Dietary metabolizable energy and crude protein levels affect pectoral muscle composition and gut microbiota in native growing chickens

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ABSTRACT The experiment aimed to study effects of dietary metabolizable energy (**ME**) and crude protein (**CP**) levels alone and in interaction on performance, pectoral muscle composition and gut microbiota in native growing chickens. A total of 648 10-wks-old *Beijing-You Chicken* (**BYC**) female chickens were randomly allocated to 9 groups with 6 replicates per group and 12 chickens per replicate, and the chickens were fed with a 3×3 factorial diets (3 levels of dietary ME: 11.31 MJ/kg, 11.51 MJ/kg, 11.71 MJ/kg; and 3 levels of dietary CP: 14%, 15%, 16%). The results showed that dietary ME and CP levels didn't affect average feed intake (**AFI**), body weight gain, feed gain ratio ($P > 0.05$), but ME level significantly affected the AFI ($P < 0.05$); mortality rate of 11.31 MJ/kg group was the highest ($P < 0.05$). Dietary ME, CP levels, and the interaction significantly affected pectoral CP and crude fat (**CF**) content of the growing chickens ($P < 0.01$). Dietary CP level had opposite effects on pectoral CP and CF content ($P < 0.01$). The 16% CP increased the pectoral CF content, which may have a negative impact on meat flavor. Dietary ME level affected 11 types of pectoral free amino acids (**FAA**) contents, including aspartic acid, L-threonine ($P < 0.05$), also amino acid classification, for example, total amino acid (**TAA**) and essential amino acid (**EAA**) content ($P < 0.05$). The 11.51 MJ/kg group had the highest TAA, EAA, delicious amino acid (**DAA**) content and EAA percentage ($P < 0.05$), while 11.31 MJ/kg group had the lowest bitter amino acid (**BAA**) content and BAA percentage and the highest fresh and sweet amino acid (**FSAA**) percentage ($P < 0.05$). Dietary CP level significantly affected glutamine and tyrosine content ($P < 0.05$). The interaction of dietary ME and CP level affected C20:3n6 content, saturated fatty acid (**SFA**), and unsaturated fatty acid (**UFA**) percentage ($P < 0.05$). The CP level significantly affected SFA percentage ($P < 0.05$). The 16% CP level increased the diversity of gut microbiota, but at the same time increased the relative abundance of *Proteobacteria* ($P < 0.05$), which is a sign of microbiota disorder. The increase of dietary ME level resulted in a gradual decrease in the diversity and relative abundance of gut microbiota. In conclusion, the present study suggested that the medium dietary ME (11.51 MJ/kg) and low CP (14−15%) levels can be helpful for enhancing pectoral muscle composition, increase meat quality such as flavor and nutritional value, and benefit for gut microbiota in native growing chickens.

Key words: crude protein, energy, meat quality, growing chicken, gut microbiota

INTRODUCTION

The gut microbiota plays an important role in the overall health and function of the host (Stanley et al., 2014; Sánchez et al., 2008). The gut microbiota of poultry is influenced by diet, age, antibiotic use, and infection by pathogenic organisms (Zimmermann et al., 2019; Pont et al., 2020). Diet is one of the most critical factors in shaping the gut microbiota (De Filippo et al.,2010; Carmody et al., 2015), and the most critical aspects are dietary metabolizable energy (**ME**) and crude protein (**CP**) levels. Low CP diets helped control faecal microbiota characteristics without affecting production of AA broilers (Laudadio et al., 2012). Variation in dietary ME levels had a significant effect on bacterial composition of animals (Daniel et al., 2014). A study on Pekin ducks showed that ME levels affected the composition and diversity of the gut microbiota (Liu et al., 2019).

Appropriate levels of ME and CP are necessary to ensure nutritional level of the diet and to promote healthy growth of poultry. When the ME and CP ratio is appropriate, poultry grow faster, have higher feed conversion and production (Musigwa et al., 2021). Previous

studies on the interaction of ME and CP levels varied depending on different environment, production system, breeds, etc.

In recent years, with the improvement of people's living standards, consumers have placed higher demands on meat quality. Dietary ME and CP levels are two most important factors affecting meat quality. Low dietary ME reduced abdominal fat of broilers (Fan et al., 2008; Abdel-Hafeez et al., 2016) and improved tenderness. Increasing CP levels significantly reduced content of abdominal fat, carcass, and breast and leg muscles, and increased pectoral CP content in broiler (Marcu et al., 2013), affecting the quality indicators (Jlali et al., 2012). Excess dietary ME can lead to increased fat deposition and reduced carcass quality, while low CP diets can reduce the energy required to break down dietary protein and excrete excess amino acids, while improving nitrogen utilization and reducing nitrogen emissions (Girish et al., 2013). Oyedeji et al. (2005) suggested that 13.38 MJ/Kg is optimal for broiler chickens; while energy level is 12.96 MJ/Kg for broilers at 0 to 4 wk of age and 13.18 MJ/Kg at 4 to 8 wk of age (Nahashon et al., 2005).

Dietary ME and CP also affect the meat composition, especially protein and fat contents and free amino acids (**FAA**), fatty acids (**FA**) composition. The content and composition not only determine its nutritional value but also affect meat quality indicators such as tenderness and flavor. Dietary ME and CP levels had significant effects on pectoral Glu content and FA content in 63-day-old yellow broilers (Jiang et al., 2013). While higher ME levels improved pectoral quality of Cobb broiler chicks and had a positive effect on pectoral FAA (Akbari et al., 2017).

Beijing You Chicken (**BYC**), a native chicken for meat and egg production in Beijing district, is generally marketed at 110 to 120 days of age with an average weight of 1.4 to 1.6 kg. The FAA, intramuscular fat, and unsaturated fatty acid (**UFA**) content of BYC meat were significantly higher than that of other chickens, with the FAA content reaching 6.4 g/kg, the intramuscular fat content reaching 10.9 g/kg and the UFA content reaching 4.9 g/kg, having delicious meat and a rich flavor (Liu, 2020). However, whether dietary ME and CP levels affect the pectoral muscle composition and gut microbiota remains unclear for the native bird. Our group compared 6 levels of dietary CP (16.0%, 15.8%, 15.6%, 15.4%, 15.2%, and 15.0%) on performance and found that 15.8% CP can meet the requirement of BYC laying hens aged from 32 to 43 wks (Geng et al., 2014). This study was conducted to compare the effects of dietary ME and CP levels alone and in interaction on performance, pectoral muscle composition and gut microbiota in growing chickens, in order to provide a reference for healthy breeding of the native chickens.

MATERIALS AND METHODS

Experimental Design and Chickens

The experiment was conducted at a BYC rearing farm, Shunyi district, Beijing. A total of 648 10-wks-old BYC female growing chickens were randomly allocated to 9 groups with 6 replicates per group and 12 chickens per replicate (one replicate three cages, 4 birds a cage) and the average weight was 581.46 g. A 3 × 3 factorial experiment was arranged, and the chickens were fed with 3 levels of dietary ME (11.31 MJ/kg, 11.51 MJ/kg, 11.71 MJ/kg) and 3 levels of dietary CP (14%, 15%, 16%). The settings of ME and CP levels were adjusted up and down according to the recommended levels in "Technical regulation of Beijing You Chicken Feed and Management" (DB11/T 1378, 2016). Each group was randomly fed with one of the 9 diets. The composition and nutrient levels of the diets were seen in Table 1.

Feeding and Management

The chickens were reared in 3-story A-type cages, and the replicate cages were evenly distributed in the house. The chickens were fed ad libitum, and immunized according to normal immunization procedures. The management were manipulated according to "Technical regulation of Beijing You Chicken Feed and Management" (DB11/T 1378, 2016). The adjustment experimental period was 3 days and formal experimental period was from 10 to 16 wks of age.

Measurement Indicators and Methods

Feed intake and body weight of each replicate cage were recorded and the average feed intake (**AFI**), body weight gain (**BWG**), feed gain ratio (**F/G**) and mortality rate were calculated at 10 to 16 wks of age. Once a chicken was found dead, the weight of the dead chicken, the time of death, and the remaining feed for the cage will be recorded in time, and the F/G were corrected from mortality.

At the end of 16 wks, 6 chickens from each group were randomly chosen and euthanized by cervical dislocation, then pectoral muscle composition were measured. The pectoral muscles were freeze-dried by using freeze dryer (LD53, Millrock Technology, Kingston, NY) and pectoral CP, CF, FAA, FA were measured. The cecum was separated and the digesta was gently extruded, stored in liquid nitrogen, and then transferred to −80°C for later analysis. 0.25 g freeze dried sample was used to measure pectoral CP by using Kjeldahl nitrogen tester (K1160, Hanon, China); 5 g freeze dried sample was used to measure pectoral CF by Soxhlet extraction method by using a CF tester (SER148, VELP, Italy); 1 g freeze dried samples with fat extracted were used for FAA analysis by using an amino acid analyzer (L8900, HITACHI, Japan); 5 g freeze dried sample was used to measure FA content by using high performance gas chromatograph (HP 6890, Agilent, Palo Alto, CA).

Cecal contents from 4 chickens in each group were used for measurement of microbiota. Microbial community genomic DNA was extracted from cecal content using E.Z.N.A. soil DNA Kit (Omega Bio-tek, Norcross, GA) according to manufacturer's instructions. The

Table 1. Composition and nutrient levels of the experimental diets.

ME, MJ/kg	11.31	11.31	11.31	11.51	11.51	11.51	11.71	11.71	11.71
CP, %	14	15	16	14	15	16	14	15	16
Corn	65.3	63.5	62.8	68.8	66	62.6	68	68	66
Wheat bran	13.5	12	9.5	9.5	9	9	9	6	5
Soybean meal	15.7	19	22.5	16.5	19.5	22.5	17	20.5	23.5
Soybean oil	0.5	0.5	0.2	0.2	0.5	0.9	1	0.5	0.5
Premix[1]	5	5	5	5	5	5	5	5	5
Total	100	100	100	100	100	100	100	100	100
Nutritional levels[2]									
ME, MJ/kg	11.31	11.31	11.32	11.51	11.52	11.51	11.72	11.71	11.70
CP, %	14.01	15.04	16.03	14.00	14.98	15.99	14.04	15.04	16.03
Lysine, %	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Methione + Cystine, %	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Calcium, %	0.73	0.72	0.75	0.74	0.72	0.73	0.74	0.76	0.72
Total phosphorus, %	0.63	0.61	0.62	0.64	0.60	0.63	0.64	0.62	0.60
Available phosphorus, %	0.33	0.32	0.34	0.32	0.33	0.31	0.35	0.31	0.31

[1]Premix provided per kilogram of diet: Vitamin A, 6000 IU; Vitamin D, 2800 IU; Vitamin E, 20 IU; Vitamin K, 2.4 mg; Vitamin B12, 0.027 mg; Thiamine, 2.0 mg; Riboflavin, 5.2 mg; D-Calcium pantothenate, 11 mg; Niacin, 30 mg; Pyridoxine, 3.6 mg; Biotin, 0.2 mg; Folic acid, 1.2 mg; Choline, 800 mg; Manganese, 90 mg; Iodine, 1.8 mg; Ferrous, 100 mg; Copper, 8 mg; Zinc, 80 mg; Selenium, 0.30 mg. Any deficiencies in the methionine and lysine content of the diets were made up in the 5% premix.

[2]CP, calcium, and available phosphorus are measured values, the rest of the nutrient levels are calculated from the data provided by Feed Database in China (2013).

DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with Nano-Drop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, NC). The hypervariable region V3 to V4 of the bacterial 16S rRNA gene were amplified with primer pairs 343F-5′-TACGGRAGGCAGCAG-3′ and the back-end primer: 798R-5′- AGGGTATC-TAATCCT-3′ by an ABI Gene Amp 9700 PCR thermocycler (ABI, CA). PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using Axy Prep DNA Gel Extraction Kit (Axy gen Biosciences, Union City, CA) according to manufacturer's instructions and quantified using Quantus Fluorometer (Promega, Madison, WI). After PCR reactions, amplification products were recovered, library construction was completed, library fragment ranges and concentrations were checked. Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The DNA sequences are publicly available in the National Center for Biotechnology Information (**NCBI**) Sequence Read Archive and are accessible under BioProject accession number PRJNA841792.

Statistical Analysis

The main effects of ME and CP levels were analyzed using the general linear model (**GLM**) in SPSS 25.0 software for Windows (SPSS Inc. Chicago, IL). Duncan's Test was used for multiple comparisons. The percentage was arcsine transformed before the normality test, with $P < 0.01$ as very statistically significant, $P < 0.05$ as statistically significant, and $0.05 < P < 0.10$ as having a significant trend.

RESULTS

Performance

Table 2 showed that the interaction of dietary ME and CP levels had no significant effects on AFI, BWG, F/G ($P > 0.05$), but ME level alone significantly affected AFI ($P < 0.05$); mortality rate of 11.51 MJ/kg and 11.71 MJ/kg group were significantly lower than that of 11.31 MJ/kg group ($P < 0.05$).

Pectoral CP, CF and Moisture Content

Table 3 showed that dietary ME and CP levels alone and in interaction affected pectoral CP and CF content ($P < 0.01$). The pectoral CP content of 11.51 MJ/kg ME group (94.80%) was lower than that of the other 2 groups ($P < 0.05$), the pectoral CF content of 11.31 MJ/kg ME group (3.31%) was lower than that of the other two groups ($P < 0.01$), the pectoral CF content of 11.51 MJ/kg group (4.79%) was the highest ($P < 0.01$).

The pectoral CP content of 15% CP group (95.94%) was significantly higher than that of the other 2 groups ($P < 0.01$), while the pectoral CF content (3.39%) was the lowest in 15% CP group ($P < 0.01$).

Pectoral FAA Content and Proportions

Table 4a showed that the interaction of dietary ME and CP levels affected most of free amino acid content including aspartic acid (**Asp**), L-threonine (**Thr**), serine (**Ser**), glutamine (**Glu**), alanine (**Ala**), methionine (**Met**), leucine (**Leu**), tyrosine (**Tyr**), lysine (**Lys**), arginine (**Arg**), total amino acid (**TAA**), essential amino acid (**EAA**), fresh and sweet amino acid (**FSAA**), bitter amino acid (**BAA**), delicious amino acid (**DAA**) ($P < 0.05$), and there was a significant

Table 2. Effect of dietary ME and CP levels on performance of 10−16 wks of age.

ME, MJ/kg	CP, %	AFI, g	BWG, g	F/G	Mortality, %
11.31	14	3,621.38	535.01	6.78	6.94
11.31	15	3,591.03	591.22	6.07	4.17
11.31	16	3,698.43	627.93	5.88	4.17
11.51	14	3,550.00	558.75	6.35	0
11.51	15	3,594.17	636.39	5.64	0
11.51	16	3,552.39	546.84	6.49	4.17
11.71	14	3,519.82	517.12	6.81	4.17
11.71	15	3,623.06	559.26	6.48	1.38
11.71	16	3,332.45	512.19	6.50	1.38
SEM		26.96	11.39	0.11	0.59
Main effects					
ME	11.31	3,636.95	584.72	6.22	5.09[a]
	11.51	3,565.21	580.66	6.14	1.39[b]
	11.71	3,491.77	529.53	6.59	2.31[b]
	P-value	0.079	0.072	0.132	0.023
CP	14	3,563.73	536.90	6.64	3.70
	15	3,602.75	595.63	6.05	1.85
	16	3,527.76	562.32	6.27	3.24
	P-value	0.493	0.089	0.082	0.365
ME × CP	P-value	0.145	0.288	0.409	0.216

Data in the same column without letters or the same letter superscripts mean no significant difference ($P > 0.05$), different letter superscripts mean significant difference ($P < 0.01$ as highly significant, $P < 0.05$ as significant), the same below.

Table 3. Effect of dietary ME and CP levels on pectoral CP, CF, and moisture content (dry matter basis).

ME, MJ/kg	CP, %	Crude protein, %	Crude fat, %	Moisture, %
11.31	14.0	95.86[b]	3.33[c]	74.77
11.31	15.0	95.01[cd]	3.60[bc]	74.80
11.31	16.0	95.57[c]	3.00[ce]	74.78
11.51	14.0	92.84[e]	6.43[a]	74.07
11.51	15.0	96.67[a]	3.27[c]	74.92
11.51	16.0	94.88[d]	4.67[b]	74.24
11.71	14.0	95.09[cd]	4.46[bc]	74.99
11.71	15.0	96.16[ab]	3.29[c]	74.70
11.71	16.0	95.31[cd]	4.90[b]	74.70
SEM	0.072	0.148	0.153	
Main effects				
ME	11.31	95.48[a]	3.31[b]	74.79
	11.51	94.80[b]	4.79[a]	74.41
	11.71	95.52[a]	4.22[a]	74.80
	P-value	<0.01	<0.01	0.51
CP	14.0	94.60[c]	4.74[a]	74.61
	15.0	95.94[a]	3.39[b]	74.81
	16.0	95.26[b]	4.19[a]	74.58
	P-value	<0.01	<0.01	0.80
ME × CP	P-value	<0.01	<0.01	0.783

Data in the same column without letters or the same letter superscripts mean no significant difference ($P > 0.05$), different letter superscripts mean significant difference ($P < 0.01$ as highly significant, $P < 0.05$ as significant)

trend for proline (**Pro**), glycine (**Gly**), and isoleucine (**Ile**) ($0.05 < P < 0.1$).

Table 4b showed that Asp, Thr, Ser, Glu, Ala, Val, Ile, Tyr, Phe, Lys, and Arg were affected by the dietary ME level ($P < 0.05$). Glu and Tyr were significantly affected by dietary CP level ($P < 0.05$). TAA, EAA, BAA, DAA contents and the EAA/TAA ratio were the highest in 11.51 MJ/kg ME group and significantly higher than in the other 2 groups ($P < 0.05$); the FSAA/TAA ratio (48.61%) was the highest in 11.31 MJ/kg group ($P < 0.05$).

Pectoral FA Composition

As shown in Tables 5 and 6, the pectoral FA mainly consisted of palmitic, oleic, linolenic, stearic, and arachidonic acids, with a total share of over 90% and a lower share of the remaining FA. Table 5a showed that there was a significant interaction of dietary ME and CP level on C20:3n6 ($P = 0.046$), and the highest content was in group with 11.31 MJ/kg ME and 15% CP; There had an influence trend for C20:1 and C20:2 ($0.05 < P < 0.1$). Table 5b showed that dietary ME level affected the C20:0 ($P = 0.050$), had an influence trend on C20:2 ($P = 0.080$); Dietary CP levels had an influence trend on C20:4n6 ($P = 0.063$). The interaction of ME and CP levels had an influence trend on C20:2 ($P = 0.055$).

Table 6a showed that C18:2n6c, SFA and UFA were significantly affected by the interaction of dietary ME and CP levels ($P < 0.05$). Table 6b showed that dietary CP level affected SFA percentage ($P < 0.05$), and had an influence on C16:0 and UFA content ($0.05 < P < 0.01$).

Alpha Diversity

As seen from Figure 1(A), all rarefraction curves of Chao1 were close to the maximum value of OTU contained in all samples, but the community richness showed some differences among samples, and the rising trend of each sample curve gradually flattened out and approaches a plateau, indicating that the amount of sequencing data of the samples was reasonable and reliable. The Good_Coverage of all samples in Figure 1(B) were close to 100%, which confirmed the reliability of the data. The rarefraction curve was shown as the number of OTUs constructed at different sampling depths. The curve provided an initial assessment of sequencing saturation, with the final curve leveling off as shown in Figure 1 (C), representing sufficient saturation of the current sequencing volume. At the same time, the Shannon of the sequenced samples was analyzed to construct a rarefraction curve, and the results were shown in Figure 1(D). It could be seen from Figure 1(D), all curves of the Shannon had a rapid rising process and then flatten out to a steady state, which indicated that the amount of sequencing data of the samples was sufficient for effective subsequent analysis.

As shown in Figure 2(A), the Rank Abundance curves for each group were wider and gradually flatter, indicating a richer and more homogeneous species composition for all samples. In Figure 2(B) the curves in the sample curves flatten out with increasing sample size, indicating that the species in each group did not increase significantly with increasing sample size, indicating that the groups were adequately sampled.

As shown in Table 7 and Figure 3, the Chao1, Observed species, and PD_whole_tree indices were higher than the rest of the groups at 11.31 MJ/kg and

Table 4a. Effect of dietary ME and CP levels on pectoral free amino acid content (dry matter basis) g/100 g.

ME, MJ/kg CP, %	11.31 14	11.31 15	11.31 16	11.51 14	11.51 15	11.51 16	11.71 14	11.71 15	11.71 16	SEM	P-value
Asp#	7.59[ab]	7.38[b]	7.42[b]	7.59[ab]	7.63[ab]	7.63[ab]	7.46[b]	7.70[a]	7.56[ab]	0.02	0.018
Thr*#	3.65[a]	3.54[ab]	3.52[b]	3.48[b]	3.48[b]	3.49[bc]	3.42[bc]	3.63[a]	3.57[ab]	0.01	0.002
Ser#	3.02[a]	2.85[b]	2.79[bc]	2.67[c]	2.67[c]	2.69[c]	2.68c	2.97[ab]	2.96[ab]	0.02	0.001
Glu#	11.72[a]	11.18[b]	11.22[b]	11.68[a]	11.82[a]	11.67[a]	11.67[a]	11.80[a]	11.55[a]	0.03	0.009
Pro*#	3.21[a]	3.00[b]	3.06[ab]	3.03[ab]	3.19[a]	3.01[b]	3.12[ab]	3.00[b]	3.07[ab]	0.02	0.063
Gly#	4.00[a]	3.71[b]	3.81[ab]	3.81[ab]	4.01[a]	3.75[ab]	3.92[ab]	3.79[ab]	3.93[ab]	0.03	0.069
Ala#	4.84[ab]	4.64[bc]	4.68[bc]	4.73[bc]	4.89[a]	4.82[ab]	4.75[ab]	4.92[a]	4.87[a]	0.02	0.004
Val*^	3.96[b]	3.87[b]	4.03[b]	4.45[ab]	4.50[a]	4.55[a]	4.34[b]	4.31[b]	4.25[b]	0.02	0.141
Met*^	2.79[a]	2.71[b]	2.70[bc]	2.65[bc]	2.69[bc]	2.68[bc]	2.62[c]	2.81[a]	2.72[b]	0.01	0.001
Ile*^	3.78[c]	3.68[c]	3.83c	4.17[ab]	4.19[ab]	4.22[a]	4.07[b]	4.13[ab]	4.01[b]	0.02	0.072
Leu*^	6.48[ab]	6.31[b]	6.37[b]	6.45[b]	6.47[ab]	6.48[ab]	6.34[b]	6.59[a]	6.46[ab]	0.02	0.009
Tyr^	2.80[a]	2.71[ab]	2.70[ab]	2.37[c]	2.37[c]	2.40[c]	2.36[c]	2.74[ab]	2.67[b]	0.01	0.000
Phe*^	4.36[ab]	4.26[b]	4.29[b]	4.38[ab]	4.39[ab]	4.45[a]	4.29[b]	4.38[ab]	4.39[ab]	0.01	0.156
Lys*	7.22[bc]	7.02[c]	7.10[c]	7.36[ab]	7.37[ab]	7.40[ab]	7.28[ab]	7.43[a]	7.26[b]	0.02	0.026
His*^	2.78[b]	2.84[ab]	2.83[ab]	3.06[ab]	2.96[ab]	3.19[a]	2.69[b]	2.97[ab]	3.14[ab]	0.04	0.481
Arg*^	5.37[a]	5.20[b]	5.23[b]	5.32[ab]	5.40[a]	5.32[ab]	5.29[ab]	5.41[a]	5.35[ab]	0.02	0.033
TAA	77.55[ab]	74.80[b]	75.55[b]	77.20[ab]	78.00[a]	77.73[ab]	76.30[b]	78.57[a]	77.75[ab]	0.19	0.004
EAA	43.58[b]	42.42[c]	42.95[bc]	44.32[a]	44.61[a]	44.77[a]	43.45[bc]	44.66[a]	44.20[ab]	0.12	0.049
FSAA	38.01[a]	36.30[b]	36.49[b]	36.99[b]	37.67[ab]	37.05[b]	37.01[b]	37.81[ab]	37.51[ab]	0.11	0.004
BAA	32.31[b]	31.56[b]	31.97[b]	32.82[ab]	32.95[a]	33.28[a]	31.99[b]	33.33[a]	32.97[ab]	0.10	0.039
DAA	70.32[b]	67.85[b]	68.46[b]	69.81[ab]	70.62[a]	70.32[ab]	69.00[b]	71.14[a]	70.48[ab]	0.17	0.003
EAA/TAA	56.20[c]	56.71[b]	56.85[b]	57.41[ab]	57.20[ab]	57.60[a]	56.94[b]	56.84[b]	56.85[b]	0.06	0.130
FSAA/TAA	49.01[a]	48.52[ab]	48.30[ab]	47.91[b]	48.29[b]	47.66[b]	48.51[ab]	48.12[b]	48.24[b]	0.08	0.331
BAA/TAA	41.66[b]	42.18[ab]	42.32[ab]	42.52[ab]	42.25[ab]	42.81[a]	41.92[b]	42.42[ab]	42.41[ab]	0.08	0.410

In the first column, "*" mean EAA, "#" mean FSAA, "^" mean BAA, "#" add "^" mean DAA.

Data in the same line without letters or the same letter superscripts mean no significant difference ($P > 0.05$), different letter superscripts mean significant difference ($P < 0.01$ as highly significant, $P < 0.05$ as significant).

15% CP group, but the difference was not significant ($P > 0.05$).

Intestinal Flora Community

Figure 4(A)(C) showed the composition of microbiota in the cecum of chickens at the phylum level. The composition of Top 15 phyla was approximately equal in each group, and the top 3 dominant phyla of most groups were *Bacteroidetes, Firmicutes, Proteobacteria*. The third dominant phylum at the ME level of *Fusobacteria* replaced *Proteobacteria* as the third dominant phylum in the two groups with ME level of 11.31 MJ/kg and CP levels of 14% and 15%. The relative abundance of *Proteobacteria* increased with dietary CP level.

Table 4b. Effect of dietary ME and CP levels on pectoral free amino acid content (dry matter basis) g/100 g.

	ME				CP				ME*CP
	11.31	11.51	11.71	P-value	14	15	16	P-value	P-value
Asp#	7.47[b]	7.62[a]	7.57[a]	0.017	7.55	7.57	7.54	0.773	0.018
Thr*#	3.57[a]	3.48[b]	3.54[a]	0.017	3.52	3.55	3.53	0.442	0.002
Ser#	2.89[a]	2.68[b]	2.87[a]	0.000	2.79	2.83	2.81	0.625	0.001
Glu#	11.37[b]	11.72[a]	11.67[a]	0.000	11.69[a]	11.60[ab]	11.48[b]	0.035	0.009
Pro*#	3.09	3.08	3.07	0.861	3.12	3.07	3.05	0.334	0.063
Gly#	3.84	3.86	3.88	0.872	3.91	3.84	3.83	0.454	0.069
Ala#	4.72[b]	4.81[a]	4.85[a]	0.006	4.78	4.81	4.79	0.602	0.004
Val*^	3.95c	4.50[a]	4.30[b]	0.000	4.25	4.23	4.28	0.491	0.141
Met*^	2.73[a]	2.68[b]	2.72[ab]	0.053	2.69[b]	2.74[a]	2.70[ab]	0.077	0.001
Ile*^	3.77c	4.19[a]	4.07[b]	0.000	4.01	4.00	4.02	0.869	0.072
Leu*^	6.39	6.47	6.47	0.116	6.42	6.46	6.44	0.651	0.009
Tyr^	2.74[a]	2.38c	2.59[b]	0.000	2.51[b]	2.61[a]	2.59[a]	0.011	0.000
Phe*^	4.30[b]	4.41[a]	4.35[ab]	0.016	4.34	4.34	4.38	0.433	0.156
Lys*	7.12[b]	7.38[a]	7.32[a]	0.000	7.29	7.28	7.25	0.706	0.026
His*^	2.82[b]	3.07[a]	2.93[ab]	0.086	2.84	2.92	3.05	0.163	0.481
Arg*^	5.27[b]	5.35[a]	5.35[a]	0.056	5.33	5.34	5.30	0.584	0.033
TAA	75.97[b]	77.64[a]	77.54[a]	0.002	77.02	77.12	77.01	0.963	0.004
EAA	42.99[b]	44.57[a]	44.10[a]	0.001	43.79	43.90	43.98	0.803	0.049
FSAA	36.93	37.24	37.45	0.168	37.34	37.26	37.02	0.444	0.004
BAA	31.95[b]	33.02[a]	32.77[a]	0.001	32.38	32.62	32.74	0.338	0.039
DAA	68.88[b]	70.25[a]	70.21[a]	0.005	69.712	69.872	69.757	0.922	0.003
EAA/TAA%	56.58[b]	57.40[a]	56.88[b]	0.000	56.85	56.91	57.00	0.178	0.130
FSAA/TAA%	48.61[a]	47.95[b]	48.29[ab]	0.013	48.48	48.31	48.06	0.136	0.331
BAA/TAA%	42.05[b]	42.52[a]	42.25[ab]	0.064	42.03[b]	42.28[ab]	42.51[a]	0.059	0.410

In the first column, "*" mean EAA, "#" mean FSAA, "^" mean BAA, "#" add "^" mean DAA.

Data in the same line without letters or the same letter superscripts mean no significant difference ($P > 0.05$), different letter superscripts mean significant difference ($P < 0.01$ as highly significant, $P < 0.05$ as significant).

Table 5a. Effect of dietary ME and CP levels on proportion of pectoral free fatty acid content (dry matter basis) g/100 g.

ME/(MJ/kg) CP/%	11.31 14	11.31 15	11.31 16	11.51 14	11.51 15	11.51 16	11.71 14	11.71 15	11.71 16	SEM	P-value
C20:4n6[345]	0.425[a]	0.360[ab]	0.250[b]	0.374[ab]	0.344[ab]	0.302[ab]	0.372[ab]	0.253[b]	0.291[ab]	0.018	0.647
C14:0[1]	0.020	0.026	0.022	0.032	0.025	0.021	0.023	0.030	0.028	0.003	0.754
C14:1[2]	0.005	0.006	0.004	0.008	0.006	0.008	0.008	0.005	0.007	0.001	0.935
C15:0[1]	0.001	0.007	0.003	0.002	0.003	0.003	0.004	0.006	0.005	0.001	0.860
C16:0[1]	0.740[b]	1.171[ab]	0.909[ab]	1.471[a]	0.846[ab]	0.930[ab]	1.038[ab]	0.916[ab]	1.179[ab]	0.074	0.184
C16:1[2]	0.070	0.122	0.101	0.199	0.079	0.102	0.137	0.108	0.181	0.016	0.350
C17:0[1]	0.005	0.007	0.003	0.007	0.008	0.006	0.005	0.003	0.006	0.001	0.753
C18:0[1]	0.370[b]	0.534[a]	0.277[b]	0.631[a]	0.418[ab]	0.440[ab]	0.456[ab]	0.399[ab]	0.484[ab]	0.028	0.163
C18: 1n9c[2]	0.750[b]	1.268[ab]	0.999[ab]	1.890[a]	0.928[ab]	1.082[ab]	1.220[ab]	0.994[ab]	1.438[ab]	0.112	0.232
C18:2n6c[345]	0.600[b]	0.910[ab]	0.804[ab]	1.085[a]	0.755[ab]	0.712[ab]	0.934[ab]	0.670[ab]	0.788[ab]	0.05	0.217
C18:3n3[346]	0.015[b]	0.029[ab]	0.050[a]	0.031[ab]	0.029[ab]	0.025[ab]	0.030[ab]	0.022[ab]	0.027[ab]	0.004	0.402
C20:1[2]	0.009[b]	0.017[ab]	0.012[b]	0.024[a]	0.009[b]	0.011[b]	0.014[b]	0.011[b]	0.016[ab]	0.001	0.072
C20:2[3]	0.011[b]	0.016[a]	0.014[ab]	0.017[a]	0.012[ab]	0.012[b]	0.013[ab]	0.009[b]	0.010[b]	0.001	0.055
C20:3n6[345]	0.031[b]	0.044[a]	0.029[b]	0.039[ab]	0.030[b]	0.033[ab]	0.037[ab]	0.026[b]	0.031[b]	0.001	0.046
C22:1n9[2]	0.006	0.003	0.003	0.003	0.005	0.003	0.004	0.003	0.003	0.001	0.712
C22:6n3[346]	0.048[a]	0.035[ab]	0.027[b]	0.038[ab]	0.036[ab]	0.031[ab]	0.036[ab]	0.027[b]	0.030[ab]	0.002	0.751
C23:0[1]	0.000[b]	0.001[ab]	0.001[ab]	0.004[a]	0.002[ab]	0.003[ab]	0.002[ab]	0.003[ab]	0.004[a]	0.001	0.384
C24:1[2]	0.006[a]	0.006[a]	0.004[ab]	0.005[ab]	0.004[ab]	0.005[ab]	0.005[ab]	0.003[b]	0.005[ab]	0.001	0.286
C18:1n9t[2]	0.004	0.008	0.005	0.009	0.006	0.006	0.006	0.007	0.008	0.001	0.446
SFA	1.131[b]	1.745[ab]	1.215[b]	2.147[a]	1.302[ab]	1.402[ab]	1.528[ab]	1.357[ab]	1.706[ab]	0.102	0.181
UFA	1.979[b]	2.822[ab]	2.301[ab]	3.721[a]	2.243[ab]	2.333[ab]	2.815[ab]	2.138[ab]	2.835[ab]	0.171	0.207
MUFA	0.846[b]	1.428[ab]	1.127[ab]	2.138[a]	1.038[ab]	1.218[ab]	1.393[ab]	1.131[ab]	1.658[ab]	0.129	0.24
PUFA	1.133[b]	1.394[ab]	1.174[ab]	1.583[a]	1.205[ab]	1.115[b]	1.422[ab]	1.007[b]	1.177[ab]	0.05	0.167
EFA	1.121[b]	1.377[ab]	1.160[ab]	1.567[a]	1.193[ab]	1.103[b]	1.409[ab]	0.998[b]	1.167[ab]	0.05	0.171
TFA	3.110[b]	4.567[ab]	3.516[ab]	5.868[a]	3.545[ab]	3.735[ab]	4.343[ab]	3.494[ab]	4.541[ab]	0.273	0.199
n6PUFA	1.059[b]	1.313[ab]	1.083[ab]	1.498[a]	1.128[ab]	1.047[b]	1.344[ab]	0.949[b]	1.110[ab]	0.048	0.164
n3PUFA	0.063	0.064	0.078	0.069	0.064	0.056	0.065	0.048	0.057	0.004	0.753
n6/n3	16.969	20.456	18.106	21.409	17.931	19.174	21.024	19.917	19.588	0.795	0.699
PUFA/SFA	1.023[a]	0.823[ab]	0.986[ab]	0.785[ab]	0.965[ab]	0.818[ab]	0.938[ab]	0.761[b]	0.708[b]	0.029	0.150

Data in the first column: the letters superscripts "1" means SFA, "2" means MFA, "3" means PUFA, "4" means EFA, "5" means n6PUFA, "6" means n3PUFA, "2" add "3" means UFA.

Data in the same line without letters or the same letter superscripts mean no significant difference ($P>0.05$), different letter superscripts mean significant difference ($P<0.01$ as highly significant, $P<0.05$ as significant).

Table 5b. Effect of dietary ME and CP levels on pectoral free fatty acid content (dry matter basis) g/100 g.

	ME				CP				ME × CP
	11.31	11.51	11.71	P-value	14	15	16	P-value	P-value
C20:4n6[345]	0.35	0.34	0.31	0.620	0.39[a]	0.32[ab]	0.28[b]	0.063	0.647
C14:0[1]	0.02	0.03	0.03	0.640	0.02	0.03	0.02	0.853	0.754
C14:1[2]	0.005	0.007	0.006	0.500	0.007	0.006	0.006	0.836	0.935
C15:0[1]	0.004	0.003	0.005	0.700	0.002	0.005	0.004	0.452	0.860
C16:0[1]	0.94	1.08	1.05	0.720	1.08	0.98	1.01	0.839	0.184
C16:1[2]	0.10	0.13	0.14	0.510	0.14	0.10	0.13	0.676	0.350
C17:0[1]	0.005	0.007	0.005	0.490	0.006	0.006	0.005	0.902	0.753
C18:0[1]	0.40	0.50	0.45	0.360	0.49	0.45	0.40	0.471	0.163
C18: 1n9c[2]	1.00	1.30	1.22	0.550	1.29	1.06	1.17	0.725	0.232
C18:2n6c[345]	0.77	0.85	0.80	0.810	0.87	0.78	0.77	0.647	0.217
C18:3n3[346]	0.03	0.03	0.03	0.850	0.03	0.03	0.03	0.607	0.402
C20:1[2]	0.01	0.02	0.01	0.750	0.02	0.01	0.01	0.572	0.072
C20:2[3]	0.01[a]	0.01[ab]	0.01[b]	0.080	0.01	0.01	0.01	0.470	0.055
C20:3n6[345]	0.04	0.03	0.03	0.560	0.04	0.03	0.03	0.394	0.046
C22:1n9[2]	0.004	0.004	0.003	0.800	0.004	0.004	0.003	0.693	0.712
C22:6n3[346]	0.04	0.04	0.03	0.540	0.04	0.03	0.03	0.134	0.751
C23:0[1]	0.001[b]	0.003[a]	0.003[ab]	0.050	0.002	0.002	0.003	0.796	0.384
C24:1[2]	0.005	0.005	0.004	0.260	0.005	0.004	0.005	0.609	0.286
C18:1n9t[2]	0.006	0.007	0.007	0.640	0.006	0.007	0.007	0.976	0.446
SFA	1.36	1.62	1.53	0.600	1.60	1.47	1.44	0.791	0.181
UFA	2.37	2.77	2.60	0.640	2.84	2.40	2.49	0.555	0.207
MUFA	1.13	1.46	1.40	0.560	1.46	1.20	1.33	0.717	0.240
PUFA	1.23	1.30	1.20	0.720	1.38	1.20	1.15	0.186	0.167
EFA	1.22	1.29	1.19	0.730	1.37	1.19	1.14	0.186	0.171
TFA	3.73	4.38	4.13	0.630	4.44	3.87	3.93	0.650	0.199
n6PUFA	1.15	1.22	1.13	0.730	1.30	1.13	1.08	0.176	0.164
n3PUFA	0.07	0.06	0.06	0.560	0.07	0.06	0.06	0.800	0.753
n6/n3	18.51	19.50	20.18	0.700	19.80	19.43	18.96	0.910	0.699
PUFA/SFA	0.94	0.86	0.80	0.170	0.92	0.85	0.83	0.516	0.150

Data in the first column: the letters superscripts "1" means SFA, "2" means MFA, "3" means PUFA, "4" means EFA, "5" means n6PUFA, "6" means n3PUFA, "2" add "3" means UFA.

Data in the same line without letters or the same letter superscripts mean no significant difference ($P > 0.05$), different letter superscripts mean significant difference ($P < 0.01$ as highly significant, $P < 0.05$ as significant).

Table 6a. Effect of dietary ME and CP levels on pectoral fatty acid proportion (dry matter basis)%.

| ME, MJ/kg | 11.31 | 11.31 | 11.31 | 11.51 | 11.51 | 11.51 | 11.71 | 11.71 | 11.71 | SEM | P-value |
CP, %	14	15	16	14	15	16	14	15	16		
C20:4n6[345]	14.30[a]	8.37[ab]	7.35[b]	7.96[b]	10.45[ab]	8.32[ab]	8.75[ab]	7.70[b]	6.70[b]	0.71	0.351
C14:0[1]	0.52[b]	0.55[ab]	0.65[ab]	0.52[ab]	0.64[ab]	0.55[ab]	0.54[ab]	0.80[a]	0.60[ab]	0.03	0.572
C14:1[2]	0.17	0.14	0.11	0.13	0.17	0.22	0.17	0.11	0.15	0.02	0.618
C15:0[1]	0.03	0.13	0.08	0.03	0.08	0.08	0.09	0.12	0.11	0.02	0.978
C16:0[1]	23.70[b]	25.47[ab]	26.85[a]	24.63[ab]	23.44[b]	24.67[ab]	23.86[b]	26.29[ab]	25.85[ab]	0.28	0.204
C16:1[2]	2.17[b]	2.72[ab]	2.94[ab]	2.95[ab]	2.11[b]	2.59[ab]	3.11[ab]	3.04[ab]	3.87[a]	0.19	0.717
C17:0[1]	0.14[ab]	0.12[ab]	0.07[ab]	0.12[ab]	0.24[a]	0.16[ab]	0.13[ab]	0.06[b]	0.13[ab]	0.02	0.492
C18:0[1]	12.00[a]	11.68[a]	7.32[ab]	11.25[ab]	11.90[a]	11.90[a]	10.54[ab]	11.64[a]	10.70[ab]	0.45	0.288
C18: 1n9c[2]	23.33[b]	26.93[ab]	29.17[ab]	30.50[a]	25.12[ab]	28.35[ab]	27.95[ab]	28.49[ab]	30.96[a]	0.80	0.395
C18:2n6c[345]	19.44[b]	19.95[ab]	23.51[a]	18.42[b]	21.33[ab]	19.55[ab]	21.48[ab]	19.40[b]	17.62[b]	0.45	0.049
C18:3n3[346]	0.47[b]	0.60[b]	1.38[a]	0.49[b]	0.84[ab]	0.66[b]	0.69[ab]	0.60[b]	0.59[b]	0.08	0.20
C20:1[2]	0.28[ab]	0.34[ab]	0.34[ab]	0.41[a]	0.26[b]	0.30[ab]	0.31[ab]	0.33[ab]	0.35[ab]	0.02	0.256
C20:2[3]	0.37	0.37	0.41	0.32	0.37	0.35	0.31	0.27	0.24	0.02	0.868
C20:3n6[345]	1.04	1.06	0.88	0.79	0.88	0.89	0.86	0.78	0.73	0.06	0.924
C22:1n9[2]	0.18	0.07	0.08	0.08	0.16	0.09	0.09	0.08	0.06	0.02	0.422
C22:6n3[346]	1.60[a]	0.84[b]	0.78[b]	0.83[b]	1.10[ab]	0.84[b]	0.84[b]	0.81[b]	0.69[b]	0.08	0.277
C23:0[1]	0.00[b]	0.02[ab]	0.05[ab]	0.09[ab]	0.07[ab]	0.06[ab]	0.03[ab]	0.08[ab]	0.09[a]	0.01	0.68
C24:1[2]	0.19	0.15	0.12	0.09	0.12	0.13	0.11	0.09	0.11	0.01	0.608
C18:1n9t[2]	0.12	0.18	0.15	0.15	0.15	0.16	0.13	0.14	0.18	0.01	0.931
SFA	36.39[b]	37.98[ab]	35.02[b]	36.63[b]	36.36[b]	37.42[b]	35.18[b]	39.00[a]	37.47[ab]	0.25	0.032
UFA	63.67[b]	61.72[b]	67.24[a]	63.12[b]	63.04[b]	62.44[b]	64.82[ab]	61.90[b]	62.23[b]	0.34	0.023
MUFA	26.44[b]	30.54[ab]	32.92[ab]	34.30[ab]	28.09[ab]	31.84[ab]	31.88[ab]	32.33[ab]	35.67[a]	0.96	0.437
PUFA	37.22[a]	31.18[ab]	34.31[ab]	28.82[ab]	34.95[ab]	30.61[ab]	32.93[ab]	29.57[ab]	26.55[b]	1.01	0.291
EFA	36.85[a]	30.81[ab]	33.90[ab]	28.50[ab]	34.58[ab]	30.25[ab]	32.62[ab]	29.30[ab]	26.32[b]	0.99	0.283
n6PUFA	34.78[a]	29.37[ab]	31.74 [ab]	27.17 [ab]	32.65 [ab]	28.76 [ab]	31.09[ab]	27.89 [ab]	25.04[b]	0.941	0.330
n3PUFA	2.07	1.44	2.16	1.33	1.94	1.50	1.53	1.41	1.28	0.107	0.314

Data in the first column: the letters superscripts "1" means SFA, "2" means MFA, "3" means PUFA, "4" means EFA, "5" means n6PUFA, "6" means n3PUFA, "2" add "3" means UFA.

Data in the same line without letters or the same letter superscripts mean no significant difference (P>0.05), different letter superscripts mean significant difference (P<0.01 as highly significant, P<0.05 as significant).

The relative abundance of the main microbial phyla for each sample was statistically analyzed, and the results were shown in Table 8. *Firmicutes* showed a significant difference by the interaction of ME and CP levels (P < 0.05), the group with 11.51 MJ/kg ME and 14% CP had the highest abundance (31.42%). The abundance of *Proteobacteria* was significantly affected by dietary CP levels (P < 0.01) and increased with the

Table 6b. Effect of dietary ME and CP levels on the proportion of pectoral fatty acid contents (dry matter basis)%.

| | ME | | | | CP | | | | ME × CP |
	11.31	11.51	11.71	P-value	14	15	16	P-value	P-value
C20:4n6[345]	10.008	8.909	7.713	0.435	10.338	8.839	7.453	0.278	0.351
C14:0[1]	0.576	0.567	0.646	0.543	0.522	0.663	0.602	0.212	0.572
C14:1[2]	0.142	0.171	0.143	0.741	0.157	0.141	0.159	0.899	0.618
C15:0[1]	0.080	0.061	0.107	0.596	0.049	0.109	0.090	0.403	0.978
C16:0[1]	25.339	24.247	25.332	0.224	24.064[b]	25.064[ab]	25.789[a]	0.070	0.204
C16:1[2]	2.61	2.553	3.34	0.192	2.743	2.627	3.133	0.527	0.717
C17:0[1]	0.112	0.174	0.104	0.355	0.130	0.142	0.119	0.904	0.492
C18:0[1]	10.333	11.687	10.96	0.477	11.264	11.742	9.973	0.270	0.288
C18: 1n9c[2]	26.479	27.988	29.132	0.416	27.259	26.846	29.494	0.368	0.395
C18:2n6c[345]	20.966	19.763	19.501	0.382	19.781	20.224	20.224	0.897	0.049
C18:3n3[346]	0.816	0.662	0.622	0.592	0.549	0.676	0.876	0.271	0.200
C20:1[2]	0.322	0.322	0.330	0.974	0.334	0.310	0.330	0.801	0.256
C20:2[3]	0.383[a]	0.347[ab]	0.273[b]	0.123	0.334	0.336	0.333	0.999	0.868
C20:3n6[345]	0.993	0.852	0.790	0.339	0.899	0.906	0.831	0.837	0.924
C22:1n9[2]	0.110	0.112	0.078	0.603	0.120	0.102	0.078	0.545	0.422
C22:6n3[346]	1.071	0.923	0.782	0.362	1.092	0.916	0.769	0.284	0.277
C23:0[1]	0.023	0.072	0.070	0.127	0.040	0.058	0.068	0.558	0.680
C24:1[2]	0.156	0.112	0.103	0.184	0.131	0.121	0.119	0.904	0.608
C18:1n9t[2]	0.151	0.151	0.167	0.831	0.136	0.169	0.164	0.483	0.931
SFA	36.462	36.808	37.219	0.489	36.070[b]	37.780[a]	36.639[ab]	0.038	0.032
UFA	64.207	62.869	62.981	0.229	63.868[ab]	62.218[b]	63.971[a]	0.086	0.023
MUFA	29.968	31.411	33.294	0.382	30.878	30.318	33.478	0.374	0.437
PUFA	34.239	31.457	29.688	0.205	32.991	31.901	30.491	0.605	0.291
EFA	33.853	31.112	29.412	0.208	32.657	31.564	30.157	0.594	0.283
n6PUFA	31.966	29.526	28.005	0.249	31.015	29.970	28.513	0.562	0.330
n3PUFA	1.889	1.586	1.406	0.207	1.642	1.595	1.644	0.978	0.314

Data in the first column: the letters superscripts "1" means SFA, "2" means MFA, "3" means PUFA, "4" means EFA, "5" means n6PUFA, "6" means n3PUFA, "2" add "3" means UFA.

Data in the same line without letters or the same letter superscripts mean no significant difference (P>0.05), different letter superscripts mean significant difference (P<0.01 as highly significant, P<0.05 as significant).

dietary CP levels, got the maximum value at 16% CP group (12.68%) ($P < 0.05$).

Figure 4(B)(D) showed the composition of gut microbiota at the genus level in each treatment group. The main dominant genus in each treatment group was *Bacreroides, Rikenellaceae_ RC9_ gut_ group, Fusobacterium, Parabacteroides*, but *Romboutsia* was the dominant genus in D4 and D8.

Table 9 indicated the relative abundance statistics at the genus level of gut microbiota for each group. It was found that the ME and CP levels of the diets did not affect the genus of top4 ($P > 0.05$). In contrast, the abundance of *Rikenellaceae_ RC9_ gut_ group* and *Parabacteroides* showed a gradual increase with the increasing dietary CP level, and both obtained maximum abundance at 16% CP level (10.62%, 3.38%).

From the above results, it was found that the top three dominant phyla in each group were *Bacteroidetes, Firmicutes* and *Proteobacteria*, and the composition of all phyla tended to be stable. The abundance of *Proteobacteria* was significantly affected by dietary CP levels ($P < 0.01$), and the abundance of *Proteobacteria* increased with the dietary CP levels.

DISCUSSION

Dietary ME and CP are two of the most critical nutritional parameters and have important impacts on growth and development of poultry (Mir et al., 2017). One study in Mexican Creole birds showed that the males with 10.67 MJ/kg diet had a high performance, without compromising carcass yield and body composition (Matus-Aragón et al., 2022). Reducing dietary CP levels did not affect the growth performance of broiler chickens (Chrystal et al., 2020), and the high ME level tended to increase body weight and daily gain and reduce feed conversion ratio of yellow-feathered male chickens (Abouelezz et al., 2019). Our group compared the performance of BYC laying hens with 3 levels of CP (16.0%, 15.5%, and 15.0%), and suggested that the egg laying rate was not significantly affected by CP levels at 19 to 55 wks of age (Geng et al., 2011). In this study, dietary ME and CP levels did not affect performance of growing chickens, but the mortality rate of 11.31 MJ/kg was the highest, probably because the 11.31 MJ/kg ME was not enough to build up the chicken's resistance.

The pectoral muscle composition is directly related to its nutritional characteristics and is a concrete expression of its nutritional value, flavor, juiciness and other
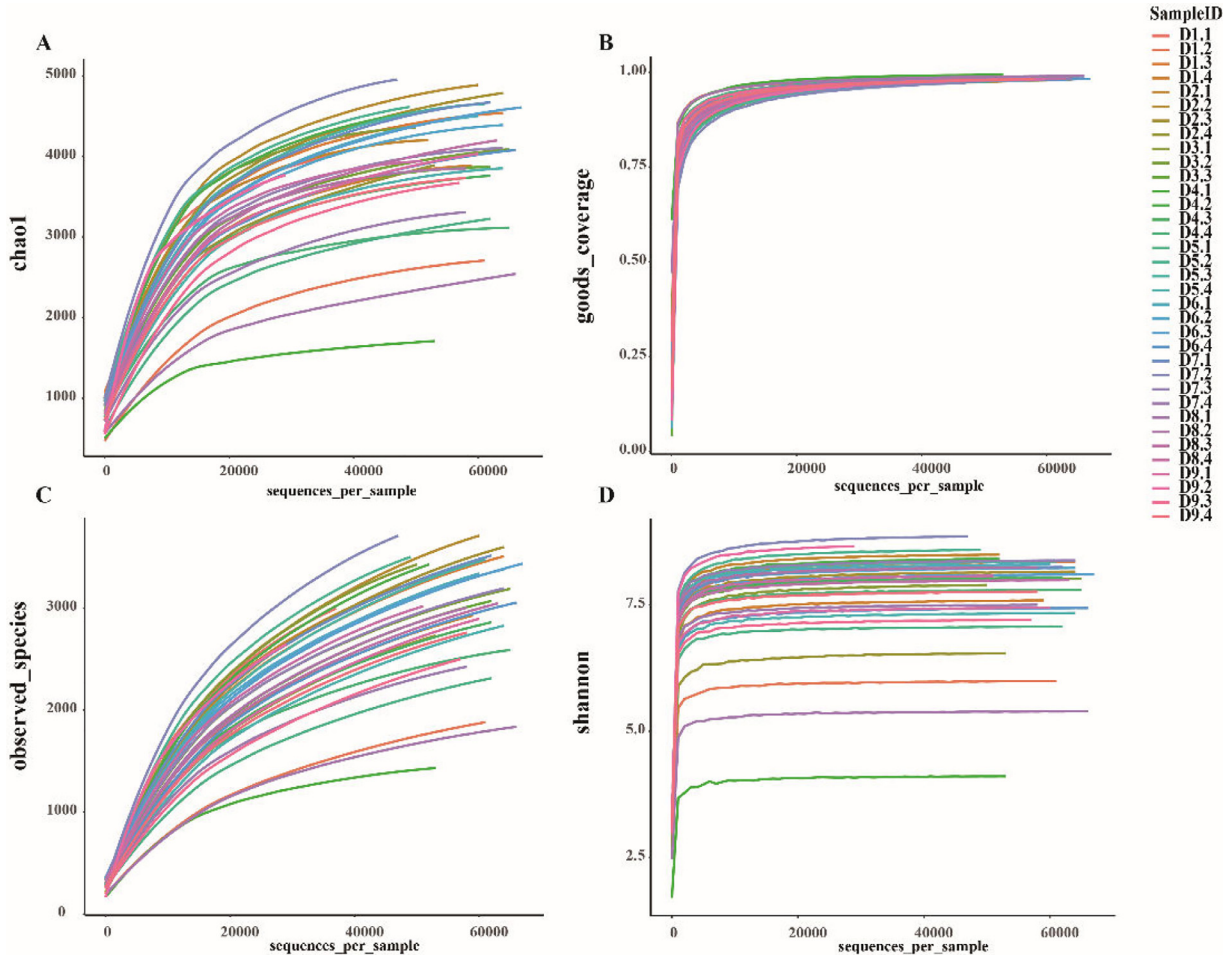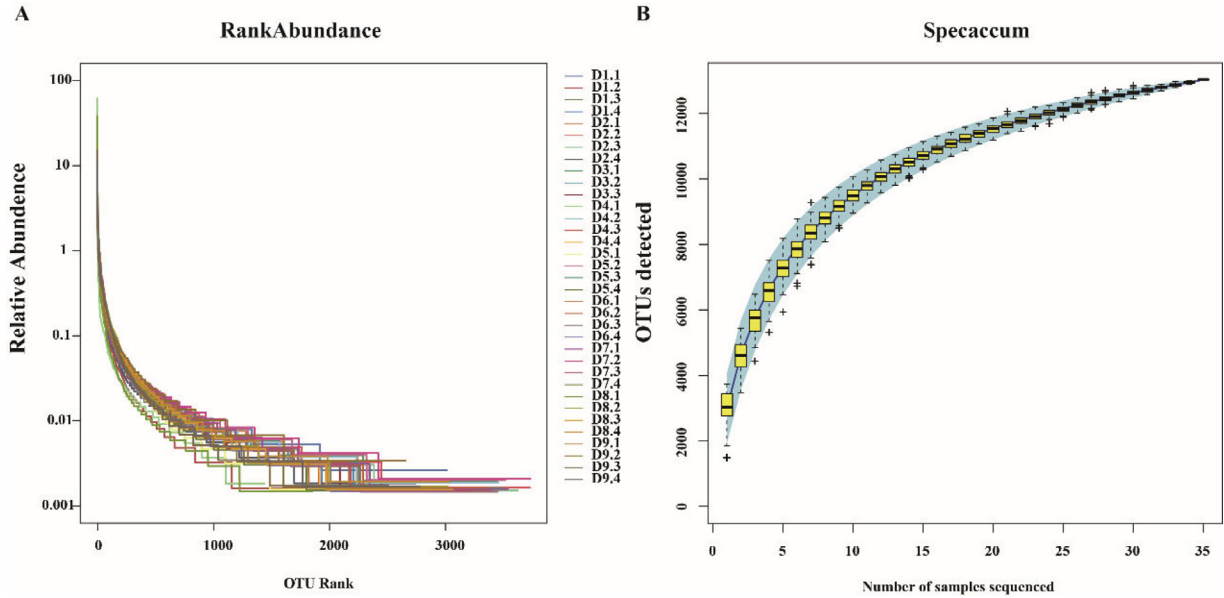

Figure 1. Alpha Index Rarefraction Curve Graph. Figure A is Chao1, B is Goods_Coverage, C is Observed and D is Shannon, showing the saturation of sequencing and the differences between samples (different Y-axis coordinates, that is, the Alpha diversity varies greatly). As well as the large differences between each sample (each line in the graph represents one sample).

Figure 2. Rank-abundance curve and Specaccum species accumulation curve, Figure A is Rank-abundance, Figure B is Specaccum.

Table 7. Effect of dietary ME and CP levels on the alpha diversity of gut microbiota.

| ME,MJ/kg	CP, %	goods_coverage	chao1	observed_species	Shannon	PD_whole_tree
11.31	14.0	96.71	3,392.23	2,235.58	7.49	87.59
11.31	15.0	96.17	3,902.84	2,557.70	7.84	97.03
11.31	16.0	96.32	3,698.12	2,482.40	7.92	95.98
11.51	14.0	97.23	2,944.02	2,018.78	7.05	81.62
11.51	15.0	96.55	3,550.55	2,305.25	7.80	87.65
11.51	16.0	96.36	3,731.72	2,431.03	7.97	92.82
11.71	14.0	96.42	3,745.85	2,495.85	8.20	91.88
11.71	15.0	96.92	3,180.32	2,079.45	7.39	85.22
11.71	16.0	96.71	3,395.64	2,182.15	7.74	88.18
SEM		0.116	112.322	77.236	0.175	2.122
ME	11.31	96.40	3,664.39	2,425.23	7.75	93.54
	11.51	96.71	3,408.76	2,251.68	7.60	87.36
	11.71	96.68	3,440.60	2,252.48	7.78	88.43
	P-value	0.501	0.615	0.59	0.909	0.457
CP	14.0	96.79	3,360.70	2,250.07	7.58	87.03
	15.0	96.54	3,544.57	2,314.13	7.67	89.97
	16.0	96.46	3,608.49	2365.19	7.87	92.33
	P-value	0.503	0.65	0.833	0.789	0.599
ME × CP	P-value	0.363	0.326	0.394	0.58	0.67

Data in the same column without letters or the same letter superscripts mean no significant difference ($P>0.05$), different letter superscripts mean significant difference ($P<0.01$ as highly significant, $P<0.05$ as significant).

traits. Pectoral nutritional indicators mainly include CP, CF, and water content (Zhang et al., 2021). The CP content is an important reflection of the nutritional value of breast meat (Irm et al., 2020), while CF is also one of the important indicators of meat quality. Proper intramuscular fat can improve the tenderness and flavor of breast meat (Aaslyng et al., 2017).

It has been found that the pectoral CP content decreased significantly and the CF content increased significantly with the increase of ME level (Peter et al., 1998). The growth rate of the BYC was slow, the pectoral CP content was the lowest and the CF content was the highest when the dietary ME level was 11.51 MJ/kg, which was similar to the study of yellow-feathered broilers from 22 to 42 d of age (Zhou et al., 2004). Ferreira et al. (2015) found that pectoral CF content of broiler appeared lower after feeding diets with lower ME levels. When ME level increased, the pectoral CF content also increased in AA broilers (Fang et al., 2002), which was similar to the present results.

Proper pectoral fat is important to maintain good palatability and flavor, but meat quality decreases when fat content exceeds a certain range, and meat with higher fat content is not always popular with consumers (Chen et al., 2008). Pectoral CF is also linked to tenderness and flavor, and moderate fat deposition can lead to juicier, tastier chicken products (Glitsch, 2000; Hocquette et al., 2010). In this study, the pectoral CF content showed a trend of increasing and then decreasing with the increase of ME level, which may be related to the CP level, at low CP levels, the CF content increased and then decreased with the increase of ME levels. The pectoral CF increased gradually with increasing ME level at 16% CP. This indicates that a certain amount of protein
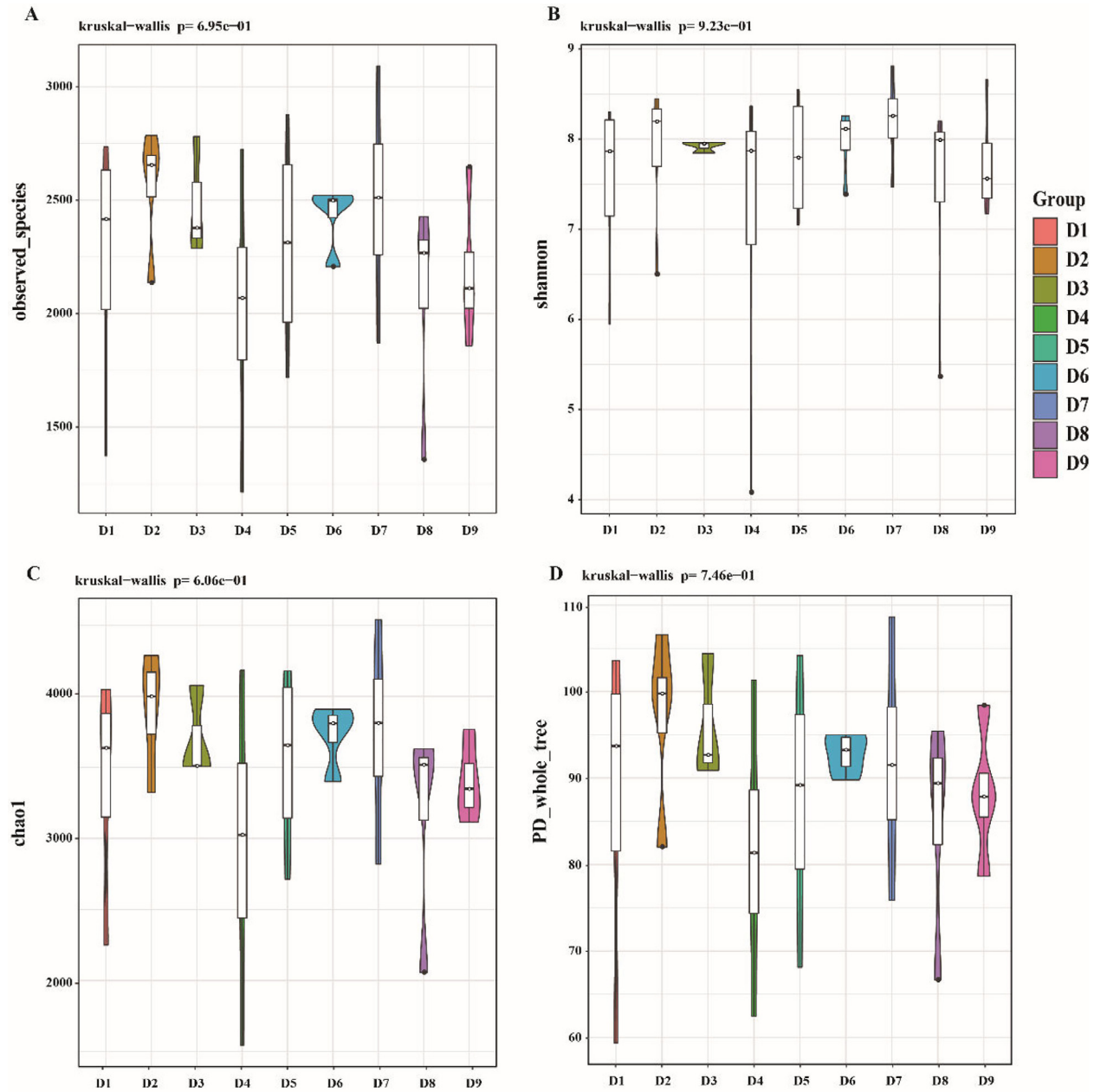
Figure 3. Comparison of OTU diversity indices between groups violin plot, Fig. A is Observed species, Fig. B is Shannon index represent the diversity of microflora in the samples tested, Fig. C is Chao1 index to measure species richness, Fig. D is PD_whole tree. The violin plot shows the degree of sample dispersion within groups and the difference in the level of indices between groups.

is beneficial for promoting the growth of breast, but excess protein is deposited as body fat, suggesting that the maximum protein utilization can be obtained at 15% CP, which is in agreement with the findings of Fang et al. (2002).

Poultry have the characteristic of "eating for energy" (Hossain et al., 2012), and there is a dynamic equilibrium between the energy conversion rate and protein conversion rate in the body (Marcu et al., 2012). In this experiment, the pectoral CP and CF contents were in dynamic equilibrium, with one indicator increasing and the other decreasing, the higher dietary CP levels may result in deposition of body fat, therefore, it is not recommended to have a higher dietary CP to avoid protein waste. Especially in the context of the worldwide shortage of protein resources, appropriate reduction of

dietary CP level can not only make greater use of limited resources, but also reduce the nitrogen content excreted from chicken manure, which is of great significance to reduce environmental pollution.

FAA is one of the important taste substances and aroma precursors of chicken meat, many of which have sweet, sour, salty, fresh and other flavors. Among them the content of amino acids with taste function has a significant impact on muscle flavor (del Olmo et al., 2015; Qi et al., 2017; Diez-Simon et al., 2019). The present study showed that dietary ME and CP levels affected the FAA, not only some amino acids content but also amino acid types, especially the flavoring amino acids. Dietary ME level significantly affected TAA and EAA content, with 11.71 MJ/kg ME group having a higher TAA and EAA content, while dietary CP level did not
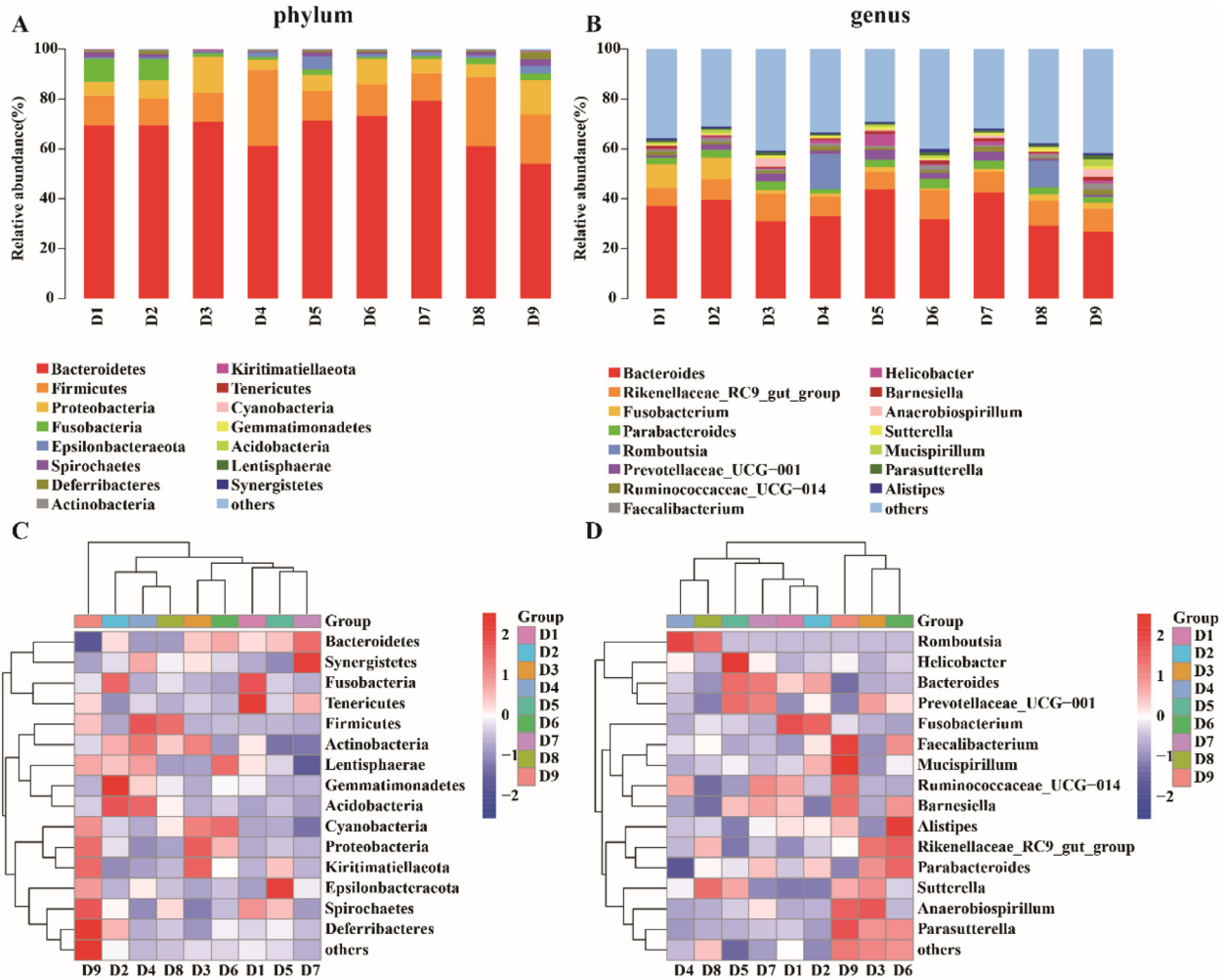
Figure 4. Relative abundance of gut microbial at phylum level and genus level, A and C show the histogram and clustering heat map at the phylum level, B and D show the histogram and clustering heat map at the genus level, showing the Top15 phyla and genus respectively.

differ, suggesting that an increase in dietary ME level could significantly increase the TAA and EAA content. About flavoring amino acids, higher dietary ME levels increased the content of flavoring amino acids and the content of FSAA and BAA. However, we found that the proportion of EAA, FSAA, and BAA in TAA, with the increase of dietary ME level, FSAA content in the low ME group accounted for the largest proportion, and the smallest proportion of BAA, while the proportion of EAA showed a trend of rising first and then declining, indicating that a tasty chicken meat can be obtained at a moderate ME level.

Dietary CP levels affected the Glu content, which was one of the fresh flavor substances (Kurihara., 2009), and at the 14% CP level, BAA accounted for a relatively small proportion of the total FAA and a higher proportion of FSAA.

Fatty acids are important chemical substances that make up fats and are part of tissue cells that determine the physicochemical properties of fats and influence the flavor of meat (Diez-Simon et al., 2019; Zhan et al., 2022). Among them, UFA contribute considerably to human health and can inhibit the development of many diseases by increasing n−3

PUFA content of food (i.e., reducing the n−6/n−3 PUFA ratio) (Kouba et al., 2003; Theodoratou et al., 2007; Simopoulos., 2008). The FA composition of the bird can be directly regulated by nutritional level of the diet (Wood et al., 2004; Simopoulos, 2006; Wood et al., 2008; Kouba et al., 2011). Diet-regulated chicken meat can be an important source of PUFA (especially n−3 PUFA) for human health (Howe et al., 2006). Jahan et al. (2005) found a correlation between α-tocopherol, PUFA, ω-3 FA content and flavor abundance in cooked chicken meat, with PUFA and α-tocopherol content strongly correlated with total flavor abundance

This study showed that the dietary ME and CP levels affect the pectoral FA composition, especially PUFA. In the group with 11.51 MJ/kg ME and 14% CP, the TFA content was increased, which had an impact on each FA content, with a significant increase in PUFA content, which is a determinant of flavor. In contrast, the highest PUFA content group had higher values for both n−6 PUFA and n−3 PUFA, but also had the highest value for n−6/n−3, indicating that the increase in n−6 PUFA content was much higher than that of n−3 PUFA under this condition.

Table 8. Relative abundance of dietary ME and CP levels at the phylum level of the gut microbiota (%).

ME, MJ/kg	CP, %	Bacteroidetes	Firmicutes	Proteobacteria	Fusobacteria	Firmicutes/Bacteroidetes
11.31	14.0	69.18	11.85[b]	6.08[b]	9.17	0.166[b]
11.31	15.0	69.04	10.59[b]	7.15[ab]	9.05	0.150[b]
11.31	16.0	70.33	11.55[b]	14.90[a]	1.49	0.173[b]
11.51	14.0	60.10	31.42[a]	4.01[b]	1.12	1.029[a]
11.51	15.0	71.77	12.03[b]	6.10[b]	1.99	0.178[b]
11.51	16.0	73.35	12.45[b]	10.30[ab]	0.76	0.177[b]
11.71	14.0	79.25	11.01[b]	5.66[b]	1.16	0.142[b]
11.71	15.0	61.86	26.81[ab]	5.12[b]	2.52	0.543[ab]
11.71	16.0	56.05	19.74[ab]	12.83[ab]	2.28	0.362[ab]
SEM		2.41	1.93	0.921	1.23	0.095
ME	11.31	69.52	11.33	9.38	6.57	0.163
	11.51	68.41	18.63	6.80	1.29	0.461
	11.71	65.72	19.18	7.87	1.99	0.349
	P-value	0.809	0.199	0.521	0.177	0.454
CP	14.0	69.51	18.09	5.25[b]	3.82	0.446
	15.0	67.56	16.47	6.12[b]	4.52	0.29
	16.0	66.58	14.58	12.68[a]	1.51	0.237
	P-value	0.884	0.758	0.005	0.578	0.653
ME × CP	P-value	0.149	0.046	0.952	0.769	0.213

Data in the same column without letters or the same letter superscripts mean no significant difference ($P>0.05$), different letter superscripts mean significant difference ($P<0.01$ as highly significant, $P<0.05$ as significant).

In this study, the pectoral n−6 PUFA content increased and then decreased with increasing ME level, while the n−3 PUFA content continued to decrease. The reason may be due to the competition between n−3 PUFA and n−6 PUFA (Woods et al., 2009). Increasing n−3 long chain polyunsaturated fatty acid content did not increase n−6 long chain polyunsaturated fatty acid content at the same time (Schmitz and Ecker, 2008). In this study the healthiness of chicken meat may gradually decrease with the increasing nutrition level, while higher ME could increase the PUFA content of flavor substance. This further supports that higher flavor chicken meats can be obtained at moderate ME levels and CP level. Yang. (2001) studied the effects of dietary ME and CP levels on Hetian chickens and found that, when fed diets with high ME levels, the pectoral SFA and MFA contents increased significantly, while PUMA content decreased, and when fed diets with high CP level, the

PUFA content increased and the SFA and MUFA content decreased, which was similar to the results of this study. Yang (2001) found that the pectoral muscle had the highest PUFA content in the group with 11.93 MJ/kg ME and 14.5% CP (energy protein ratio was 0.823), while the group with 11.51 MJ/kg ME and 14% CP had the closest energy protein ratio (0.822) in this present study.

The diversity of the gut microbiota and the number of dominant microbiota are important to improve the immunity of the organism and improving intestinal health. In adult chickens, the phyla *Bacteroidetes, Firmicutes*, and *Proteobacteria* were found to be the dominant phyla in the gut microbiota (Oakley et al., 2014), Their abundance accounted for more than 90% of the total sequences (Wei et al., 2013), with *Firmicutes* accounting for 70%, *Bacteroidetes* 12.3%, and *Proteobacteria* 9.3%. This study showed that the dominant

Table 9. Relative abundance of dietary ME and CP levels at the genus level of the gut microbiota (%).

ME, MJ/kg	CP, %	Bacteroides	Rikenellaceae_RC9_gut_group	Fusobacterium	Parabacteroides
11.31	14.0	35.65[ab]	7.40	9.17	2.53[ab]
11.31	15.0	39.15[ab]	8.23	9.05	3.19[ab]
11.31	16.0	30.23[ab]	11.31	1.49	3.66[ab]
11.51	14.0	31.87[ab]	8.14	1.12	1.73[b]
11.51	15.0	43.65[a]	7.14	1.98	2.92[ab]
11.51	16.0	31.75[ab]	11.67	0.76	3.92[a]
11.71	14.0	42.28[ab]	8.44	1.16	3.24[ab]
11.71	15.0	29.57[ab]	10.15	2.52	3.02[ab]
11.71	16.0	27.64[b]	8.89	2.27	2.55[ab]
SEM		1.82	0.63	1.23	0.21
ME	11.31	35.01	8.98	6.57	3.13
	11.51	35.75	8.98	1.29	2.86
	11.71	33.16	9.16	1.98	2.94
	P-value	0.83	0.991	0.177	0.885
CP	14.0	36.60	7.99	3.82	2.50
	15.0	37.45	8.51	4.52	3.04
	16.0	29.87	10.62	1.51	3.38
	P-value	0.195	0.222	0.578	0.303
ME × CP	P-value	0.256	0.599	0.769	0.339

Data in the same column without letters or the same letter superscripts mean no significant difference ($P>0.05$), different letter superscripts mean significant difference ($P<0.01$ as highly significant, $P<0.05$ as significant).

phyla for each group was *Bacteroidetes, Firmicutes, Proteobacteria* and *Fusobacteria*, which was consistent with Oakley et al. (2014).

The gut microbiota consists mainly of two dominant phyla, *Firmicutes* and *Bacteroidetes* (Qin et al., 2010; Lei et al., 2022). *Bacteroidetes* are associated with carbohydrate metabolism in the body, breaking down sugars to produce volatile fatty acid, which are absorbed and utilized by the intestine (Lozupone et al., 2012; Johnson et al., 2015). *Firmicutes* help the host absorb energy from the diet, effectively promoting calorie absorption as well as weight gain (Ley et al., 2006; De Filippo et al., 2010; Krajmalnik et al., 2012; Zhang et al., 2020). The *Firmicutes/Bacteroidetes* (**F/B**) values are closely associated with obesity (De Bandt et al., 2011; Koliada et al., 2017; Zou et al., 2020). The ability of the *Bacteroidetes* to promote nutrient absorption and the *Firmicutes* to inhibit nutrient absorption in chicken intestines explains the relationship between *Bacteroidetes* and *Firmicutes* and growth (Islam et al., 2019; Magne et al., 2020). In this study, the abundance of *Bacteroidetes* continued to decrease with increasing dietary ME levels and CP levels, while the abundance of *Firmicutes* didn't increase, the F/B value increased relatively, and there was a tendency for the growing chickens to become obese. This suggests that a higher ME level and CP level can affect energy absorption by altering the F/B value in the gut microbiota, which was consistent with that in humans (Dong et al., 2020).

Proteobacteria are the largest phylum of bacteria and include many pathogenic bacteria (Rizzatti et al., 2017), whereas the abundance of the *Proteobacteria* is associated with obesity (Zhang et al., 2012; Zhu et al., 2013) and diabetes (Geurts et al., 2011; Karlsson et al., 2013). Its increased abundance often represents an imbalance in the composition of the gut microbiota (Shin et al., 2015) and brings about metabolic disorders (Fei et al., 2013; Suez et al., 2014). The increasing dietary CP level increase the abundance of *Proteobacteria* in this study. Experiments in the mouse also confirmed that with the increase of *Proteobacteria* abundance, the energy imbalance and unstable microbiota increased in the host (Shin et al., 2015). Majority of microorganisms in gut microbiota are obligate anaerobe, but members of the *Proteobacteria* are facultative anaerobe. This unique oxygen requirement of the *Proteobacteria* may affect the relationship between the abundance of the *Proteobacteria* and the oxygen homeostasis or concentration in the gut microbiota (Litvak et al., 2017). In contrast, *Proteobacteria* is more abundant in the gut of dogs and cats fed a high-protein diets (Moon et al., 2018). Therefore, excessive dietary CP may lead to a certain degree of imbalance in the gut microbiota, which in turn may affect health.

Bacteroides promote the maturation of the immune system, but also facilitate the absorption of UFA, accelerate fat deposition and regulate the structure and function of gut microbiota through nutritional intervention. This explains why *Bacteroides* is the dominant genus (Oh et al., 2016). The present study found that the relative abundance of the *Rikenellaceae_RC9_gut_group* increased with increasing ME and CP levels, which is consistent with Ahmad et al. (2020) on rumen microbial diversity in yaks. The relative abundance of *Fusobacterium* tended to decrease with increasing ME and CP levels, whereas the abundance of *Parabacteroides* indicated a gradual increase with increasing dietary CP level, and a decreasing trend with increasing dietary ME level. The *Parabacteroides* and its strains have been shown to be negatively associated with obesity, and high protein diets could improve lipid metabolism and have a preventive effect on obesity brought about by diet (Wu et al., 2019).

The stability and dynamic balance of the gut microbiota is important for disease resistance and nutrient uptake in animals (Annuk et al., 2010), and the diversity of gut microbiota plays an important role in animal health (Diaz Carrasco et al., 2019; Chen et al., 2021). Dietary CP level plays an important role in regulating microbial diversity (Ma et al., 2017), with different CP levels can change diversity of gut microbiota, and the composition of animal gut microbiota becomes simpler under low level protein diet (Cho et al., 2015). In this study, the diversity and relative abundance of gut microbial tended to decrease as dietary ME level increased, while an increase in dietary CP level led to a gradual increase in gut microbial diversity and relative abundance.

CONCLUSION

a. Dietary ME level significantly affected mortality rate, the mortality rate of 11.31 MJ/kg group was the highest.

b. The 11.31 MJ/kg ME significantly increased the proportion of pectoral FSAA and decreased the proportion of BAA. Dietary CP level significantly affected glutamate content, the BAA to TAA ratio was relatively small and FSAA ratio was higher at the 14% CP level.

c. The 16% CP level increased gut microbiota diversity and relative abundance, but at the same time increased *Proteobacteria* abundance, which may result in unstable gut microbiota and metabolic disturbances.

d. The present study suggested that the medium dietary ME (11.51 MJ/kg) and low CP (14−15%) levels can be helpful for enhancing pectoral muscle composition, increase meat quality such as flavor and nutritional value, and benefit for gut microbiota in native growing chickens.

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DISCLOSURES

The authors declared that we have no conflicts of interest to this work.

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