

Effect of Dietary Yeast Culture Supplementation on the Growth Performance and Cecal Microbiota Modulation of Geese

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Research Article

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Abstract

Background

One of the most extensively applied animal feed additives is yeast culture (YC), which can increase production efficiency by altering gastrointestinal tract (GIT) microbiota. However, its use is still limited in waterfowl. Geese are the ideal model to study the interaction between dietary and GIT, due to their adaptation to consume different roughage sources. Therefore, the effect of YC supplementation at different concentrations (0%, 0.5%, 1.0%, 2.0% and 4.0%) on the GIT microbiota of geese was investigated in the present study.

Results

Three hundred Sichuan white geese with healthy and similar body weight (BW: 95.57 ± 2.42 g) were randomly divided into five groups: i) basal diet (control), ii) basal diet+0.5%YC (treat1), iii) basal diet+1.0% YC (treat2), iv) basal diet+2.0% YC (treat3), and v) basal diet+4.0% YC (treat4). After 10 weeks, slaughter and collected the cecum contents, then analysis GIT microbiota by high-throughput sequencing. The results showed that YC supplementation did not significantly affect α -diversity ($P \geq 0.05$). Principal coordinates analysis showed an obvious separation between control and treat4. The dominant phyla were *Firmicutes* and *Bacteroidetes* whereas the predominant genera were *Alistipes* and *Desulfovibrionaceae*. The relative abundance of *Firmicutes* significantly increased in the treat1 group, whereas that of *Bacteroidetes* significantly decreased in the treat4 group. Dietary YC increased the proportion of beneficial bacteria, such as *Parabacteroides*, *Enterococcus*, *Streptococcus* and *Pseudomonas*, particularly in group treat2. Furthermore, treat3 significantly improved the body weight and feed utilization of geese.

Conclusion

Collectively, these findings demonstrate that dietary YC supplementation tends to increase species diversity and richness of GIT microbiota in geese. This increases the proportion of beneficial bacteria which improves amino acid and carbohydrate metabolism. Moreover, YC increases the relative abundance of *Firmicutes* that promote energy utilization and nutrition absorption, thereby improving the growth performance of geese. This dietary strategy based on feed additives is an effective method to maintain the health of the geese GIT and to improve growth efficiency.

Introduction

Antibiotics combat disease in animals and improve production efficiency and quality, resulting ultimately in economic profits. Therefore, antibiotics have been widely used as feed additives for animal husbandry. However, their overuse has caused many problems, such as presence of antibiotic residue, drug resistance and others^[1]. Therefore, it is imperative to explore feed additives with similar effects to antibiotics, but which are safer and more effective. Yeast cultures (Ycs) are micro-ecological products

formed by yeast after sufficient anaerobic fermentation on specific media and are mainly composed of yeast metabolites, fermented medium and a few yeast cells. YCs are rich in vitamins, saccharides, minerals, enzymes, growth-promoting factors and amino acids, which may benefit animal growth, metabolism, and health. YC is one of the most important feed additives [2] as it can improve yield and quality of animal by-products, such as milk [3] and eggs [4]. YC can also enhance animal growth performance, as measured by average daily gain, body weight and average daily intake [5–7], and also can strengthen immunity and disease resistance [8–10]. The constituents of YC are live yeast and various yeast metabolites. These may act on the gastrointestinal tract (GIT) directly to maintain the balance of the microorganism and promote development of the GIT. For example, YC can significantly affect community structure and composition of gut microbiota of grass carp, increasing the abundance of beneficial bacteria [11]. Addition of YC in the diet resulted in an increase in beneficial bacteria, making greater compensatory weight gains in weanling pigs [12]. The effect of YC on hindgut microbial communities was evaluated in horse [13] where it increased the abundance of fiber-degrading bacteria. Geese are important poultry in China (90% of the world's production), and their cecum are more developed compared with other poultry, so it is the ideal model to study the interaction between GIT microbiota and diet [14]. Supplementation of geese diets with yeast fermented feed regulate cecal microflora that is beneficial of growth performance [15]. Addition of yeast supplement in the diets improve intestinal microflora of goose and lead to better carcass hygiene [16]. However, few studies have investigated the effect of YC on the GIT microbiota of geese. Furthermore, the variety in GIT microbiota composition and structure can reflect the effect of dietary on the host. Thus, the present study was conducted to investigate the effect of YC supplementation on the GIT microbiota of geese.

Methods

Animals, diets, feeding and experimental design

Three hundred Sichuan white geese with healthy and similar body weight (BW: 95.57 ± 2.42 g) were randomly divided into five groups: i) basal diet (control), ii) basal diet + 0.5%YC (treat1), iii) basal diet + 1.0% YC (treat2), iv) basal diet + 2.0% YC (treat3), and v) basal diet + 4.0% YC (treat4). The commercial YC (Baihuibang, Beijing Enhalar Biotechnology Co., Ltd., Beijing, China) was a fermented product composed of *Saccharomyces cerevisiae* grown on a medium, which contains ~ 15.0% CP, ~ 3.5% crude fat, ~ 8.7% crude fiber, ~ 14.2% amino acid, ~ 3.3% mannan, ~ 14.0% β -glucan and other micro-components. Each group was fed four times a day (at 7:30, 12:30, 17:00 and 21:00) for 10 weeks. Two corn-soybean based basal diets were formulated to be fed during starter (0–4 weeks) and grower (5–10 weeks; Table 1) periods. Each group consisted of 3 replicates pens with 20 geese per pen. Geese were allowed access to feed (granule form) and water *ad libitum* throughout the experimental period. The daily temperature inside the house throughout the experimental period was (21.32 ± 2.39) °C, and the relative humidity was (84.03 ± 5.15)%.

Table 1
The composition of the basal diet of geese.

Items	0–4 weeks	5–10 weeks
Ingredients (%)		
Corn	63.80	53.60
Wheat bran	2.99	14.50
Soybean meal	20.00	11.50
Rapeseed meal	4.00	/
Rice bran	/	13.40
Silkworm chrysalis	4.30	1.79
Calcium hydrogen phosphate	1.59	0.90
Stone powder	0.87	0.75
Salt	0.20	0.20
L-Lysine (98%)	0.15	0.18
DL-methionine	0.05	0.07
Choline chloride	0.05	0.12
Premix	2.00	2.00
Sand	/	1.00
Total	100	100
Nutrient content		
Metabolizable Energy (MJ·kg ⁻¹)	11.97	11.21
Crude protein (%)	20.43	14.81
Crude fiber (%)	4.12	8.04
Calcium (%)	0.87	0.80
Available P (%)	0.43	0.40
Lysine (%)	1.14	0.85
Methionine (%)	0.36	0.30
Note: Premix provides (per kilogram of diet): vitamin A 2000 IU, vitamin D 3350 IU, vitamin E 3000 mg, vitamin K 3200 mg, vitamin B1 100 mg, vitamin B21 200 mg, vitamin B6 200 mg, vitamin B12 2.5 mg, niacin 6000 mg, pantothenic acid 200 mg, folic acid 200 mg, biotin 20 mg, choline 7.0 g, iron 6 g, copper 0.2 g, manganese 15 g, zinc 8 g, iodine 10 mg, selenium 30 mg.		

Growth performance measurements

The BW was measured on days 1 and 70 with empty stomach, and feed consumption was recorded every morning before the feeding. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated for the total period.

Intestinal content collection

Three 70 day old geese were randomly selected from each pen and killed. Nine cecum contents samples from each group were extracted and pooled by 3 (n = 3). The cecum was dissected with sterile instruments and washed in 70% ethanol and sterile water to avoid transient bacteria. The content from cecum was squeezed out and mix thoroughly, then collected into sterilized tubes and immediately stored in liquid nitrogen.

16S rRNA PCR amplification and sequencing

Bacterial genomic DNA was extracted from 100 mg of each sample using the QIAamp DNA Stool Mini Kit (Qiagen, Germany) following the manufacturer's protocol. The bacterial V3-V4 region of the 16S rRNA was amplified using the following primers: 319F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTC TAAT-3'), and a sequencing adapter was added to the end of the primers. Then the PCR products were purified, quantified, and homogenized to establish a sequencing library. The constructed library was subjected to library quality inspection, and the qualified library was sequenced as described in Fadrosh, Ma, Gajer, Sengamalay, Ott, Brotman and Ravel^[17] using Illumina HiSeq 2500 (Illumina, San Diego, CA, USA). All control and YC-treated samples were included in the same sequencing run.

Sequences analysis

According to the overlap relationship between paired-end (PE) reads, the double-end sequences obtained from Hi-seq sequencing were merged into a single sequence tag, to control the quality of reads and filter the effect of merge. This included the following three main steps: 1) PE reads merging: used FLASH v1.2.7 to merge the reads of each sample through the overlap, obtaining the original tags data (raw tags); 2) tags filtering: used Trimmomatic v0.33 to filter the raw tags obtained by merging to obtain high quality tags data (clean tags); 3) chimera removal: used UCHIME v4.2 to identify and remove the chimera sequence to obtain the final effective data (effective tags).

Bioinformatics analysis

Usearch^[18] was used to cluster tags at a similarity level of 97% to identify operational taxonomic units (OTUs). OTUs were annotated based on the Silva (bacterial) and UNITE (fungi) taxonomy databases. Taxonomic assignment was achieved using RDP classifier, clustering the sequences at 97% similarity with a confidence threshold of 0.8.

Microbiota diversity analysis

The α - and β -diversity of each sample was evaluated based on the OTU data (Supplementary Material S1). Mothur (version v.1.30) and QIIME were used to calculate α - and β -diversity, respectively. PICRUSt^[19] was used to detect the composition of functional genetic of each group by comparing species information and analyzing differences between groups in KEGG Orthologs (KO) composition including function. This involved metabolism, genetic information processing, human diseases, environmental information processing, cellular processes and organismal systems. Bugbase was used to detect the potential pathogenic bacteria abundance at the phylum and genus level.

Statistical analysis

The data analyses were performed with SPSS 22.0 software (IBM Corporation, 2014) using one-way ANOVA with LSD multiple comparison tests. All data are shown as means \pm SEM unless otherwise indicated. Differences were considered statistically significant when $P < 0.05$ throughout.

Results

Sequencing data analysis

A total of 1,195,635 couples of effective reads were obtained from 16S rRNA gene V3 + V4 amplicons with an average of 79,709 reads per sample (ranging from 76,541 to 80,376). Detailed data is shown in Supplementary Table S1. At a similarity level of 97%, we found a total of 484 OTUs common to all groups, where the number of OTUs in different groups (Fig. 1a) is: control, 477; treat1, 479; treat2, 481; treat3, 480; treat4, 475). A heat map with the 50 most abundant bacteria at the family level in the five groups (Fig. 1b) suggests that bacterial abundance in the control was different from the YC groups. A total of 462 common OTUs were shared among the five groups, and the most abundant taxa of the shared OTUs (Fig. 1c) indicate that *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia* and *Cyanobacteria* form the six most dominant phyla. Seven unique OTUs were found in the YC-group compared with the control (Fig. 1d). Among them, four OTUs at the genus level, *Butyrivibrio*, *Ruminococcus_1*, *DTU014*, and *Bacteroides* were found in the YC-group, *Caproiciproducens* and *Clostridiales_vadinBB60_group* were found in the YC-group except treat1, and [*Eubacterium*]₋*coprostanoligenes_group* was found in the treat3 and treat4.

Variation in microbiota diversity

The addition of YC in the diet produced no significant differences ($P > 0.05$) in α -diversity, as measured using Shannon, Simpson, Ace and Chao1 indices (Table 2). Using the Simpson's diversity index, the sequence is the same but in opposite order. These results indicate that the diet supplemented with 0.5% and 4% of YC caused the highest and the lowest species diversity, respectively. The Chao1 and Ace indices indicating that the diets supplemented with 2% and 4% YC had the highest and lowest microbial richness, respectively. The β -diversity, based on the overall community composition, indicated that the similarity between clustered GIT microbiota in the five groups was due to the YC supplement (Fig. 2). This figure shows that group treat4 clearly separated from the other groups, using both weighted and

unweighted PCoA analysis, whereas treat1 and treat3 formed a cluster. In addition, the replicates in treat 2 show a large variation. These results are significant since the sum of PC1 and PC2 was more than 50%, therefore these results likely represent the differences in GIT microbial community structure among these five groups.

Table 2
Effect of dietary yeast culture supplementation on the gut microbiota α -diversity of geese.

Item	control	treat1	treat2	treat3	treat4
Shannon	3.48 ± 0.29	3.83 ± 0.18	3.55 ± 0.34	3.56 ± 0.35	2.84 ± 0.58
Simpson	0.144 ± 0.013	0.129 ± 0.005	0.136 ± 0.006	0.130 ± 0.021	0.156 ± 0.033
Ace	460.85 ± 12.71	466.61 ± 4.31	467.45 ± 2.25	469.30 ± 5.53	450.53 ± 8.53
Chao1	463.55 ± 13.23	467.44 ± 6.84	469.58 ± 21.16	470.85 ± 9.17	452.74 ± 7.37
All data represent means ± SEM.					

Microbial analysis at the phylum and genus level

At the phylum level, a total of 13 phyla were identified (control: 13; treat1: 12; treat2: 11; treat3: 13; treat4: 12). *Firmicutes* (19.90 ~ 54.60%), *Bacteroidetes* (21.60 ~ 59.20%), *Proteobacteria* (8.09 ~ 23.50%), *Actinobacteria* (1.77 ~ 4.65%), *Cyanobacteria* (1.01 ~ 2.52%) and *Verrucomicrobia* (0.55 ~ 5.46%) constituted the dominant phyla (Fig. 3a). At the genus level, a total of 189 genera were identified (control: 186; treat1: 188; treat2: 189; treat3: 187; treat4: 184). *Alistipes* (17.6 ~ 49.7%), *Desulfovibrionaceae* (4.57%~18.20%), *Lachnospiraceae* (1.49%~7.64%), CHKCI001 (1.23%~14.10%), *Blautia* (1.42%~5.30%), *Bacteroides* (2.30%~8.24%) were predominant (Fig. 3b).

Comparison of the relative abundance of bacteria

YC treatment produced a significant effect on the relative abundance at both phylum and genus levels (Fig. 4). Compared with the control, the relative abundance of phyla *Bacteroidetes* and *Firmicutes* in treat4 significantly increased or decreased, respectively ($P < 0.05$), whereas *Firmicutes* significantly increased in treat1 treat2 and treat3 ($P < 0.05$). In treat1, the relative abundance of genus *Shuttleworthia*, *Christensenellaceae_R-7_group*, *Ruminococcaceae_UCG-005*, *Faecalibacterium*, *Eisenbergiella*, *Blautia* and *Sellimonas* significantly increased ($P < 0.05$), whereas in treat 4, genus *Alistipes* and *Bacteroides* also increased ($P < 0.05$).

Predicted functions of microbiota

Based on functionality prediction, we investigated the KO composition in the five groups. We found no significant differences between control, treat2, treat3 and treat4. However, neurodegenerative diseases, substance dependence, cancers: specific types, carbohydrate metabolism, amino acid metabolism, global

and overview maps, aging and sensory system achieve a statistical significant difference ($P < 0.05$) between control and treat1 (Fig. 5a). To determine whether the YC supplementation has a positive or negative effect on the GIT, and what levels are optimal, we predicted what bacteria were pathogenic and beneficial in the five groups (Fig. 5b). At the genus level, the proportion of potential beneficial bacteria in treat2 was higher than in other groups, whereas the proportion of potential pathogenic bacteria in treat4 was higher than in other groups. Among these bacteria, the dominant potential pathogenic genera were *Rikenellaceae*, *Bacteroides* and *Ruminococcus*, whereas the prominent potential beneficial genera were *Parabacteroides*, *Enterococcus*, *Streptococcus* and *Pseudomonas*.

Growth performance

There was no significant difference in the initial BW of geese with different YC dietary groups. Dietary YC supplementation significantly affected the final BW, ADG, ADFI, and F/G (Table 3). Compared with the control, the YC dietary significantly increased the final BW and ADG, while significantly decreased the F/G, with treat3 being the most effective. Moreover, ADFI in treat3 was significantly reduced, and no significant differences were found among other groups.

Table 3
Effect of dietary yeast culture supplementation on growth performance of geese.

Item	control	treat1	treat2	treat3	treat4
Initial BW (g)	94.50 ± 1.32	96.33 ± 0.76	94.67 ± 3.06	95.67 ± 3.06	96.67 ± 4.16
Final BW (g)	2868.00 ± 49.15 ^c	3024.67 ± 157.03 ^b	3026.67 ± 66.01 ^b	3474.00 ± 12.17 ^a	3063.33 ± 36.02 ^b
ADG (g)	39.62 ± 0.68 ^c	41.83 ± 2.24 ^b	41.89 ± 0.90 ^b	48.26 ± 0.17 ^a	42.38 ± 0.46 ^b
ADFI (g)	157.25 ± 1.36 ^a	156.19 ± 0.43 ^a	156.51 ± 2.09 ^a	148.01 ± 1.71 ^b	157.16 ± 1.41 ^a
FCR	3.97 ± 0.08 ^a	3.74 ± 0.21 ^b	3.74 ± 0.11 ^b	3.07 ± 0.04 ^c	3.71 ± 0.02 ^b
All data represent means ± SEM. In the same row, means with different letters are significantly different ($P < 0.05$), means with the same letters are not significantly different ($P > 0.05$). Absence of letters indicates no significant difference between treatments.					
BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio					

Discussion

The GIT microbiota is a community of microorganisms inhabiting the animal gastrointestinal tract, which can be beneficial or harmful. In general, there is a balance between beneficial and harmful microbiota, and this has relevance for the immune system, metabolism [20] and diseases [21]. The composition of the GIT microbiota evolves throughout an individual's lifetime, and the balance may be altered by both

endogenous and exogenous factors [22], such as the diet [23]. Thus, the GIT microbial composition depends on the interaction between the microorganisms and their host, and with the diet of the host. Therefore, studying the GIT microbiota composition and its structure can be used to better understand how dietary YC affects growth performance of goose at the microbial level. In the present study, we chose five commonly used YC diet supplementation levels (0, 0.5, 1.0, 2.0, and 4.0%) to study how YC modulates GIT microbiota. We used high-throughput sequencing of the V3 + V4 region of the 16S rRNA gene to explore the diversity and composition of GIT microbiota in geese.

The α -diversity analysis showed that there is no significant differences in species diversity or richness between these treatments, whereas β -diversity analysis based on PCoA showed a clear separate microbiota structure among the groups. Further, the α -diversity analysis suggested that some microorganisms are essential for the growth of the host. In addition, the presence or absence of microorganisms is not easily affected by the rearing environment or changes in diet, although the relative abundance may be affected. This is consistent with a report indicating that there are no significant differences in α -diversity when gilthead sea bream is supplemented with different mannan-oligosaccharide levels [24]. However, laying hens with the higher fermentation yeast addition into diets had the lower total bacterial count [25]. The discrepancies between these and the present study may be caused by differences in yeast product used, host species and rearing conditions.

In the present study, there were clear differences in microbial composition between the different YC treatments. *Firmicutes* and *Bacteroidetes* were the two most dominant phyla in the bacterial communities of the goose. Similar results have been observed in chicken [26], buffalo [27] and goose [28], where the predominant genera were *Alistipes* and *Desulfovibrionaceae*. Phylum *Firmicutes* and *Bacteroidetes* are beneficial to the host. *Firmicutes* are tightly related to nutrition and to energy absorption [29]. In particular, the genera of this phylum degrade fiber and cannot stand extremely acidic environments. In contrast, phylum *Bacteroidetes* plays a role in maintaining a healthy GIT, adapting to changing and extreme environments [30]. Furthermore, we found that as the amount of YC increased, the relative abundance of the phyla *Firmicutes* and *Bacteroidetes* decreased and increased, respectively, and a similar trend can be found in their respective genera (Fig. 3). These results are consistent with similar studies performed on gut microbiota in humans [31]. These results may be explained by considering that YC diet supplementation can enhance microbiota fermentation, resulting in an increase in the concentration of volatile fatty acids and a reduced pH of the GIT [32]. In contrast to *Firmicutes*, *Bacteroidetes* can adapt to this acidic environment.

The dominant bacteria in the GIT may play many important functions essential to the goose [33]. Our study indicates that 0.5% YC can significantly enhance carbohydrate and amino acid metabolism and global and overview maps (Fig. 5a). These biochemical processes might promote utilization of energy, absorption of nutrients and growth [34]. Remarkably, the proportion of potential beneficial bacteria after 1.0 and 2.0% YC treatment was higher than in the control (Fig. 5b). These results are caused by the presence of oligosaccharides in YC, which can minimize GIT pathogen concentration and stimulate the

growth of beneficial bacteria ^[35], thus alleviating the competition for diet between the host and the pathogenic bacteria. However, the proportion of potential beneficial bacteria in the sample treated with the highest amount of YC (treat4) was the lowest (Fig. 5b). High concentration of YC may induce the goose to develop immune tolerance, leading to wastage of energy and nutrients and suppressing the growth of both host and microbiota ^[36]. A similar result was obtained for growth performance, where low YC concentration (0.5%, 1% and 2%) improved average daily gain and feed conversion rate in goose, whereas high YC concentration (4%) did not.

In conclusion, dietary YC modulates the composition and relative abundance of GIT microbiota in goose and lead to better growth performance. In general, dietary YC is beneficial to GIT microbiota and its effect depends on concentration. Specifically, 0.5% YC increased species diversity, 1% YC increased the proportion of beneficial bacteria and 2% YC improved species richness.

Abbreviations

YC
yeast culture
GIT
gastrointestinal tract

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The authors declare that the data of this research are not deposited in any official repository. All sequences have been deposited in the NCBI's Sequence Read Archive with the accession number PRJNA616162.

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Figures

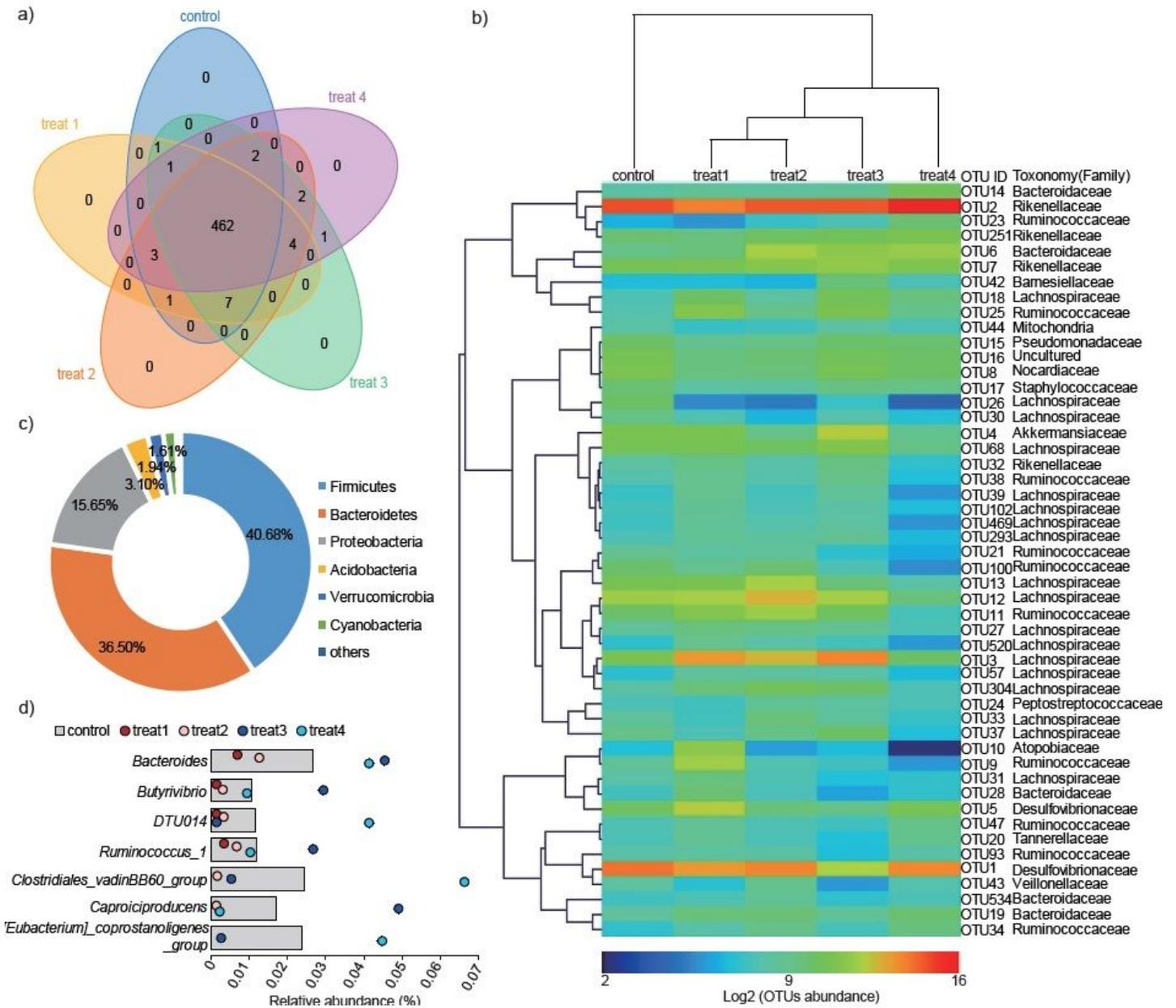


Figure 1

Effect of dietary yeast culture supplementation on bacterial community composition in goose gut. Number of shared OTUs among five groups (a); heat map of changes in relative abundance of the 50 most abundant OTUs summarized, at the family level (b); composition of shared gut microbiota in the five groups, at the phylum level (c); relative abundance of gut microbiota that is uniquely present in the YC group, at the genus level (d).

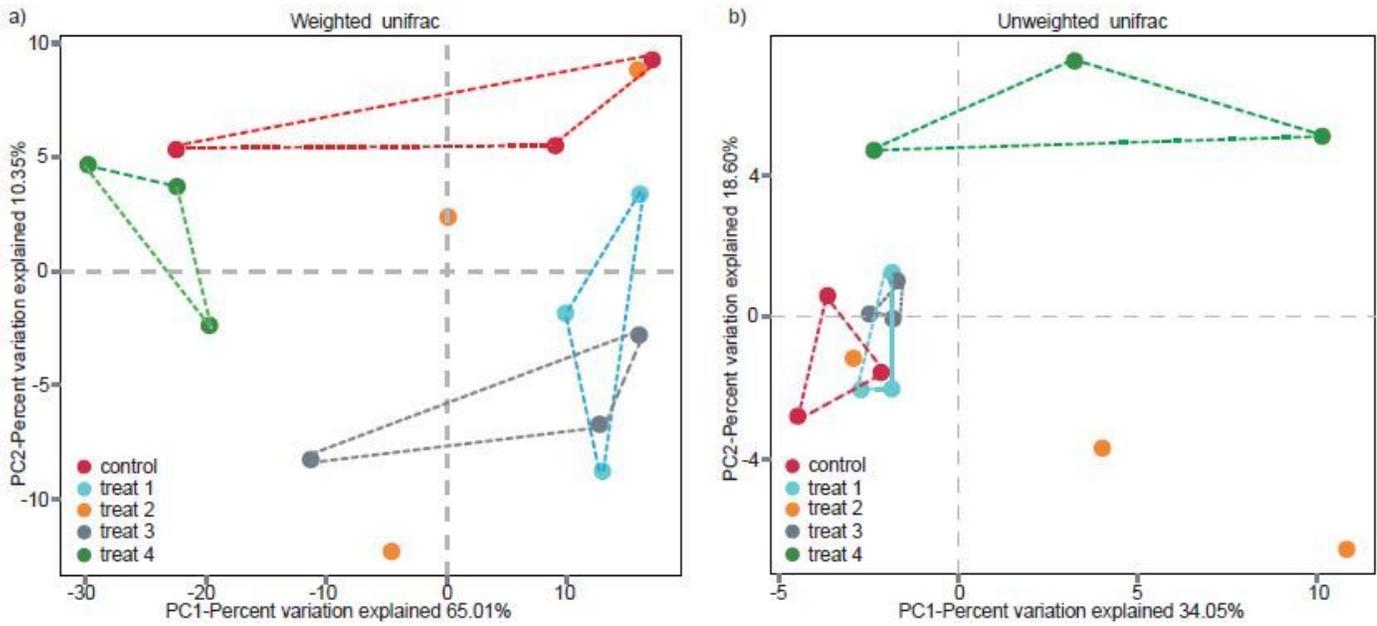


Figure 2

Effect of dietary yeast culture supplementation on microbiota community structure in goose gut. Principal coordinates analysis (PCoA) using weighted (a) or unweighted (b) UniFrac distances.

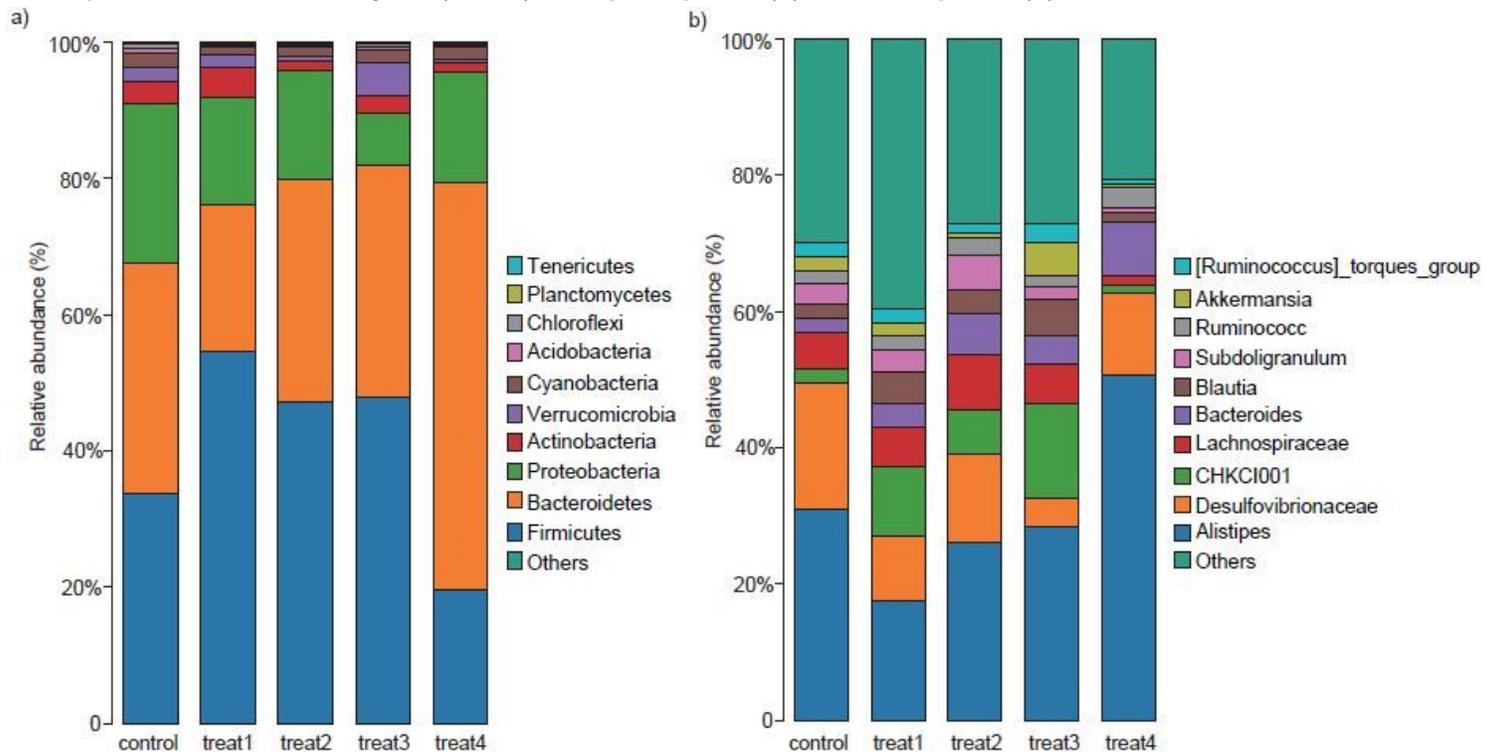


Figure 3

Effect of dietary yeast culture supplementation on the relative abundance of gut microbial population in geese. Relative abundance of bacterial phyla (a) and genera (b).

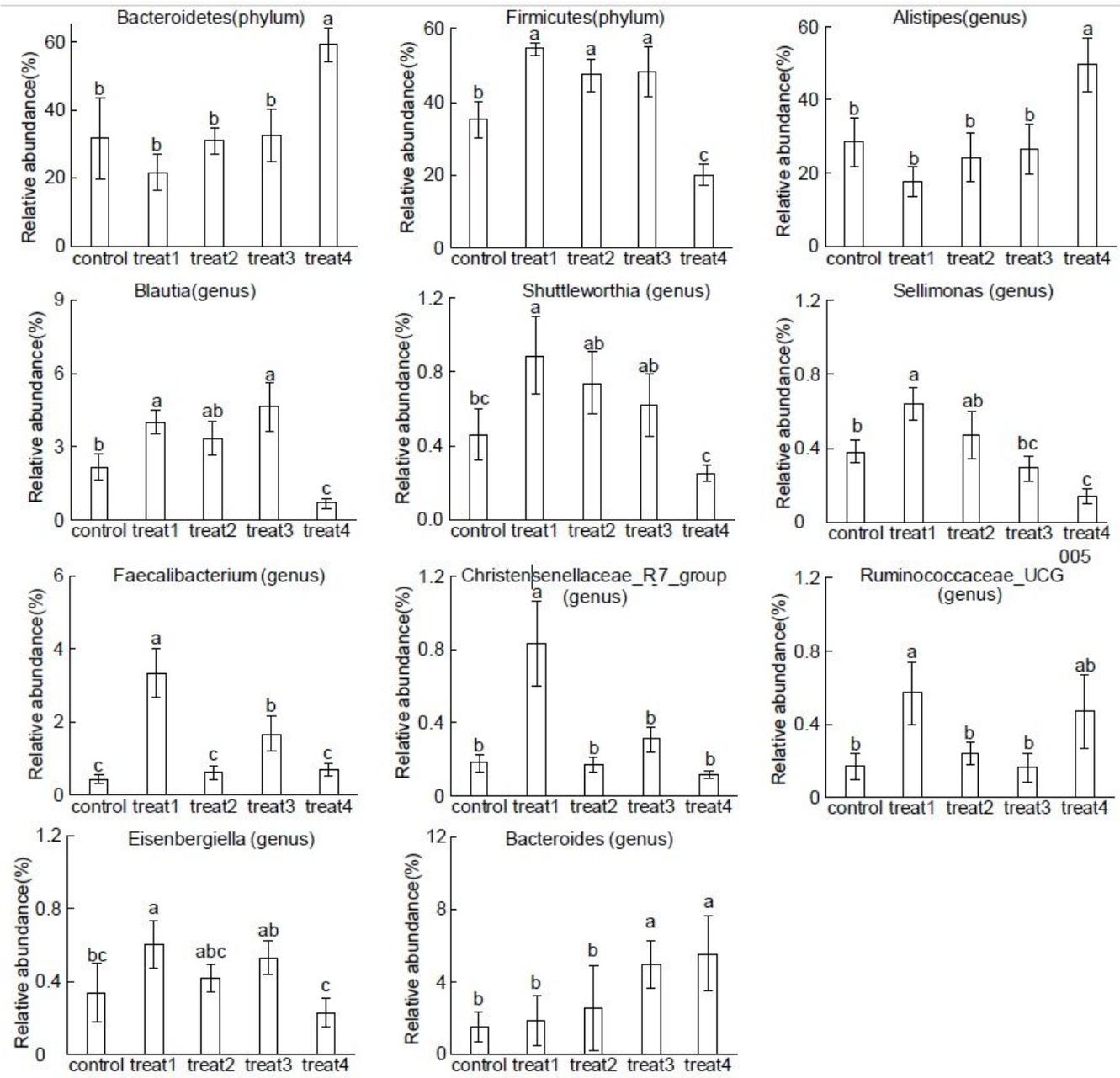


Figure 4

Effect of dietary yeast culture supplementation on relative abundance of gut bacteria in geese which were significantly affected by YC treatment.

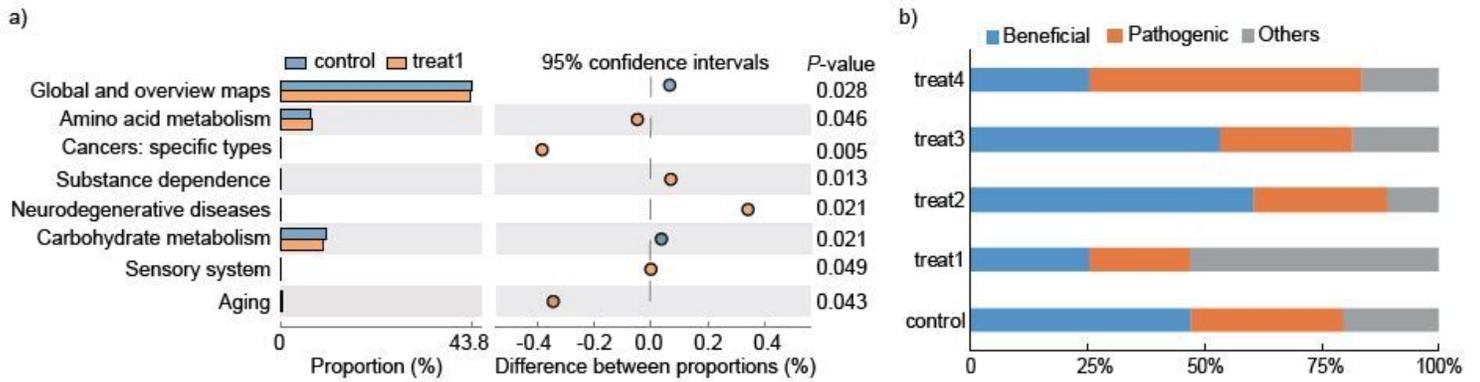


Figure 5

Effect of YC dietary yeast culture supplementation on the function of gut bacteria in geese. Predicted function of gut microbiota (a); proportion of potential beneficial and pathogenic bacteria at the genus level (b).

Supplementary Files

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