Effects of rearing system and narasin on growth performance, gastro-intestinal development and gut microbiota of broilers

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Research

Keywords: rearing system, narasin, growth performance, gastro-intestine development, intestinal microbiota, broilers

Posted Date: July 29th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-49565/v1

Version of Record: A version of this preprint was published on November 19th, 2020. See the published version at https://doi.org/10.1016/j.psj.2020.10.073.

Abstract

Background

This study was conducted to evaluate the effects of three rearing systems (FL: flooring litter rearing, MC: multilayer cage rearing, PN: plastic net rearing) and narasin inclusion or not on growth performance, gastro-intestine development and health of broilers. A total of 2400 one-day-old Ross 308 mixed-sex broilers (1:1 ratio of males and females) were used in a completely randomized design utilizing a 3 × 2 factorial arrangement of treatments, with 12 replicates per treatment. In each replicate for FL, MC and PN consisted of: 34 birds per pen, 30 birds per cage, and 36 birds per pen, respectively, ensuring that the density of each rearing system was the same (12 birds/m^2).

Results

Lower ADG (average daily gain), ADFI (average daily feed intake) and FCR (feed conversation ratio) observed in MC group than those of the other two systems from 1 to 36 days of age ($P < 0.05$). Narasin inclusion in diets decreased ADFI and FCR significantly ($P < 0.05$). MC and PN rearing systems reduced the relative weight of the gizzard significantly ($P < 0.05$). Compared with FL rearing, MC reduced the relative weight of the duodenum, jejunum and ileum ($P < 0.05$). The mRNA expression levels of the ileal IL-1β and IFN-γ in FL was higher than those in PN and MC groups ($P < 0.05$). Narasin decreased the mRNA expressions of TNF-α in the ileum ($P < 0.05$). Different rearing systems changed the ileal microflora structure of broilers. The FL system increased ileal microbial diversity of broilers and relative abundance of *Actinobacteria*. Narasin combined with MC increased the relative abundance *Proteobacteria* of broilers.

Conclusion

birds reared in PN had higher body weight. MC birds had poorer intestinal development and health condition, higher abundance of *Proteobacteria*, but better FCR. FL rearing appeared to be propitious for gastro-intestinal development and health. Narasin inclusion in diets improved FCR and changed the relative abundance *Proteobacteria* of broilers.

Background

In China, floor litter system and plastic net rearing are two traditional systems for rearing broilers. With the development of intensive farming, multilayer cage rearing is becoming widespread, which effectively prevents broilers from having direct contact with their excreta, with one clear benefit where coccidiosis and intestinal diseases are largely eliminated, saving resources and facilitating automated management. Rearing systems are a crucial factor affecting bird comfort, welfare, health and production efficiency [1]. Several studies have been conducted to evaluate the effect of different systems on broiler performance and health of broilers. Thamilvanan et al. [2] reported that the cage rearing system produces better performance and a higher survival rate than the floor rearing system, whereas Swain et al. [3] found no significant effect for either the cage or the floor rearing system on live weight gain and feed intake. Santos et al. [4] revealed that birds raised on floors had better average weight gain and FCR than those reared in cages. Contrarily, Mariam et al.[5] reported that cage rearing improved the growth performance of Cobb broilers. Thus, the literature findings on different rearing systems are equivocal for bird performance. There are numerous underlining issues for the differences. One is the effect of bedding materials on bird health and performance [6] and the other is coccidiosis. Indeed, coccidiosis is a major disease in poultry that causes intestinal lesions, depresses growth, and reduces feed conversion efficiency [7]. Coccidiostats are usually used to counter the negative effects of Eimeria in poultry. Narasin, an ionophore coccidiostat, is used to prevent coccidiosis and necrotic enteritis in broiler chicks [8]. Although there have been numerous studies either on rearing systems or on coccidiostats for their efficacy in broiler diets, the combination of the two in some rearing systems has not extensively examined. This study evaluated the effects of three rearing systems on growth performance, gastro-intestinal development and gut microbiota of broilers with or without narasin.

Methods

The study was approved by the Animal Care and Experiment Committee of New Hope Liuhe Corporation. The management and husbandry of the birds strictly followed the Chinese government regulation on animal welfare.

Experimental design and dietary treatments

A total of 2400 one-day-old Ross 308 mixed-sex broilers (1:1 ratio of males and females) were used in a completely randomized design utilizing a 3 × 2 factorial arrangement of treatments, with 12 replicates in each treatment. In each replicate for FL, MC and PN: there are 34 birds per floor pen, 30 birds per cage, and 36 birds per net pen, respectively, ensuring that the density of each rearing system was the same (12.5 birds/m^2). Narasin was supplemented at 75 ppm in diets. Table 1 shows the experimental design.

Table 1 Experimental design

Treatment	Systems	Narasin	birds/pen(cage)
Treatment 1	Flooring litter rearing(FL)	+	34
Treatment 2	Flooring litter rearing(FL)	-	34
Treatment 3	Multilayer cage rearing(MC)	+	30
Treatment 4	Multilayer cage rearing(MC)	-	30
Treatment 5	Plastic net rearing(PN)	+	36
Treatment 6	Plastic net rearing(PN)	-	36

Birds were fed crumble-pellet diets from d 1-12, and pellet diets from d 13-36. Broiler starter (d 1 to 12), grower (d 13 to 23) and finisher (d 24 to 36) diets were formulated to meet Ross 308 strain recommendations (Table 2).

Table 2 Composition and nutrient levels of basal diets (as is basis, %)

	1 to 12 days of age	13 to 23 days of age	24 to 36 days of age
Ingredients			
Corn	51.95	55.42	61.42
Soybean meal	36.90	30.20	19.20
Corn DDGS	4.00	6.00	8.00
Peanut meal	2.00	3.00	4.00
Corn protein powder	-	-	2.00
Soybean oil	1.20	1.57	1.77
$CaHPO_4$	1.38	1.17	0.72
Limestone	1.23	1.09	1.09
Premix[1]	0.50	0.50	0.50
L-Lys·H_2SO_4	0.26	0.42	0.66
DL-Met	0.22	0.23	0.21
L-Thr	0.06	0.1	0.13
NaCl	0.30	0.30	0.30
Total	100.00	100.00	100.00
Nutrients levels[2]			
CP	22.52	21.00	19.50
ME MJ/kg	10.70	11.10	11.50
Ca	0.90	0.80	0.70
TP	0.55	0.50	0.45

1. The premix provides following per kg diet：I 0.65 mg，Se 0.35 mg，Vitamin A 9000 IU，Vitamin D_3 2000 IU，Vitamin E11 IU，Vitamin K1.0 mg，Vitamin $B_1$2mg，Vitamin $B_2$5.8 mg，Niacin 66mg，Pantothenic acid10 mg，Vitamin B_6 2.6 mg，Biotin 0.10 mg，Folic acid 0.7 mg，Vitamin B_{12} 0.012 mg.
2. All the values are calculated.

Management and husbandry

Rice husk was used as a litter material and was uniformly distributed to cover the floor area to a depth of 5 cm in the FL system. The metal frame is covered with a plastic mesh in the net rearing system. Broiler type cage house of 3 vertical tiers was used in the present study. The brooding temperature was maintained at 33 °C for the first day and was gradually decreased by 2 °C per week until 21 °C and maintained at that level thereafter. During the whole experimental period, chickens had free access to feed and water. Birds were immunized with normal procedures. The indexes of temperature, humidity, light and hygiene in the chicken house accord with the hygienic requirements of broilers (GB 14925-1994).

Sample and data collection

Growth performance

Body weights (BW) and feed intake by pen were recorded on d 12, 23 and 36, and mortality was recorded daily. Average weight gain (ADG), average daily feed intake (ADFI), and FCR were calculated for starter, grower, finisher and overall periods.

Relative digestive organ weights

At 37d of age, 10 chickens around average BW were selected from each treatment, weighed and killed by exsanguinations after CO_2 stunning. After the abdominal incision, the length and weight of proventriculus, gizzard, duodenum, jejunum and ileum were measured, to calculate relative weight of proventriculus, gizzard, duodenum, jejunum and ileum.

Intestinal lesion score

At 37d of age, 10 chickens around average BW were selected from each treatment, weighed and killed by exsanguinations after CO_2 stunning. After the abdominal incision, the small intestine from each bird was opened and scored blindly by three independent observers. Briefly, lesions were scored using a scale from 0 to 4, in which 0 was apparently normal intestinal appearance, no lesion; 1 = thin walled and friable intestines with small red petechiae (>5); 2 = focal necrotic lesions; 3 = patches of necrosis (1 to 2 cm long); and 4 = diffused necrosis typical of field cases.

mRNA expression of ileum immune factors

At 37d of age, 10 chickens around average BW were selected from each treatment, weighed and killed by exsanguinations after CO_2 stunning. After the abdominal incision, a middle section of ileum mucosa were collected, for detecting mRNA expression of ileum IL-1β、TNF-α、IL-8 and IFN-γ.

Total RNA was extracted from intestinal segments using Trizol reagent (Invitrogen Life Technologies, Carlsbad, California, USA) following the manufacturer's protocol. The concentration of extracted RNA was measured using a NanoDrop spectrophotometer (ND-1000, NanoDrop Products, Wilmington, Dela[1]ware, USA) at an optical density of 260 nm, and RNA purity was verified by the ratio of absorbance at 260 nm/280 nm. Then, 1μg of total RNA was used for reverse transcription by a reverse transcription kit (Takara Bio Inc., Dalian, China) following the manufacturer's protocol. All the cDNA preparations were stored at -20 °C until further use.

Expression levels of the following genes were analyzed by real-time quantitative PCR (RT-PCR):IL-1β, IL-8, TNF-α, IFN-γand an endogenous reference gene GAPDH. Gene-specific primer sequences are shown in Table 3. The RT-PCR was performed on the 7500-fluorescence detection system (Applied Biosystems, Foster City, California, USA) using a commercial SYBR-Green PCR kit (Takara Bio Inc.). According to the manufacturer's protocol, the following PCR conditions were employed: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 34 s, and followed by the stage of melting curve. At the end of each run, melting curve analysis and subsequent agarose gel electrophoresis of the PCR products were subjected to confirm the amplification specificity. Relative gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method as developed by Livak and Schmittgen[9].

Table 3 RT-PCR primers and Genbank accession numbers of chicken

Target	Primer sequence (5'–3')[a]	Accession no.	Product size, bp
IL-1β	F:ACTGGGCATCAAGGGCTA R:GGTAGAAGATGAAGCGGGTC	NM_204524	131
TNF-α	F: GAGCGTTGACTTGGCTGTC R: AAGCAACAACCAGCTATGCAC	NM_204267	64
IL-8	F: ATGAACGGCAAGCTTGGAGCTG R:TCCAAGCACACCTCTCTTCCATCC	AJ_009800	103
IFN-γ	F: AGCTGACGGTGGACCTATTATT R:GGCTTTGCGCTGGATTC	Y07922	259
GAPDH	F:TGCTGCCCCAGAACATCATCC R: ACGGCAGGTCAGGTCAACAA	NM_204305.1	108

Intestinal flora

At d 37, 10 broilers were chosen from each treatment. After execution, intestine was taken out and separated by germ free cotton. Ileal digesta was collected and then stored at -80°C after snap freezing with liquid nitrogen for further analysis.

Statistical analyses

Effects of treatments were analyzed as a 3 × 2 factorial arrangement by two-way analysis of variance. Experimental data were analyzed using the GLM procedures of SAS 9.3 (SAS Inc., Cary, NC). If the test showed significant differences ($P < 0.05$), ranked scores were separated by the least significant difference procedure. Results in tables were reported as means ± SEM.

Results

Growth performance

The effects of rearing system and narasin on the growth performance of broilers were shown in table 4. The main effect analysis showed that, from d 1 to d 12, PN birds had higher ADG and ADFI, also higher body weight on d 12 than those of the other two systems(P<0.05). FCR of MC birds was significant lower than that of PN birds (P<0.05). While, narasin inclusion reduced ADG, ADFI and body weight significantly (P<0.05). From d 13 to d 23, MC birds had lower ADG and ADFI, and lower body weight on d 23 than those of the other two systems (P<0.05). Narasin inclusion reduced ADG, ADFI and body weight on d 23 significantly (P<0.05). From d 24 to d 36, PN birds had higher ADG and FCR, also body weight than those of CM birds (P<0.05). Narasin decreased ADFI and FCR significantly (P<0.05). From d 1 to d 36, MC birds had lower ADG and ADFI than FL and PN birds (P<0.05). There was no significant difference between FL and PN treatments (P>0.05). Narasin inclusion reduced ADFI and FCR significantly (P<0.05).

Table 4 Effects of raising system and narasin on growth performance of broilers

	FL		MC		PN		P□	SEM	System			Narasin		P-va
	+	-	+	-	+	-			FL	CM	PN	+	-	Sys
12dBW/(g)	439de	444^{cd}	434^{e}	448^{bc}	455^{b}	467^{a}	<0.001	1.718	441^{b}	441^{b}	461^{a}	443^{b}	453^{a}	<0.(
23dBW/(g)	1348^{b}	1355^{b}	1304^{c}	1330^{b}	1347^{b}	1385^{a}	<0.001	4.559	1351^{a}	1317^{b}	1366^{a}	1333^{b}	1357^{a}	<0.(
36dBW/(g)	2509^{ab}	2509^{ab}	2457^{b}	2488^{b}	2510^{ab}	2544^{a}	0.025	7.477	2509^{a}	2473^{b}	2527^{a}	2492	2514	<0.(
1~12d														
ADG/(g/d)	32.6^{de}	33.0^{cd}	32.2^{e}	33.3^{bc}	33.9^{b}	34.9^{a}	<0.001	0.143	32.8^{b}	32.8^{b}	34.4^{a}	32.9^{b}	33.8^{a}	<0.(
FCR	1.131	1.130	1.126	1.116	1.142	1.131	0.070	0.002	1.1309^{ab}	1.121^{b}	1.136^{a}	1.133	1.126	0.0
ADFI/(g/d)	36.8^{cd}	37.3^{c}	36.2^{d}	37.2^{c}	38.7^{b}	39.5^{a}	<0.001	0.161	37.0^{b}	36.7^{b}	39.1^{a}	37.2^{b}	38.0^{a}	<0.(
Survival rate/ (%)	99.8	99.8	99.5	99.8	99.1	99.3	0.724	0.143	99.8	99.6	99.2	99.5	99.6	0.3
13~23d														
ADG/(g/d)	82.6^{ab}	82.8^{ab}	79.1^{c}	80.3^{c}	81.1^{bc}	83.4^{a}	<0.001	0.339	82.7^{a}	79.7^{b}	82.3^{a}	80.9^{b}	82.2^{a}	<0.(
FCR	1.335^{b}	1.356^{a}	1.337^{b}	1.355^{a}	1.358^{a}	1.342^{ab}	<0.001	0.002	1.345	1.346	1.350	1.343	1.351	0.6
ADFI/(g/d)	110.3^{ab}	112.3^{a}	105.7^{c}	108.7^{b}	110.2^{ab}	112.0^{a}	<0.001	0.430	111.3^{a}	107.2^{b}	111.1^{a}	108.7^{b}	111.0^{a}	<0.(
Survival rate/ (%)	99.5	99.8	99.5	99.7	98.4	99.3	0.193	0.172	99.6	99.6	98.8	99.1	99.6	0.1(
24~36d														
ADG/(g/d)	89.3	88.8	88.7	89.1	89.5	89.2	0.992	0.370	89.1	88.9	89.3	89.2	89.0	0.9(
FCR	1.778^{cd}	1.831^{ab}	1.763^{d}	1.813^{b}	1.804^{bc}	1.851^{a}	<0.001	0.006	1.804^{ab}	1.788^{b}	1.827^{a}	1.782b	1.832a	<0.(
ADFI/(g/d)	158.8^{bc}	162.4^{ab}	156.4^{c}	161.4^{ab}	161.2^{ab}	164.9^{a}	<0.001	0.598	160.6^{ab}	158.9^{b}	163.1^{a}	158.8b	162.a	<0.(
Survival rate/ (%)	99.2	98.7	99.4	99.2	98.6	98.1	0.560	0.224	99.0	99.3	98.3	99.1	98.7	0.2
1~36d														
ADG/(g/d)	68.4^{ab}	68.4^{ab}	66.9^{b}	67.8^{b}	68.4^{ab}	69.3^{a}	0.0257	0.207	68.4^{a}	67.4^{b}	68.9^{a}	67.9	68.5	<0.(
FCR	1.511^{b}	1.542^{a}	1.507^{b}	1.533^{a}	1.532^{a}	1.542^{a}	<0.001	0.003	1.527^{b}	1.520^{b}	1.537^{a}	1.517^{b}	1.539^{a}	<0.(
ADFI/(g/d)	103.3^{b}	105.4^{ab}	100.8^{c}	103.9^{b}	104.8^{b}	106.9^{a}	<0.001	0.353	104.4^{b}	102.4^{c}	105.9^{a}	103.0^{b}	105.4^{a}	<0.(
Survival rate/ (%)	98.5	98.3	98.4	98.6	96.1	96.8	0.125	0.335	98.4^{a}	98.5^{a}	96.4^{b}	97.7	97.9	<0.(

FL=flooring litter rearing, MC= multilayer cage rearing, PN= plastic net rearing,BW= body weight, ADG = average daily gain, ADFI = average daily feed intake, FCR= feed conversion ratio

[a, b] Within a row, numbers with different superscripts differ statistically at P <0.05.

[1] n= 12 replications.

Digestive organ development

The effect of rearing system and narasin on gizzard and proventriculus development of broilers is shown in Figure 1. FL birds gizzards were brighter and plumper than those of PN and MC rearing in appearance, and the proventriculus was and isthmus was moderate. Both cage and net rearing broilers had small gizzards, swollen proventriculi and looked unthrift.

The effects of rearing systems and narasin on relative weight of digestive organs of broilers were shown in Tables 5 and 6. Broiler chickens on FL treatment had heavier gizzards than those of CM and PN treatment (P<0.05). CM significantly reduced the relative weights and of the duodenum, jejunum and ileum compared to the other two systems (P<0.05).

Table 5 Effects of raising system and narasin on relative weight digestive organ of broilers

System	Narasin	Relative weight of gizzard	Relative weight of proventriculus
FL	+	1.24^{a}	0.25^{b}
FL	-	1.15^{a}	0.26^{b}
MC	+	0.90^{b}	0.39^{a}
MC	-	0.96^{b}	0.39^{a}
PN	+	0.86^{b}	0.35^{a}
PN	-	0.87^{b}	0.32^{ab}
P-value		<0.001	0.0004
SEM		0.026	0.012
Main effect			
System	FL	1.19^{a}	0.26^{b}
	MC	0.93^{b}	0.39^{a}
	PN	0.87^{b}	0.34^{a}
Narasin	+	1.00	0.33
	-	0.99	0.32
P-value	System	<0.001	<0.001
	Narasin	0.881	0.757
	Interaction	0.240	0.724

FL=flooring litter rearing, MC= multilayer cage rearing, PN= plastic net rearing

$^{a, b}$ Within a row, numbers with different superscripts differ statistically at P <0.05.

n= 10 replications.

Fig. 1 Development of proventriculus and gizzard

Intestinal lesion score and mRNA expression of ileum immune factors

The effects of rearing systems and narasin on intestinal lesion score and mRNA expression in the ileum of broilers were shown in Table 7. There are no significant difference in intestinal lesion score among those systems (P>0.05).

Cage rearing significantly reduced the gene expression of IL-1β and IFN-γ in intestinal tract (P<0.05). The mRNA expression levels of the ileal IL-1β and IFN-γ in FL birds were higher than those in PN and MC groups (P < 0.05). Narasin decreased the mRNA expressions of TNF-α in the ileum (P < 0.05). Different rearing systems and narasin adding showed a significant interaction in the expression level of ileal IL-1β and IL-8(P < 0.05). Among all groups, the FL + narasin treatment had the highest expression level of IL-1β and the FL + non-narasin treatment had the highest expression level of IL-8 whereas The MC + narasin treatment had the lowest level of expression of IL-1β.

Table 6 Effects of raising system and narasin on intestine development of broilers

System	Narasin	Relative weight（%）			Intestine weight length ratio（g/cm）		
		Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
FL	+	0.64^{ab}	1.34^{a}	1.02^{a}	0.51^{a}	0.41^{ab}	0.31^{a}
FL	-	0.65^{a}	1.27^{ab}	0.97^{a}	0.56^{a}	0.42^{a}	0.30^{ab}
MC	+	0.44^{d}	0.91^{d}	0.72^{b}	0.43^{b}	0.32^{c}	0.27^{bc}
MC	-	0.53^{c}	1.07^{c}	0.75^{b}	0.52^{a}	0.37^{b}	0.26^{c}
PN	+	0.58^{abc}	1.13^{bc}	0.92^{a}	0.50^{a}	0.40^{ab}	0.33^{a}
PN	-	0.57^{bc}	1.08^{c}	0.95^{a}	0.52^{a}	0.41^{ab}	0.33^{a}
P-value		<0.001	<0.001	0.0002	0.003	0.001	0.001
SEM		0.014	0.027	0.024	0.010	0.007	0.006
Main effect							
System	FL	0.65^{a}	1.30^{a}	0.99^{a}	0.53^{a}	0.42^{a}	0.31^{a}
	MC	0.48^{c}	0.98^{c}	0.74^{b}	0.47^{b}	0.34^{b}	0.27^{b}
	PN	0.57^{b}	1.10^{b}	0.94^{a}	0.51^{ab}	0.41^{a}	0.33^{a}
Narasin	+	0.55	1.12	0.89	0.48^{b}	0.38	0.30
	-	0.58	1.14	0.90	0.53^{a}	0.40	0.30
P-value	System	<0.001	<0.001	<0.001	0.026	<0.001	<0.001
	Narasin	0.219	0.679	0.921	0.004	0.077	0.543
	Interaction	0.118	0.031	0.697	0.210	0.419	0.879

FL=flooring litter rearing, MC= multilayer cage rearing, PN= plastic net rearing

$^{a, b}$ Within a row, numbers with different superscripts differ statistically at $P<0.05$.

n= 10 replications.

Table 7 Effects of raising system and narasin on mRNA expression of ileum immune factors of broilers

System	Narasin	Lesion score	IL-1β	TNF-α	IL-8	IFN-γ
FL	+	0.26	1.08[a]	1.03	1.05[c]	1.05[a]
FL	-	0.35	0.85[b]	1.08	1.59[a]	0.84[b]
MC	+	0.33	0.60[c]	0.95	1.38[abc]	0.42[c]
MC	-	0.30	0.83[b]	1.18	1.48[ab]	0.48[c]
PN	+	0.27	0.82[b]	1.03	1.54[ab]	0.77[b]
PN	-	0.33	0.87[b]	1.29	1.23[bc]	0.82[b]
P-value			0.683	0.194	0.010	<0.001
SEM			0.018	0.041	0.050	0.040
Main effect						
System	FL	0.30	0.97[a]	1.06	1.32	0.94[a]
	MC	0.32	0.71[c]	1.06	1.43	0.45[c]
	PN	0.30	0.84[b]	1.16	1.39	0.79[b]
Anticoccidial drug	+	0.29	0.83	1.00[b]	1.32	0.75
	-	0.33	0.85	1.19[a]	1.43	0.71
P-value	System	0.931	0.001	0.504	0.629	<0.001
	Narasin	0.290	0.777	0.030	0.222	0.511
	Interaction	0.407	0.002	0.510	0.002	0.068

FL=flooring litter rearing, MC= multilayer cage rearing, PN= plastic net rearing

[a, b] Within a row, numbers with different superscripts differ statistically at $P < 0.05$.

n= 8 replications.

Intestinal microbiota

As shown in Figure 2, a total of 3,250 OTUs were identified based on >97% sequencing similarity. Wherein 2,061 OTU's were common in all three rearing systems and 2940 OTU's were common among adding narasin or not. Respectively, 399, 78 and 94 OTU's were exclusive in the FL, NP and MC gorups whereas154 and 156 OTU's were exclusive in narasin-adding group and narasin-free group. The specaccum curves and rank abundance curves indicated that sufficient sequencing coverage was achieved (Shown in Figures 3 and 4).

As presented in Table 8, narasin didn't affect the alpha diversity of ileal microbiota. Floor rearing numerically increased both richness index (Chao1 and ACE) and diversity index (Shannon and Simpson). Shannon index was significantly improved in FL feeding ($P<0.05$). PLS-DA in Figure 5 indicated that there was differentiation of the microbial community structure among the treatments.

Intestine microbiota at the phylum level are shown in Table 9, Figures 6 and 7. At the phylum level, the ileal microbiota was dominated by *Firmicutes* (65.18~93.01%), *Proteobacteria* (3.71~13.93%), *Actinobacteria* (0.04~2.18%) and Cyanobacteria (0.14~0.50%). FL rearing markedly increased *Actinobacteria* abundance than other rearing modes ($P<0.05$). MC rearing increased *Proteobacteria*, *Thermi* and decrease *Bacteroidetes* abundances than other rearing modes and increased *Cyanobacteria* abundance compared to floor rearing ($P<0.05$). However, narasin increased *Proteobacteria* abundances than control chicks ($P<0.05$).

As shown in Table 10, Figures 8 and 9, FL rearing improve *Corynebacterium*, *Facklamia*, *Dietzia*, *Brevibacterium*, *Staphylococcus* abundances than other treatments ($P<0.05$). MC rearing markedly increased *Bacillus* abundance than floor rearing and increased *Pseudomonas* and *Bacillus* than PN rearing ($P<0.05$). Increase was observed after narasin adding in *Ochrobactrum* abundance.

Table 11 presents the predicted microbial functions at level 1 of the KEGG pathways. Compared to other rearing systems, MC had significantly more abundance of KEGG pathways affiliated with cellular processes, and less abundance of KEGG pathways belonging to genetic information processing ($P<0.05$). Net rearing had significantly less abundance of KEGG pathways affiliated with organismal systems than other feeding modes. Narasin had remarkably larger abundance of KEGG pathways belonging to cellular processes and organismal systems.

Table 12 shows the top 10 predicted microbial functions at level 2 of the KEGG pathways. PN rearing had significantly less abundance of KEGG pathways affiliated with amino acid metabolism ($P<0.05$). MC rearing had significantly less abundance of KEGG pathways affiliated with replication and repair, translation and nucleotide metabolism and remarkably larger abundances of KEGG pathways belonging to lipid metabolism and xenobiotics biodegradation

and metabolism (P<0.05). FL rearing had less abundance of KEGG pathways affiliated with carbohydrate metabolism compared with other rearing condition and had more abundance of KEGG pathways affiliated with energy metabolism compared with cage feeding (P<0.05). Narasin adding markedly decreased abundance of KEGG pathways affiliated with replication and repair, translation, nucleotide metabolism and increased abundance of KEGG pathways belonging to amino acid metabolism (P<0.05).

Table 8 α-diversity of ileal microflora

System	Narasin	Simpson	Chao1	ACE	Shannon
FL	+	0.93	994.87	1012.88	6.46[a]
FL	-	0.94	988.88	1001.31	6.60[a]
MC	+	0.93	986.84	1007.66	6.08[ab]
MC	-	0.89	903.69	914.62	5.68[ab]
PN	+	0.86	800.88	818.97	5.35[b]
PN	-	0.89	935.17	930.9	5.75[ab]
P-value		0.612	0.617	0.408	0.063
SEM		0.012	35.704	35.868	0.140
Main effect					
System	FL	0.93	991.87	1007.09	6.53[a]
	MC	0.91	945.27	961.14	5.88[a]
	PN	0.88	968.03	911.28	5.55[b]
Narasin	+	0.91	927.53	946.50	5.96
	-	0.91	942.58	973.18	6.01
P-value	System	0.184	0.604	0.547	0.013
	Narasin	0.918	0.571	0.706	0.847
	Interaction	0.48	0.228	0.271	0.462

FL=flooring litter rearing, MC= multilayer cage rearing, PN= plastic net rearing

[a, b] Within a row, numbers with different superscripts differ statistically at P <0.05.

n = 8 replications.

Table 9 Microflora structure at phylum level

System	Narasin	Firmicutes	Proteobacteria	Actinomycetes	Cyanobacteria	Bacteroidetes
FL	+	71.03	5.69^{b}	1.90^{a}	0.21	0.04^{abc}
FL	-	70.72	4.11^{b}	2.18^{a}	0.14^{b}	0.06^{ab}
MC	+	65.18	13.93^{a}	0.25^{b}	0.50^{a}	0.02^{bc}
MC	-	93.01	6.43^{b}	0.12^{b}	0.40^{ab}	0.02^{c}
PN	+	71.67	7.78^{b}	0.08^{b}	0.44^{ab}	0.07^{a}
PN	-	73.77	3.71^{b}	0.04^{b}	0.20^{ab}	0.02^{bc}
P-value		0.683	0.004	0.001	0.126	0.036
SEM		4.614	0.877	0.209	0.047	0.007
Main effect						
System	FL	70.87	4.90b	2.04a	0.16b	0.06^{a}
	MC	79.09	10.18a	0.20b	0.45a	0.02^{b}
	PN	72.72	5.89b	0.06b	0.31^{a}b	0.05^{a}
Narasin	+	69.11	8.97b	0.75	0.37	0.05
	-	76.10	4.88a	0.86	0.24	0.04
P-value	System	0.669	0.029	<0.001	0.064	0.032
	Narasin	0.501	0.021	0.779	0.210	0.418
	Interaction	0.359	0.300	0.757	0.624	0.024

Table 10 Microflora structure at genus level

System	Narasin	Lactobacillus	Pseudomonas	Corynebacterium	Bacillus	Facklamia	Ochrobactrum	Enterococcus	Dietzia	Brevil
FL	+	86.99^{ab}	2.48^{b}	2.02^{a}	0.07^{c}	0.51^{a}	0.07^{b}	0.11^{ab}	0.21^{b}	0.10^{a}
FL	-	87.16^{ab}	1.97^{b}	2.15^{a}	0.10^{bc}	0.25^{ab}	0.04^{b}	0.02^{ab}	0.42^{a}	0.12^{a}
MC	+	80.08^{b}	5.32^{a}	0.02^{b}	0.48^{a}	<0.01^{b}	0.24^{a}	0.17^{a}	<0.01^{c}	0.02^{b}
MC	-	89.69^{a}	2.31^{b}	0.01^{b}	0.29^{b}	<0.01^{b}	0.11^{b}	<0.01^{b}	<0.01^{c}	0.01^{b}
PN	+	90.66^{a}	1.72^{b}	0.01^{b}	0.13^{bc}	<0.01^{b}	0.16^{ab}	0.01^{b}	<0.01^{c}	0.01^{b}
PN	-	89.90^{a}	1.42^{b}	0.01^{b}	0.11^{bc}	<0.01^{b}	0.09^{b}	<0.01^{b}	<0.01^{c}	<0.01
P-value		0.149	<0.001	<0.001	0.001	0.002	0.015	0.099	<0.001	0.003
SEM		1.258	0.267	0.208	0.032	0.046	0.017	0.022	0.032	0.012
Main effect										
System	FL	87.08	2.25^{ab}	2.09^{a}	0.09^{b}	0.37^{a}	0.06^{b}	0.07	0.32^{a}	0.11^{a}
	MC	84.89	3.81^{a}	0.02^{b}	0.41^{a}	<0.01^{b}	0.18^{a}	0.14	<0.01^{b}	0.01^{b}
	PN	90.28	1.57^{b}	<0.01^{b}	0.12^{b}	<0.01^{b}	0.13^{a}	<0.01	<0.01^{b}	<0.01
Narasin	+	89.01	2.76	0.67	0.22	0.16	0.16^{a}	0.10	0.07	0.05
	-	89.08	1.88	0.68	0.15	0.09	0.08^{b}	0.01	0.09	0.03
P-value	System	0.515	0.005	0.001	<0.001	0.001	0.017	0.062	<0.001	<0.00
	Narasin	0.981	0.095	0.983	0.269	0.418	0.048	0.082	0.723	0.115
	Interaction	0.506	0.153	0.999	0.150	0.550	0.277	0.049	0.891	0.363

Table 11 Predicted Functional Changes at level 1

System	Narasin	Cellular Processes	Environmental Information Processing	Genetic Information Processing	Human Diseases	Metabolism	Organismal Systems
FL	+	5.02^{b}	13.95	23.54^{a}	0.86^{b}	51.09	0.44^{ab}
FL	-	4.97^{b}	13.76	23.83^{a}	0.86^{b}	51.02	0.43^{ab}
MC	+	5.94^{a}	14.62	21.32^{b}	0.93^{a}	51.61	0.47^{a}
MC	-	5.24^{b}	14.25	22.74^{a}	0.84^{b}	51.54	0.41^{bc}
PN	+	5.15^{b}	14.5	23.32^{a}	0.88^{ab}	50.68	0.40^{bc}
PN	-	5.14^{b}	14.25	23.87^{a}	0.84^{b}	50.40	0.39^{c}
P-value		0.041	0.643	0.001	0.020	0.576	0.002
SEM		0.097	0.164	0.212	0.008	0.217	0.007
Main effect							
System	FL	5.00^{b}	13.87	23.68^{a}	0.85	51.06	0.44^{a}
	MC	5.59^{a}	14.52	22.03^{b}	0.88	51.58	0.44^{a}
	PN	5.15^{b}	14.38	23.73^{a}	0.86	50.54	0.39^{b}
Narasin	+	5.37^{a}	14.36	22.73	0.89	51.13	0.44^{a}
	-	5.00^{b}	14.05	23.68	0.84	51.05	0.41^{b}
P-value	System	0.003	0.282	<0.001	0.165	0.258	0.002
	Narasin	0.025	0.361	0.098	0.258	0.849	0.006
	Interaction	0.311	0.860	0.606	0.002	0.896	0.121

FL=flooring litter rearing, MC= multilayer cage rearing, PN= plastic net rearing

$^{a, b}$ Within a row, numbers with different superscripts differ statistically at $P<0.05$.

n = 8 replications.

Table 12 Predicted Functional Changes at level 2

System	Narasin	Membrane Transport	Carbohydrate Metabolism	Replication and Repair	Amino Acid Metabolism	Translation	Energy Metabolism	Nucleotide Metabolism	Lipid Metabolism	Xenobiot Biodegra and Metaboli
FL	+	12.21	11.23^{b}	9.37^{a}	8.54^{a}	6.22^{a}	5.01^{ab}	4.84^{a}	3.55^{ab}	3.39
FL	-	12.12	11.16^{b}	9.51^{a}	8.34^{ab}	6.35^{a}	5.04^{a}	4.84^{a}	3.42^{b}	3.25
MC	+	12.57	11.30^{b}	8.25^{b}	8.87^{a}	5.39^{b}	4.90^{ab}	4.22^{b}	3.82^{a}	3.93
MC	-	12.84	12.55^{a}	9.00^{a}	7.85^{ab}	6.02^{a}	4.89^{b}	4.54^{ab}	3.67^{ab}	3.86
PN	+	12.82	11.99^{ab}	9.18^{a}	7.87^{ab}	6.15^{a}	4.99^{ab}	4.68^{a}	3.49^{ab}	3.36
PN	-	12.64	11.95^{ab}	9.46^{a}	7.64^{c}	6.28^{a}	4.96^{ab}	4.81^{a}	3.44^{ab}	3.3
P-value		0.660	0.025	0.002	0.002	0.006	0.142	0.012	0.176	0.114
SEM		0.151	0.146	0.106	0.106	0.087	0.020	0.061	0.051	0.093
Main effect										
System	FL	12.17	11.19^{b}	9.44^{a}	8.45^{a}	6.35^{a}	5.03^{a}	4.90^{a}	3.49^{b}	3.33^{b}
	MC	12.71	11.98^{a}	8.67^{b}	8.35^{a}	5.73^{b}	4.88^{b}	4.39^{b}	3.75^{a}	3.89^{a}
	PN	12.73	11.97^{a}	9.32^{a}	7.76^{b}	6.22^{a}	4.97^{ab}	4.79^{a}	3.46^{b}	3.33^{b}
Narasin	+	12.53	11.50	8.93^{b}	8.43^{a}	5.92^{b}	4.97	4.58^{b}	3.62	3.56
	-	12.53	12.00	9.41^{a}	7.97^{b}	6.31^{a}	4.96	4.82^{a}	3.59	3.60
P-value	System	0.249	0.046	0.001	0.016	0.003	0.019	<0.001	0.004	0.003
	Narasin	0.999	0.081	0.011	0.021	0.013	0.81	0.022	0.767	0.823
	Interaction	0.821	0.097	0.367	0.107	0.416	0.811	0.619	0.720	0.741

Discussion

Growth performance

Growth performance is the most direct index for assessing poultry production and can be affected by rearing systems [10]. Li et al. [11] reported that cage rearing broilers had poorer growth performance than floor and net rearing broilers in the early phase of broilers, but caged broilers have the highest feed conversion and slaughter weight at the later phase. Wang et al. [12] reported that there was no significant difference in body weight, average daily feed intake, mortality rate and average daily gain between the net rearing and floor rearing, while FCR of the net reared birds is significantly higher than that of floor reared counterparts. Similarly, Wang et al. [13] reported that there was no significant difference in the growth performance of broilers among the three rearing systems, but feed intake in the floor rearing system was lower than cage rearing system and net rearing system. The results of the current study showed that the body weight of broilers in cage rearing group was the lowest in all phases which might be caused by a series of problems as associated with the immune function, intestinal health and gut mircoflora. The body weight of birds in the net rearing group is the highest in each stage, with correspondingly higher FCR and feed intake and lower survival rate. However, FCR of cage reared broilers was the lowest in the early and late growth phases. Overall, the bird in cage rearing group had the best FCR. This may be related to a lower energy consumption on activity, and a better hygiene environment for the birds. Net rearing can also prevent broilers from directly contacting with excreta, and more conducive to the growth of broilers than floor rearing, while the range of activities for floor broilers is increased, thus increasing energy consumption and the probability of foot pad dermatitis [14, 15]. But net rearing is similar to cage rearing which is more hygienic and may be conducive for preventing coccidiosis. To test this hypothesis, the current study examined the effects of a coccidiosis in the three systems. Narasin is an ionophore coccidiostat widely used in the poultry industry. The research shows that narasin was effective in reducing mortality and suppression of growth and feed efficiency associated with necrotic enteritis (NE) among broiler chickens challenged with *C. perfringens*[16, 17]. Anticoccidial (narasin) diet increased BW gain and decreased feed conversion ratio of male broilers with subclinical coccidia challenge [18]. Narasin is not only used for their anticoccidial effect, but also as growth promoters in Eimeria-free environment, due to its effect in improving feed conversion efficiency [19]. This experiment showed that narasin addition can reduce the daily average feed intake of broilers and improve FCR. However, Karimi [20] showed that under coccidial and necrotic enteritis free environment, the prophylactic effect of narasin was insignificant for broiler chicks housed in floor pens using wood shavings as bedding material.

Digestive organ development

This experiment showed that floor rearing was beneficial to the development of broiler's gizzard, a effect which was clearly better than net and cage rearing. Broilers raised on floor had directly contact with rice hulls on the ground, consuming an amount of rice hulls that could increase increase the bulk of the digesta, produce physical dilation of the gizzard walls, and increase the development of the muscular layers and the size of this organ [21]. A well-developed

gizzard promotes the secretion of digestive enzymes, reduce the rate of proventriculitis, and enhance nutrient digestion. Similarly, Hetland et al. [22] found that the intake of wood shavings from the litter accounted for 4% of the feed intake, pushing up the gizzard and proventriculus weights of laying hens by 50%.

Studies have shown that the growth rate of gastrointestinal tract of chicks is faster than that of other organs and tissues after hatching [23]. The current study showed that the body weight of caged broilers is lower than that of the other two rearing systems. The intestinal tract development followed a similar trend. In addition, cage rearing reduced the relative weight and unit weight of each intestinal tract of broilers. However, the floor reared broilers ate rice husks and absorbed more crude fiber, which was beneficial to the development and full physical abrasion of the gizzard, leading to improved physical abrasion, and stimulation of the secretion of digestive juice from proventriculus. The fiber of the kind present in rice hulls is known as structural components, which, in an appropriate particle size, plays an important role to stimulate gizzard activity and enhances gut development [21].

In the current study, the addition of narasin had no significant effect on the relative weight of digestive organs in broilers, but significantly reduced the unit weight of the duodenum. Studies have shown that the addition of narasin to the diet reduces the length and relative weight of the duodenum, jejunum and ileum; the duodenum is the main organ to produce and release digestive enzymes into the broiler gastrointestinal tract, and hence the reduction of the unit weight of duodenum may be caused by the reduction of inflammation [18].

Intestinal lesion score and Intestinal immunity

The current study did not detect any significant difference in the lesion score among different rearing systems. But the use of narasin markedly reduced intestinal damage in broilers, in particular, in caged birds.

Intestinal tract is not only the main organ for digestion and absorption, but also the largest immune organ of broilers. Interleukin (IL) plays an important role in the regulation of immune cell differentiation and immune response[24, 25]. IFN-γ is a soluble glycoprotein produced by a variety of cells and has a wide range of antiviral, antitumor and immunoregulatory effects. It can affect the activity of host immune cells and has a wide range of antiviral effects [26]. There are few reports on the expression of intestinal immune factors in broilers under different rearing systems. Wang [12] found that the relative expression of proinflammatory factors IL-6 and IFN-γ in jejunal mucosa of broilers in net rearing and floor rearing systems was significantly lower than that in low-density free range system, and the immune level of intestinal mucosa of broilers in net rearing was higher. The current study showed that the expression of intestinal mucosal immune factors in broilers was different between net and floor rearing system, while cage rearing significantly reduced the gene expression of pro-inflammatory factors IL-1β and IFN-γ in broiler intestinal tract, indicating that the response to intestinal inflammatory factors by cage reared broilers was not as good as that in floor reared and net reared broilers. In addition, the intestinal lesion score of broilers in cage reared birds was the worst, which may be related to the low content of immune factors. Similar to our findings, Li et al. [27] reported that there were lower levels of intestinal mucosal sIgA and IL-2 in cage rearing broilers raised in cages. Although cage rearing does not directly contact with excreta the possibly reducing the potential exposure to pathogenic bacteria, it does not afford the birds any priming effects of microbes for the immune system nor the benefits of the injection of litter material that can aid the development of gizzard. The end result may be poorer disease resistance and less robust birds compared with those reared on floors and in pens.

We also found that the expression level of TNF-α in intestinal mucosa of broiler chickens without narasin was significantly increased, which may be due to the fact that the body is in the stage of inflammatory reaction, and TNF-α produced by monocytes and macrophages is increased to promote cell proliferation and differentiation and repair body injury. Kaldhusdal et al. [28] reported that narasin supplementation tended to reduce gizzard lesions in broilers ($P < 0.1$). However, in our case, narasin supplementation did not affect intestinal lesion score , which agrees with the findings of Scheurer et al. [29].

Intestinal microbiota

The gastrointestinal tract of broilers has a very complex microflora. Intestinal microflora plays an important role in nutrient digestion and absorption, modulation of the immune system, prevention of diseases, and maintenance of physiological functions [30, 31] . The diversity and composition of the broiler intestinal microflora are regulated by many factors, such as diet, age, antibiotics, genetics, immune response and pathogen infection [32]. Today the role of gut microbiota on health, wellbeing and performance of animals, including broilers, is an exciting area of research and commercial development[33, 34].

In relation to the effect of rearing, it is generally shown that floor rearing usually leads to a more rich and diverse gut microbiota compared to other systems. For instance, when laying hens are raised on free range settings, they are exposed to a lot of environmental microbes, which enrich their intestinal microflora during pecking litter, scratching and dust-bathing [35, 36]. Wang [12] reported that birds reared on floors had a much more diverse range of microorganisms in the duodenum, jejunum and ileum than those raised on nets, although the difference diminished in the ceca. Our results mirrored their findings. Indeed, floor reared broilers had more unique ’s which were 411% and 324% higher than that in net and cage reared birds, respectively. The results were also proved in α-diversity that the Shannon Index is significantly increased in floor rearing, and other α-diversity indicators were also increased numerically. The results indicate that free-rang rearing can increase the diversity of microorganisms in the intestine, while the higher diversity of the intestinal microflora could improve the homeostasis of the body, the digestion and absorption of nutrients, and the resistance against pathogens [37].

At a phylum level, the relative abundance of *Firmicutes* is the highest, and other relatively high abundance phyla were *Cyanobacteria*, *Proteobacteria*, *Actinomycetes* etc., which was consistent with previous studies [38]. *Proteobacteria* belong to a Gram-negative phylum, including many important pathogens, including *Salmonella*, *Vibrio*, *Helicobacter* as well as some species in the *Cyanobacteria*, which can produce a variety of neurotoxins leading to diseases [39]. In our study, the abundance of *Proteobacteria* and Cyanobacteria in the intestines of floor reared broilers was reduced. It was probably due to the richness and diversity of the intestinal microflora of floor reared broilers that may competitively excluded some of the harmful bacteria. *Actinomycetes* are also Gram-positive bacteria, most of which are saprophytic. In our study, the abundance of *Actinomycetes* in the floor reared chicks increased significantly, probably because the birds picked up environmental *Actinomycetes* from the litter. Studies [35] have also shown an increase in the abundance of *Streptomyces* belonging to *Actinomycetes* in the intestine of floor reared laying hens. The findings suggested that *Actinomycetes* were major contributors to biological

buffering of soils, which can resist the invasion of pathogene [40]. Besides, bacteria of *Actinomycetes* like *Streptomyces* can produce a variety of antibacterial, antifungal, and antiparasitic substances, which work against harmful bacteria [41]. In our study, flooring litter and plastic net rearing increased the abundance of ileal *Bacteroidetes* of broilers. Literature findings indicate that bacteria of *Bacteroidetes* can hydrolyze a variety of polysaccharides, including cellulose which cannot usually be digested by monogastric animals, and produce organic acids such as propionic acid and succinic acid as the major end-products [42, 43]. These organic acids have anti-inflammatory, bacteriostatic, intestinal-protection and many other beneficial effects [44-46]. In the current study, the abundance of ileal *Bacteroidetes* of cage reared broilers reduced, which coincided with a lower level of organic acid production. Furthermore, the expression of intestinal mucosal inflammatory factor like IL-2 and IFN-γ was lower in cage reared broilers compared with birds raised in other systems.

At genus level, *Corynebacterium*, *Facklamia*, *Dietzia*, *Brevibacterium* and *Staphylococcus* of FL broilers had relatively higher abundance, most of which belong to *Actinomycetes*; genus with lower abundance like *Pseudomonas* and *Ochrobactrum* belong to *Proteobacteria*. These changes at genus level are in accord with the results at phylum level. *Corynebacterium* is usually harmless and exists in the host symbiotically. Some species can produce glutamate for the host to utilize (*C. glutamicum*), but some species are pathogens which could cause diseases such as diphtheria and pseudotuberculosis [47]. *Facklamia* and *D. maris* in *Dietzia* have been reported to be pathogens in humans [48, 49]. *Brevibacterium* could secretion aminopeptidases to hydrolyze protein, leading to improved digestion of dietary protein [50]. Staphylococcus is mostly saprophytic and may also enter the intestine due to more contact with litter and excreta. But *S. aureus* in *Staphylococcus* is more pathogenic. Similar with our research, Wang et al. [36] reported that *Corynebacterium, Facklamia* and *Staphylococcus* of broilers which used fresh litter had higher abundance compared with those with resued litter. *Pseudomonas* and *Bacillus* are more abundant in the intestines of caged reared broilers. *Pseudomonas* includes the opportunistic pathogen *P. aeruginosa*. Ccicks infected *Pseudomonas* show symptoms of diarrhoea, ruffled feather and drooping wings [51]. *Bacillus* includes the probiotic *B. subtilis* and also includes the pathogenic *B. anthracis* [52]. Ileal *Ochrobactrum* had higher abundance in MC and PN chicks. Study reported that *Ochrobactrum* was found in the gut lymphoid tissue and associated with systemic inflammation [53]. Although floor rearing enriches the intestinal flora of the broilers at phylum level, the abundance of many potential pathogens and probiotics generally increase at genus level. We found that the expression levels of IL-1β and IFN-γ of the ileal mucosa of FL broilers were higher while the expression levels of caged broilers were lower.

Conclusion

Net and floor raised broilers had higher body weight, whereas cage reared broilers had better FCR. In addition, cage rearing reduced the relative weight of the gizzard and intestine, together with a higher intestinal lesion score and a lower expression of intestinal immune factors.

Floor rearing with rice husk is beneficial to the development of gizzard and led to a more rich and diverse gut microbiota. Narasin supplementation improved FCR of broilers in general and increased the abundance of *Proteobacteria.*

Declarations

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

All procedures complied with the Beijing Regulations of Laboratory Animals, and the study was approved by The Laboratory Animal Ethical Committee of China Agricultural University (permit number SYXK20130013).

Consent for publication

Not applicable.

Competing interests

The authors confirm they have read Biomed Central's guidelines on competing interests and declare no competing interests.

Acknowledgements

This research was funded by the Shandong Taishan Industry Leading Talent Project (LJNY2015006) and Key technology research and development program of Shandong (2019JZZY020602).

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Figures

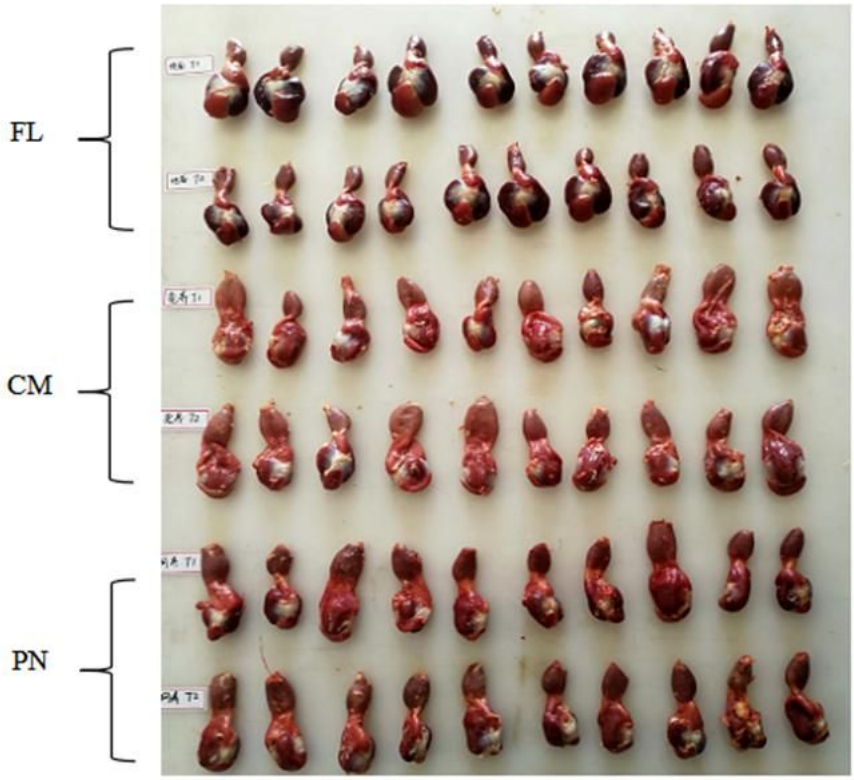

Figure 1

Development of proventriculus and gizzard

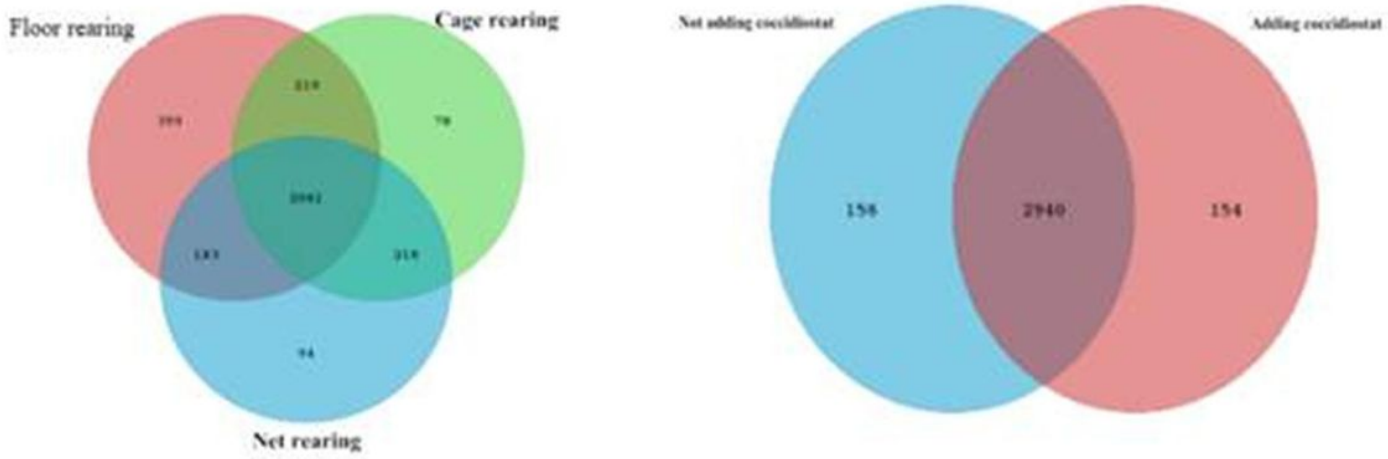

Figure 2

Venn

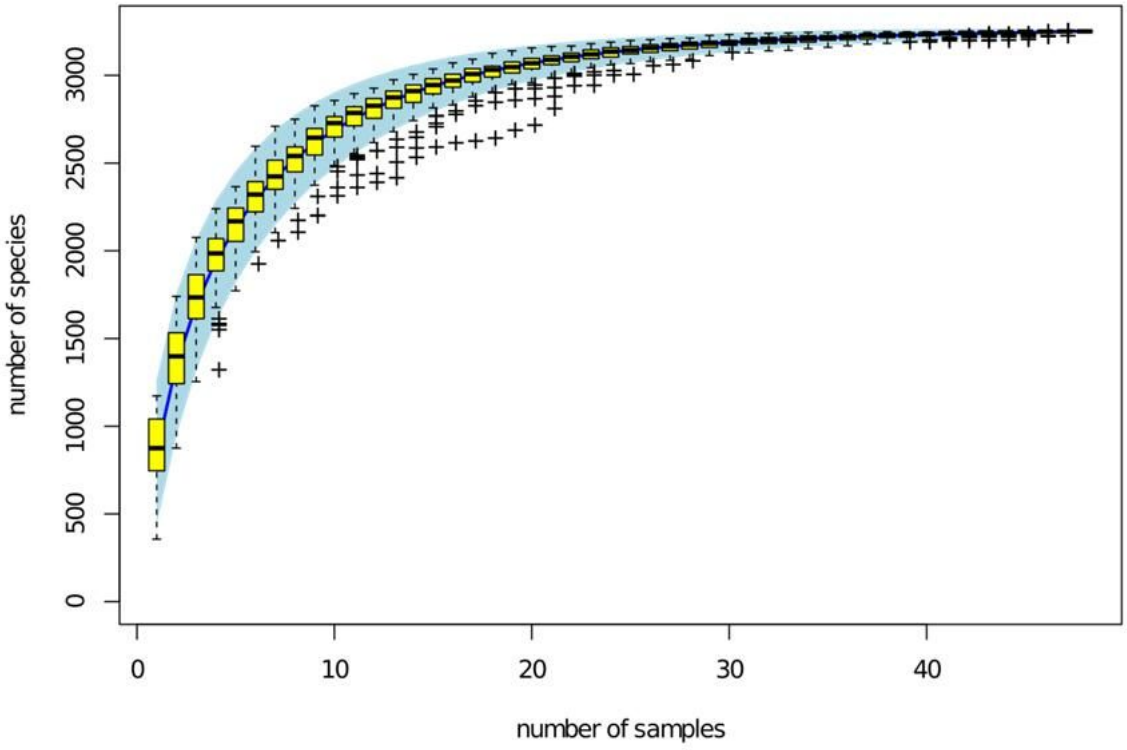

Figure 3

Species accumulation curves

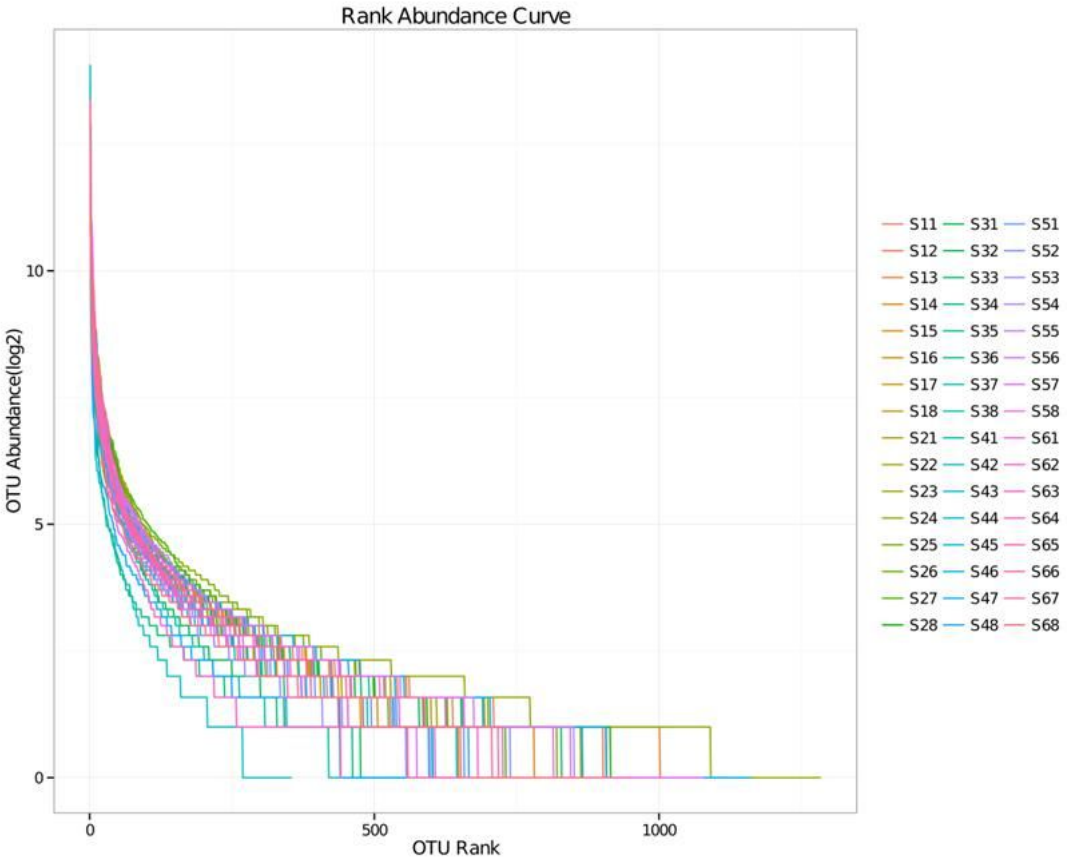

Figure 4

Rank abundance curve

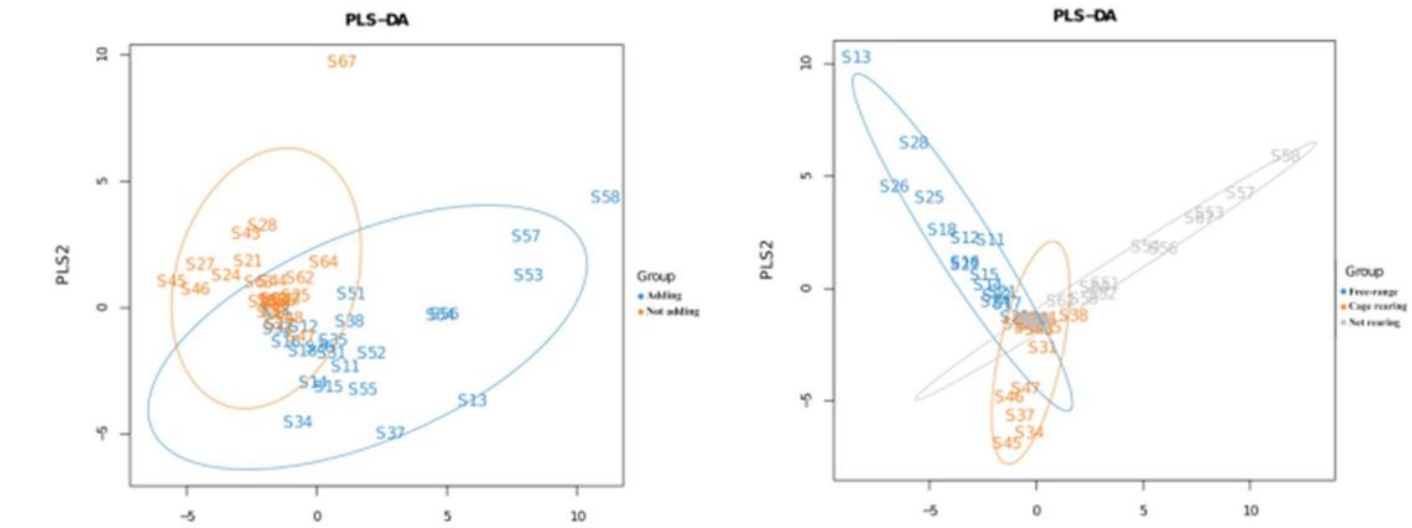

Figure 5

PLS-DA

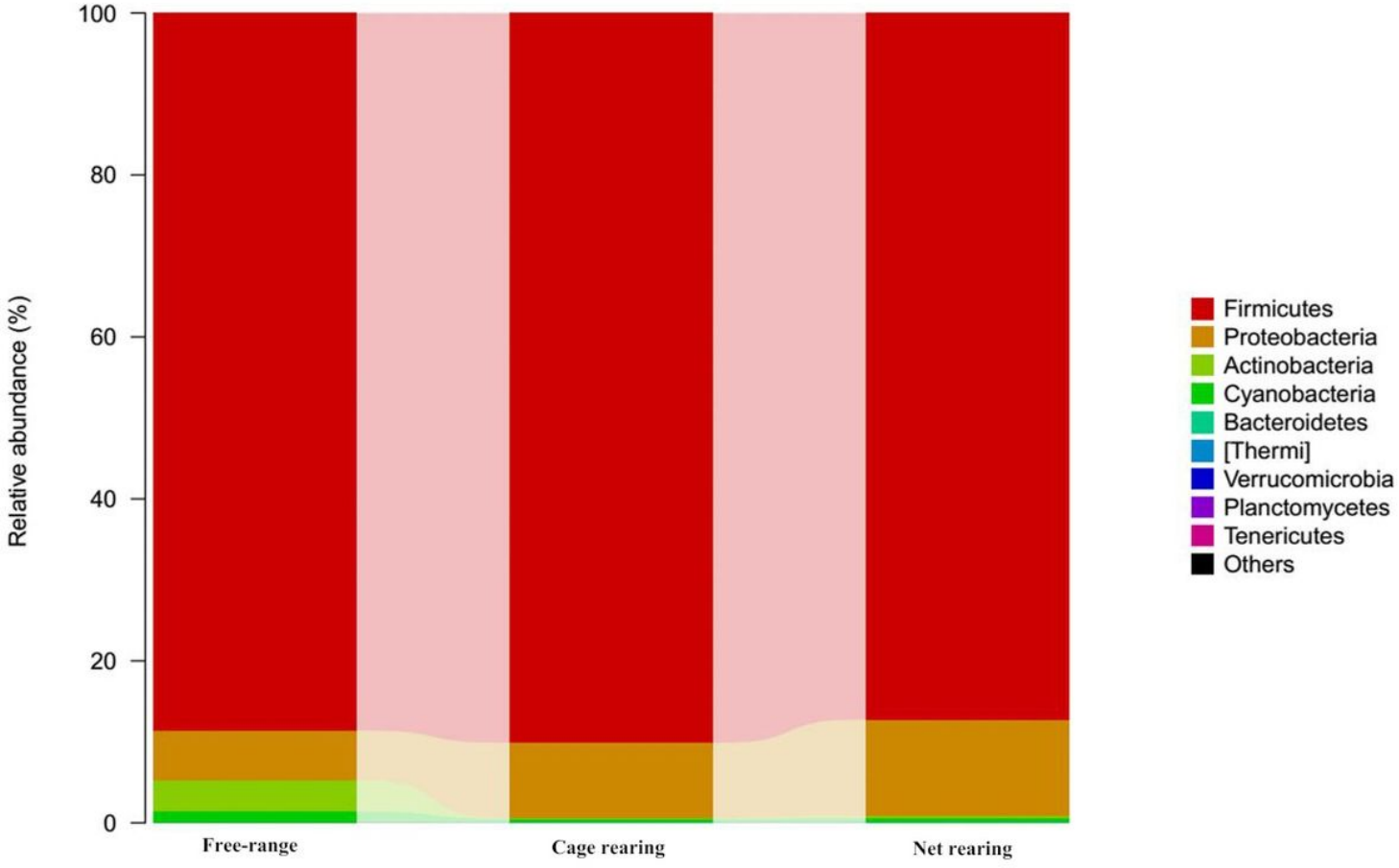

Figure 6

Effects of rearing condition on ileal microflora at phylum level

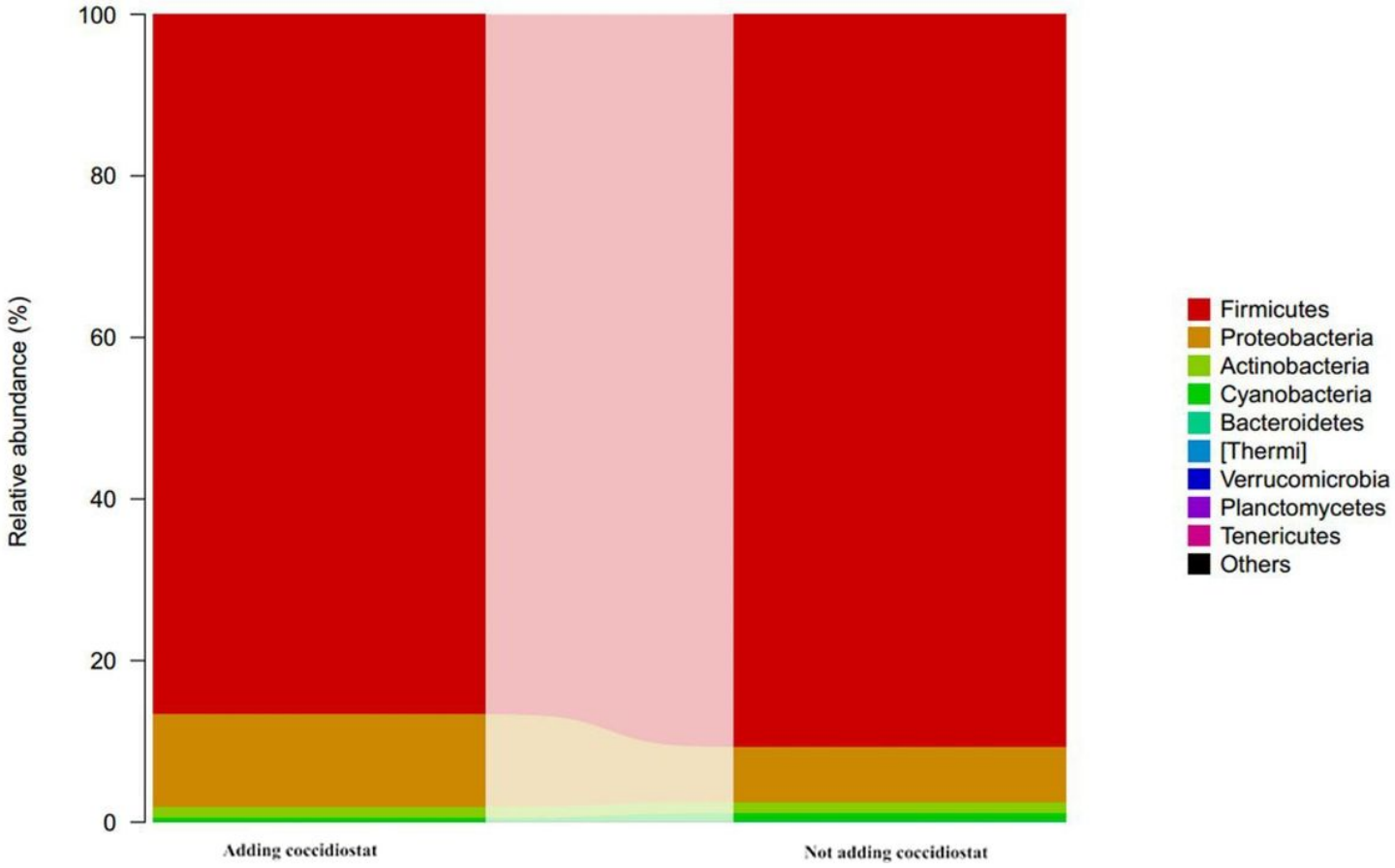

Figure 7

Effects of narasin on ileal microflora at phylum level

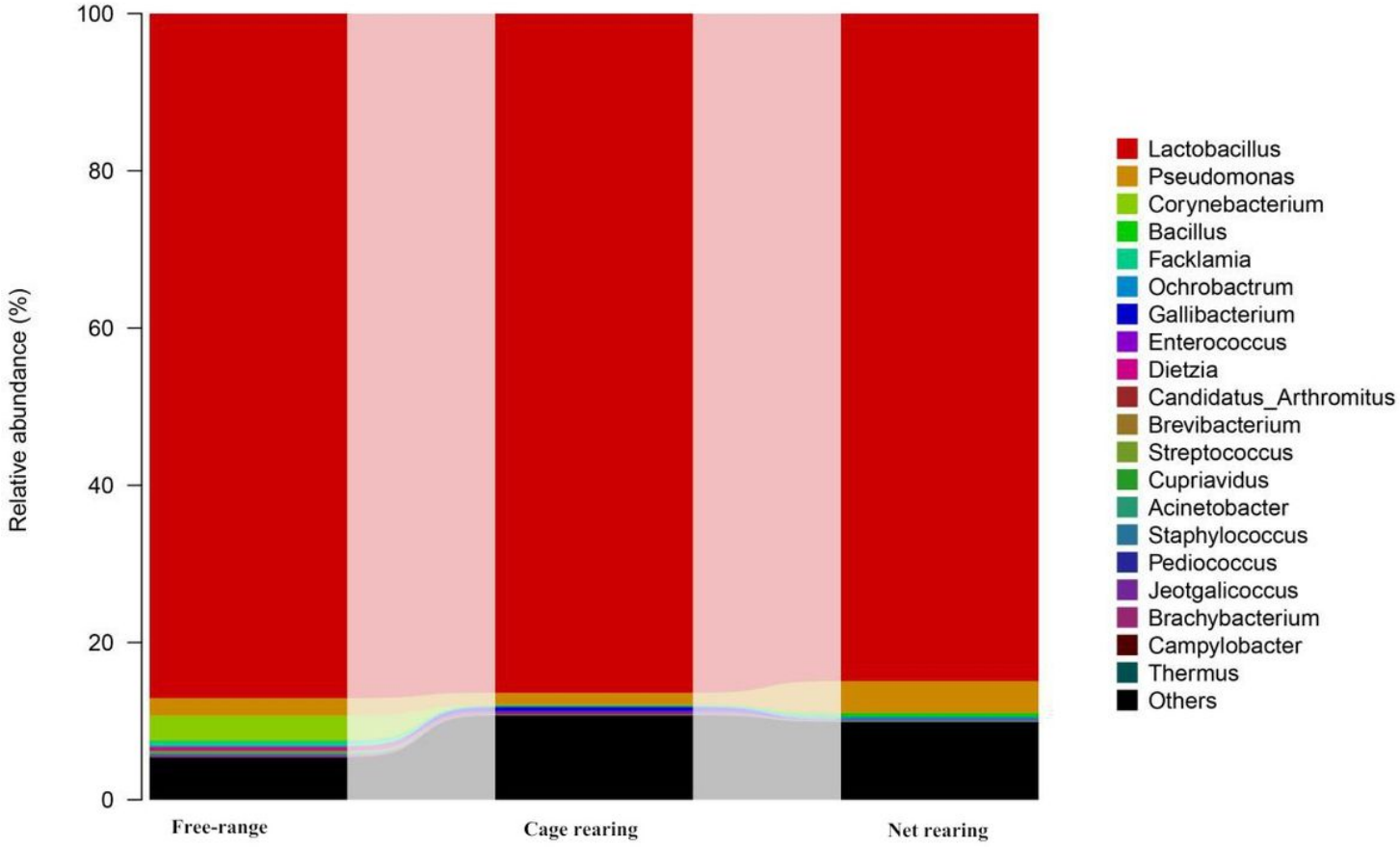

Figure 8

Effects of rearing condition on ileal microflora at genus level

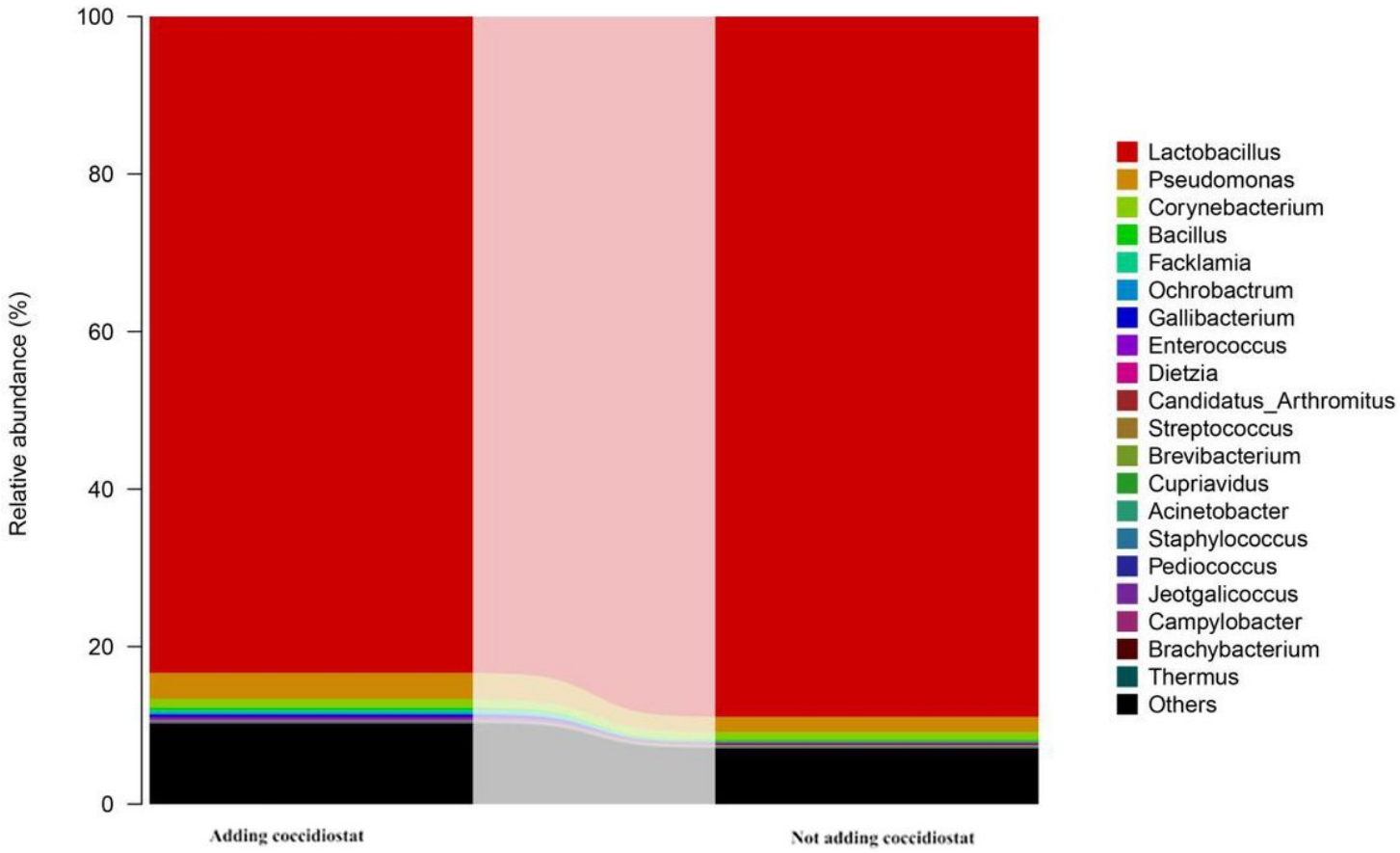

Figure 9

Effects of narasin on ileal microflora at genus level