Effects of *Enterococcus faecium* L3 on production performance, egg quality and intestinal microflora in Hyline-brown laying hens

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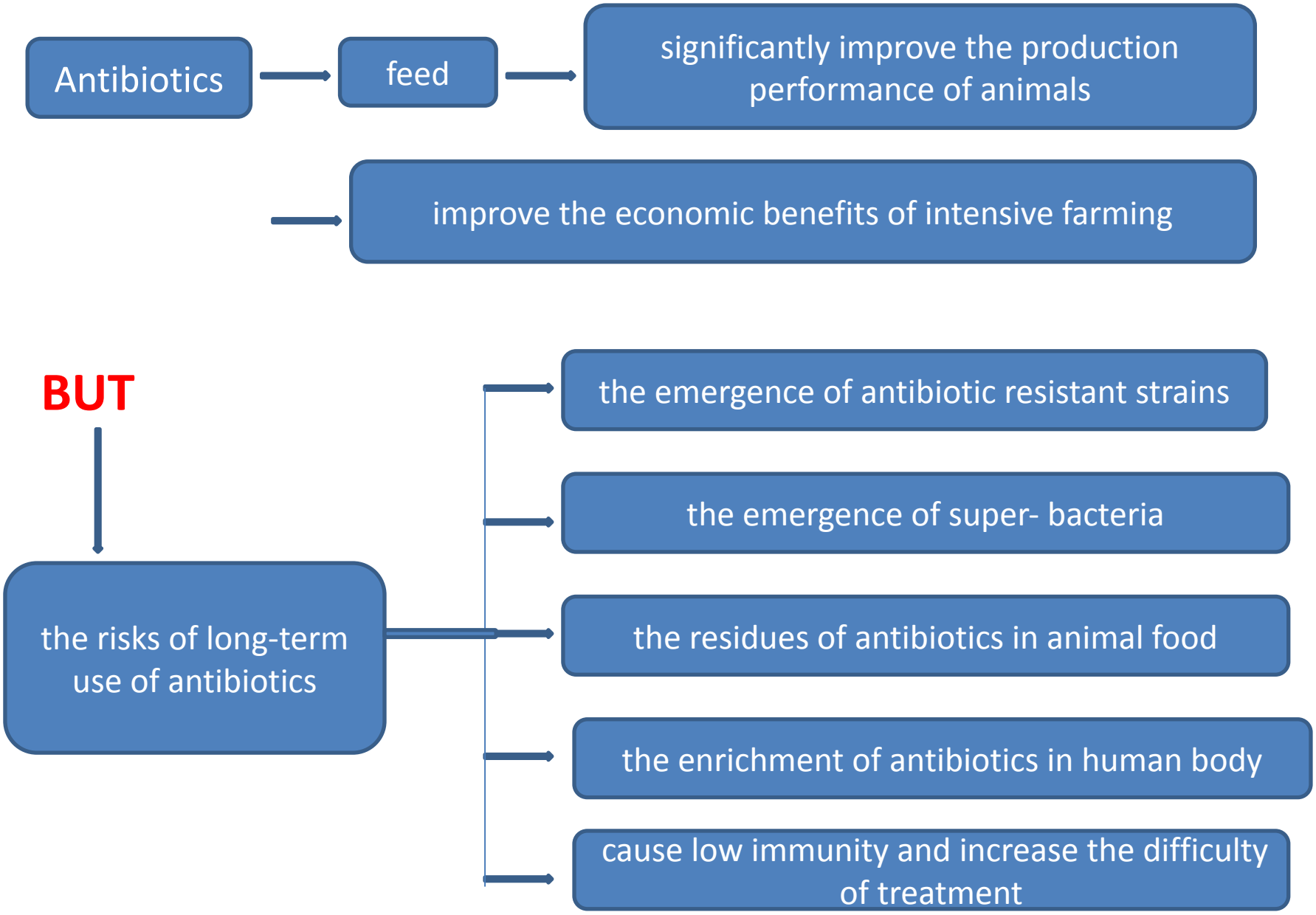
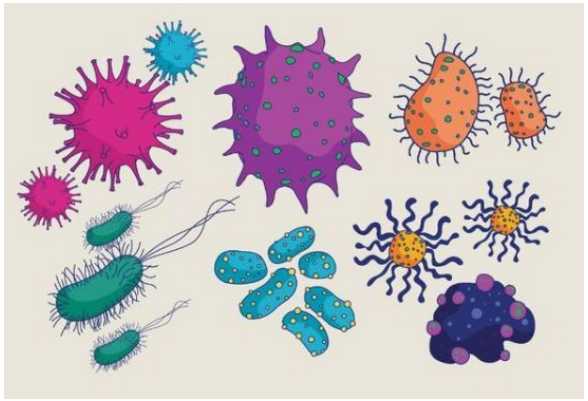

Antibiotics
feed
significantly improve the production performance of animals
improve the economic benefits of intensive farming
BUT
the risks of long-term use of antibiotics
the emergence of antibiotic resistant strains
the emergence of super- bacteria
the residues of antibiotics in animal food
the enrichment of antibiotics in human body
cause low immunity and increase the difficulty of treatment

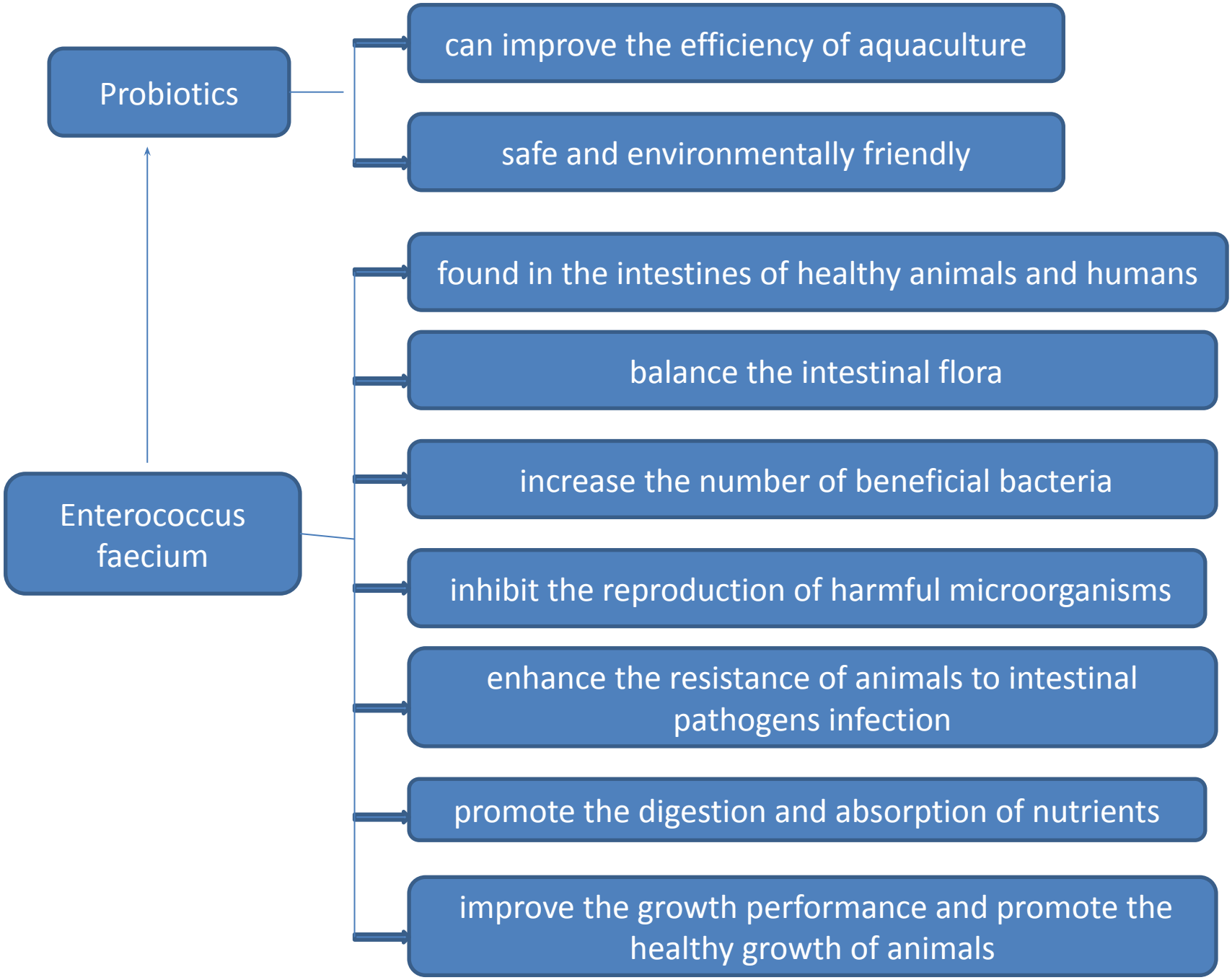


January 1, 2006, the European Union has completely banned the use of antibiotics in animal feed

The United States planed to ban the use of prophylactic antibiotics in animal feed for three years from 2014

Since July 1, 2020, China has completely banned the use of antibiotics in feed production,

Research and development of safe, green and efficient antibiotic substitutes has become a hot spot .


Probiotics
can improve the efficiency of aquaculture
safe and environmentally friendly
Enterococcus faecium
found in the intestines of healthy animals and humans
balance the intestinal flora
increase the number of beneficial bacteria
inhibit the reproduction of harmful microorganisms
enhance the resistance of animals to intestinal pathogens infection
promote the digestion and absorption of nutrients
improve the growth performance and promote the healthy growth of animals

Research contents

In this study, the effects of *Enterococcus faecium* L3 on the production performance, egg quality and intestinal flora of laying hens were studied by adding home made *Enterococcus faecium* L3 in feed.

The basal diet was supplemented with 4×10^9 CFU / kg *Enterococcus faecium* L3 for 28 days .

Composition and nutrient levels of the basal diet(air-dry basis)

Ingredients	Content(%)	Nutrient levels	Content(%)
Corn	62.00	ME（MJ/kg）	11.29
Soybean meal	24.00	Pro.	16.50
Soybean oil	1.00	Ca	3.50
Limestone	8.00	P	0.58
Premix	5.00	Nacl	0.30
Totle	100.00	Met	0.48
		Lys	1.02
		Met+Cys	0.70

1. Effect of *Enterococcus faecium* on L3 production performance of laying hens

Items	A	B	P
Average daily feed intake (g)	125.7±0.79	122.3±1.90	0.100
Laying rate / %	0.93±0.01	0.93±0.004	0.956
Average egg weight (g)	63.7±0.80	63.04±0.60	0.506
Ratio of feed to egg(g/g)	2.16±0.02	2.14±0.03	0.575
Broken egg rate(%)	0.03±0.01	0.02±0.01	0.303
Soft egg rate(%)	0.02±0.001	0.004±0.002	0.412
malformed egg rate(%)	0.02±0.01	0.02±0.01	0.798

2. Effects of *Enterococcus faecium* L3 on egg quality of laying hens

Items	A	B	P
Egg weight(g)	62.7±0.86	61.3±0.88	0.252
Egg shape index	1.31±0.01	1.30±0.01	0.721
Eggshell thickness(mm)	0.36±0.03	0.38±0.02	0.726
Eggshell strength(N/cm^2)	37.31±0.74	36.91±0.94	0.745
Albumen height(mm)	6.92±0.25	6.51±0.31	0.311
Yolk color	5.55±0.25	5.48±0.44	0.896
Haugh unit(HU)	81.75±1.88	80.96±1.79	0.764
Yolk weight(g)	18.10±0.35	17.64±0.27	0.303
Albumen weight(g)	37.48±0.66	36.76±0.82	0.507

3.Effect of *Enterococcus faecium* L3 on blood biochemical indexes of laying hens

Items	A	B	P
Leukocyte counts(10^9/L)	91.65±2.60	92.46±2.27	0.815
Lymphocyte ratio (%)	75.68±0.90	76.83±0.89	0.373
Intermediate cell ratio (%)	8.92±0.21	9.04±0.23	0.690
Granulocyte ratio (%)	14.87±0.65	14.13±0.71	0.454
lymphocyte (10^9/L)	69.90±2.12	71.87±1.51	0.453
Intermediate cell (10^9/L)	8.18±0.28	8.55±0.36	0.434
granulocyte (10^9/L)	13.54±0.73	14.27±0.66	0.469
red blood cell counts (10^{12}/L)	2.23±0.16	2.33±0.06	0.540
Hematocrit (%)	29.95±0.91	29.27±0.77	0.571
mean red cell volume (fL)	126.67±0.99	125.70±1.17	0.535
Red blood cell distribution width SD (fL)	38.09±2.30	37.15±0.76	0.360
Red blood cell distribution width CV(%)	10.71±0.13	10.53±0.16	0.413
platelet count (10^9/L)	41.64±3.83	39.58±3.63	0.701
Mean platelet volume (fL)	10.35±0.18	10.00±0.10	0.103
platelet distribution width (%)	10.39±0.32	10.18±0.51	0.740
platelet crit (%)	0.04±0.005	0.03±0.004	0.211
Platelet large cell ratio (%)	1.78±0.13	1.86±0.26	0.801

Effect of *Enterococcus faecium* L3 on serum biochemical indexes of laying hens

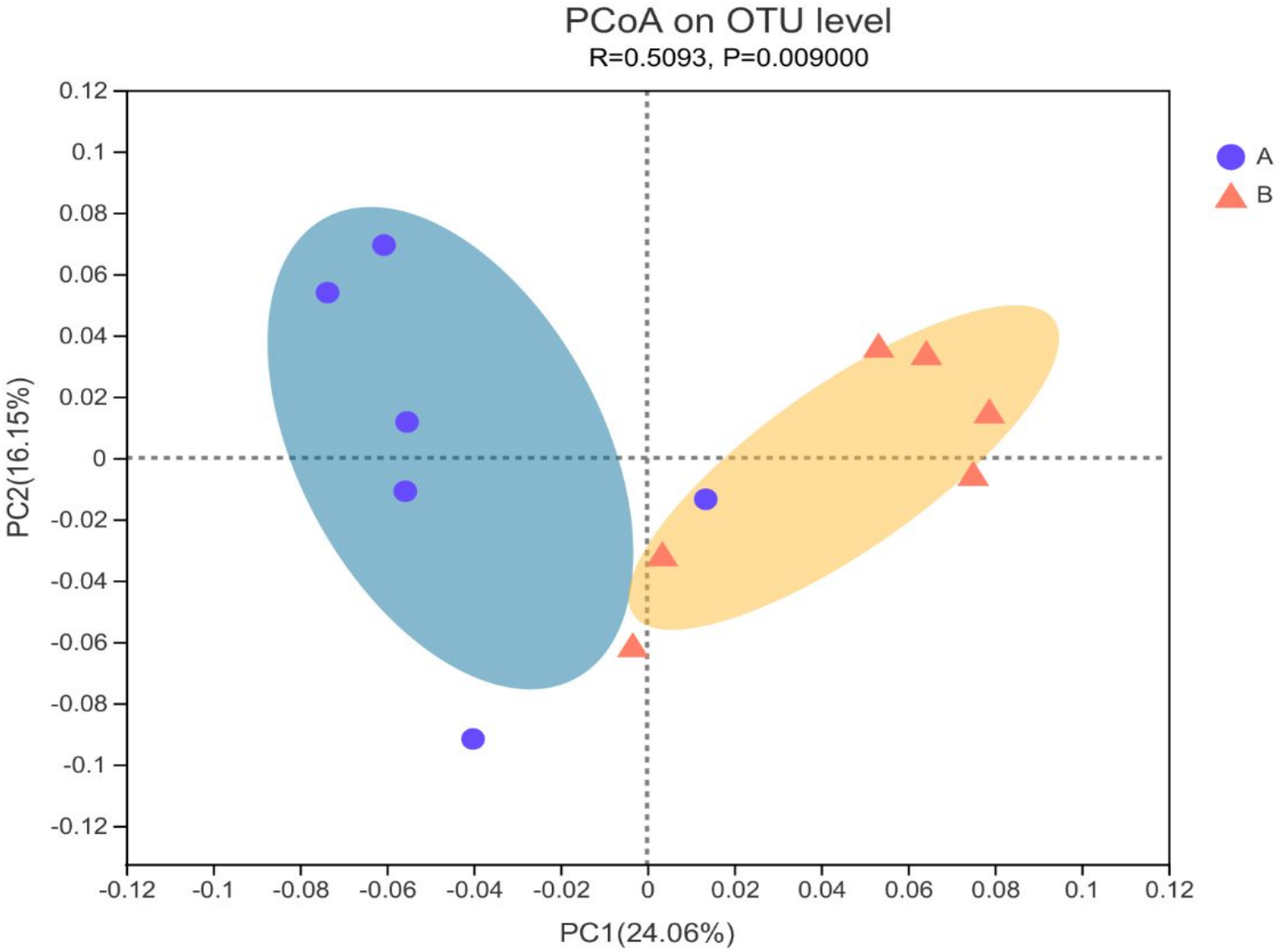
Items	A	B	P
triglycerides（TG,mmol/L）	20.33±0.90	22.93±0.81	0.039
total cholesterol (TC,mmol/L)	3.71±0.16	4.25±0.18	0.028
IL1（pg/mL）	181.82±8.21	134.93±11.06	0.004
IL4（pg/mL）	98.32±7.48	91.14±7.87	0.533
IL6（pg/mL）	26.96±1.77	27.28±2.67	0.919
IgA（pg/mL）	16.94±0.85	15.27±1.13	0.252
C-reactive protein（CRP, ug/mL）	160.02±13.76	138.81±9.92	0.245

4. Effects of Enterococcus faecium on cecal content microflora of 60-week-old Hyline -brown laying hens

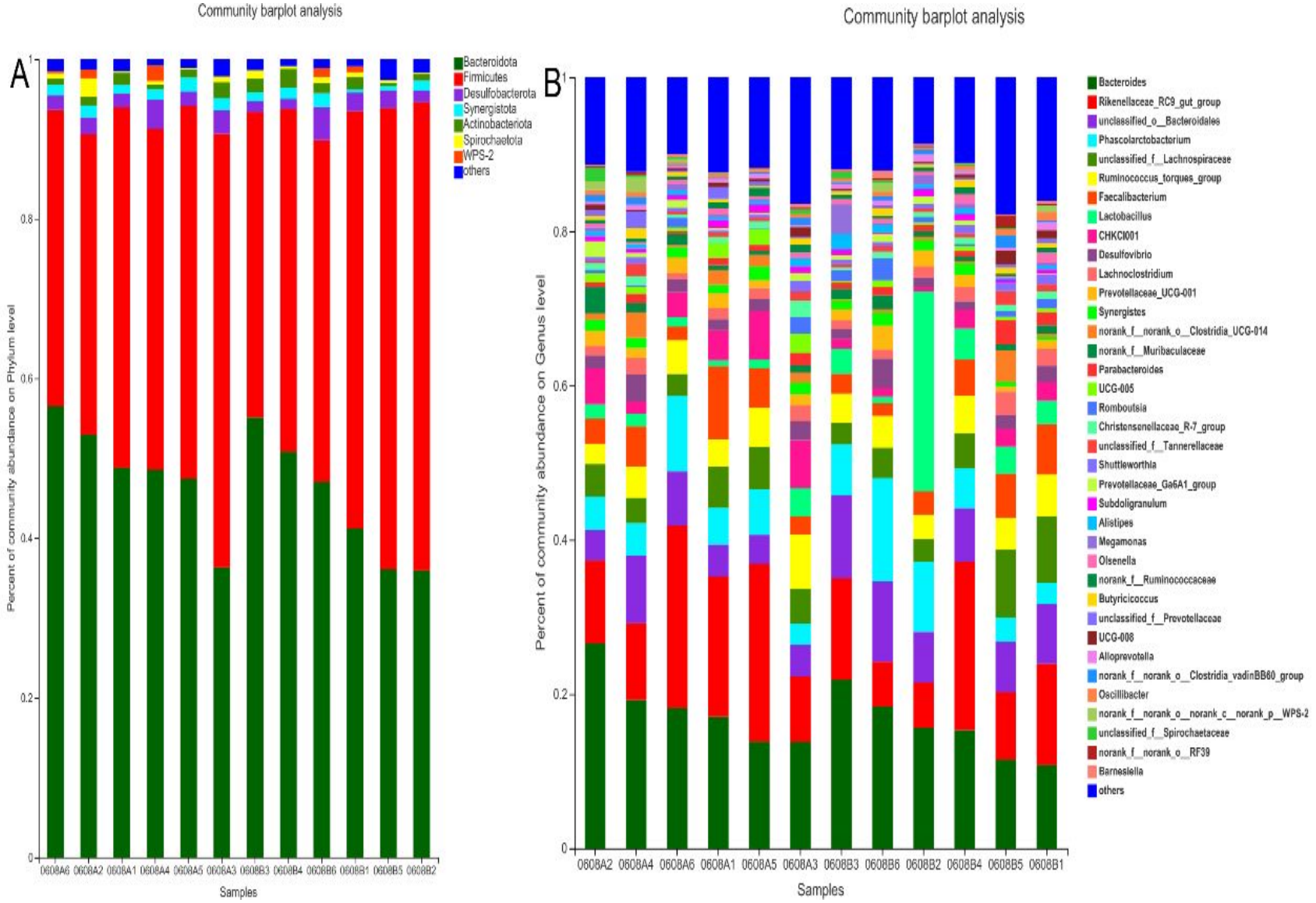
The data were analyzed on the free online platform of Majorbio Cloud Platform

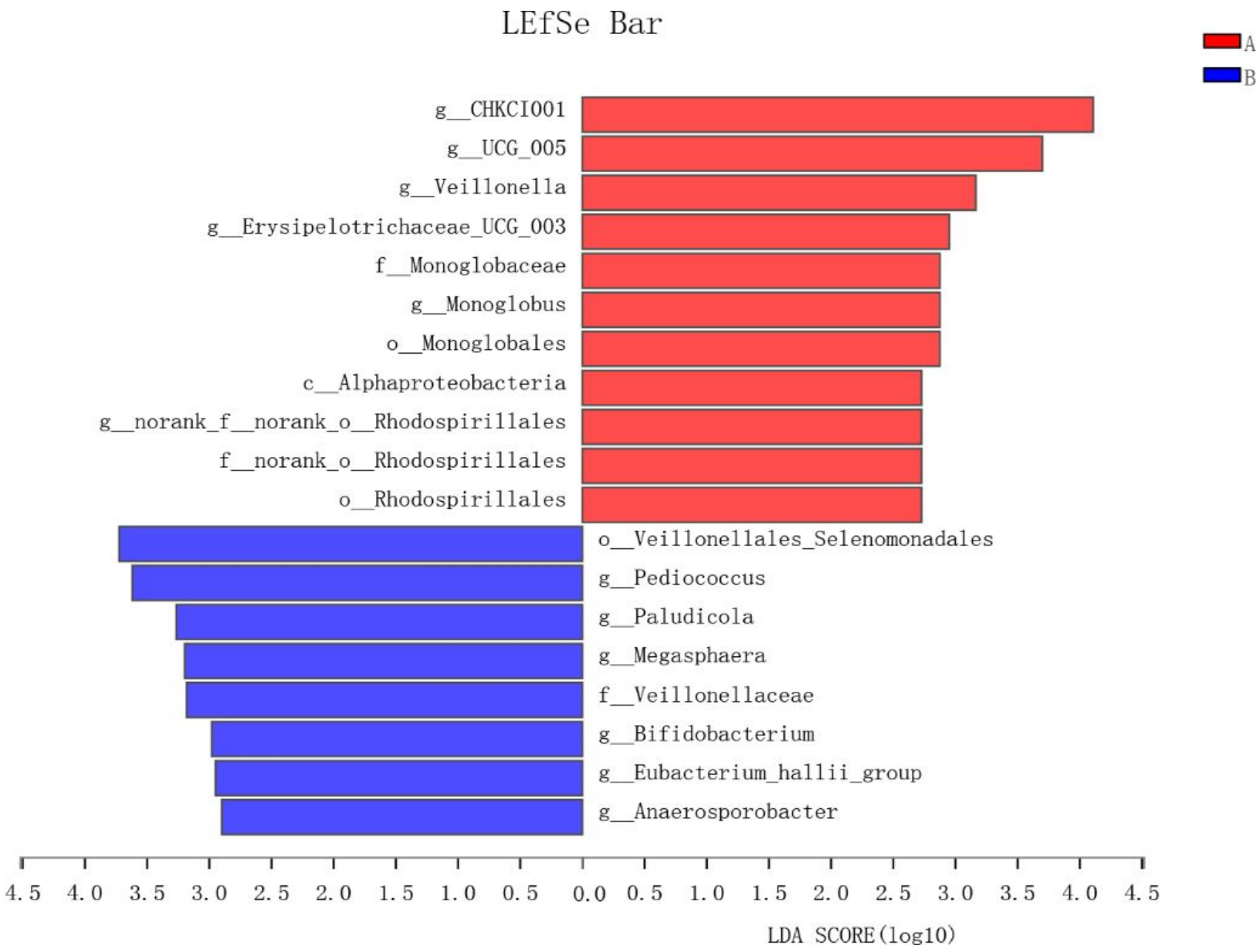
Effects of *Enterococcus faecium L3* on alpha diversity indexes of cecal content microflora

Estimators	A group	B group	P -value
Sobs	441.17±8.35	454±10.66	0.04267
Ace	459.35±8.03	477.0±11.58	0.0119
Chao	464.44±11.74	485.13±16.94	0.03378
Shannon	4.62±0.22	4.65±0.22	0.7964
Simpson	0.02±0.01	0.03±0.01	0.7669

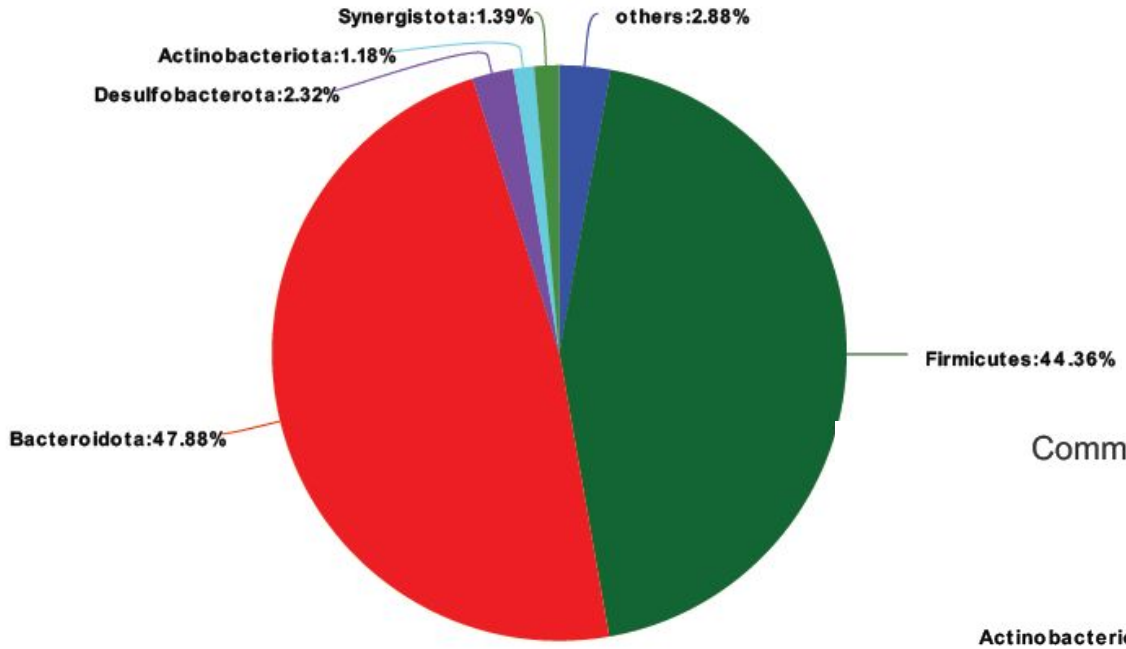


Effect of *Enterococcus faecium* L3 on the structure of cecal flora in laying hens

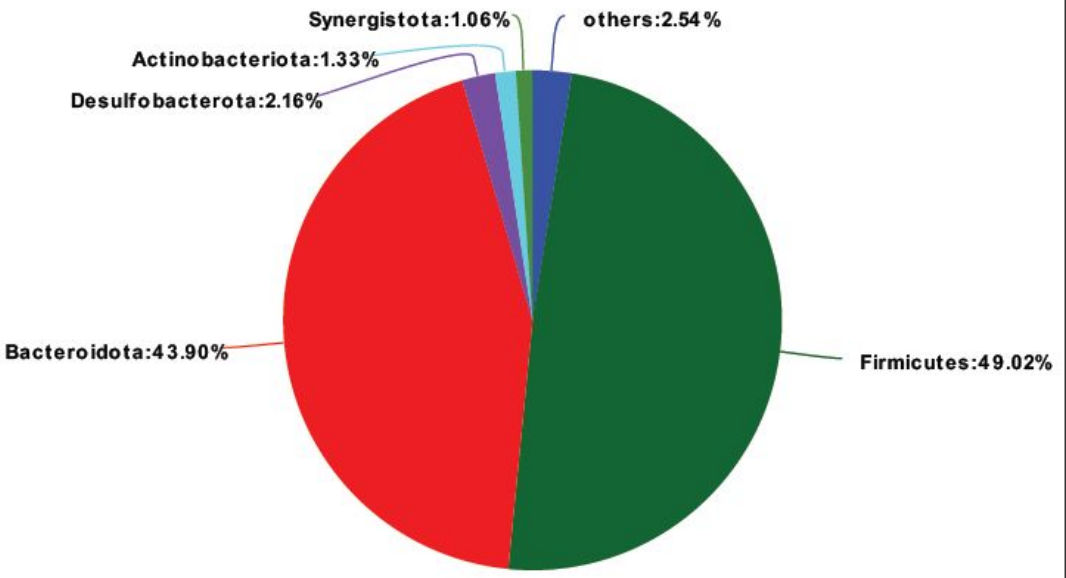

Community barplot analysis
A
Percent of community abundance on Phylum level
Samples
0608A6 0608A2 0608A1 0608A4 0608A5 0608A3 0608B3 0608B4 0608B6 0608B1 0608B5 0608B2
Bacteroidota
Firmicutes
Desulfobacterota
Synergistota
Actinobacteriota
Spirochaetota
WPS-2
others
Community barplot analysis
B
Percent of community abundance on Genus level
Samples
0608A2 0608A4 0608A6 0608A1 0608A5 0608A3 0608B3 0608B6 0608B2 0608B4 0608B5 0608B1
Bacteroides
Rikenellaceae_RC9_gut_group
unclassified_o__Bacteroidales
Phascolarctobacterium
unclassified_f__Lachnospiraceae
Ruminococcus_torques_group
Faecalibacterium
Lactobacillus
CHKCI001
Desulfovibrio
Lachnoclostridium
Prevotellaceae_UCG-001
Synergistes
norank_f__norank_o__Clostridia_UCG-014
norank_f__Muribaculaceae
Parabacteroides
UCG-005
Romboutsia
Christensenellaceae_R-7_group
unclassified_f__Tannerellaceae
Shuttleworthia
Prevotellaceae_Ga6A1_group
Subdoligranulum
Alistipes
Megamonas
Olsenella
norank_f__Ruminococcaceae
Butyricicoccus
unclassified_f__Prevotellaceae
UCG-008
Alloprevotella
norank_f__norank_o__Clostridia_vadinBB60_group
Oscillibacter
norank_f__norank_o__norank_c__norank_p__WPS-2
unclassified_f__Spirochaetaceae
norank_f__norank_o__RF39
Barnesiella
others


LEfSe Bar
A
B
g__CHKCI001
g__UCG_005
g__Veillonella
g__Erysipelotrichaceae_UCG_003
f__Monoglobaceae
g__Monoglobus
o__Monoglobales
c__Alphaproteobacteria
g__norank_f__norank_o__Rhodospirillales
f__norank_o__Rhodospirillales
o__Rhodospirillales
o__Veillonellales_Selenomonadales
g__Pediococcus
g__Paludicola
g__Megasphaera
f__Veillonellaceae
g__Bifidobacterium
g__Eubacterium_hallii_group
g__Anaerosporobacter
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5
LDA SCORE(log10)

Community analysis pieplot on Phylum level :a



Community analysis pieplot on Phylum level :b



Effects of *Enterococcus faecium*L3 on relative abundance of cecal content microflora in phylum level of laying hens (relative abundance>1%)

Species name	A group	B group	P -value
Bacteroidota	48.10±7.00	45.97±7.59	0.5228
Firmicutes	44.13±6.40	46.66±7.79	0.7842
Desulfobacterota	2.32±0.74	2.24±1.13	0.9273
Actinobacteriota	1.15±0.51	1.45±0.76	0.4113
Synergistota	1.40±0.23	1.03±0.56	0.4113

Conclusion

- *Enterococcus faecium* L3 has obvious effect on improving the digestion and metabolism of nutrients, improving feed utilization and reducing feed consumption.
- *Enterococcus faecium* L3 can improve the intestinal flora structure and prolong the laying period of laying hens.

Thank you for your attention!