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Effects of N-Carbamylglutamate supplementation on cecal morphology, microbiota composition, and short-chain fatty acids contents of broiler breeder roosters

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The objective of this study was to assess the effects of N-Carbamylglutamate (NCG) supplementation on cecal morphology, microbiota composition, and short-chain fatty acids (SCFAs) contents in broiler breeder roosters. A total of 72 11-week-old Zhuanghe Dagu broiler breeder roosters with a similar initial body weight (1.53 ± 0.06 kg) were randomly allocated into two groups. Each group had 3 replicates with 12 birds per replicate. The experimental period lasted 42 days. All birds underwent the same production practices, except for the dietary conditions. It was found that an increase in cecal muscularis thickness and villi epithelium thickness. The NCG supplementation was found to have regulatory effects on the composition of cecal microbiota. Additionally, the study observed an increase in the content of butyric acid in the cecum of broiler breeder roosters fed with the NCG-containing control diet compared to those fed with the basal diet. Spearman correlation analysis showed that the variation of cecal microbiota was closely related to the production of butyric acid as well as the improvement of muscularis and villi epithelium thickness in cecum. The increase of butyric acid content in cecum was positively correlated with the improvement of cecal muscularis and villi epithelium thickness. In conclusion, the findings of this study indicate that dietary supplementation of NCG in broiler breeder roosters can positively influence cecal morphology, microbiota composition, and butyric production.

Keywords N-Carbamylglutamate, Broiler chick, Intestinal health, Intestinal microbiota, Hindgut fermentation

The reproductive performance and general health of broiler breeder roosters are crucial for the success of poultry operations. Various factors, including intestinal microbiota, intestinal morphology, and hindgut fermentation, significantly impact the performance of broiler breeder roosters by influencing gut health and intestinal integrity[1].

Diet plays a vital role in affecting the intestinal microbiota, intestinal morphology, and hindgut fermentation activities. Supplementing exogenous additives is an effective approach to improving the intestinal microbiota, intestinal morphology, and hindgut fermentation activities of broiler chicks, thereby enhancing overall performance[2,3]. For example, adding an unsaturated fat source to broiler chicken diets improves the composition of thigh fatty acids and improves immune function in broiler chickens[4]. The addition of zinc to the diet improves feed intake, egg weight and optimal tissue absorption of zinc in laying hens. The addition of methionine-chelated zinc improves the mechanical properties of the shins at the end of the production cycle without affecting the eggshell quality in laying hens[5]. Moharreri et al.[6], Moharreri et al.[7] found that microcapsules containing thyme, savory, peppermint and black pepper seeds as feed additives increased final body weight, total feed intake, FCR, antioxidant status in vivo, ileum morphological structure and gut microbial counts, and increased the expression of antioxidants and Microbes regulated inflammatory genes in ileal tissue. N-carbamylglutamate (NCG), a synthetic analog of N-acetylglutamate, promotes the synthesis of endogenous arginine. Several studies have

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reported the regulatory effects of NCG on the intestinal microbiota. For instance, Zhang et al.[8] demonstrated that NCG supplementation can mitigate colonic barrier injury, oxidative stress, and inflammation by modulating colonic microbiota in intrauterine growth retardation-suckling lambs. Similarly, Li et al.[9] found that NCG supplementation enhances amino acid biosynthesis by regulating gut microbiota and contributes to the growth promotion of tilapia. The widespread use of antibiotic growth promoters (AGPs) in animal production has played a large role in increasing animal production, but certain drug-borne diseases, immunosuppression, and other phenomena caused by the toxic side effects of antibiotics may result in animal health impairment[10]. Over the years, various feed additives have been launched that have been specifically developed to help the poultry industry achieve its goal of being antimicrobial by optimizing poultry's healthy digestive tract. Studies have shown that ESBM replacement for EFS improves the immune response, antioxidant status, intestinal morphology and barrier function in pigs that are fed an antibiotic-free diet, thereby improving their performance[11]. Feng et al.[12] reported that NCG supplementation during gestation improves the intestinal microbiome in sows, ultimately enhancing placental and fetal development. The addition of NCG maintains optimal poultry performance and productivity without the need for antibiotics. Zeng et al.[13] showed that oral administration of NCG to newborn piglets improves growth performance by regulating intestinal microbiota. Additionally, Zhu et al.[14] observed that NCG can improve rumen fermentation, increase rumen microbial diversity, and enhance the production performance of Holstein dairy cows. SCFA is the main metabolite formed during microbial fermentation in the intestine and plays an important role in intestinal health. NCG supplementation increased propanoic acid content while decreasing acetic acid and hexanoic acid in the hindgut[15]. The relative abundance of Lactobacillus and Streptococcus normalizes, and both arginine and NCG can attenuate colonic barrier damage, oxidative stress (OS), and inflammation by modulating the colonic microbiota of IUGR dairy lambs[8,16]. As a feed additive, the application of NCG in mammal production shows that it has the functions of improving production performance, promoting intestinal development and strengthening immunity. However, the application of NCG in poultry production is rarely reported, and most of them focus on broilers. Zhang et al.[17] suggested that NCG treatment of amniotic membranes in broiler embryos at the late incubation stage could increase arginine content, improve nutritional properties, increase antioxidant capacity and improve meat quality of broiler pectoral muscles. Hu et al.[18] found that the effect of NCG diet on the growth of broilers may be related to the homeostatic balance of arginine metabolism, lipid deposition, protein synthesis and immune response. However, studies investigating the effect of NCG supplementation on the intestinal morphology, microbiota composition, and hindgut fermentation activities of broiler chicks are still lacking.

In poultry, the cecum plays a significant role in affecting overall performance. Despite chickens having a relatively smaller cecum compared to herbivorous animals, it serves important functions. The cecum is a specialized pouch located at the junction of the small and large intestines in poultry and other monogastric animals[19]. Within the cecum resides a diverse population of microorganisms, including bacteria, protozoa, and fungi, collectively known as the cecal microbiota. These microorganisms possess the necessary enzymes to break down complex carbohydrates that the bird's own digestive enzymes cannot efficiently digest[20]. Through fermentation, the cecal microbiota metabolizes complex carbohydrates into short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. SCFAs serve as important energy sources for the bird, contributing to overall energy availability and nutrient utilization[21]. They are absorbed by the cecal epithelium and act as energy substrates for the bird's metabolism. Thus, maintaining the health and balance of the cecal microbiota is crucial for optimal nutrient absorption, gut health, and overall performance in poultry[22].

Based on previous research and understanding, we hypothesized that dietary NCG supplementation can improve cecal morphology, microbiota composition, and hindgut SCFAs contents in broiler breeder roosters. Therefore, the objective of this study was to assess the effects of NCG supplementation on cecal morphology, microbiota composition, and SCFAs contents of broiler breeder roosters.

Materials and methods

Animals and experimental design

Seventy-two 11-week-old breeding chickens from Zhuanghe with large bones and similar initial weight (1.53 ± 0.06 kg), good appetite, good development, normal fecal morphology, and artificially reared, except for the parasites, which should be excluded from cleaning. Grade experimental birds that did not carry serious potential infections or parasites that severely interfered with scientific experiments and did not carry zoonoses and infectious disease pathogens were randomly divided into two groups. Each group had 3 replicates with 12 birds per replicate. The experimental period lasted 42 days. The test animals were obtained from the Big Bone Chicken Experimental Plant of Jinzhou Medical University. All birds underwent the same production practices except for the dietary conditions. The dietary treatments consisted of a basal diet and a control diet (basal diet supplemented with 0.08% NCG). The dosage of NCG was determined based on a prior study conducted by Ma et al.[23]. Research showed that supplementation with NCG significantly increased testicular weight on both sides in roosters aged 16 weeks ($p < 0.05$). Both sides of the testicular indices of birds aged 16 weeks were higher in groups N1 (basal diet supplemented with 0.08% NCG) than in groups N2 (basal diet supplemented with 0.12% NCG), N3 (basal diet supplemented with 0.16% NCG) and C (basal diet supplemented with 0% NCG) ($p < 0.05$). Secondary sex characteristics N1 were the most pronounced. The NCG used in this study was provided by Anhui Pusheng Pharmaceutical Co., Ltd, Anhui, China. Throughout the experimental period, the birds had ad libitum access to feed and water. The diets were formulated to meet the nutritional requirements recommended by the NRC[24], as presented in Table 1.

The birds were housed in a naturally ventilated room with programmable lighting. Each bird was individually reared in an adjacent steel cage equipped with nipple drinkers and a common trough feed. The dimensions of the steel cage were $36 \times 55 \times 60$ cm. The average ambient temperature during the experimental period was 23 °C. The daily photoperiod consisted of 16 h of light (from 05:00 to 21:00) with a light intensity of 5.2 lx, followed

Ingredients (%)	
Corn	63.82
Soybean meal	16.00
Dried distiller's grains with solubles	8.00
Wheat bran	8.00
Stone powder	1.20
Dicalcium phosphate	1.20
Baking soda	0.20
Salt	0.25
Methionine	0.09
Trytophan	0.02
Lysine	0.22
Mineral and vitamin mixture[1]	1.00
Total	100.00
Calculated value	
Metabolism energy, MJ/kg	11.09
Crude protein, %	15.50
Calcium, %	0.75
Available phosphorus, %	0.34
Lysine, %	0.80
Methionine, %	0.25
Methionine + cysteine, %	0.61
Tryptophan, %	0.16
Crude fiber, %	3.53
Crude ash, %	6.11

Table 1. Composition and nutrient levels of the experimental basal diet, (%, as-fed basis). [1]Provided per kg of complete diet: Fe, 80 mg; Cu, 8 mg; Mn, 100 mg; Zn, 80 mg; I, 0.7 mg; Se, 0.3 mg; Vit. A, 6,500 IU; Vit. D_3, 850 IU; Vit. E, 12.5 IU; Vit. K_3, 0.85 mg; Vit. B_1, 0.7 mg; Vit. B_2, 4.5 mg; pantothenic acid, 9.2 mg; niacin, 28 mg; Vit. B_6, 2.8 mg; biotin, 0.14 mg; folic acid, 0.6 mg, Vit. B_{12}, 0.012 mg; choline, 950 mg.

by 8 h of darkness. All methods and procedures were approved by the Ethics Committee of Jinzhou Medical University and conducted in accordance with the relevant guidelines formulated by the Ministry of Agriculture of the People's Republic of China. This study was conducted in accordance with the ARRIVE guidelines (https://arriveguidelines.org).

Sample collection

On the final day, one bird was selected from each replicate cage and euthanized with 1 cc of Euthasol administered intravenously. The cecal tissue and contents were then collected. The cecal tissue samples were cut into small pieces, fixed with formalin, dehydrated using increasing concentrations of alcohol and xylene, and embedded in paraffin to prepare solidified paraffin blocks. Fresh cecal contents were placed in sterile bags and duplicated, then stored at − 20 °C until analysis.

Body weight

The body weights of the birds were evaluated for each set of replicate cages on the initial and final day of the study.

Cecal morphology analysis

Serial tissue sections were excised using a cryostat. The samples were stained using the hematoxylin and eosin staining protocol[25] after removing any excess paraffin. Muscularis thickness and villi epithelium thickness were measured using an optical microscope (Olympus, BX53F, Tokyo, Japan) at 10X magnification. A minimum of ten measurements per slide were performed for each parameter, and the average values were calculated.

Cecal SCFAs contents analysis

The contents of SCFAs in the cecum were analyzed using an Agilent Technologies Inc. 7890–5977 GC/MS (Agilent Technologies Inc., CA, USA). The data were manually processed after export from the instrument. The SCFA content of the sample was calculated as μg/mg = (sample concentration read by the instrument × final constant volume of the sample × the dilution factor)/the sample weight.

Cecal microbiota analysis

Total DNA was extracted from 0.5 g of cecal content using a magnetic Soil and Stool DNA kit (cat# DP712, TIANGEN Biotech Co., Ltd., Beijing, China). The concentration and purity of the extracted DNA were

Items, kg	Dietary NCG levels, %	
	0.00	0.08
Initial day	1.52 ± 0.04	1.53 ± 0.06
Final day	2.60 ± 0.38	2.67 ± 0.24

Table 2. Effects of N-Carbamylglutamate (NCG) supplementation on body weight of broiler breeder roosters.

Items, μm	Dietary NCG levels, %	
	0.00	0.08
Muscularis thickness	274.24 ± 31.06[b]	423.73 ± 49.16[a]
Villi epithelium thickness	259.64 ± 33.23[b]	311.02 ± 35.00[a]

Table 3. Effects of N-Carbamylglutamate (NCG) supplementation on cecal morphology of broiler breeder roosters. [a,b]Different superscripts within a row indicate a significant difference ($P < 0.05$).

determined using a Qubit 2.0 spectrophotometer (Invitrogen, Carlsbad, CA) and 1% (w/v) agarose gel electrophoresis. The DNA samples were diluted with sterile water to a concentration of 1 ng/μL and stored at − 20 °C before analysis. The V3–V4 hypervariable regions of the extracted DNA were amplified using quantitative PCR with specific full-length universal forward (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primers (5′-GGACTACGVGGGTVVTCTAAT-3′). The PCR products were further purified using a Qiagen Gel Extraction Kit (cat# 28706, Qiagen, Germany). The purity of the PCR mixture was evaluated using a Qubit 2.0 dsDNA HS Assay Kit (cat# Q32854, Invitrogen). The cecal microbial community structures were analyzed by sequencing the 16S rRNA gene on the NovaSeq 6000 platform (Illumina, San Diego, CA) at Majorbio Co., Ltd. (Shanghai, China). Raw data were processed by cutting low-quality reads using Cutadapt software version 1.9.1. Chimeric sequences were trimmed by alignment and detection. High-quality reads were clustered into operational taxonomic units (OTUs) at 97% sequence identity using Uparse v7.0.1001. The taxonomic assignment of the representative sequences was performed using QIIME v1.9.1. Alpha-diversity was estimated using Chao1, ACE, Shannon, and Simpson indices. Beta-diversity was estimated using the P-value of Adonis and Anosim analyses. The LEfSe analysis was conducted using LEfSe software, and the screening value (linear discriminant analysis score; LDA) was set at 3.5.

Statistical analysis

All data were analyzed using the Student's t-test procedure in SAS software (SAS Inst. Inc., Cary, NC, USA). The normality of the data was assessed using the Shapiro–Wilk test and QQ plots. The replicate cage was considered the experimental unit. Spearman analysis was used to evaluate the correlations among cecal microbiota and cecal SCFAs content and cecal morphology as well as the correlations among cecal SCFAs content and cecal morphology. The results were presented as means ± standard deviation. A probability value below 0.05 was considered statistically significant.

Results

Dietary supplementation of NCG had no significant effects on the body weight (Table 2).

The results of the cecal morphology analysis showed that supplementation of 0.08% NCG in the basal diet increased ($P < 0.05$) cecal muscularis thickness and villi epithelium thickness in Zhuanghe Dagu broiler breeder roosters (Table 3).

In terms of cecal SCFAs contents, the supplementation of NCG led to an increase in the levels of butyric acid in the cecum compared to the basal diet group (Table 4). This difference was also statistically significant ($P < 0.05$).

However, the dietary supplementation of NCG did not have a significant impact on the alpha-diversity (Table 5) and beta-diversity (Table 6) of the cecal microbiota. There were no significant differences in the number of unique OTUs between the two groups, but there were 435 OTUs shared among both groups (Fig. 1A).

To examine the similarity or difference in sample community composition, the cecal contents of two groups of large-boned chickens were subjected to PCoA analysis. As shown in Fig. 1B, the two groups of microflora were obviously different and there was no intersection between them up to a certain distance, indicating that the main components of the two groups were significantly different and the addition of NCG had a certain influence on it the community structure of the cecum of large-boned chickens.

The predominant bacteria at the genus level in the cecal microbiota of Zhuanghe Dagu broiler breeder roosters were *Alistipes*, *Faecalibacterium*, *Barnesiella*, *norank_f_noranl_o_Clostridia_vadinBB60_group*, *Ruminococcus_torques_group*, *Bifidobacterium*, *norank_f_norank_o_Clostridia_UCG-014*, *Bacteroides*, *unclassified_f_Lachnospiraceae*, and *Subdoligranulum* (Fig. 1C).

Based on the LDA scores, certain taxons were identified as potential biomarkers for the cecal microbiota of the two groups. *Actinobacteriota* phylum, *Tannerellaceae* family, *Parabacteroides* genus, *Sellimonas* genus, *Coriobacteriiia* class, *Coriobacteriales* order, *Ruminococcus_torques_group* genus, and *norank_f_Oscillospiraceae*

Items, μg/mg	Dietary NCG levels, %	
	0.00	0.08
Acetic acid	1.08 ± 0.05	0.98 ± 0.02
Propionic acid	0.36 ± 0.04	0.39 ± 0.07
Isobutyric acid	0.04 ± 0.00	0.05 ± 0.00
Butyric acid	0.27 ± 0.01[b]	0.40 ± 0.01[a]
Isovaleric acid	0.05 ± 0.00	0.07 ± 0.00
Valeric acid	0.09 ± 0.00	0.07 ± 0.00
Isocaproic acid	0.02 ± 0.00	0.02 ± 0.00
Caproic acid	0.04 ± 0.00	0.02 ± 0.00

Table 4. Effects of N-Carbamylglutamate (NCG) supplementation on cecal short-chain fatty acids contents of broiler breeder roosters. [a,b]Different superscripts within a row indicate a significant difference ($P < 0.05$).

Items	Dietary NCG levels, %	
	0.00	0.08
ACE index	430.57 ± 28.00	420.34 ± 30.20
Chao1 index	429.73 ± 32.39	417.98 ± 24.29
Shannon index	3.73 ± 0.22	3.52 ± 0.24
Simpson index	0.07 ± 0.02	0.10 ± 0.04

Table 5. Effects of N-Carbamylglutamate (NCG) supplementation on alpha-diversity of cecal microbiota of broiler breeder roosters.

Adonis	Anosim
0.50	0.59

Table 6. The *P*-value of beta-diversity in cecal microbiota of broiler breeder roosters as affected by N-Carbamylglutamate supplementation.

genus served as biomarkers for the cecal microbiota of broiler breeder roosters fed the basal diet. On the other hand, *Clostridia_UCG-014* order, *Eubacterium_coprostanoligenes_group* family, *norank_f_Eubacterium_coprostanoligenes_group* genus, *norank_f_noranl_o_Clostridia_UCG-014* genus, *norank_o_Clostridia_UCG-014* family, and *Tuzzerella* genus served as biomarkers for the cecal microbiota of broiler breeder roosters fed the control diet (Fig. 2).

At the genus level, there were statistical differences among the groups in terms of the abundance of certain bacteria. The abundance of *norank_f__Eubacterium_coprostanoligenes_group* ($P < 0.05$), *Tuzzerella* ($P < 0.05$), *norank_f__norank_o__RF39* ($P < 0.05$), *Candidatus_Soleaferrea* ($P < 0.05$), and *Gordonibacter* ($P < 0.05$) in the cecal microbiota of broiler breeder roosters fed the basal diet supplemented with 0.08% NCG was higher compared to the basal diet group. Conversely, the abundance of *Christensenellaceae_R-7_group* ($P < 0.05$), *Butyricimonas* ($P < 0.05$), *Flavonifractor* ($P < 0.05$), *norank_f__Erysipelotrichaceae* ($P < 0.05$), *Holdemania* ($P < 0.05$), and *unclassified_p__Firmicutes* ($P < 0.05$) in the cecal microbiota of broiler breeder roosters fed the basal diet was lower than in the control diet (Fig. 3).

Spearman correlation analysis among cecal microbiota and cecal butyric acid content revealed that the abundance of *Tuzzerella* ($P < 0.05$), *Candidatus_Soleaferrea* ($P < 0.05$), and *Gordonibacter* ($P < 0.05$) positively correlated with cecal butyric acid content, cecal muscularis thickness, and cecal villi epithelium thickness. Moreover, the abundance of *Christensenellaceae_R-7_group* ($P < 0.05$), *Butyricimonas* ($P < 0.05$), *Flavonifractor* ($P < 0.05$), and *Holdemania* ($P < 0.05$) negatively correlated with cecal butyric acid content and cecal muscularis thickness. Furthermore, the content of butyric acid in cecum positively correlated to the cecal muscularis ($P < 0.05$) and villi epithelium thickness ($P < 0.05$) (Table 7).

Discussion

Because of growing interest in advanced nutritional supplements and their mechanistic role in improving animal health and performance, NCG research is part of the growing field of functional feed additives for animal nutrition[26]. NCG is inexpensive, costing only 10% of Arg, and can be frequently utilized in actual production. NCG can enhance protein conversion rates[27], and research on broiler chickens and pigs has shown that it can substitute for soy flour[28,29], which is crucial for reducing costs and boosting efficiency. NCG has

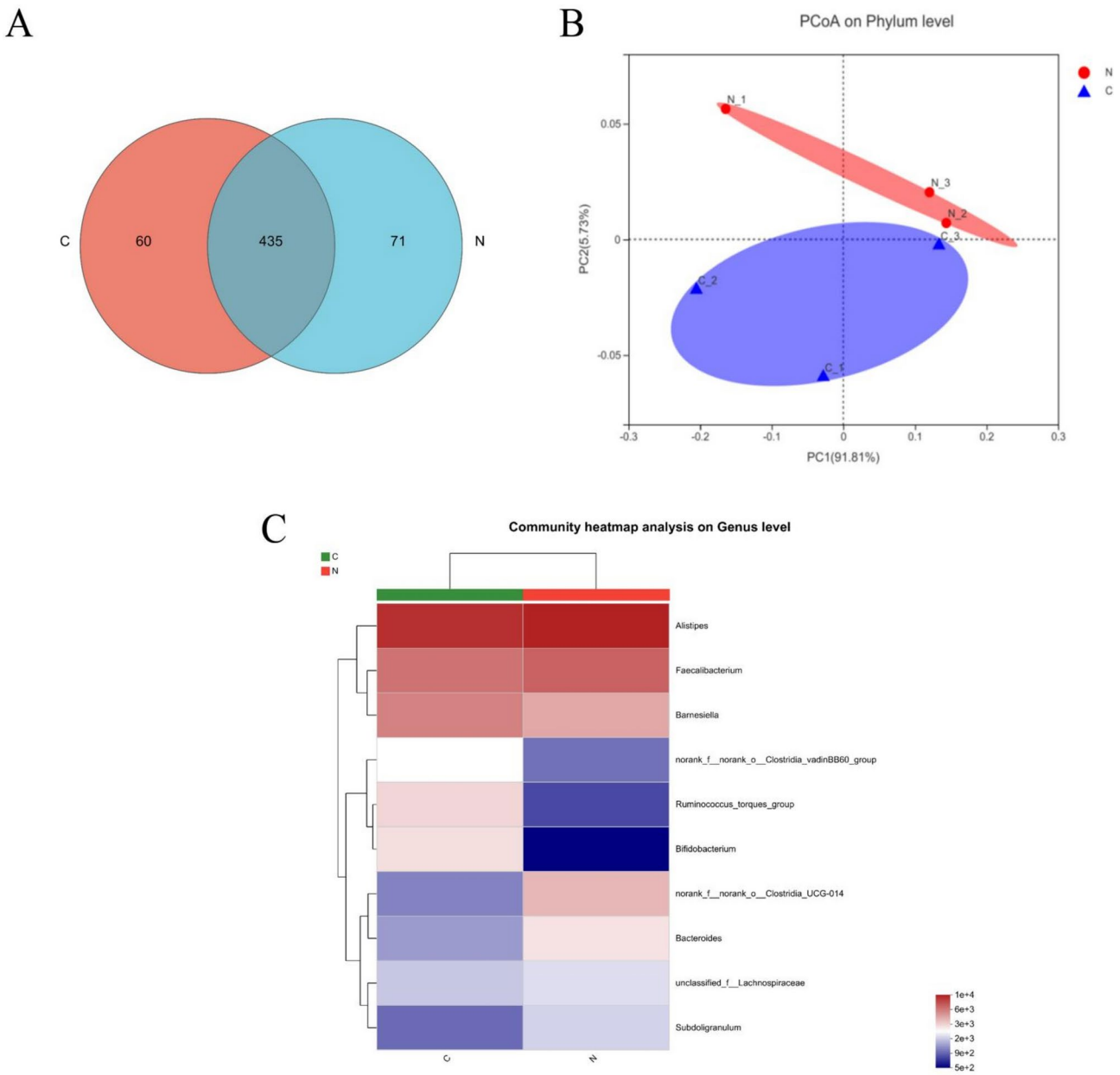

Fig. 1. (**A**) Venn graph for cecal microbiota of broiler breeder roosters in different groups. (**B**) PCoA analysis chart. (**C**) Community heatmap analysis for cecal microbiota of broiler breeder roosters on Genus level in different groups. C was defined as the cecal microbiota from broiler breeder rooster fed with basal diet. N was defined as the cecal microbiota from broiler breeder rooster fed with basal diet supplemented with 0.08% N-Carbamylglutamate.

good compatibility with existing feed formulations because it is stable under typical feed processing conditions and does not interact negatively with common feed ingredients such as proteins, carbohydrates or minerals. Its solubility in water allows easy integration into liquid or solid feed matrices, while its stability over a wide pH range ensures effectiveness in various digestive environments. However, compatibility testing is recommended to confirm that NCG maintains its bioactivity and does not degrade when exposed to high temperatures during pelleting or extrusion processes. Overall, NCG is a useful additive for improving nitrogen utilization and promoting animal growth in feed formulations. As reported by Hu et al.[18], dietary supplementation of NCG is an effective measure to increase the body weight of broiler chicks. In our previous work in our research group, it was found that weekly weight gain in the 0.08% NCG group was higher than in the basal diet group and a lower mass-to-weight ratio than in the basal diet group. Studies have shown that broilers gain weight at different rates in different weeks of life and that weight gain gradually slows at the growth tipping point[30]. Therefore, we speculate that the effect of NCG on body weight is also closely related to the stages associated with chicken development. However, in our study, NCG supplementation did not exhibit a positive effect on body weight improvement and

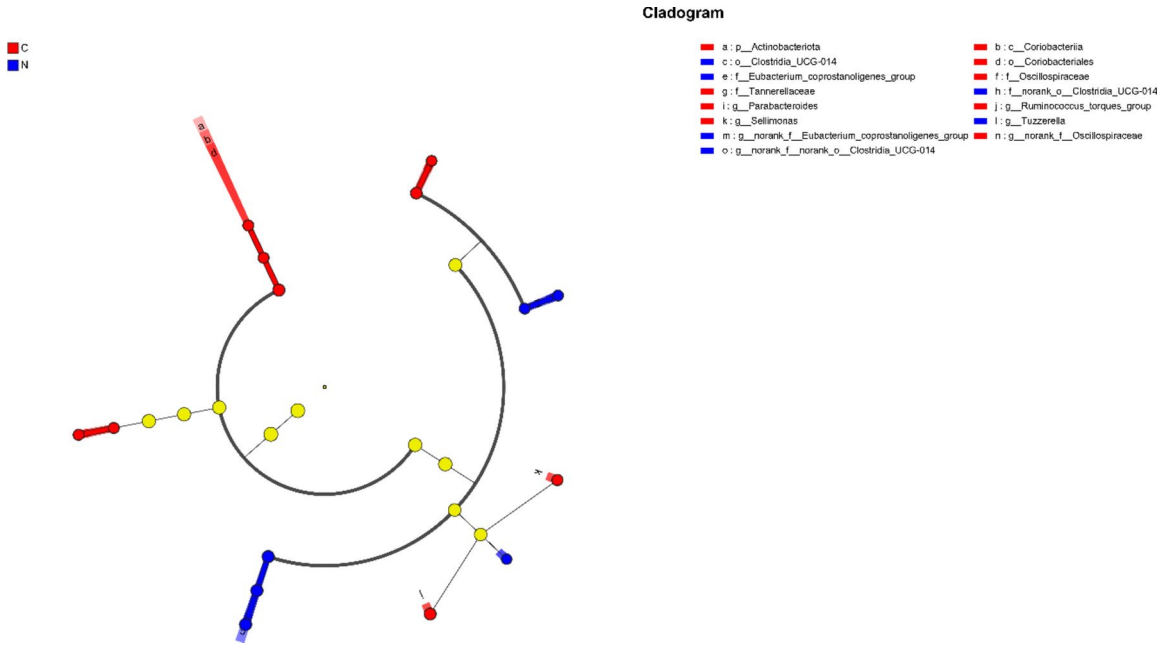

Fig. 2. The cladogram presenting the phylogenetic distribution of the lineages for cecal microbiota of broiler breeder roosters in different groups. The lineages in the values of least discriminant analysis higher than 3.5 are displayed. C was defined as the cecal microbiota from broiler breeder rooster fed with basal diet. N was defined as the cecal microbiota from broiler breeder rooster fed with basal diet supplemented with 0.08% N-Carbamylglutamate. The different color nodes represent microbial groups that are significantly enriched in the corresponding groups and have a significant influence on the differences between the groups. Pale yellow nodes indicate microbial taxa that do not differ significantly in different groups or do not significantly influence differences between groups. When the number of species with significant differences is ≤ 50, the legend position is displayed in one column, and when the number of species with significant differences is > 50, the legend position is displayed in two columns when the number of species with significant differences is shown on the right.

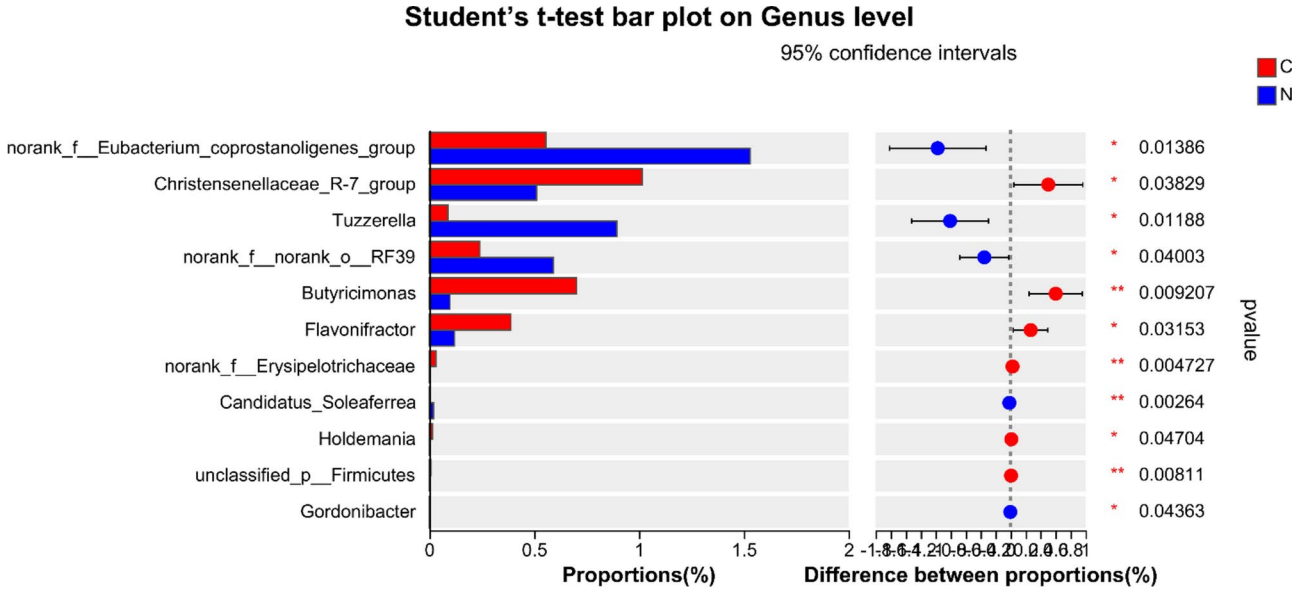

Fig. 3. Statistical difference between groups analyzed by t-test on Genus level. C was defined as the cecal microbiota from broiler breeder rooster fed with basal diet. N was defined as the cecal microbiota from broiler breeder rooster fed with basal diet supplemented with 0.08% N-Carbamylglutamate. Note: The X-axis represents the different groups, the boxes in different colors represent the different groups, and the Y-axis represents the average relative abundance of a species in the different groups.

	Items	Muscularis thickness, μm	Villi epithelium thickness, μm	Butyric acid, μg/mg
Variables	*Tuzzerella*	0.911***	0.751**	0.890***
	Candidatus_Soleaferrea	0.931***	0.744**	0.942***
	Gordonibacter	0.867***	0.767**	0.815**
	Christensenellaceae_R-7_group	− 0.661*	− 0.371	− 0.822**
	Butyricimonas	− 0.771**	− 0.489	− 0.906***
	Flavonifractor	− 0.683*	− 0.431	− 0.867***
	Holdemania	− 0.657*	− 0.370	− 0.794**
Short-chain fatty acids	Butyric acid, μg/mg	0.894***	0.670*	

Table 7. Spearman correlations analysis among cecal microbiota and cecal morphology. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

also no negative effects. Therefore, any changes observed in hindgut morphology, microbiota composition, and fermentation parameters in response to NCG supplementation can be considered a direct consequence of NCG intake, rather than effects induced by changes in body weight. This emphasizes the potential of NCG to influence hindgut health independently of body weight alterations. In addition to intestinal effects, Ma et al.[31] found that adding NCG to the diet can promote ovarian angiogenesis in chickens and therefore follicular development. Ma et al.[23] found that supplementation with NCG had a positive effect on the reproductive characteristics of roosters. NCG supplementation improves the development of reproductive traits in roosters by regulating the expression of genes in testicular tissue, thereby improving the synthesis of reproductive hormones in the body.

The intestinal microbiota-modulating properties of NCG have been widely reported in non-ruminant animals[12,13]. In this study, we also observed a regulatory effect of NCG supplementation on the cecal microbiota. In our study, we identified the *Tuzzerella* genus as a biomarker for the cecal microbiota of broiler breeder roosters fed a diet containing NCG. *Tuzzerella* has been found in the intestines of mice and is considered a beneficial bacterium[32]. Previous research has shown a positive correlation between *Tuzzerella* and body weight in mice[33]. Furthermore, Lo et al.[34] reported that an increased abundance of *Tuzzerella* was beneficial in alleviating cancer.

Differential analysis of bacteria revealed that the cecal microbiota of broiler breeder roosters fed with an NCG-containing diet showed an enrichment of *Tuzzerella*, *Candidatus_Soleaferrea*, and *Gordonibacter*. As mentioned earlier, *Tuzzerella* may serve as a probiotic. Additionally, Zhao et al.[35] and Toney et al.[36] considered *Gordonibacter* to be a probiotic, capable of transforming dietary ellagic acid into anti-inflammatory urolithins[37]. Ability to convert polyphenols from foods into bioavailable metabolites called urolithins[38]. The health effects of urolithin (cardiovascular, anti-inflammatory and anti-cancer properties) have been confirmed by various bioassays, and urolithin can be used in the development of functional foods and nutraceuticals[39]. Tang et al.[40] found that the concentration of LPC was positively correlated with *Gordonibacter*. In vitro and in vivo, LPC has been shown to promote the release of pro-inflammatory cytokines and disrupt the epithelial barrier. McCurry et al.[41] found that *Gordonibacter pamelaeae* and *Eggerthella lenta* converted a class of immune and metabolism-modulating steroids into a class of sex hormones and neurosteroids by converting abundant cholecorticoids into gestagens through 21-dehydroxylation. May affect host physiology, particularly during pregnancy and in women. The *Candidatus Soleaferrea* genus has been shown to exert anti-inflammatory effects through the secretion of metabolites and protection of intestinal homeostasis[42]. Conversely, the abundance of *Christensenellaceae_R-7_group*, *Butyricimonas*, *Flavonifractor*, and *Holdemania* was decreased in the cecum of broiler breeder roosters fed with an NCG-containing diet. Mao et al.[43] found that the *Christensenellaceae_R-7_group* is associated with allergenicity. Toprak et al.[44] reported that *Butyricimonas* is a potential pathogen that can result in bacteremia. *Flavonifractor* has been shown to depress Th2 immune responses in mice[45] and has also been associated with insulin resistance[46]. *Holdemania* is considered a conditional pathogen[47]. Therefore, NCG supplementation is capable of regulating the composition of the cecal microbiota, promoting the enrichment of beneficial bacteria such as *Tuzzerella*, *Candidatus_Soleaferrea*, and *Gordonibacter*, while decreasing the abundance of potentially problematic bacteria including *Christensenellaceae_R-7_group*, *Butyricimonas*, *Flavonifractor*, and *Holdemania*.

The intestinal microbiota plays a significant role in shaping intestinal morphology which is supported by the correlation analysis conducted in this study. Dysbiosis can disrupt the barrier function, allowing harmful substances and pathogens to penetrate the intestinal wall. This can lead to inflammation, tissue damage, and morphological changes in the intestine[48]. The thickness of the intestinal muscularis and the villi epithelium are crucial parameters that reflect the overall structural integrity and functionality of the intestine. Adequate muscularis thickness ensures the smooth movement of food through the intestinal tract, facilitating nutrient uptake[49]. Additionally, a healthy intestinal muscularis provides support to the intestinal lining, preventing the entry of harmful substances into the bloodstream and minimizing the chances of inflammation or infection[50]. The thickness of the villi epithelium reflects the level of cellular activity and secretory function within the intestine. These cells are responsible for the secretion of various enzymes, mucus, and other substances that aid in the digestion and absorption of nutrients[51]. Adequate villi epithelium thickness indicates the presence of active and functional cells that contribute to efficient nutrient processing. Reduced villi epithelium thickness may indicate compromised digestive capacity, impaired nutrient absorption, or underlying intestinal health issues[52]. In this study, we observed an increase in muscularis thickness and villi epithelium thickness in the cecum induced by NCG supplementation. Similarly, Xu et al.[53] reported that dietary supplementation of probiotic improved cecal myometrial and mucosal thickness by regulating cecal microbiota. Therefore, NCG supplementation is also an

effective measure to increase the myometrial and mucosal thickness in cecum of broiler breeder roosters, which is attributed to the variation of cecal microbiota composition.

The mechanism by which variation in cecal microbiota affect cecal morphology involve changes in cecal SCFAs content. Zhang et al.[16] supplemented soy protein concentrate in the diet of broiler chicks and observed an increased cecal butyric acid induced by regulating intestinal microbiota, resulting in a healthy intestinal morphology. SCFAs are produced through the microbial fermentation of dietary fiber and other fermentable carbohydrates in the hindgut of birds[54]. Therefore, the intestinal microbiota has a significant impact on the production of SCFAs in the intestine. Correlation analysis between cecal microbiota and cecal SCFAs contents revealed that the production of butyric acid in cecum is closely related to the variation of cecal microbiota. Similarly, Chen et al.[55] reported a negative correlation between the abundance of *Christensenellaceae_R-7_group* in the intestine and butyric acid levels. Xiao et al.[56] observed that supplementation of microencapsulated cinnamaldehyde increased the abundance of the *Gordonibacter* genus, which was accompanied by an increase in butyric acid content in mouse feces. Bjørkhaug et al.[57] found that patients with chronic alcohol overconsumption had higher levels of the *Holdemania* genus and lower concentration and percentage of butyric acid. Therefore, the reduction of *Christensenellaceae_R-7_group*, *Butyricimonas*, *Flavonifractor*, and *Holdemania*, as well as the increase in *Tuzzerella*, *Candidatus_Soleaferrea*, and *Gordonibacter* genera induced by NCG supplementation, may have certain effects on altering the content of butyric acid in the hindgut. Studies have found that butyric acid is most effective against salmonella and pathogenic bacteria such as E. coli and stimulates the number of beneficial intestinal bacteria. It is the main source of energy for colon cells and promotes the differentiation and maturation of intestinal cells. Butyric acid promotes the absorption of sodium and water from the large intestine and has a nutritional effect on intestinal cells[58]. Sodium can promote the digestive and absorption function of the gastrointestinal tract of animals, improve the digestibility and utilization of feed, and thus promote healthy animal growth. butyric acid or butyrate sodium can enhance gut innate immune function through G-protein-mediated signaling pathways while mitigating the overactive inflammatory responses by inhibiting histone deacetylase[59]. Overall, butyric acid should be considered as an alternative to antibiotic growth promoters as it reduces pathogenic bacteria and their toxins, improves intestinal health and therefore improves the digestibility of nutrients, thereby improving the growth performance and immunity of bird[60].

Liao et al.[61] found that a high content of butyric acid in intestine is beneficial to optimal intestinal morphology. Furthermore, butyric acid possesses anti-inflammatory properties and can help regulate immune responses in the intestine, reducing inflammation and promoting a balanced immune system[62]. Butyric acid also contributes to the maintenance of a favorable microbial population in the gut, supporting the growth of beneficial bacteria and inhibiting the growth of potentially harmful pathogens[63], which is essential for nutrient digestion, absorption, and overall gut health[64]. Deng et al.[65] also noted that dietary supplementation of xylo-oligosaccharide is able to improve intestinal morphology by increasing the contents of butyric acid in cecum. Therefore, the improvement in cecal morphology can be attributed to the increase in cecal butyric acid content, which is supported by the correlation analysis conducted in this study.

Conclusion

The findings of this study revealed that NCG supplementation led to an increase in the thickness of the cecal muscularis and the villi epithelium. These parameters are crucial indicators of the overall health and functionality of the cecum, ensuring smooth food movement, efficient nutrient uptake, and protection against harmful substances and inflammation.

Furthermore, NCG supplementation influenced the composition of the cecal microbiota. It resulted in the enrichment of beneficial bacteria such as *Tuzzerella*, *Candidatus_Soleaferrea*, and *Gordonibacter*, while reducing the abundance of potentially problematic bacteria including *Christensenellaceae_R-7_group*, *Butyricimonas*, *Flavonifractor*, and *Holdemania*. This modulation of the microbiota is significant as it plays a vital role in hindgut fermentation and the production of SCFAs. In particular, NCG supplementation increases the content of butyric acid in the cecum, which has a positive effect on the body's immune function and the maintenance of normal intestinal health.

Overall, the findings suggest that dietary supplementation of NCG is an effective approach to enhance the structural integrity, functionality, and microbial composition of the cecum in broiler breeder roosters. These positive effects contribute to improved gut health, nutrient utilization, and overall well-being of the birds.

Data availability

The datasets generated and analysed during the current study are available in the Figshare (https://figshare.com) repository, https://doi.org/10.6084/m9.figshare.28070726.

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References

1. Barko, P. C., McMichael, M. A., Swanson, K. S. & Williams, D. A. The gastrointestinal microbiome: a review. *J. Vet. Intern. Med.* **32**, 9–25. https://doi.org/10.1111/jvim.14875 (2018).
2. Ali, A. et al. Cinnamon: A natural feed additive for poultry health and production—A review. *Animals.* **11**(7), 2026. https://doi.org/10.3390/ani11072026 (2021).
3. Dang, D. X. et al. Feeding broiler chicks with bacillus subtilis, *Clostridium butyricum*, and *Enterococcus faecalis* mixture improves growth performance and regulates cecal microbiota. *Probiotics Antimicrob. Proteins.* **1**, 1–12. https://doi.org/10.1007/s12602-022-10029-3 (2022).

4. Vakili, R. & Ebrahimnezhad, Y. Impact of dietary supplementation of unsaturated and saturated fatty acids on bone strength, fatty acids profile of thigh muscle and immune responses in broiler chickens under heat stress. *Vet. Med. Sci.* **9**(1), 252–262. https://doi.org/10.1002/vms3.1035 (2023).
5. Niknia, A. D., Vakili, R. & Tahmasbi, A. M. Role of zinc-methionine chelate on bone health and eggshell quality in late–phase laying hens. *All Life* https://doi.org/10.1080/26895293.2022.2162609 (2023).
6. Moharreri, M., Vakili, R., Oskoueian, E. & Rajabzadeh, G. Phytobiotic role of essential oil-loaded microcapsules in improving the health parameters in *Clostridium perfringens*-infected broiler chickens. *Ital. J. Anim. Sci.* **20**, 2075–2085. https://doi.org/10.1080/1828051X.2021.1993093 (2021).
7. Moharreri, M., Vakili, R., Oskoueian, E. & Rajabzadeh, G. Evaluation of microencapsulated essential oils in broilers challenged with *Salmonella enteritidis*: a focus on the body's antioxidant status, gut microbiology, and morphology. *Arch Razi Inst.* **77**(2), 629–639. https://doi.org/10.22092/ARI.2021.354334.1634 (2022).
8. Zhang, H. et al. Dietary L-Arginine or N-carbamylglutamate alleviates colonic barrier injury, oxidative stress, and inflammation by modulation of intestinal microbiota in intrauterine growth-retarded suckling lambs. *Antioxidants.* **11**(11), 2251. https://doi.org/10.3390/antiox11112251 (2022).
9. Li, W. et al. Dietary supplementation of N-carbamylglutamate promotes growth performance by modulating the homeostasis of gut microbiota in tilapia (Oreochromis niloticus). *Aquac. Rep.* **20**, 100750. https://doi.org/10.1016/j.aqrep.2021.100750 (2021).
10. Suresh, G. et al. Alternatives to antibiotics in poultry feed: molecular perspectives. *Crit. Rev. Microbiol.* **44**(3), 318–335. https://doi.org/10.1080/1040841X.2017.1373062 (2018).
11. Long, S. et al. Enzyme-treated soybean meal enhanced performance via improving immune response, intestinal morphology and barrier function of nursery pigs in antibiotic free diets. *Animals* **11**(9), 2600. https://doi.org/10.3390/ani11092600 (2021).
12. Feng, T. et al. N-Carbamylglutamate improves reproductive performance and alters fecal microbiota and serum metabolites of primiparous sows during gestation after fixed-time artificial insemination. *Biology.* **11**, 1432. https://doi.org/10.3390/biology11101432 (2022).
13. Zeng, X. et al. Oral administration of N-carbamylglutamate might improve growth performance and intestinal function of suckling piglets. *Livestock Sci.* **181**, 242–248. https://doi.org/10.1016/J.LIVSCI.2015.09.004 (2015).
14. Zhu, J. et al. Effects of dietary N-carbamylglutamate on rumen fermentation parameters, and bacterial community diversity of Holstein dairy cows in Tibet. *Front. Microbiol.* **14**, 1101620. https://doi.org/10.3389/fmicb.2023.1101620 (2023).
15. Ma, W., Yuan, M., Chang, S. & Wang, C. N-carbamylglutamate supplementation regulates hindgut microbiota composition and short-chain fatty acid contents in Charollais and Small Tail Han crossbred sheep. *Front. Vet. Sci.* **10**, 1230190. https://doi.org/10.3389/fvets.2023.1230190 (2023).
16. Zhang, Q., Zhang, S., Wu, S., Madsen, M. H. & Shi, S. Supplementing the early diet of broilers with soy protein concentrate can improve intestinal development and enhance short-chain fatty acid-producing microbes and short-chain fatty acids, especially butyric acid. *J. Anim. Sci. Biotechnol.* **13**, 97. https://doi.org/10.1186/s40104-022-00749-5 (2022).
17. Zhang, F. D. et al. Effect of amniotic injection of N-carbamylglutamate on meat quality of broilers. *Animals* **10**(4), 576. https://doi.org/10.3390/ani10040576 (2020).
18. Hu, Y. et al. Effects of dietary N-carbamylglutamate supplementation on growth performance, tissue development and blood parameters of yellow-feather broilers. *Poult. Sci.* **98**, 2241–2249. https://doi.org/10.3382/ps/pey591 (2019).
19. Clench, M. H. The avian cecum: update and motility review. *J. Exp. Zool.* **283**, 441–447 (1999).
20. Józefiak, D., Rutkowski, A. & Martin, S. A. Carbohydrate fermentation in the avian ceca: a review. *Anim. Feed Sci. Technol.* **113**, 1–15. https://doi.org/10.1016/J.ANIFEEDSCI.2003.09.007 (2004).
21. Den Besten, G. et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J. Lipid Res.* **54**, 2325–2340. https://doi.org/10.1194/jlr.R036012 (2013).
22. Seifi, K. et al. Short chain fatty acids may improve hepatic mitochondrial energy efficiency in heat stressed-broilers. *J. Therm. Biol.* **89**, 102520. https://doi.org/10.1016/j.jtherbio.2020.102520 (2020).
23. Ma, W., Dang, X., Zhang, J., Wang, C. & Li, D. Effects of dietary supplementation of N-carbamylglutamate on the haematology parameters, secondary sexual characteristics and testicular gene expression in roosters. *J. Anim. Physiol. Anim. Nutr.* **107**(2), 621–630. https://doi.org/10.1111/jpn.13714 (2023).
24. NRC. *Nutrient Requirements of Poultry*. https://doi.org/10.17226/2114 (National Academic Press, 1994).
25. Felício, A. M. et al. Heritability and genetic correlation estimates for performance, meat quality and quantitative skeletal muscle fiber traits in broiler. *Livestock Sci.* **157**, 81–87. https://doi.org/10.1016/J.LIVSCI.2013.08.005 (2013).
26. Ghassemi Nejad, J. et al. Worldwide research trends for chelates in animal science: a bibliometric analysis. *Animals* **13**(14), 2374. https://doi.org/10.3390/ani13142374 (2023).
27. Yang, J. et al. Effects of dietary N-carbamylglutamate on growth performance, apparent digestibility, nitrogen metabolism and plasma metabolites of fattening holstein bulls. *Animals* **11**(1), 126. https://doi.org/10.3390/ani11010126 (2021).
28. Elahi, U. et al. The response of broiler chickens to dietary soybean meal reduction with glycine and cysteine inclusion at marginal sulfur amino acids (SAA) deficiency. *Animals* **10**(9), 1686. https://doi.org/10.3390/ani10091686 (2020).
29. Wang, C. et al. Effectiveness and safety evaluation of graded levels of N-carbamylglutamate in growing-finishing pigs. *Anim. Nutr.* **10**, 412–418. https://doi.org/10.1016/j.aninu.2022.04.012 (2022).
30. Şengül, T., Çelik, Ş, Şengül, A. Y., İnci, H. & Şengül, Ö. Investigation of growth curves with different nonlinear models and MARS algorithm in broiler chickens. *PLoS ONE.* **19**(11), e0307037. https://doi.org/10.1371/journal.pone.0307037 (2024).
31. Ma, Y. et al. Effect of dietary N-carbamylglutamate on development of ovarian follicles via enhanced angiogenesis in the chicken. *Poult. Sci.* **99**(1), 578–589. https://doi.org/10.3382/ps/pez545 (2020).
32. Cardilli, A. et al. Impact of high salt-intake on a natural gut ecosystem in wildling mice. *Nutrients.* **15**(7), 1565. https://doi.org/10.3390/nu15071565 (2023).
33. Qiao, Y. et al. Apigenin alleviates obesity-associated metabolic syndrome by regulating the composition of the gut microbiome. *Front. Microbiol.* **12**, 805827. https://doi.org/10.3389/fmicb.2021.805827 (2022).
34. Lo, E. K. K. et al. Mechanistic insights into zearalenone-accelerated colorectal cancer in mice using integrative multi-omics approaches. *Comput. Struct. Biotechnol. J.* **21**, 1785–1796. https://doi.org/10.1016/j.csbj.2023.02.048 (2023).
35. Zhao, D., Yang, Y., Lee, P. K. & Wong, H. C. Effect of oral supplementation of *Gordonibacter urolithinfaciens* on gut microbiota and bioavailability of ellagic acid and urolithins in mice. *Curr. Dev. Nutr.* **6**, 6009344. https://doi.org/10.1093/cdn/nzac053.085 (2022).
36. Toney, A. et al. Introducing *Gordonibacter urolithinfaciens* into gut ecosystems to study its role in mediating the metabolic benefits of dietary polyphenols. *Curr. Dev. Nutr.* **4**, 4141594. https://doi.org/10.1093/cdn/nzaa062_051 (2020).
37. Romo-Vaquero, M. et al. Interindividual variability in the human metabolism of ellagic acid: Contribution of Gordonibacter to urolithin production. *J. Funct. Foods.* **17**, 785–791. https://doi.org/10.1016/J.JFF.2015.06.040 (2015).
38. Ngom, I. I. et al. Taxono-genomics and description of *Gordonibacter massiliensis* sp. Nov., a new bacterium isolated from stool of healthy patient. *New Microbes New Infect.* **33**, 100624. https://doi.org/10.1016/j.nmni.2019.100624 (2019).
39. Selma, M. V., Beltrán, D., García-Villalba, R., Espín, J. C. & Tomás-Barberán, F. A. Description of urolithin production capacity from ellagic acid of two human intestinal Gordonibacter species. *Food Funct.* **5**(8), 1779–1784. https://doi.org/10.1039/c4fo00092g (2014).
40. Tang, X. et al. Gut microbiota-mediated lysophosphatidylcholine generation promotes colitis in intestinal epithelium-specific Fut2 deficiency. *J. Biomed. Sci.* **28**(1), 20. https://doi.org/10.1186/s12929-021-00711-z (2021).

41. McCurry, M. D. et al. Gut bacteria convert glucocorticoids into progestins in the presence of hydrogen gas. *Cell.* **187**(12), 2952-2968.e13. https://doi.org/10.1016/j.cell.2024.05.005 (2014).
42. Cai, J. et al. Alteration of intestinal microbiota in 3-deoxyglucosone-induced prediabetic rats. *BioMed Res. Int.* **2020**, 8406846. https://doi.org/10.1155/2020/8406846 (2020).
43. Mao, J. H. et al. Molecular structure, IgE binding capacity and gut microbiota of ovalbumin conjugated to fructose and galactose: A comparative study. *Int. J. Biol. Macromol.* **234**, 123640. https://doi.org/10.1016/j.ijbiomac.2023.123640 (2023).
44. Toprak, N. U., Bozan, T. U. R. G. U. T., Birkan, Y. A. ŞA. R., Isbir, S. & Soyletir, G. Butyricimonas virosa: the first clinical case of bacteraemia. *New Microbes New Infect.* **4**, 7. https://doi.org/10.1016/j.nmni.2014.12.004 (2015).
45. Ogita, T. et al. Oral administration of *Flavonifractor plautii* strongly suppresses Th2 immune responses in mice. *Front. Immunol.* **11**, 379. https://doi.org/10.3389/fimmu.2020.00379 (2020).
46. Angeles, L. Gut Bacteria May Play a Role in Diabetes. https://www.cedars-sinai.org/newsroom/gut-bacteria-may-play-a-role-in-diabetes/ (2023).
47. Jang, J. H. et al. Acupuncture inhibits neuroinflammation and gut microbial dysbiosis in a mouse model of Parkinson's disease. *Brain, Behav. Immunity.* **89**, 641–655. https://doi.org/10.1016/j.bbi.2020.08.015 (2020).
48. Grosheva, I. et al. High-throughput screen identifies host and microbiota regulators of intestinal barrier function. *Gastroenterology.* **159**, 1807–1823. https://doi.org/10.1053/j.gastro.2020.07.003 (2020).
49. Treuting, P. M., Arends, M. J. & Dintzis, S. M. Upper gastrointestinal tract. In *Comparative Anatomy and Histology*, 191–211 https://doi.org/10.1016/B978-0-12-802900-8.00011-7 (Academic Press, 2018).
50. Alexander, J. S., Ganta, V. C., Jordan, P. A. & Witte, M. H. Gastrointestinal lymphatics in health and disease. *Pathophysiology.* **17**, 315–335. https://doi.org/10.1016/j.pathophys.2009.09.003 (2010).
51. Van Dijk, J. E., Huisman, J. & Koninkx, J. F. J. G. Structural and functional aspects of a healthy gastrointestinal tract. *Nutr. Health Gastrointest. Tract.* **1**, 71–96 (2002).
52. McWhorter, T. J., Caviedes-Vidal, E. & Karasov, W. H. The integration of digestion and osmoregulation in the avian gut. *Biol. Rev.* **84**, 533–565. https://doi.org/10.1111/j.1469-185X.2009.00086.x (2009).
53. Xu, L. et al. Dietary supplementation with *Clostridium butyricum* improves growth performance of broilers by regulating intestinal microbiota and mucosal epithelial cells. *Anim. Nutr.* **7**, 1105–1114. https://doi.org/10.1016/j.aninu.2021.01.009 (2021).
54. Szabó, R. T., Kovács-Weber, M., Zimborán, Á., Kovács, L. & Erdélyi, M. Effects of short-and medium-chain fatty acids on production, meat quality, and microbial attributes—a review. *Molecules.* **28**, 4956. https://doi.org/10.3390/molecules28134956 (2023).
55. Chen, P. et al. Structure elucidation and in vitro rat intestinal fermentation properties of a novel sulfated glucogalactan from *Porphyra haitanensis*. *Food Sci. Hum. Wellness.* **12**, 596–606. https://doi.org/10.1016/j.fshw.2022.07.062 (2023).
56. Xiao, Y. et al. Cinnamaldehyde microcapsules enhance bioavailability and regulate intestinal flora in mice. *Food Chem. X.* **15**, 100441. https://doi.org/10.1016/j.fochx.2022.100441 (2022).
57. Bjørkhaug, S. T. et al. Characterization of gut microbiota composition and functions in patients with chronic alcohol overconsumption. *Gut Microbes.* **10**(6), 663–675. https://doi.org/10.1080/19490976.2019.1580097 (2019).
58. Manrique Vergara, D. & González Sánchez, M. E. Ácidos grasos de cadena corta (ácido butírico) y patologías intestinales [Short chain fatty acids (butyric acid) and intestinal diseases]. *Nutr. Hosp.* **34**(4), 58–61. https://doi.org/10.20960/nh.1573 (2017).
59. Zhang, M. et al. Mechanistic basis and preliminary practice of butyric acid and butyrate sodium to mitigate gut inflammatory diseases: a comprehensive review. *Nutr. Res.* **95**, 1–18. https://doi.org/10.1016/j.nutres.2021.08.007 (2021).
60. El-Saadony, M. T. et al. Applications of butyric acid in poultry production: the dynamics of gut health, performance, nutrient utilization, egg quality, and osteoporosis—CORRIGENDUM. *Anim. Health Res. Rev.* **24**(1), 40. https://doi.org/10.1017/S1466252323000038 (2023).
61. Liao, X. et al. The relationship among gut microbiota, short-chain fatty acids, and intestinal morphology of growing and healthy broilers. *Poult. Sci.* **99**, 5883–5895. https://doi.org/10.1016/j.psj.2020.08.033 (2020).
62. Gulcan, H. O., Kupeli, E., Unlu, S., Yesilada, E. & Sahin, M. F. 4-(5-Chloro-2 (3H)-benzoxazolon-3-yl) butanoic acid derivatives: synthesis, antinociceptive and anti-inflammatory properties. *Archiv der Pharmazie Int. J. Pharm. Med. Chem.* **336**, 477–482. https://doi.org/10.1002/ardp.200300722 (2003).
63. Pickard, J. M., Zeng, M. Y., Caruso, R. & Núñez, G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol. Rev.* **279**, 70–89. https://doi.org/10.1111/imr.12567 (2017).
64. Melaku, M. et al. Butyric and citric acids and their salts in poultry nutrition: Effects on gut health and intestinal microbiota. *Int. J. Mol. Sci.* **22**, 10392. https://doi.org/10.3390/ijms221910392 (2021).
65. Deng, F. et al. Combined effects of sodium butyrate and xylo-oligosaccharide on growth performance, anti-inflammatory and antioxidant capacity, intestinal morphology and microbiota of broilers at early stage. *Poult. Sci.* **102**, 102585. https://doi.org/10.1016/j.psj.2023.102585 (2023).

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Author contributions

NL, ZZ: writing—original draft, investigation, writing—review and editing. NL, ZZ, JZ: formal analysis, investigation. MW, CW: conceptualization, methodology, supervision, writing—review and editing.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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