

Effects of floor- and net-reared systems on the intestinal growth and microbial diversity in the cecum of ducks

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Abstract

Background

Rearing systems can affect livestock production directly, but the effects of floor-reared systems (FRSs) and net-reared systems (NRSs) on the intestinal growth states and microbial diversity in the cecum of ducks are largely unclear.

Methods

The ducklings in this study were randomly divided into FRS and NRS groups, weighed at 4, 8 and 13 weeks, respectively, then the duodenum, jejunum, ileum and cecum were sampled and measured, and the content of cecum were analyzed by 16S RNA.

Results

The values of relative weight (RW), relative length (RL) and RW/ RL of four intestinal segments in FRS were significantly higher than that in the NRS during week 4, 8 and 13 ($p < 0.05$). A total of 157 genus were identified from ducks under the two systems, the dominant microorganisms in both groups were Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria at phyla level. The distribution of microorganisms in cecum of two groups showed significant separation in three time periods, and the value of Simpson index in FRS was significantly higher than NRS at 13 weeks ($p < 0.05$). Five differential microorganisms and 25 differential metabolic pathways were found in the cecum at week 4, 7 differential microorganisms and 25 differential metabolic pathways were found in the cecum at week 8, and 4 differential microorganisms and 2 differential metabolic pathways were found in the cecum at week 13.

Conclusions

There were differences in intestinal growth and microorganism between FRS and NRS ducks.

Introduction

Digestion and nutrient absorption are the basic functions of the intestine and mainly occur in the small intestine, which is also the longest part of the digestive tract. The mucosa is a crucial component of the small intestine wall with many finger-like villi extending from the mucosal layer to the lumen, increasing the surface area of the small intestine 600 times compared to that of the whole intestinal cavity, and the nutrients in intestinal content can be easily absorbed because the villi have tight blood capillaries with thin vascular walls [1–3]. Premature mice with poor weight have lower villus height, crypt depth, cell proliferation, goblet cells and Pan's cells than normal mice [4]. Thus, increasing the length and weight of

the intestinal tract helps to expand the digestive area of food and promote the digestion and absorption of nutrients.

Intestinal microorganisms are known as the "second genome" of the host, and approximately 35% of microbial enzymes in the intestine can be utilized by the host. Intestinal microorganisms play an important role in body growth and health by impacting intestinal villus and crypt morphology, nutrient metabolism regulation, mucosal immune activation, energy-rich short-chain fatty acid production, host behavior regulation, intestinal epithelial cell repair and pathogenic microorganism resistance[5–9]. Food rapidly passes through the front of the intestinal tract but stays for several hours in the tail end of the tract [10]. The cecum, as the main site of intestinal microbial colonization and the main area of microbial anaerobic fermentation with the highest content of short-chain fatty acids, has a higher fermentation ability than the small intestine [11], is considered to be of highest importance in poultry health and is a major pathogen reservoir [12–14]. The abundance and diversity of cecum microorganisms are influenced by many factors [15–17], and rearing systems are an important factor. The microbial diversity in the cecum of wild red-crowned cranes is lower than that of captive and artificial cranes, and the microorganisms composition is also significantly variable [18], which is consistent with the results of Kakapo parrots, Antarctic seals and wild captured rodents [19, 20]. However, different from the above consequences, the microbial abundance of the cecum of outdoor Dagu chickens is higher than that of cage chickens [21].

After China's accession to the WTO, the export share of duck's main and by-products, such as duck meat, duck egg and down, has been greatly increased. According to the statistics of FAO, the number of ducks raised in China and the number of ducks in stock have ranked first in the world in recent years. The floor-reared system (FRS) and net-reared system (NRS) are the two main systems of intensive duck production; the FRS is beneficial for muscle growth and product quality [22], while the NRS can allow excreta to be removed through metal nets, thus keeping the environment clean. In this study, we aim to perform a comprehensive assessment of the intestinal growth and microorganisms in the cecum of ducks in an FRS and NRS. The results of this study will help to offer useful information for selecting an appropriate and healthy rearing system for ducks and provide a theoretical and practical reference for the further study of ducks in rearing systems.

Methods

Laboratory Animals

The Nonghua sheldrake ducks used in this experiment were provided by the poultry raising experimental farm of Sichuan Agricultural University. A total of 180 healthy ducks were randomly divided into an FRS and NRS equally after brooding with a feeding density of 4-5/m², and the intake of feed and water for each duck were ensured ad libitum during the experiment to meet the National Research Council requirements. Table 1 shows the nutritional standards of the different stages of the duck. Thirty ducks in each group were randomly selected at 4, 8 and 13 weeks, respectively, and euthanized by cervical

dislocation after fasting for 12 h. All animal handling procedures were reviewed and approved by the Animal Ethics Committee of Sichuan Agricultural University (Ya'an, China).

Table 1 The nutrition standard of the diets for different stages of duck

nutrition	week	
	0~3	3~10
Moisture (%)	≤14.0	≤14.0
Crude protein (%)	≥19.0	≥15.0
Crude fiber (%)	≤6.0	≤7.0
Coarse ash (%)	≤8.0	≤10.0
Calcium (%)	0.8~1.5	0.8~1.5
Total phosphorus (%)	≥0.60	≥0.60
Sodium chloride (%)	0.3~0.8	0.3~0.8
Methionine (%)	≥0.35	≥0.30

Measurement of Intestinal Growth

The intestine of FRS and NRS was taken out and tissues such as pancreas and fat attached to the intestine were removed; one end of the intestine was fixed on a glass plate wetted with distilled water and gently straightened to measure the length of the duodenum, jejunum, ileum and cecum when the intestine no longer retracted. The contents of the intestine were removed, and the aforementioned four intestinal segments were weighed with an electronic balance. RW/RL relative length (RL), relative density (RW) and RW/RL were calculated.

RL = intestinal length (cm)/live weight (kg)

RW = intestinal weight (g)/live weight (kg)

Determination of Cecum Microorganisms

The intestinal contents of 5 ducks in each reraring system were randomly selected at 4, 8 and 13 weeks respectively. Place the obtained contents of intestines in the marked EP tubes and put them into liquid nitrogen immediately. The 16S rRNA gene library was constructed by extracting total microbial DNA from intestinal contents and amplified 16S rRNA sequences of all microorganisms. PCR products were purified using Agencourt Ampure XP beads (Beckman, USA) accounting to the instructions of manufacturer and quantified using the PicoGreen dsDNA Assay kit (Invitrogen, USA), equally combined and followed by gel purification using a QIAquick Gel Extraction Kit (Qiagen, USA), and then requantified by PicoGreen. The prepared DNA library was then sequenced using the MiSeq platform (Illumina, USA). An

operational taxonomic units (OTUs) table was generated using the Uparse clustering method (97 % cutoff), and all samples were rarefied to the same sequencing depth by resampling OTUs prior to downstream analysis.

Data Statistic Analysis

Mothur software (version v.1.30) and QIIME software (version 1.8) were used to evaluate the alpha diversity and beta diversity of the samples. The beta diversity distance matrix was calculated by Qiime (version 2), and the differences were reflected on the two-dimensional coordinate map for NMDS analysis. In LEfSe analysis (version 1.5.3), the nonparametric factor Kruskal Wallis rank sum test and Wilcoxon rank sum test were used to detect the difference between the two groups, and the biomarker was obtained based on $LDA > 4$. The figures were drawn using the R language tool (version 3.6.0). SPSS 21.0 software (IBM, USA) was used to analyze the data, and a t-test was used to analyze the significance of the sample data. The data are expressed as the mean \pm S.D. Statistically, $p < 0.05$ represents a significant difference, and $p < 0.01$ is an extremely significant difference.

Results

The Effects of Rearing Systems on the Growth of the Small Intestine

The values of relative weight (RW)/ relative length (RL) of the duodenum, jejunum, and ileum and the value of jejunal RW in FRS were significantly higher than that in the NRS at 4 weeks ($p < 0.05$), and cecal RW and RW/RL in the FRS were great significantly higher ($p < 0.01$). The RW/ RW of cecum in FRS was significantly higher than that in the NRS at 8 weeks ($p < 0.05$), and all other intestinal growth related indexes, including RL, RW and RW/RW, in FRS were great significantly higher ($p < 0.01$). The values of RW/RL and RW of the duck small intestine and the RL of ileum in the FRS were still great significantly higher than those in the NRS at 13 weeks ($p < 0.01$), and cecal RW/RL in NRS was also significantly lower than that in FRS ($p < 0.05$). In addition, the body weight of ducks in the two systems was also statistically analyzed and it was found that the value in FRS was significantly higher than NRS at 4 weeks ($p < 0.05$), while significantly lower at 8 weeks ($p < 0.05$). However, there was no significant difference in body weight between the two systems at week 13 ($p > 0.05$) (Table 2).

Table 2
The effects on the body weight and intestinal growth of ducks

week	system	Body	segment	system	relative	relative	relative
		weight			length	weight	length/ relative weight
		(kg)			(cm/kg)	(g/kg)	(cm/g)
4	FRS	1.07 ± 0.12	Duodenum	FRS	26.05 ± 1.86	4.34 ± 0.34	0.17 ± 0.02
	NRS	1.02 ± 0.10		NRS	26.01 ± 1.35	4.16 ± 0.66	0.15 ± 0.02
	<i>P</i> value	0.03		<i>P</i> value	0.90	0.12	0.02
			Jejunum	FRS	63.59 ± 4.08	11.48 ± 1.09	0.18 ± 0.02
				NRS	63.88 ± 3.09	10.86 ± 1.43	0.17 ± 0.02
				<i>P</i> value	0.71	0.03	0.02
			Ileum	FRS	60.78 ± 4.07	10.62 ± 0.78	0.18 ± 0.01
				NRS	61.38 ± 3.31	10.33 ± 0.99	0.17 ± 0.02
				<i>P</i> value	0.46	0.13	0.01
			Cecum	FRS	13.76 ± 1.43	1.80 ± 0.58	0.13 ± 0.05
				NRS	13.77 ± 1.06	1.20 ± 0.32	0.09 ± 0.02
				<i>P</i> value	0.98	0.00	0.00
8	FRS	2.11 ± 0.21	Duodenum	FRS	12.98 ± 0.88	2.66 ± 0.21	0.21 ± 0.02
	NRS	2.35 ± 0.24		NRS	11.94 ± 0.65	2.33 ± 0.18	0.20 ± 0.01
	<i>P</i> value	0.19		<i>P</i> value	0.00	0.00	0.00

Note: FRS represent floor-reared system, and NRS represent net-reared system. n = 30

week	system	Body	segment	system	relative	relative	relative
		weight			length	weight	length/ relative
		(kg)			(cm/kg)	(g/kg)	weight (cm/g)
			Jejunum	FRS	32.46 ± 3.32	6.31 ± 0.50	0.20 ± 0.01
				NRS	28.95 ± 1.62	4.97 ± 0.36	0.18 ± 0.01
				<i>P value</i>	0.00	0.00	0.00
			Ileum	FRS	31.68 ± 2.11	5.91 ± 0.34	0.19 ± 0.01
				NRS	27.77 ± 1.74	4.81 ± 0.37	0.18 ± 0.01
				<i>P value</i>	0.00	0.00	0.00
			Cecum	FRS	14.83 ± 1.09	1.43 ± 0.16	0.10 ± 0.01
				NRS	13.36 ± 0.86	1.23 ± 0.13	0.10 ± 0.01
				<i>P value</i>	0.00	0.00	0.03
13	FRS	2.39 ± 0.26	Duodenum	FRS	13.77 ± 1.06	2.29 ± 0.23	0.21 ± 0.01
	NRS	2.37 ± 0.26		NRS	10.91 ± 0.91	1.93 ± 0.23	0.18 ± 0.01
	<i>P value</i>	0.73		<i>P value</i>	0.28	0.00	0.00
			Jejunum	FRS	11.16 ± 0.82	5.50 ± 0.58	0.20 ± 0.01
				NRS	27.63 ± 2.55	4.19 ± 0.66	0.17 ± 0.01
				<i>P value</i>	0.11	0.00	0.00
			Ileum	FRS	27.59 ± 1.99	5.20 ± 0.56	0.19 ± 0.01

Note: FRS represent floor-reared system, and NRS represent net-reared system. n = 30

week	system	Body	segment	system	relative	relative	relative
		weight			length	weight	length/ relative
		(kg)			(cm/kg)	(g/kg)	weight (cm/g)
				NRS	25.82 ± 2.15	4.05 ± 0.68	0.17 ± 0.01
				<i>P value</i>	0.00	0.00	0.00
			Cecum	FRS	12.28 ± 1.06	1.27 ± 0.18	0.11 ± 0.01
				NRS	11.84 ± 1.00	1.25 ± 0.16	0.11 ± 0.01
				<i>P value</i>	0.10	0.59	0.03

Note: FRS represent floor-reared system, and NRS represent net-reared system. n = 30

The Effects Of The Rearing System On Cecum Microorganisms

A total of 4,612,553 clean tags were generated from 104 samples of duck cecal contents after splicing and filtering for quality, and each sample produced at least 25,925 clean tags. The rarefaction curve with the number of OTUs based on sequencing tended to reach a saturation plateau, suggesting that the 104 samples were adequate to estimate the phenotype richness and microbial community diversity of cecum microorganisms at a 97% similarity threshold, and broadly, the microbial abundance in the FRS was higher than that in the NRS (Fig. 1A). To investigate the microbial community of the cecum in the FRS and NRS, pairwise comparisons of microbial similarity among the two systems were performed, and analyses of the common and unique OTUs were conducted. A total of 157 genus were identified from ducks under the two systems. However, no specific microorganism was found in each intestinal segment at 4,8 and 13 weeks (Fig. 1B). The bacterial phyla of the top 10 most abundant microorganisms of the cecum were determined, and the dominant microorganisms in both groups were Firmicutes(43.87%~ 49.61% vs 41.58%~ 57.40%), Bacteroidetes(20.54%~ 28.06% vs 14.14%~ 18.51%), Actinobacteria(9.79%~ 22.67% vs 12.34%~ 33.89%) and Proteobacteria(5.93%~ 6.41% vs 3.66%~ 6.58%); the abundance of Bacteroidetes at 13 weeks was higher than that of Actinobacteria in the FRS(27.56% vs 9.79%), which was the exact opposite of the NRS (14.14% vs 23.69%) (Fig. 1C).

Simpson index had no significant difference between FRS and NRS at 4 and 8 weeks by analyzing the microbial diversity of cecal contents ($p > 0.05$), while the value of FRS was significantly higher than NRS at 13 weeks ($p < 0.05$) (Fig. 2A). Besides, the distribution of cecal microorganisms in the two rearing systems were obviously separated in the three time periods of the experiment (stress1 = 0.1314, stress1 =

0.1226, stress1 = 0.1441) (Fig. 2B). LEfSe analysis was carried out to determine the specific microorganisms at species level responsible for microorganism diversity. The abundances of *Ruminococcaceae-uncultured-bacterium*, *Ruminococcaceae-UCG-014* and *Desulfovibrio* were higher in FRS than in NRS at 4 weeks, while *Brachybacterium* and *Lactobacillus* genera hold higher abundances. The genera *Brevibacterium*, *Brachybacterium* and *Bacteroides* were enriched in the FRS at 8 weeks, while *Subdoligranulum*, *Akkermansia*, *Blautia* and *Collinsella* were enriched in the NRS. The abundances of the genera *Bacteroides* and *Ruminococcaceae-uncultured-bacterium* were higher in the FRS at 13 weeks, while *Subdoligranulum* and *Brachyspira* were more abundant in the NRS (Table 3).

Table 3
LEfSe analysis of the cecum microorganisms

week	microorganism	abundance	system	LDA	p value
4	Lactobacillus	4.63	NRS	4.08	0.03
	Desulfovibrio	4.71	FRS	4.21	0.00
	Brachybacterium	4.91	NRS	4.29	0.01
	uncultured_bacterium_f_Ruminococcaceae	4.63	FRS	4.03	0.01
	Ruminococcaceae_UCG_014	4.41	FRS	4.06	0.00
8	Bacteroides	5.32	FRS	4.72	0.00
	Collinsella	4.66	NRS	4.19	0.00
	Blautia	4.59	NRS	4.02	0.00
	Akkermansia	4.82	NRS	4.57	0.00
	Subdoligranulum	4.93	NRS	4.14	0.02
	Brevibacterium	4.77	FRS	4.45	0.00
	Brachybacterium	4.88	FRS	4.57	0.00
13	Brachyspira	4.58	NRS	4.18	0.02
	Bacteroides	5.3	FRS	4.75	0.00
	Subdoligranulum	4.99	NRS	4.41	0.01
	uncultured_bacterium_f_Ruminococcaceae	4.92	FRS	4.39	0.00

Note: FRS represent floor-reared system, NRS represent net-reared system.

Further analysis of the microorganisms in the cecum was conducted to study the different functional pathways at class 2 level among the FRS and NRS and the results showed that Carbohydrate metabolism (16.36%~16.59% vs 16.52%~16.72%), Global and overview maps(14.00%~14.29% vs 14.14%~14.37%) and Amino acid metabolism (11.46%~ 11.71% vs 10.80%~10.93%) hold top 3 abundance in both

systems (Fig. 3A). There were 25 significantly different pathways between the two systems at 4 weeks, and 9 pathways were more abundant in the FRS, including drug resistance, environmental adaptability, energy metabolism and cell motility pathways (Fig. 3B). Similar to the results at 4 weeks, 25 different pathways, including 11 functional pathways, such as immune diseases, cofactors and vitamin metabolism, endocrine system and amino acid metabolism, were more abundant in the FRS at 8 weeks (Fig. 3C). However, only cofactors and vitamin metabolism were more abundant in the FRS at 13 weeks, while the abundances of the translation pathway were higher in the NRS (Fig. 3D).

Discussion

The host and environment both influence the microorganisms of the cecum, and environmental factors are more significant than the host [23, 24]. The FRS and NRS are two main methods of intensive farming of ducks. The FRS is the most primitive method of duck farming in China due to its low cost and high meat quality. However, FRS require a particular rearing area and frequent replacement of cushions, which are still challenges, and diseases occur easily in FRS due to direct contact with feces. Currently, most farmers build grid structures approximately 60 cm above the ground and lay metal nets to remove excreta, but the cost is relatively high, and the cleaning and disinfection of the nets are inconvenient. In this study, we reared ducks with the same density, intake and drink under unified management in both an FRS and NRS to determine the differences in the intestinal growth and microorganisms of the cecum.

The growth of the small intestine in the outdoor environment, such as grazing and artificial grassland, was significantly higher than that in the cage [21, 25], and a similar situation appeared in this experiment. Both intestinal relative length, which can reflect the intestinal capacity, and the ratio of relative weight to relative length, which could reflect intestinal motility, were higher in FRS, suggesting that ducks reared in floor system have stronger intestinal peristalsis ability and larger food digestion areas, and it may result from increased activity in a swimming pool. Many reports have shown that the length and weight of poultry intestines are affected by the level of fiber, for example, dietary sunflower hulls could increase the length of the small intestine of broilers [26]. Considering the specific situation of this experiment, it may be because in addition to artificial feeding, ducks in the FRS also consume mattresses on the ground and algae in ponds, which increase their fiber levels. Therefore, it can be concluded that FRSs is more conducive to duck intestinal growth than NRSs.

The comprehensive characterization of duck intestinal microbial communities is a critical precondition to understand and predict how rearing systems alter these communities. In order to further explore the differences between the FRS and NRS, the sequences of the cecum content were detected. Although there are no specific microorganisms in cecum of ducks in both systems at genus level, the diversity showed a significant difference at 13 weeks. In general, the diversity of intestinal microbial composition of poultry gradually increases with the increase of age after birth. All kinds of microorganisms rise and fall one after another, and then tend to a relatively stable state in youth[15]. Therefore, the difference in diversity of duck intestinal microorganism in this study is convincing. Intestinal microorganisms were affected by many factors, including age, gender and environment[27–29]. Different rearing systems provided

different growth environment for ducks, which made the diversity in ground-based ducks higher, and the result is consistent with that of Dagu chicken [21]. Considering that long-term stress could reduce the diversity of intestinal microbiota [30], this result may be because the ducks were raised on a net and unable to contact the natural environment. Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria were dominant in the cecum in both systems, and this result coincides with wild turkeys, captive broilers, caged Beijing ducks and floor-raised Landes geese [31–34].

The environmental adaptability pathways of the microorganisms in the cecum of ducks in the FRS at 4 weeks were higher, and the metabolic-related pathways were lower, including xenobiotic biodegradation and metabolism, amino acid metabolism and lipid metabolism, suggesting that the change in the living environment after the brooding period caused stress. The abundance of most pathways related to diseases, including cardiovascular disease pathways, substance dependence and viral infectious diseases, in the NRS was higher than that in the FRS at 4, 8 or 13 weeks, and these diseases cause serious harm to the body, which is consistent with previous studies [35, 36]. Of the 8 different metabolic pathways at 8 weeks, the abundance of 5 of them were greater in the FRS than in the NRS, suggesting that ducks in the FRS had adapted to the environment and needed more substance, improving the meat quality. Studies have shown that the meat of outdoor chickens is darker and has a better water-holding capacity [37, 38]. In addition, of the 25 functional pathways at 8 weeks, 16 pathways appeared at 4 weeks, and their abundances changed between 4 weeks and 8 weeks. Only the cofactors and vitamin metabolic pathways in the FRS were more abundant due to the higher abundance of *Bacteroides* and *Ruminococcaceae-uncultured bacteria*, while the abundance of the translation pathway in the NRS was higher at 13 weeks due to the presence of *Subdoligranulum* and *Brachyspira*, implying that the differences in the functional pathways of the microorganisms in the cecum of ducks in the FRS and NRS gradually decreased with time. As discussed before, the colonization of intestinal microflora is a process that changes with age [39], thus the differential metabolic pathways and microorganisms are also a gradually stable process. When ducks enter the youth period and the intestinal environment is relatively stable, the influence of FRS and NRS on duck intestinal development and microorganisms can be revealed.

Conclusion

There were differences in intestinal development and microorganism between floor-reared systems (FRS) and net-reared systems (NRS) ducks. The values of intestinal relative length, relative weight and relative weight / relative length in FRS were higher than NRS. The cecum microorganisms of ducks were obviously separated under the two rearing systems, and the diversity of cecal microorganisms was higher in FRS at 13 weeks. The differential metabolic pathways of cecal microorganisms decreased with the increase of age, and the abundances of the translation pathway were higher in the NRS at week 13, while cofactors and vitamin metabolism were more abundant in FRS.

Abbreviations

Abbreviation	Full name
FRS	floor-reared systems
NRS	net-reared systems
RL	Relative length
RW	Relative weight
OTUs	Operational taxonomic units
LEFse	Linear Discriminant Analysis effect size

Declarations

Ethics approval and consent to participate

The experimental protocol utilized in this research were complied with the Chinese guidelines for animal welfare and approved by the Institutional Animal Care and Use Committee (IACUC) of Sichuan Agricultural University (DKY-B20141401).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

Not applicable.

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Authors' contributions

JW, SH and LL put forward the idea and method of the experiment. BH, LH, JH and LC participated in the incubation, breeding and sample collection of the experimental animals. XC, CH and BK participated in the 16S RNA analysis of intestinal contents. HL, HX and HH analyzed and interpreted the collected data. LL

was the supervisor of the project. XC was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Figures

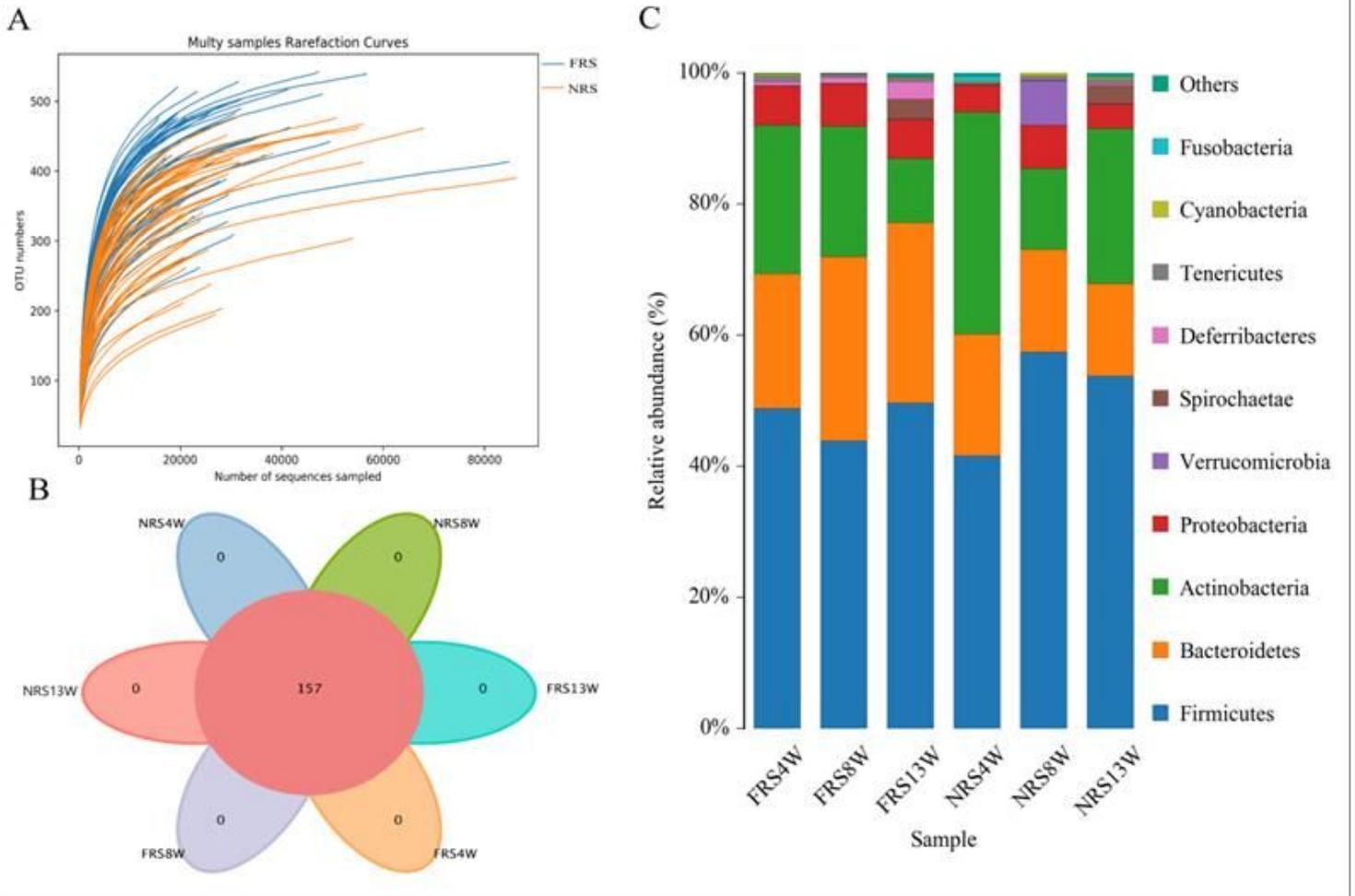


Figure 1

A: Multy sample rarefaction curves of microorganisms in cecal contents of ducks. B: Venn map of cecal microorganisms at genus level at week 4, 8 and 13. C: Distribution of cecal microorganisms at phylum level. All the microorganisms are expressed as percentages, and only the top 10 microbial phyla are shown. In A, B and C, FRS represents floor-reared systems, and NRS represents net-reared systems. 4W, 8W and 13W represent 4 weeks of age, 8 weeks of age and 13 weeks of age.

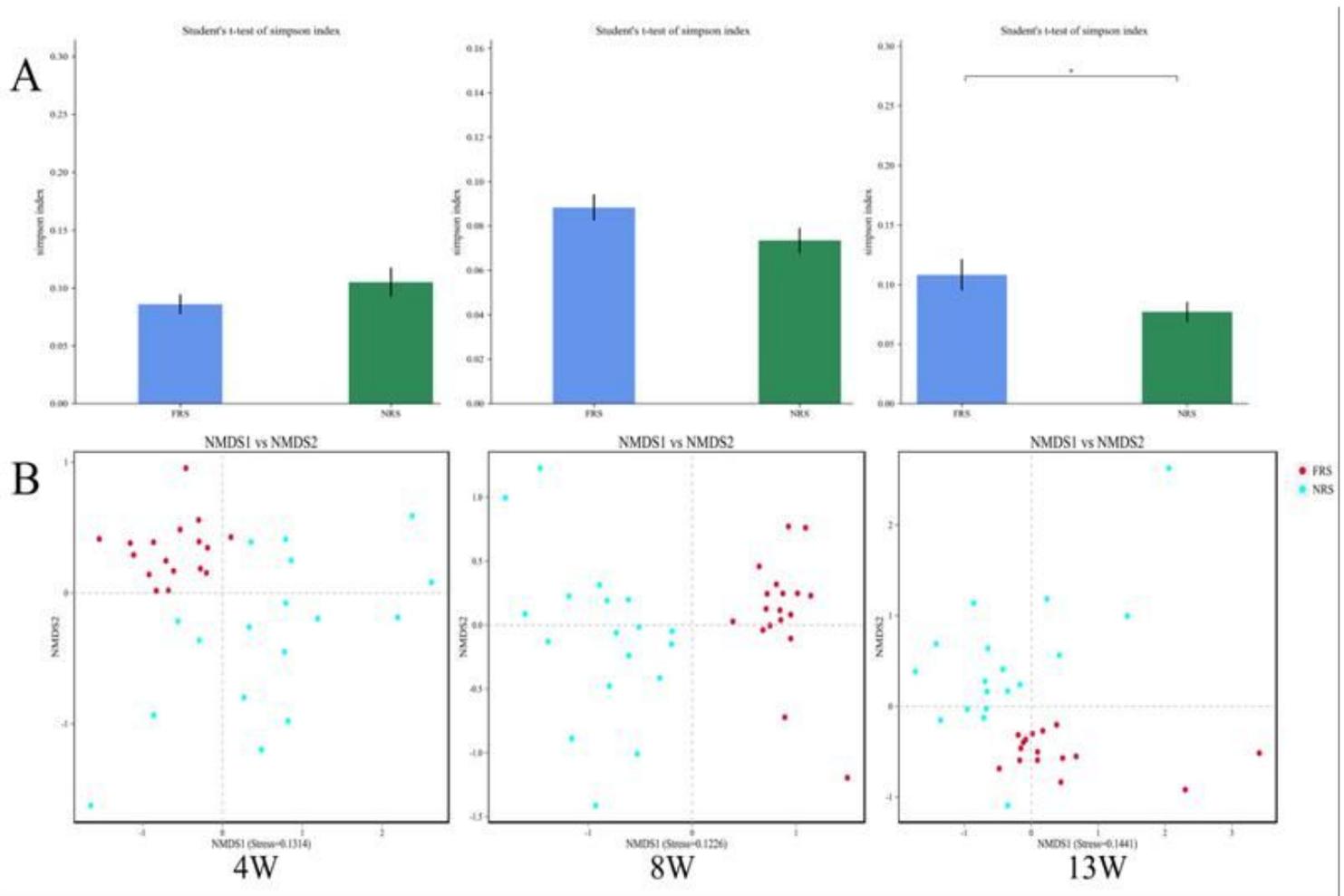


Figure 2

A: Simpson's index at genus level of microorganisms in cecal contents of ducks. B: NMDS analysis of cecal microorganisms at genus level. In A and B, FRS represents floor-reared systems, and NRS represents net-reared systems. 4W, 8W and 13W represent 4 weeks of age, 8 weeks of age and 13 weeks of age.

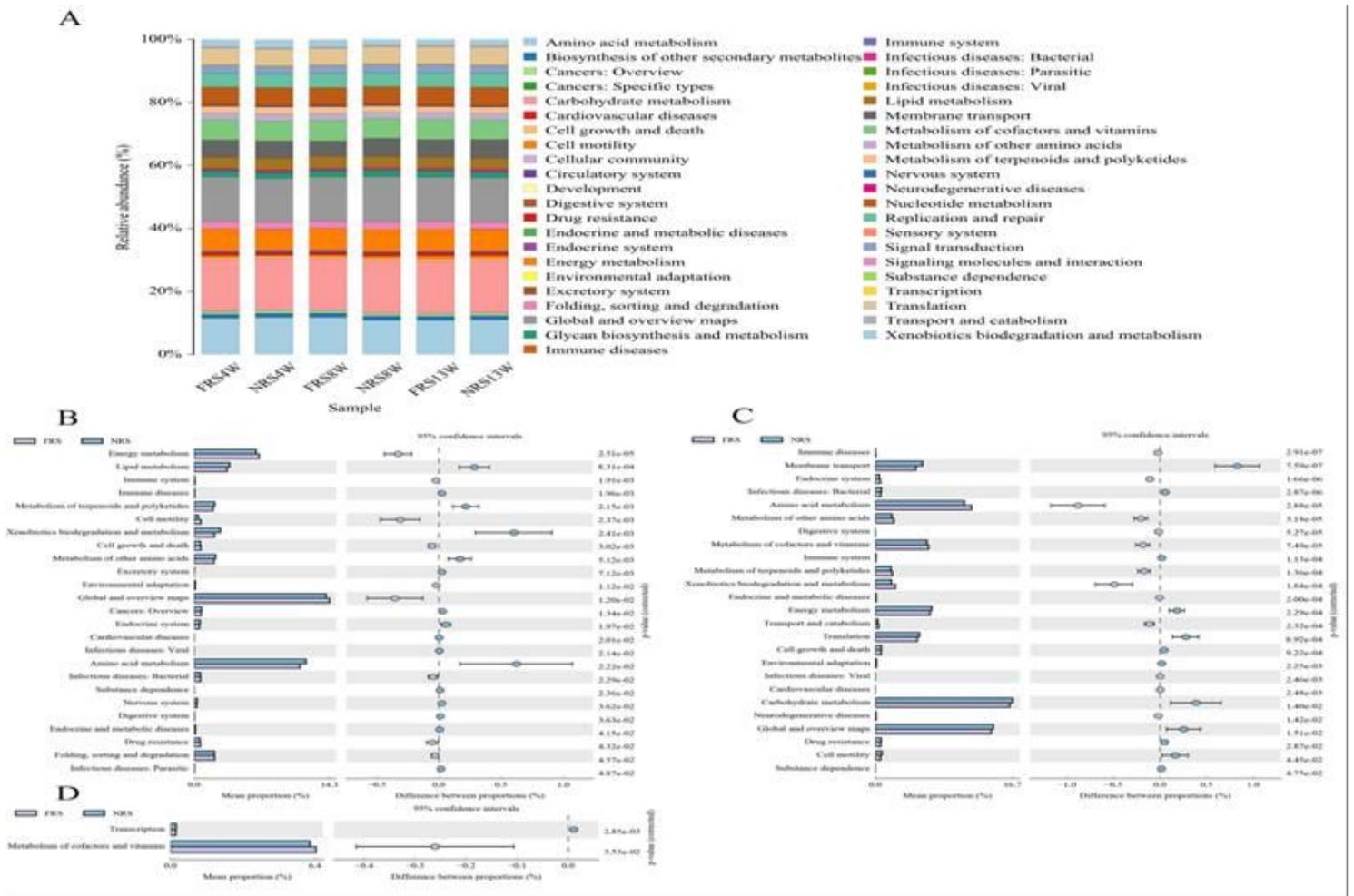


Figure 3

A: Distribution of functional pathways of microorganisms in cecal contents of ducks. All the microorganisms are expressed as percentages. B: Differential function pathways at 4 weeks. C: Differential function pathways at 8 weeks. D: Differential function pathways at 13 weeks. In A, B, C and D, FRS represents floor-reared systems, and NRS represents net-reared systems. 4W, 8W and 13W represent 4 weeks of age, 8 weeks of age and 13 weeks of age.