Counts	Total OTUs
CT	CT.F1	female	22128	19233	844
	CT.F2	female	28338	26282	1095
	CT.F3	female	30860	25879	1155
	CT.M1	male	14350	13370	692
	CT.M2	male	14056	13372	538
	CT.M3	male	19576	17791	821
3% TE	3%TE.F1	female	34921	32532	1242
	3%TE.F2	female	30075	28430	939
	3%TE.F3	female	35477	33002	1258
	3%TE.M1	male	17749	16779	789
	3%TE.M2	male	28293	25013	968
	3%TE.M3	male	20685	18731	816
6% TE	6%TE.F1	female	17552	16604	778
	6%TE.F2	female	11130	10485	733
	6%TE.M1	male	21864	20146	759
	6%TE.M2	male	22151	20367	862
	6%TE.M3	male	24735	21618	942

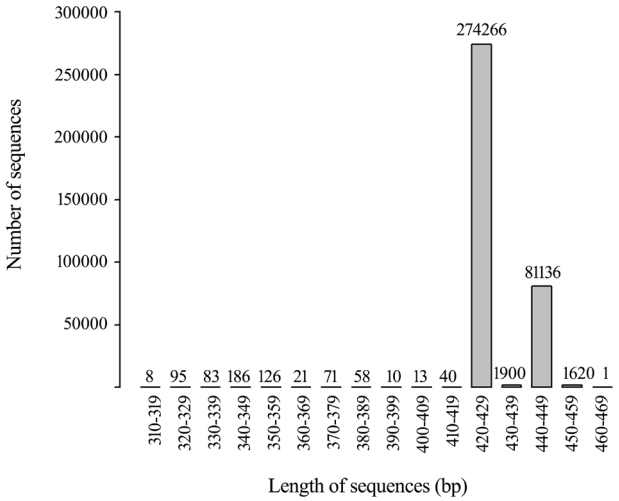

Fig. 1. Distribution of the length of the sequences obtained.

Microbial communities in the control and two TA-treated groups

At the phylum level, the 10 most relatively abundant bacterial phyla were Firmicutes, Bacteroidetes, Proteobacteria, Spirochaetes, Actinobacteria, Candidate division TM7, Cyanobacteria, Deferribacteres, Tenericutes, and Verrucomicrobia (Fig. 2). Firmicutes and Bacteroidetes dominated, accounting for 78.4±2.1% and

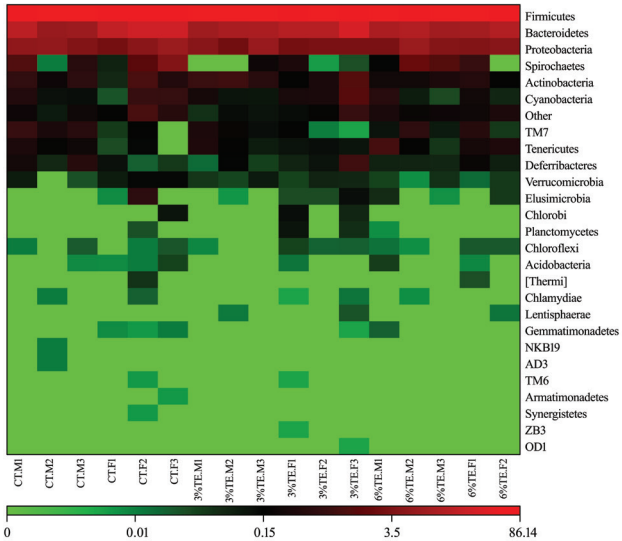

Fig. 2. The relative abundance of community of the top 30 phyla.

14.4±1.7% of sequences, respectively. Only less than 0.3% of sequences could not be classified at the phylum level.

The most relatively abundant classes (orders) were Clostridia (Clostridiales) and Bacteroidia (Bacteroidales), accounting for 77.1±2.0% and 14.2±1.7%, respectively. Ruminococcaceae, Lachnospiraceae, and S24-7 constituted the major family of bacteria detected, accounting for 21.76±0.78%, 21.54±1.6%, and 10.20±1.26%, respectively. *Oscillospira* was the most relatively abundant genus accounting for 10.36±0.52% of all sequences followed by *Ruminococcus* (7.27±0.52%), *Coprococcus* (4.85±0.68%), *Desulfovibrio* (3.23±0.31%), and *Roseburia* (2.27±0.49%). The sequences were assigned into 329 different genera, with an average of 28.3% of sequences being unclassified at the genus level.

Influences of dietary TA and gender on the bacterial compositions

Our results showed that the composition of caecal bacteria in Brandt's voles was significantly affected by dietary TA (Fig. 3). The majority of them came from four phyla (Firmicutes, Proteobacteria, Cyanobacteria, and Bacteroidetes). In most cases, these significantly altered bacteria responded to 6% TA administration by reducing their relative abundances. Three families (Enterococcaceae, Planococcaceae, and Ruminococcaceae) didn't follow the pattern. Their distributions after 6% TA treatment were significantly elevated. Accordingly, one genus from the Planococcaceae family (*Lysinibacillus*) and two genera from the Ruminococcaceae family (*Oscillospira* and

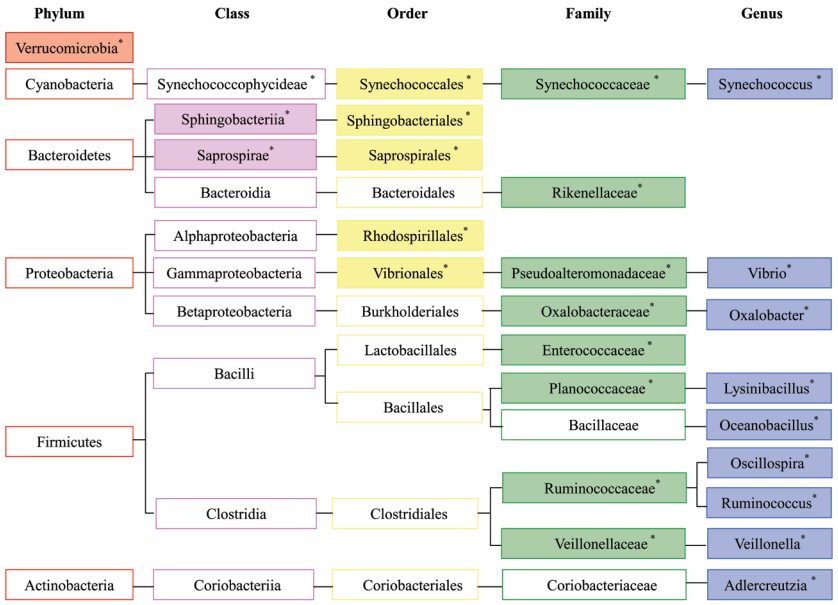

Fig. 3. Significantly altered bacterial compositions caused by the treatment of TA in voles. *Indicated significant changes ($p<0.05$) in the bacterial compositions.

Ruminococcus) exhibited significantly increased abundances. Furthermore, Most of the significant alterations in the bacterial compositions caused by 6% TA treatment were happened in the minor bacteria that accounted for less than 1% of the total caecal bacterial microbiome. The only exception was the Ruminococcaceae family, which was the major family detected in the caecum of Brandt's voles. The distribution of the Ruminococcaceae family was significantly elevated from 20.46±1.05% (0% dietary TA) to 25.46±0.45% (treated with 6% TA). Similarly, the *Ruminococcus* genus followed the same trend, its relative composition increased significantly from 5.34±0.43% (0% dietary TA) to 8.76±0.60% due to the administration of 6% dietary TA. The relative composition of another genus that belonged to the family Ruminococcaceae, *Oscillospira*, also exhibited an increase after the treatment of 6% TA, from 10.86±0.93% to 11.82±0.69%. However, the difference was not significant. On the other hand, the influence of 3% TA on the bacterial composition seemed to be bi-directional. It significantly increased the compositions of three orders (Rhodospirillales, Vibrionales, and Synechococcales), two families, Pseudoalteromonadaceae (order Vibrionales) and Synechococcaceae (order Synechococcales), as well as two genera, *Vibrio* and *Synechococcus*, which belonged to family Pseudoalteromonadaceae and Synechococcaceae, respectively. It also significantly reduced the compositions of the Rikenellaceae family, *Oscillospira* genus, and *Oxalobacter* genus. Our results indicated that under the influence of relatively low concentration of TA (3% TA) some bacteria nurtured while others were inhibited. When the concentration of dietary TA increased to 6%, most of the bacteria were inhibited.

Significant effects of gender (Fig. 4) and gender × TA interaction were also detected (Tab. 2). The structure of the bacterial community in male and female voles exhibited significant difference. Female voles were significantly higher in the composition of phylum Bacteroidetes (Fig. 5), as well as in class Bacteroidia and order Bacteroidales. At the genus level, the composition of *Oxalobacter*, *Veillonella*, *Lysinibacillus*, *Synechococcus*, and *Vibrio* were significantly affected by TA treatment, gender, and gender × TA interaction.

Table 2 Bacteria significantly affected by the gender × TA interaction

Level	Bacteria
Phylum	Verrucomicrobia ($p = 0.046$)
Class	Sphingobacteriia ($p = 0.046$), Saprospirae ($p = 0.022$)
Order	Sphingobacteriales ($p = 0.045$), Synechococcales ($p = 0.022$), Syntrophobacterales ($p = 0.034$), Vibrionales ($p = 0$), Saprospirales ($p = 0.022$)
Family	Oxalobacteraceae ($p = 0.037$), Pseudoalteromonadaceae ($p = 0.007$), Rikenellaceae ($p = 0.024$), Streptococcaceae ($p = 0.048$), Synechococcaceae ($p = 0.003$), Syntrophobacteraceae ($p = 0.033$), Veillonellaceae ($p = 0.039$)
Genus	*Oxalobacter* ($p = 0.008$), *Veillonella* ($p = 0.039$), *Lysinibacillus* ($p = 0.012$), *Paenibacillus* ($p = 0.050$), *Paludibacter* ($p = 0.047$), *Synechococcus* ($p = 0.003$), *Vibrio* ($p = 0.012$)

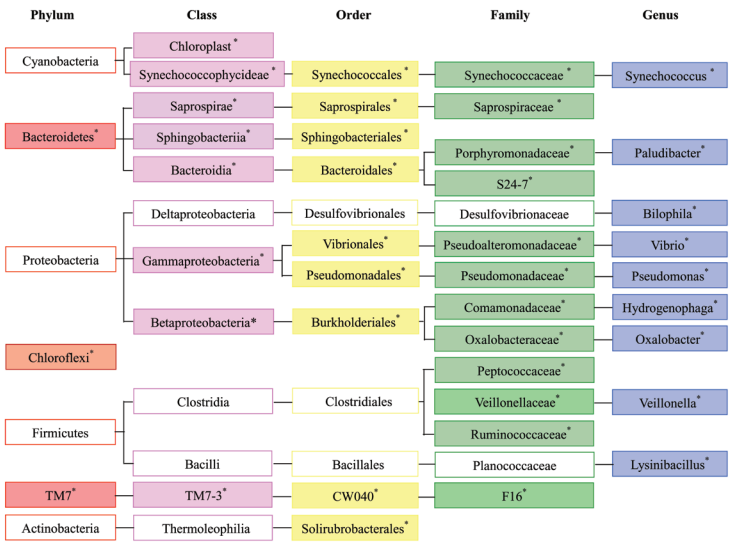

Fig. 4. Bacterial compositions significantly different between two genders in voles. *Indicated significant difference ($p<0.05$) in the bacterial compositions.

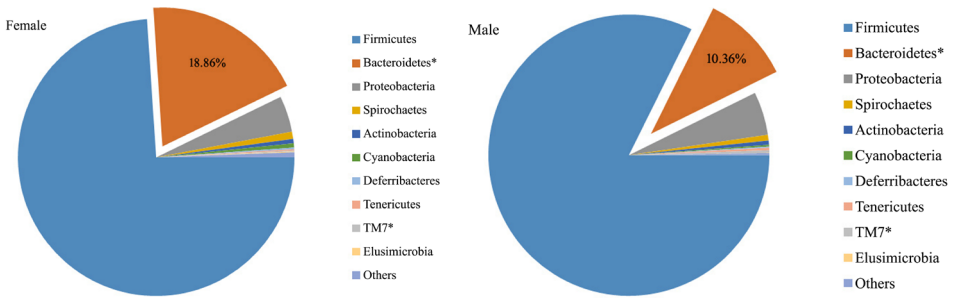

Fig. 5 Significant sex-associated distribution of bacteria in voles at the phylum level. *Indicated significant difference ($p<0.05$) in the bacterial composition.

Microbial diversity of the caecal microbiota

Ecological features of caecal bacterial communities were evaluated (Tab. 3). The average values for the Shannon and Simpson indices, which measure the community diversity, were 6.97±0.08 and 0.974±0.002, respectively. The overall average Chao1 (the number of estimated OTUs) of all samples, a nonparametric estimator of species richness, was 1292.32±80.36. The Good's coverages of all the samples were greater than 0.98, indicating good sequencing depth for reliable investigation of differences in caecal microbiota between TA-treated groups and the control group.

Our results showed that gender had significant effects on alpha diversity indices. Compared with male voles, female voles were significantly higher in Chao 1($p = 0.012$),

Table 3 Comparison of alpha diversity indices in different groups

Groups	Observed_species	Chao1	Shannon	Simpson	Goods_coverage
CT_F	1023.27±92.92	1619.84±135.20	7.26±0.20	0.981±0.002	0.983±0.002
CT_M	675.13±79.81	960.39±97.82	6.64±0.20	0.964±0.005	0.984±0.001
3%TE_F	1137.50±101.39	1669.92±181.47	7.20±0.13	0.978±0.001	0.987±0.001
3%TE_M	849.33±59.81	1189.33±55.91	6.73±0.12	0.964±0.010	0.985±0.001
6%TE_F	744.50±25.80	977.58±25.54	7.19±0.03	0.981±0.000	0.981±0.005
6%TE_M	849.20 ± 50.69	1231.96±92.19	6.89±0.09	0.976±0.003	0.986±0.001

observed species ($F_{(1,11)} = 7.555$, $p = 0.019$), Shannon and the Simpson indices ($p = 0.004$ and p=0.015, respectively). Significant interaction of gender × TA was also detected in Chao1 and observed species (p=0.008 and p=0.037, respectively). Compared to the treatment of 3% dietary TA, 6% TA significantly reduced Chao1 and observed species in female Brandt's voles, with no significant influences on the male ones.

Using high-throughput sequencing, the present study revealed that the caecal bacterial microbiota in Brandt's voles was dominated by Firmicutes and Bacteroidetes (92.8%), while Proteobacteria represented 4.6% and other bacterial phyla by approximately 2%. The caecal microbiota of the Brandt's vole was generally consistent with those from other rodents with Firmicutes and Bacteroidetes being the most abundant bacterial phyla [Kreisinger *et al*. 2015; Weldon *et al*. 2015].

Significant changes in gut micobiota due to the intake of dietary tannins (both condensed and hydrolysable tannins), such as proanthocyanidin [Choy *et al*. 2014], anthocyanin [Vendrame *et al*. 2011], ellagitannins [Li *et al*. 2015], gallic acid [Hidalgo *et al*. 2012], and gallotannins [Čandek-Potokar *et al*. 2015] have been previously reported. We studied the responses of caecal microbiota to different concentrations of dietary TA in Brandt's voles. Our results showed that TA, one member of hydrolyzed tannins, could modulate the composition of caecal bacterial microbiota community in Brandt's voles. As reviewed by Goel *et al*. [2005], a number of bacteria capable of tolerating tannins have been identified in both adapted domesticated and wild animals. Here, a group of TA-sensitive microflora, whose growth was significantly affected under the supplement of TA, was detected in the Brandt's vole. Most of them was characterized by a relatively low abundance (0.1% or less of the entire caecal bacterial microbiome) and came mainly from two phyla, Proteobacteria and Firmicutes, which indicated that some species belonging to these phyla might play important roles in the adaption of intestinal metabolism to the alteration of dietary TA concentration. However, at present, we are still unable to define the exact characters of these dynamically changed bacteria. Further researches are required to clarify the mechanisms how their growth is affected by TA.

Gender differences in the gastrointestinal tract have been observed in human [Mueller *et al*. 2006; Freire *et al*. 2011], animal [Fushuku and Fukuda 2008, Martin *et al*. 2010, Kovacs *et al*. 2011, Coretti *et al*. 2017], and insect [Han *et al*. 2017]. The identification of sexually dimorphic genes expressed in the small intestine of mice reinforced the dissimilarities between males and females at the molecular level [Steegenga *et al*. 2014]. Here, we reported a significant sex-associated difference in the

caecal microbial composition in Brandt's voles with females having higher microbial diversity than did the males. Since male and female voles within the same group were housed in the same environment and consumed the same type of laboratory chow, the observed sex-related differences in microbial communities were more likely due to sex-specific host-microbe interaction rather than differential exposure. As suggested by Markle *et al*. [2013] and Yurkovetskiy *et al*. [2013], this process of within-gut selection may be driven by pronounced hormonal differences in adult animals.

Results from the present study also suggested a role for gender in the sensitivity of caecal microbiota to dietary TA in Brandt's voles. Compared with the males, female voles exhibited much more significant alterations in the composition of caecal microbiota in response to dietary TA. Furthermore, the relative abundances of two phyla, Firmicutes and Bacteroidetes, were altered due to the administration of dietary TA. Female voles in group CT were characterized by significantly higher abundance of Bacteroidetes than the male ones. Supplemented dietary TA (both 3% and 6% TA) reduced the relative proportion of Bacteroidetes, while increased the propotion of Firmicutes. As a result, the ratio of Bacteroidetes to Firmicutes changed from 0.34 (group CT) to 0.29 (3% TE group) and 0.14 (6% TE group). However, in male voles, the ratio of Bacteroidetes to Firmicutes exhibited only little fluctuation due to TA administration (0.13, 0.12, and 0.14 for group CT, 3% TE, and 6% TE groups, respectively). Although Firmicutes and Bacteroidetes were always the two dominant taxa in the caecum of all the tested Brandt's voles, the relative abundances of these phyla in female voles were more sensitive to TA treatment than that in male ones. Sex-associated differences in the alterations of gut microbiota due to dietary treatments had been observed in other reports. Shang *et al*. [2016a] reported that keratan sulfate exerted a differing effect on male and female microbiota with much more drastic increase in the abundance of *Lactobacillus* spp. induced in female mice. They [Shang *et al*. 2016b] also revealed that the caecal microbiota in female mice were more vulnerable than that of male mice to the treatment of chondroitin sulfate and its oligosaccharide. We speculated that microbial communities in female Brandt's voles shifted more rapidly in response to the intake of dietary TA and exhibited more flexibility in the relationship between host and its microbial consortia.

Furthermore, an increase in the relative abundance of Firmicutes with a concordant decrease in Bacteriodetes was documented to be positively associated with obesity in mice [Ley *et al*. 2005; Turnbaugh *et al*. 2008], plasma glucose concentration in type II diabetic patients [Larsen *et al*. 2010], and increased fat deposition in rat treated with chronic olanzapine [Davey *et al*. 2012]. The proportional increase of Firmicutes was observed to be associated with ingestion of food [Costello *et al*. 2010], while the reduced proportional representation of the Bacteroidetes was accompanied with the development of obesity [Turnbaugh *et al*. 2008]. Thus, our findings, while preliminary, bring more insights into the significant role dietary TA may play during the lifetime of voles, especially to the female ones. It was extremely interesting as the shift in the predominant phyla of the caecal microbiota in female Brandt's voles might be directly

or indirectly linked to energy regulation and/or different nutritional requirements of the host. However, given the small sample size, additional studies of Brandt's voles consuming TA-containing diets will be necessary to determine the detailed effects of dietary TA on the variations in the composition and abundance of gut microbial taxa. Since the gut microbiota is vital to host metabolism, the potential interplay of the altered composition and diversity of the caecal microbiota in Brandt's voles due to the intake of dietary TA are worthy of further elucidation.

The present study characterized the microbial community in the caeca of Brandt's voles, as well as the modification of microbiota compositions due to the supplementation of dietary TA. We also demonstrated a sex-dependent effect of dietary TA on caecal microbiota with female voles being more sensitive to the influences of dietary TA. To our knowledge, this is the first non-culture based microbiota analysis in Brandt's voles showing that the composition and abundance of caecal microbial taxa varies due to the administration of dietary TA. Our results also provided suggestive evidence for differences in microbiota composition between sexes. Further investigations are needed to elaborate the potential consequences of the observed TA-responsive shifts in the caecal community structure in Brandt's voles.

Acknowledgements. *We thank Dr. Zining Wang in MAB and Traits Chromatin Inc., Lubbock, T.X., USA for his valuable advice on the whole manuscript.*

REFERENCES

1. BARKER C.J., GILLETT A., POLKINGHORNE A., TIMMS P., 2013 – Investigation of the koala (*Phascolarctos cinereus*) hindgut microbiome via 16S pyrosequencing. ***Veterinary Microbiology*** 167, 554-564.
2. BESSEGATTO J.A., PAULINO L.R., LISBÔA J.A.N., ALFIERI A.A., MONTEMOR C.H., MEDEIROS L.P., KOBAYASHI R.K.T., WEESE J.S., COSTA M.C., 2017 – Changes in the fecal microbiota of beef cattle caused by change in management and the use of virginiamycin as a growth promoter. ***Research in Veterinary Science*** 114, 355-362.
3. BOUKI E., DIMITRIADIS V.K., KALOYIANNI M., DAILIANIS S., 2013 – Antioxidant and pro-oxidant challenge of tannic acid in mussel hemocytes exposed to cadmium. ***Marine Environmental Research*** 85, 13-20.
4. ČANDEK-POTOKAR M., ŠKRLEP M., BATOREK LUKAČ N., ZAMARATSKAIA G., PREVOLNIK POVŠE M., VELIKONJA BOLTA Š., KUBALE V., BEE G., 2015 – Hydrolysable tannin fed to entire male pigs affects intestinal production, tissue deposition and hepatic clearance of skatole. ***The Veterinary Journal*** 204, 162-167.
5. CHOY Y.Y., QUIFER-RADA P., HOLSTEGE D.M., FRESE S.A., CALVERT C.C., MILLS D.A., LAMUELA-RAVENTOS R.M., WATERHOUSE A.L., 2014 – Phenolic metabolites and substantial microbiome changes in pig feces by ingesting grape seed proanthocyanidins. ***Food & Function*** 5, 2298-2308.
6. CORETTI L., CRISTIANO C., FLORIO E., SCALA G., LAMA A., KELLER S., CUOMO M., RUSSO R., PERO R., PACIELLO O., MATTACE RASO G., MELI R., COCOZZA S., CALIGNANO A., CHIARIOTTI L., LEMBO F., 2017 – Sex-related alterations of gut microbiota composition in the BTBR mouse model of autism spectrum disorder. ***Scientific Reports*** 7, 45356.
7. COSTELLO E.K., GORDON J.I., SECOR S.M., KNIGHT R., 2010 – Postprandial remodeling of the gut microbiota in Burmese pythons. ***The ISME Journal*** 4, 1375-1385.

8. DAVENPORT E.R., MIZRAHI-MAN O., MICHELINI K., BARREIRO L.B., OBER C., GILAD Y. 2014 – Seasonal variation in human gut microbiome composition. ***PLoS One*** 9, e90731.
9. DAVEY K.J., O'MAHONY S.M., SCHELLEKENS H., O'SULLIVAN O., BIENENSTOCK J., COTTER P.D., DINAN T.G., CRYAN J.F., 2012 – Gender-dependent consequences of chronic olanzapine in the rat: effects on body weight, inflammatory, metabolic and microbiota parameters. ***Psychopharmacology*** 221, 155-169.
10. DAVID L.A., MAURICE C.F., CARMODY R.N., GOOTENBERG D.B., BUTTON J. E., WOLFE B.E., LING A.V., DEVLIN A.S., VARMA Y., FISCHBACH M.A., BIDDINGER S.B., TURNBAUGH P.J., 2014 – Diet rapidly and reproducibly alters the human gut microbiome. ***Nature*** 505, 559-563.
11. ESPLEY R.V., BUTTS C.A., LAING W.A., MARTELL S., SMITH H., MCGHIE T.K., ZHANG J., PATURI G., HEDDERLEY D., BOVY A., SCHOUTEN H.J., PUTTERILL J., ALLAN A.C., HELLENS R.P. 2014 – Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice. ***The Journal of Nutrition*** 144, 146-154.
12. FREIRE A.C., BASIT A.W., CHOUDHARY R., PIONG C.W., MERCHANT H.A., 2011 – Does sex matter? The influence of gender on gastrointestinal physiology and drug delivery. ***International Journal of Pharmaceutics*** 415, 15-28.
13. FUSHUKU S., FUKUDA K., 2008 – Gender difference in the composition of fecal flora in laboratory mice, as detected by denaturing gradient gel electrophoresis (DGGE). ***Experimental Animals*** 57, 489-493.
14. GOEL G., PUNIYA A.K., AGUILAR C.N., SINGH K., 2005 – Interaction of gut microflora with tannins in feeds. ***Naturwissenschaften*** 92, 497-503.
15. GUASS O., HAAPANEN L.M., DOWD S.E., SIROVIC A., MCLAUGHLIN R.W., 2016 – Analysis of the microbial diversity in faecal material of the endangered blue whale, *Balaenoptera musculus*. ***Antonie Van Leeuwenhoek*** 109, 1063-1069.
16. GÜLÇIN İ., HUYUT Z., ELMASTAŞ M., ABOUL-ENEIN H.Y., 2010 – Radical scavenging and antioxidant activity of tannic acid. ***Arabian Journal of Chemistry*** 3, 43-53.
17. HAN G., LEE H.J., JEONG S.E., JEON C.O., HYUN S., 2017 – Comparative analysis of *Drosophila melanogaster* gut microbiota with respect to host strain, sex, and age. ***Microbial Ecology*** 74, 207-216.
18. HIDALGO M., ORUNA-CONCHA M.J., KOLIDA S., WALTON G.E., KALLITHRAKA S., SPENCER J.P., DE PASCUAL-TERESA S., 2012 – Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. ***Journal of Agricultural and Food Chemistry*** 60, 3882-3890.
19. JANDHYALA S.M., TALUKDAR R., SUBRAMANYAM C., VUYYURU H., SASIKALA M., NAGESHWAR REDDY D., 2015 – Role of the normal gut microbiota. ***World Journal of Gastroenterology*** 21, 8787-8803.
20. JARAMILLO A., BRIONES L., ANDREWS M., ARREDONDO M., OLIVARES M., BRITO A., PIZARRO F., 2015 – Effect of phytic acid, tannic acid and pectin on fasting iron bioavailability both in the presence and absence of calcium. ***Journal of Trace Elements in Medicine and Biology*** 30, 112-117.
21. KOHL K.D., BRUN A., MAGALLANES M., BRINKERHOFF J., LASPIUR A., ACOSTA J.C., CAVIEDES-VIDAL E., BORDENSTEIN S.R., 2017 – Gut microbial ecology of lizards: insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. ***Molecular Ecology*** 26, 1175-1189.
22. KOVACS A., BEN-JACOB N., TAYEM H., HALPERIN E., IRAQI F.A., GOPHNA U., 2011 – Genotype is a stronger determinant than sex of the mouse gut microbiota. ***Microbial Ecology*** 61, 423-428.

23. KREISINGER J., BASTIEN G., HAUFFE H.C., MARCHESI J., PERKINS S.E., 2015 – Interactions between multiple helminths and the gut microbiota in wild rodents. ***Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*** 370 (1675). doi: 10.1098/rstb.2014.0295
24. LARSEN N., VOGENSEN F.K., VAN DEN BERG F.W., NIELSEN D.S., ANDREASEN A.S., PEDERSEN B.K., AL-SOUD W.A., SORENSEN S.J., HANSEN L.H., JAKOBSEN M., 2010 – Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. ***PLoS One*** 5, e9085.
25. LEE S.H., SHINDE P.L., CHOI J.Y., KWON I.K., LEE J.K., PAK S.I., CHO W.T., CHAE B.J., 2010 – Effects of tannic acid supplementation on growth performance, blood hematology, iron status and faecal microflora in weanling pigs. ***Livestock Science*** 131, 281-286.
26. LEY R.E., BACKHED F., TURNBAUGH P., LOZUPONE C.A., KNIGHT R.D., GORDON J.I., 2005 – Obesity alters gut microbial ecology. ***Proceedings of the National Academy of Sciences of the United States of America*** 102, 11070-11075.
27. LI J.G., WANG C.D., TANG Z,H., GUO Y,Q., ZHENG T.C., LI Y.Z., YOU Z.Q., 2017 – The gut bacterial community composition of wild *cervus albirostris* (white-lipped deer) detected by the 16S ribosomal RNA gene sequencing. ***Current Microbiology***. doi: 10.1007/s00284-017-1288-9.
28. LI J.N., LIU J.K., TAO S.L., 2007 – Effects of hunger and tannic acid on food intake and foraging behavior in *Microtus fortis*. ***Acta Ecologica Sinica*** 27, 4478-4484. In Chinese.
29. LI X.S., WANG D.H., 2005 – Regulation of body weight and thermogenesis in seasonally acclimatized Brandt's voles (*Microtus brandti*). ***Hormones and Behavior*** 48, 321-328.
30. LI Z., HENNING S.M., LEE R.P., LU Q.Y., SUMMANEN P.H., THAMES G., CORBETT K., DOWNES J., TSENG C.H., FINEGOLD S.M., HEBER D., 2015 – Pomegranate extract induces ellagitannin metabolite formation and changes stool microbiota in healthy volunteers. ***Food & Function*** 6, 2487-2495.
31. MANSOORI B., MODIRSANEI M., 2012 – The effect of tannic acid and polyethylene glycol on the absorption capacity of chicken intestine for d-xylose and beta-carotene. ***Journal of Animal Physiology and Animal Nutrition*** 96, 47-51.
32. MARKLE J.G., FRANK D.N., MORTIN-TOTH S., ROBERTSON C.E., FEAZEL L.M., ROLLE-KAMPCZYK U., VON BERGEN M., MCCOY K.D., MACPHERSON A.J., DANSKA J.S., 2013 – Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. ***Science*** 339, 1084-1088.
33. MARTIN M.O., GILMAN F.R., WEISS S.L., 2010 – Sex-specific asymmetry within the cloacal microbiota of the striped plateau lizard, Sceloporus virgatus. ***Symbiosis*** 51, 97-105.
34. MASEK T., STARCEVIC K., 2017 – Lipogenesis and lipid peroxidation in rat testes after long-term treatment with sucrose and tannic acid in drinking water. ***Andrologia*** 49 (4). doi: 10.1111/and.12632
35. MAURICE C.F., KNOWLES S.C., LADAU J., POLLARD K.S., FENTON A., PEDERSEN A.B., TURNBAUGH P.J., 2015 – Marked seasonal variation in the wild mouse gut microbiota. ***The Isme Journal*** 9, 2423-2434.
36. MCLAUGHLIN R.W., COCHRAN P.A., DOWD S.E., 2015 – Metagenomic analysis of the gut microbiota of the Timber Rattlesnake, *Crotalus horridus*. ***Molecular Biology Reports*** 42, 1187-1195.
37. MUELLER S., SAUNIER K., HANISCH C., NORIN E., ALM L., MIDTVEDT T., CRESCI A., SILVI S., ORPIANESI C., VERDENELLI M.C., CLAVEL T., KOEBNICK C., ZUNFT H.J.F., DORÉ J., BLAUT M., 2006 – Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. ***Applied and Environmental Microbiology*** 72, 1027-1033.

38. SHANG Q., LI Q., ZHANG M., SONG G., SHI J., JIANG H., CAI C., HAO J., LI G., YU G., 2016a – Dietary keratan sulfate from shark cartilage modulates gut microbiota and increases the abundance of *Lactobacillus* spp. ***Marine Drugs*** 14(12). doi: 10.3390/md14120224
39. SHANG Q., SHI J., SONG G., ZHANG M., CAI C., HAO J., LI G., YU G., 2016b – Structural modulation of gut microbiota by chondroitin sulfate and its oligosaccharide. ***International Journal of Biological Macromolecules*** 89, 489-498.
40. SPOR A., KOREN O., LEY R., 2011 – Unravelling the effects of the environment and host genotype on the gut microbiome. ***Nature Reviews Microbiology*** 9, 279-290.
41. STEEGENGA W.T., MISCHKE M., LUTE C., BOEKSCHOTEN M.V., PRUIS M.G., LENDVAI A., VERKADE H.J., BOEKHORST J., TIMMERMAN H.M., PLÖSCH T., MÜLLER M., 2014 – Sexually dimorphic characteristics of the small intestine and colon of prepubescent C57BL/6 mice. ***Biology of Sex Differences*** 5, 11. doi: 10.1186/s13293-014-0011-9
42. TORREGROSSA A.M., NIKONOVA L., BALES M.B., VILLALOBOS LEAL M., SMITH J.C., CONTRERAS R.J., ECKEL L.A., 2014 – Induction of salivary proteins modifies measures of both orosensory and postingestive feedback during exposure to a tannic acid diet. ***PLoS One*** 9, e105232.
43. TURNBAUGH P.J., BACKHED F., FULTON L., GORDON J.I., 2008 – Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. ***Cell Host & Microbe*** 3, 213-223.
44. USSAR S., GRIFFIN N.W., BEZY O., FUJISAKA S., VIENBERG S., SOFTIC S., DENG L., BRY L., GORDON J.I., KAHN C.R., 2015 – Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome. ***Cell Metabolism*** 22, 516-530.
45. VENDRAME S., GUGLIELMETTI S., RISO P., ARIOLI S., KLIMIS-ZACAS D., PORRINI M. 2011 – Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. ***Journal of Agricultural and Food Chemistry*** 59, 12815-12820.
46. WELDON L., ABOLINS S., LENZI L., BOURNE C., RILEY E.M., VINEY M., 2015 - The gut microbiota of wild mice. ***PLoS One*** 10, e0134643.
47. YANG K., WEI C., ZHAO G.Y., XU Z.W., LIN S.X., 2017 – Effects of dietary supplementing tannic acid in the ration of beef cattle on rumen fermentation, methane emission, microbial flora and nutrient digestibility. ***Journal of Animal Physiology and Animal Nutrition*** 101, 302-310.
48. YATSUNENKO T., REY F.E., MANARY M.J., TREHAN I., DOMINGUEZ-BELLO M.G., CONTRERAS M., MAGRIS M., HIDALGO G., BALDASSANO R.N., ANOKHIN A.P., HEATH A.C., WARNER B., REEDER J., KUCZYNSKI J., CAPORASO J.G., LOZUPONE C. A., LAUBER C., CLEMENTE J.C., KNIGHTS D., KNIGHT R., GORDON J.I., 2012 – Human gut microbiome viewed across age and geography. ***Nature*** 486, 222-227.
49. YE M.H., NAN Y.L., DING M.M., HU J.B., LIU Q., WEI W.H., YANG S.M., 2016 – Effects of dietary tannic acid on the growth, hepatic gene expression, and antioxidant enzyme activity in Brandt's voles (*Microtus brandti*). ***Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology*** 196-197, 19-26.
50. YURKOVETSKIY L., BURROWS M., KHAN A.A., GRAHAM L., VOLCHKOV P., BECKER L., ANTONOPOULOS D., UMESAKI Y., CHERVONSKY A.V., 2013 – Gender bias in autoimmunity is influenced by microbiota. ***Immunity*** **39**, 400-412.
51. ZHANG D., JI H., LIU H., WANG S., WANG J., WANG Y., 2016 – Changes in the diversity and composition of gut microbiota of weaned piglets after oral administration of *Lactobacillus* or an antibiotic. ***Applied Microbiology and Biotechnology*** 100, 10081-10093.
52. ZHAO L. 2013 – The gut microbiota and obesity: from correlation to causality. ***Nature Reviews Microbiology*** 11, 639-647.