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RESEARCH PAPER

Evaluation of the Intestinal Bacterial Community of Local Omani and Cobb 500 Broiler Chickens Raised in an Open-Sided House Using 16S rDNA-Based Analysis

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تقييم المجتمع البكتيري المعوي للدجاج العماني المحلي ودجاج الكوب 500 اللاحم الذي تم تربيته في منزل مفتوح باستخدام التحليل القائم على 16S rDNA

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Abstract. Little is known about how the intestinal bacterial microbiota differs among different strains of chickens raised in an open sided house, predominantly those with lower growth rates, such as Indigenous chickens. Ninety-one-day-old chicks of each strain of chickens were raised in an open-sided house system and fed a conventional corn-soybean meal diet from Day 0–35 days of age. The objective of this study was to assess the relative abundance of bacteria microbiota identified in the intestinal tract of local Omani and Cobb 500 broiler chickens raised in an open-sided house system using 16S rDNA-based analysis. The results obtained showed the diversity of bacterial populations in different intestinal regions of two chicken strains. Bacilli were found in higher numbers and reached 98.8% of the bacteria in the duodenum on Day 5 in Cobb 500 versus 72.5% in the Omani chickens. Local Omani chickens had significantly higher numbers of Clostridia at an early age period. On Day 5 Clostridia comprised 13.1% of the bacteria in the duodenum of local Omani chickens, versus only 0.062% in the Cobb 500. The relative abundance of the bacterial microbiota differed significantly ($p < 0.05$) across different intestinal segments of the two strains of chickens, suggesting that each region generated its bacterial community with different relative abundances.

Keywords: Omani, chicken, 16S rDNA, intestine, microbiota, house.

الملخص: لا يعرف سوى القليل عن كيفية اختلاف الميكروبات البكتيرية المعوية بين سلالات مختلفة من الدجاج التي تربى في منزل مفتوح ، في الغالب تلك التي لديها معدلات نمو أقل ، مثل الدجاج المحلي. تم تربية واحدا وتسعين من الكتاكيت البالغة من العمر يوما من كل سلالة من الدجاج في نظام منزل مفتوح الجانب وتغذية نظام غذائي تقليدي لوجبة الذرة وفول الصويا من اليوم 0–35 يوما من العمر. كان الهدف من هذه الدراسة هو تقييم الوفرة النسبية للميكروبات البكتيرية التي تم تحديدها في الأمعاء للدجاج العماني المحلي ودجاج Cobb 500 اللاحم الذي تم تربيته في نظام منزل مفتوح الجانب باستخدام التحليل القائم على 16S rDNA. أظهرت النتائج التي تم الحصول عليها تنوع المجموعات البكتيرية في مناطق معوية مختلفة من سلالتين من الدجاج. تم العثور على العصيات بأعداد أكبر ووصلت إلى 98.8٪ من البكتيريا في الاثني عشر في اليوم 5 في كوب 500 مقابل 72.5٪ في الدجاج العماني. كان لدى الدجاج العماني المحلي أعداد أكبر بكثير من كلوستريديا في فترة مبكرة من العمر. في اليوم الخامس ، شكلت كلوستريديا 13.1٪ من البكتيريا في الاثني عشر من الدجاج العماني المحلي ، مقابل 0.062٪ فقط في Cobb 500. اختلفت الوفرة النسبية للميكروبات البكتيرية اختلافا كبيرا ($p < 0.05$) عبر شرائح معوية مختلفة من سلالتين من الدجاج ، مما يشير إلى أن كل منطقة لديها مجتمعها البكتيري الخاص بها بوفرة نسبية مختلفة.

الكلمات المفتاحية: عماني، دجاج، 16S rDNA، أمعاء، ميكروبيوتا، منزل.

Introduction

Local chicken production has contributed significantly as sources of protein, food security and source of income in communities with limited resources (Al-Jumaili et al., 2020). Local chicken production is among the farming activities in the rural communities of Oman that provides opportunities for food security and income for many rural families (MAF, 2013). The Cobb 500 broiler chickens are the world's foremost broiler breed, adapting well to warmer weathers; has the best growth rate and an ability to thrive on low density and less costly diets (Dessie et al., 2017). However, Cobb 500 broiler chickens has become very popular in Oman where feed costs are inflated saving poultry farmers a fortune. An improvement in local chicken productivity would be highly valuable in the enhancement of the socioeconomic and nutritional status of farmers. Several studies have shown the beneficial effect of the microbial community in the gastrointestinal tract of the host and their important contributions in many roles such as in nutrient absorption, feed digestion, and immune system (Gong et al., 2002).

Chickens' gastrointestinal (GI) tracts are home to a rich and complex microbiota that aids in digestion and nutrition absorption, as well as immune system development and pathogen exclusion (Shang et al., 2018). The symbiotic relationship between the host and the microbiota is critical for the health and production of chickens. The age of the chickens and their position in the digestive tract have a big impact on the diversity of their gut

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microbiota. Normal gut morphology and integrity are important in the maintenance of intestinal microbial homeostasis in the prevention of infection and promoting digestion and absorption of nutrients (Jimoh et al., 2017).

Evaluation of the microbial diversity and intestinal development of different strains of chickens has become widely recognized (Glendinning et al., 2019; Richards-Rios et al., 2020). However, there is a limited information about the effect of house type on the composition and succession of intestinal microbial communities in birds raised in open-sided housing systems. The majority of small and medium scale farmers in Middle East countries, such as Oman uses an open-sided housing system to grow their chickens. A greater understanding of the chicken gut function and microbiology can provide an opportunity for the improvement of chicken's health and production especially those with various growth rates, such as the local Omani chickens.

The use of modern high-throughput sequencing approaches, that involve analyzing the structure of bacterial communities by determining the characteristic features of the microbial DNA extracted from the community samples, are a powerful tool that has led to important new insights into the biological and ecological roles of the GI Microbiota (Shang et al., 2018; McLaren et al., 2019). The objective of this study was to assess the relative abundance of bacteria microbiota identified in the intestinal tract of local Omani and Cobb 500 broiler chickens raised in an open-sided house system from 0 to 35 days of age using 16S rDNA-based analysis.

Materials and Methods

Ethical Approval

All experimental work was conducted at the Poultry Research Unit at the Agricultural Experiment Station in accordance with the experimental unit policy on animal welfare and the requirements of the procedures involving animals/birds and their care were conducted in conformity with international laws and policies at Sultan Qaboos University.

Birds Housing, Diets and Sample Collection

One hundred and fifty 1-day-old chicks of two strains of chickens: local Omani and Cobb 500 broiler chicken were obtained from commercial hatcheries at Barka. On arrival, all chicks were scrutinized to ensure that they were free of abnormalities and early signs of sickness. Before the trial, open-sided house unit, cages, feeders, and drinkers were disinfected through fumigation. In addition, strict hygiene and biosecurity measurements were implemented. The open-sided house was built from a galvanized iron shed with profiled steel shed roofing which was naturally ventilated. Chicken mesh panels were put on all sides, along with one-meter-high block work protection. To assist circulate the air, four sets of electric wall fans were used. Shade cloth were used to screen direct sunrays during midday. There were 15 replicates for each strain of chicken with each replicate cage containing six birds (a total of 90 birds/strain). Birds per replicate combinations were randomly allocated. Chicks of both strains were fed a nonmedicated conventional corn-soybean meal diet from Day 0–35 days of age. The composition of experimental diet is as described by Al-Marzooqi et al. (2019). Feed was available ad libitum. The house temperature maintained at 33 °C on day 1 and reduced by 3 °C each week to reach a constant 22 °C. The lighting program was 23L: 1D.

At 5, 15, 25, and 35 days of age, one bird per cage for each strain of chicken was randomly selected. Birds with the body weight closest to the average from each cage were selected, marked, and kept in their cage until being euthanized. Then, the selected bird was injected with a mixed dose of ketamine 10% and xylazine 20% intramuscularly to put the bird into a deep sedation and anaesthesia. After dissection, intestinal tracts were removed from the carcasses immediately and luminal contents of the duodenum, jejunum, ileum and cecum were aseptically collected into a labeled sterile 15-mL tube. The entire process of collecting intestinal contents was performed on a thoroughly cleaned workbench and required less than 30 min. Samples were placed on ice and immediately transported to the laboratory and stored in −80 °C freezer until analysis.

DNA Extraction, 16S rDNA Gene Amplicon Production and High-Throughput Sequencing

Total DNA was extracted from contents of each luminal content samples (duodenum, jejunum, ileum, and cecum) using a QIAamp DNA Stool Mini Kit (QIAGEN, CA, Hamburg, Germany) according to the manufacturer's instructions. The DNA concentration was evaluated by measuring optical density using Nano-Drop 2000 (Thermo Electron Corporation, Waltham, MA, USA) at a wavelength of 260 and 280 nm. The integrity of the DNA extracts was assessed visually using 1.0% agarose gel (containing ethidium bromide) electerophoresis.

The variable regions V3-V4 of the 16S rDNA gene were amplified and sequenced. The PCRs were performed in triplicate in a total volume of 20 µL containing 5 µM of each primer, 10 ng of DNA template, 4 µL 1× FastPfu buffer, 2.5 mM dNTPs, and 0.4 µL of FastPfu polymerase (TransGen Biotech, Beijing, China). PCR conditions were as follows: Initial denaturation at 95 °C for 2 min; followed by 25 cycles of denaturation 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s and then, a final extension at 72 °C for 5 min. PCR products were separated on 2% agarose gels, and purified using the DNA gel extraction kit (Axygen Scientific Inc., Union City, CA, USA). Amplicons produced form different intestinal luminal content samples were sent to a commercial company (BGI Genomic Lab, Tai Po Industrial Zone, New Territories, Hong Kong, China) for sequencing on the Illumina MiSequencing platform.

Sequencing Analysis

All the raw sequences obtained from Illumina Miseq were firstly filtered for quality control to get operational sequences. The quality control and analysis of the sequences were performed using the software Quantitative Insights into Microbial Ecology (QIIME, v1.8.0) (Caporaso et al., 2010). The paired-end reads from the DNA fragments were merged using FLASH (Magoc and Salzberg, 2011). Sequences data was treated by read trimming and identification of V3-V4 sequences and set of sequences with ≥97% identity were defined as an operational taxonomic unit (OTU). The UCLUST (Edgar, 2010), clustering method was used to cluster operational taxonomic units. The defined OTUs were assigned to different taxonomic levels (phylum, class, genus and families) at a cutoff of 97%. The clustered OTUs were also used to construct the rarefaction curves and calculate the Shannon and Simpson diversity indices, abundance-based coverage estimators (ACE), Chao 1 richness, and coverage percentage by Good's method.

Bioinformatics and Statistical Analysis

Bioinformatics and statistical analyses were performed using the QIIME and R package (v3.1.1). The alpha-diversity indices (Chaol, ACE, Shannon diversity index, and Simpson index) were calculated to establish the relative abundance and diversity of the sequences. Beta diversity was determined using unweighted Unifrac distance metrics to evaluate the structure and distribution of the microbial genetic communities among the samples. Differences in the Unifrac distances for pairwise comparisons among groups were calculated using Student's t-test and the Monte Carlo permutation test with 1000 permutations. Metastats and R package (v3.1.1) (James et al., 2009) were used to compare and determine which taxonomic groups were significantly different between groups of samples based on intestinal segments and age period. The differences were considered to be significant at $p < 0.05$. The obtained p-value was adjusted by a Benjamini–Hochberg false discovery rate correction (Function 'p.adjust' in the stats package of R (v3.1.1)).

Results

Bacterial Taxonomic Composition of Duodenum

Bacteria were classified according to their respective Phylum and Class, found in the duodenum of broiler chickens at different age periods, are presented in Table 1. Seventeen bacterial florae at the class level were detected in the duodenum. Of the 30248 detected sequences, the most abundant Class was Bacilli, at 93.4 % of the total sequences. Clostridia accounted for 2.9 % out of the total detected sequences. Actinobacteria and Proteobacteria sequences represent 1.01 % and 0.694 %, respectively of the total sequences. Across different age periods Bacilli were considered the dominant group with 98.9 % at Day 5, 94.6 % at Day 15, 89.9 % at Day 25 and 88.7 % at Day 35 of sequences. Clostridia sequences fluctuated from 0.062 % at Day 5, 1.78 % at Day 15, 4.61% at Day 25, and 6.00 % at Day 35. Chloroplast were detected at very small percentage across all age periods except at Day 25 of age was detected at 3.39 %. Both Actinobacteria and Proteobacteria group-related sequences were detected at smaller percentage across all age periods.

Bacterial Taxonomic Composition of the Jejunum

Bacteria were classified according to their respective Phylum and Class, found in the jejunum of broiler chickens at different ages are presented in Table 2. Seventeen bacterial florae at the Class level were detected in the jejunum. Of the 28678 reads, Bacilli were the most abundant, at 79.6 % of the total sequences. Clostridia accounted for 7.61% of the total sequences. Actinobacteria and Proteobacteria sequences represented 4.46 % and 4.44%, respectively of the total sequences. Across different age periods Bacilli were the dominant group, representing 86.4 % at Day 5, 93.3 % at Day 15, 91.5 % at Day 25 and 40.1 % at Day 35 of sequences. Clostridia sequences varied from Day 5: 4.20%, Day 15: 4.33%, Day 25: 0.859% and Day 35: 23.60%. Actinobacteria sequences were Day 5: 2.33%, Day 15: 0.318%, Day 25: 0.109%, and Day 35: 17.4%, while sequences related to Gammaproteobacteria were Day 5: 0.015%, Day 15: 0.625%, Day 25: 4.94%, and Day 35: 12.0%.

Bacterial Taxonomic Composition of the Ileum

Bacteria were classified according to their respective Phylum and Class, found in the ileum of broiler chickens at different ages are presented in Table 3. Seventeen bacterial florae at the Class level were detected in the ileum. Of the 31007 reads, Bacilli were the most abundant, at 96.1 % of the total sequences. Only few of Clostridia (0.239%) related to the total sequences were detected. Actinobacteria and Proteobacteria represented a very small percentage of 0.893% and 0.232%, respectively, of the total sequences. Across different age periods Bacilli were the dominant group, representing 91.1 % at Day 5, 99.0 % at Day 15, 99.6 % at Day 25 and 95.2 % at Day 35 of sequences. Clostridia sequences fluctuated from 0.565% at Day 5, 0.140% at Day 15, 0.107% at Day 25, and 0.050% at Day 35. Proteobacteria group-related sequences were detected at smaller percentages across all age periods.

Bacterial Taxonomic Composition of the Cecum

Bacteria were classified according to their respective Phylum and Class, found in the cecum of broiler chickens at different ages are presented in Table 4. Seventeen bacterial florae at the Class level were detected in the cecum. Of the 28035 reads, Clostridia were the most abundant, at 63.4 % of the total sequences. Bacilli were detected at 13.1% of the total sequences. Actinobacteria and Proteobacteria sequences represented 6.01% and

Table 1. Abundance of bacterial 16S rDNA sequences (n=30248) identified from the duodenum flora of 500 Cobb broiler chicken.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	43 (0.531)	191 (2.32)	46 (0.531)	0 (0)
	Coriobacteriia	0 (0)	3 (0.036)	0 (0)	15 (0.285)
	Thermoleophilia	0 (0)	0 (0)	0 (0)	0 (0)
Bacteroidetes	Bacteroidia	0 (0)	0 (0)	0 (0)	8 (0.152)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	2 (0.023)	175 (3.32)
	Chloroplast	34 (0.420)	20 (0.243)	293 (3.39)	0 (0)
Firmicutes	Bacilli	8005 (98.9)	7777 (94.6)	7785 (89.9)	4675 (88.7)
	Clostridia	5 (0.062)	146 (1.78)	399 (4.61)	316 (6.00)
	Erysipelotrichi	0 (0)	2 (0.024)	0 (0)	42 (0.797)
Proteobacteria	Alphaproteobacteria	5 (0.062)	36 (0.438)	25 (0.289)	0 (0)
	Betaproteobacteria	1 (0.012)	27 (0.328)	6 (0.069)	0 (0)
	Deltaproteobacteria	0 (0)	0 (0)	0 (0)	1 (0.019)
	Epsilonproteobacteria	3 (0.037)	11 (0.134)	84 (0.970)	0 (0)
	Gammaproteobacteria	0 (0)	5 (0.061)	6 (0.069)	0 (0)
Tenericutes	Mollicutes	0 (0)	3 (0.036)	0 (0)	32 (0.607)
Thermi	Deinococci	1 (0.012)	3 (0.036)	11 (0.127)	0 (0)
	Other	0 (0)	0 (0)	0 (0)	6 (0.114)
Total		8097	8224	8657	5270

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

1.75%, respectively of the total sequences. Across different age periods Clostridia were the dominant group, representing 40.8% at Day 5, 73.5% at Day 15, 75.0% at Day 25 to 62.6% at Day 35 of the sequences. Bacilli sequences fluctuated from 16.3% at Day 5, 21.0% at Day 15, 11.8% at Day 25, and 2.32% at Day 35. Actinobacteria sequences were Day 5: 22.0%, Day 15: 0.809%, Day 25: 1.18%, and Day 35: 0.0%. Proteobacteria group-related sequences were detected at smaller percentages across age periods.

Table 2. Abundance of bacterial 16S rDNA sequences (n=28678) identified from the jejunum flora of Cob 500 broiler chickens.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	154 (2.33)	27 (0.318)	8 (0.109)	1090 (17.4)
	Coriobacteriia	0 (0)	0 (0)	0 (0)	0 (0)
	Thermoleophilia	0 (0)	0 (0)	0 (0)	0 (0)
Bacteroidetes	Bacteroidia	0 (0)	0 (0)	0 (0)	0 (0)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	0 (0)	0 (0)
	Chloroplast	427 (6.45)	64 (0.755)	175 (2.39)	0 (0)
Firmicutes	Bacilli	5718 (86.4)	7906 (93.3)	6705 (91.5)	2504 (40.1)
	Clostridia	278 (4.20)	367 (4.33)	63 (0.859)	1475 (23.6)
	Erysipelotrichi	0 (0)	13 (0.153)	1 (0.014)	429 (6.87)
Proteobacteria	Alphaproteobacteria	38 (0.574)	38 (0.448)	16 (0.218)	0 (0)
	Betaproteobacteria	5 (0.076)	10 (0.118)	0 (0)	0 (0)
	Deltaproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Epsilonproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Gammaproteobacteria	1 (0.015)	53 (0.625)	362 (4.94)	751 (12.0)
Tenericutes	Mollicutes	0 (0)	0 (0)	0 (0)	0 (0)
Thermi	Deinococci	0 (0)	0 (0)	0 (0)	0 (0)
	Other	0 (0)	0 (0)	0 (0)	0 (0)
Total		8584	7872	7582	7217

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

Table 3. Abundance of bacterial 16S rDNA sequences (n=31007) identified from the ileum flora of Cobb 500 broiler chicken.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	220 (2.44)	56 (0.655)	1 (0.013)	0 (0)
	Coriobacteriia	0 (0)	0 (0)	0 (0)	87 (1.46)
Bacteroidetes	Thermoleophilia	0 (0)	0 (0)	0 (0)	0 (0)
	Bacteroidia	3 (0.033)	2 (0.023)	0 (0)	40 (0.673)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	0 (0)	7 (0.118)
	Chloroplast	466 (5.16)	7 (0.082)	17 (0.227)	0 (0)
Firmicutes	Bacilli	8222 (91.1)	8458 (99.0)	7467 (99.6)	5659 (95.2)
	Clostridia	51 (0.565)	12 (0.140)	8 (0.107)	3 (0.050)
	Erysipelotrichi	1 (0.011)	0 (0)	0 (0)	137 (2.30)
Proteobacteria	Alphaproteobacteria	55 (0.610)	5 (0.059)	1 (0.013)	0 (0)
	Betaproteobacteria	4 (0.044)	3 (0.035)	0 (0)	0 (0)
	Deltaproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Epsilonproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Gammaproteobacteria	1 (0.011)	1 (0.012)	0 (0)	2 (0.034)
Tenericutes	Mollicutes	0 (0)	0 (0)	0 (0)	11 (0.185)
Thermi	Deinococci	0 (0)	0 (0)	0 (0)	0 (0)
	Other	0 (0)	0 (0)	0 (0)	0 (0)
Total		9023	8544	7494	5946

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

Differences of Bacterial Communities among Samples from Different Intestinal Segments

The p-value distribution of 16S rDNA gene sequence libraries used to compare the quantitative differences of microbial communities among samples from different intestinal segments of broiler chickens are presented in Table 5. Statistical comparisons of the libraries showed that the composition of the Duodenum-Jejunum, Duodenum-Ileum, Cecum-Duodenum, Cecum-Ileum Cecum-Jejunum bacterial microbiota differed significantly ($p < 0.05$), implying that each region established its own bacterial community. The number of Actinobacteria, Alphaproteobacteria, Bacilli, Bacteroidia, Betaproteo-

Table 4. Abundance of bacterial 16S rDNA sequences (n=28035) identified from the cecum flora of Cobb 500 broiler chicken.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	1535 (22.0)	57 (0.809)	93 (1.18)	0 (0)
	Coriobacteriia	25 (0.359)	21 (0.298)	0 (0)	114 (1.85)
	Thermoleophilia	1 (0.014)	2 (0.028)	0 (0)	0 (0)
Bacteroidetes	Bacteroidia	35 (0.502)	32 (0.454)	2 (0.025)	2010 (32.65)
Cyanobacteria	4C0d-2	12 (0.172)	13 (0.185)	0 (0)	27 (0.439)
	Chloroplast	1103 (15.8)	92 (1.31)	855 (10.9)	0 (0)
Firmicutes	Bacilli	1134 (16.3)	1480 (21.0)	925 (11.8)	143 (2.32)
	Clostridia	2839 (40.8)	5177 (73.5)	5902 (75.0)	3851 (62.6)
	Erysipelotrichi	24 (0.344)	24 (0.341)	2 (0.025)	6 (0.097)
Proteobacteria	Alphaproteobacteria	165 (2.37)	63 (0.895)	83 (1.05)	0 (0)
	Betaproteobacteria	78 (1.12)	58 (0.824)	5 (0.064)	0 (0)
	Deltaproteobacteria	0 (0)	1 (0.014)	0 (0)	5 (0.081)
	Epsilonproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Gammaproteobacteria	11 (0.158)	22 (0.312)	1 (0.013)	0 (0)
Tenericutes	Mollicutes	0 (0)	1 (0.014)	0 (0)	1 (0.016)
Thermi	Deinococci	5 (0.072)	0 (0)	0 (0)	0 (0)
	Other	0 (0)	0 (0)	0 (0)	0 (0)
Total		6967	7043	7868	6157

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

Table 5. P-value distribution of 16S rDNA gene sequence libraries compared the abundance differences of microbial communities among samples from different segments for cobb 500 broiler chicken.

Class	P-Value					
	Duodenum-Jejunum	Duodenum-Ileum	Jejunum-Ileum	Caecum-Duodenum	Caecum-Jejunum	Caecum-Ileum
4C0d-2	0.270	0.581	0.447	0.507	0.415	0.463
Actinobacteria	0.921	0.797	0.682	0.029	0.015	0.052
Alphaproteobacteria	0.575	0.724	0.745	0.303	0.025	0.108
Bacilli	0.901	0.673	0.630	0.000	0.000	0.000
Bacteroidia	0.026	0.051	1.000	0.011	0.007	0.029
Betaproteobacteria	0.191	0.153	0.879	0.018	0.042	0.005
Chloroplast	0.327	0.203	0.737	0.218	0.142	0.083
Clostridia	0.512	0.826	0.543	0.002	0.002	0.002
Coriobacteriia	0.828	0.542	0.514	0.058	0.071	0.070
Deltaproteobacteria	0.501	0.778	0.322	0.192	0.183	0.285
Epsilonproteobacteria	0.479	0.289	0.684	0.270	0.600	0.766
Erysipelotrichi	0.726	0.108	0.603	0.003	0.004	0.003
Flavobacteriia	0.233		0.236		0.501	
Gammaproteobacteria	0.699	0.427	0.405	0.069	0.096	0.362
Lentisphaeria				0.096	0.107	0.098
Mollicutes	0.124	0.124		0.146		
Sphingobacteriia	0.328	0.614	1	0.403		1
Verrucomicrobiae	0.483		0.485	0.074	0.103	0.117

bacteria, Clostridia and Erysipelotrichia differed significantly across different intestinal segments ($p < 0.05$). Bacilli were the dominant 16S rDNA sequences in the duodenum, jejunum, and ileum libraries, whereas Clostridia were the dominant 16S rDNA sequences in the cecum libraries.

Table 6. P-value distribution of 16S rDNA gene sequence libraries compared the abundance differences of microbial communities among sample from different ages period for Cobb 500 broiler chickens.

Class	P-Value					
	Day 5-Day 15	Day 5-Day 25	Day 5 - Day 35	Day 15-Day 25	Day 15-Day35	Day 25-Day 35
4C0d-2		0.247	0.198	0.226	0.199	0.191
Actinobacteria	0.005	0.046	0.004	0.016	0.748	0.012
Alphaproteobacteria	0.054	0.235	0.036	0.112	0.178	0.304
Bacilli	0.678	0.924	0.914	0.669	0.695	0.913
Bacteroidia	0.299	0.317	0.344	0.750	0.738	0.678
Betaproteobacteria	0.165	0.158	0.051	0.614	0.532	0.922
Chloroplast	0.027	0.746	0.283	0.031	0.147	0.464
Clostridia	0.871	0.875	0.854	0.848	0.806	0.856
Coriobacteriia	0.130	0.226	0.290	0.733	0.659	0.556
Deltaproteobacteria	1	0.288	0.171	0.342	0.248	0.660
Epsilonproteobacteria		0.035	0.069	0.043	0.080	0.193
Erysipelotrichi	0.592	0.758	0.511	0.652	0.907	0.648
Flavobacteriia	0.501			0.501	0.501	
Gammaproteobacteria	0.123	0.621	0.111	0.556	0.217	0.332
Lentisphaeria		0.497	0.249	0.475	0.228	1
Mollicutes	0.342	0.347	0.676		0.369	0.347
Sphingobacteriia	0.342		0.499	0.361	0.561	1.000
Verrucomicrobiae		0.347	0.299	0.361	0.337	0.760

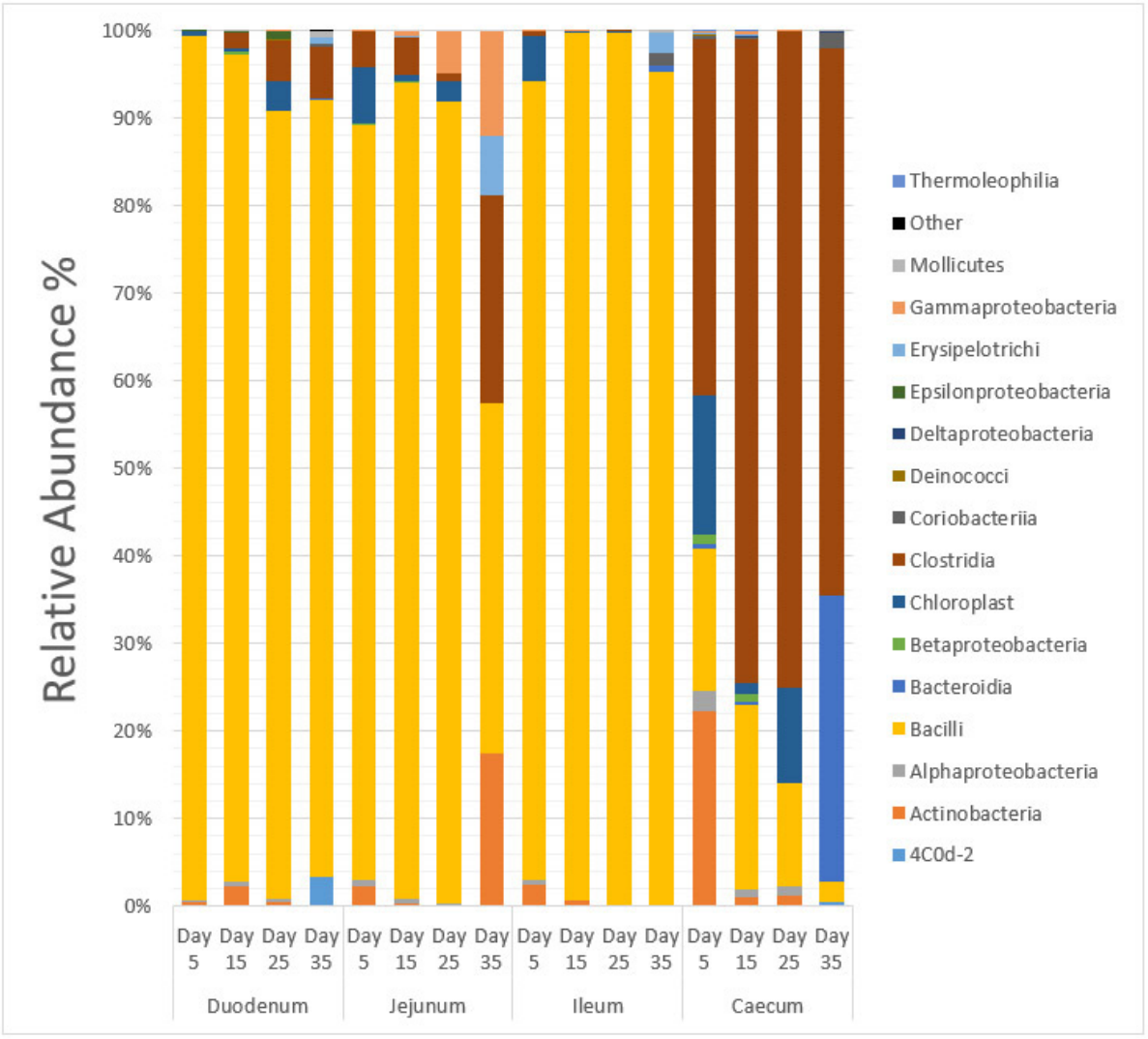

Figure 3. Percentage of relative abundance of bacterial community of Cobb 500 broiler chickens determined from different intestinal segments at different age periods from 16S rDNA libraries.

Differences of Microbial Communities among Samples

The p-value distribution of 16S rDNA gene sequence libraries comparing the quantitative differences of microbial communities among samples from broiler chickens at different age groups are presented in Table 6. Statistical comparisons of the libraries revealed that there were no significant differences ($p > 0.05$) between the microbial compositions at different age groups: Day 5–15, Day 5–25, Day 5–35, Day 15–25, Day 15–35, and Day 25–35. The results of the statistical evaluation at certain age groups revealed that the percentage of bacterial microbiota of Actinobacteria, Alphaproteobacteria, Chloroplast and Epsilonproteobacteria varied significantly ($p< 0.05$). The average percentage of Actinobacteria detected at Day 5 (6.83%) of age was significantly higher ($p < 0.05$) than at Day 15 (1.03%), at Day 25 (0.458%) and at Day 35 (4.35%) of age. The average percentage of Alphaproteobacteria was detected at significantly higher level ($p < 0.05$) at Day 35 (0.904%) of age than at Day 5 (0.458%) of age. The average percentage of Chloroplast detected at Day 15 (0.598%) was significantly lower ($p< 0.05$) than at Day 25 (4.23%) of age. The average percentage of Epsilonproteobacteria at Day 25 (0.243%) was significantly higher ($p < 0.05$) from those of the other age groups.

Taxonomic Composition Distribution of the Bacterial Community in Intestinal Segments

Percentage of relative abundance of bacterial community of Cobb 500 broiler chickens determined from dif-

Table 7. Abundance of bacterial 16S rDNA sequences (n=33443) identified from the duodenum flora of local Omani chickens.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	213 (2.55)	0 (0)	21 (0.235)	144 (1.82)
	Coriobacteriia	6 (0.072)	0 (0)	2 (0.022)	0 (0)
Bacteroidetes	Bacteroidia	2 (0.024)	0 (0)	2 (0.022)	3 (0.038)
	Flavobacteriia	0 (0)	0 (0)	0 (0)	0 (0)
	Sphingobacteriia	4 (0.048)	0 (0)	0 (0)	0 (0)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	2 (0.022)	5 (0.063)
	Chloroplast	502 (6.00)	3 (0.037)	23 (0.257)	50 (0.630)
Firmicutes	Bacilli	6065(72.5)	8023 (97.7)	8667 (97.0)	7618 (96.1)
	Clostridia	1096 (13.1)	150 (1.83)	121 (1.35)	29 (0.366)
	Erysipelotrichi	6 (0.072)	10 (0.122)	2 (0.022)	1 (0.013)
Lentisphaerae	Lentisphaeria	0 (0)	0 (0)	0 (0)	0 (0)
Proteobacteria	Alphaproteobacteria	419 (5.01)	4 (0.049)	11 (0.123)	15 (0.189)
	Betaproteobacteria	39 (0.466)	2 (0.024)	11 (0.123)	12 (0.151)
	Deltaproteobacteria	1 (0.012)	0 (0)	1 (0.011)	0 (0)
	Epsilonproteobacteria	0 (0)	0 (0)	71 (0.795)	15 (0.189)
	Gammaproteobacteria	9 (0.108)	8 (0.097)	1 (0.011)	0 (0)
Tenericutes	Mollicutes	0 (0)	15 (0.183)	0 (0)	39 (0.492)
Thermi	Verrucomicrobiae	0 (0)	0 (0)	0 (0)	0 (0)
Total		8362	8215	8935	7931

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

ferent intestinal segments at different age periods from 16S rDNA libraries are presented in Figure 1. From Figure 1, it can be seen that the diversity of the bacterial community in the intestinal segments of broiler chickens changed from one age period to the next. Species that exhibited an abundance less than 0.5% in all sam-

Table 8. Abundance of bacterial 16S rDNA sequences (n=31255) identified from the jejunum flora of local Omani chickens.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	200 (2.33)	2 (0.025)	49 (0.646)	109 (1.51)
	Coriobacteriia	4 (0.047)	0 (0)	1 (0.013)	1 (0.014)
Bacteroidetes	Bacteroidia	1 (0.012)	0 (0)	0 (0)	0 (0)
	Flavobacteriia	2 (0.023)	0 (0)	0 (0)	0 (0)
	Sphingobacteriia	0 (0)	0 (0)	0 (0)	0 (0)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	0 (0)	1 (0.014)
	Chloroplast	1578 (18.4)	18 (0.229)	268 (3.53)	178 (2.47)
Firmicutes	Bacilli	6484 (75.5)	7616 (96.8)	7092 (93.5)	6889 (95.5)
	Clostridia	215 (2.50)	225 (2.86)	101 (1.33)	10 (0.139)
	Erysipelotrichi	3(0.035)	9 (0.114)	0 (0)	0 (0)
Lentisphaerae	Lentisphaeria	0 (0)	0 (0)	0 (0)	0 (0)
Proteobacteria	Alphaproteobacteria	92 (1.07)	1 (0.013)	29(0.382)	25 (0.346)
	Betaproteobacteria	4 (0.047)	0	11 (0.145)	3 (0.042)
	Deltaproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Epsilonproteobacteria	0 (0)	0 (0)	23 (0.303)	0 (0)
	Gammaproteobacteria	1 (0.012)	1 (0.013)	8 (0.106)	0 (0)
Tenericutes	Mollicutes	0 (0)	0 (0)	0 (0)	0 (0)
Thermi	Verrucomicrobiae	0 (0)	0 (0)	0 (0)	1 (0.014)
Total		8584	7872	7582	7217

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

Table 9. Abundance of bacterial 16S rDNA sequences (n=31337) identified from the ileum flora of local Omani chicken.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	116 (1.29)	2 (0.026)	12 (0.155)	147 (1.72)
	Coriobacteriia	1 (0.011)	0 (0)	0 (0)	2 (0.023)
Bacteroidetes	Bacteroidia	1 (0.011)	0 (0)	0 (0)	1 (0.012)
	Flavobacteriia	0 (0)	0 (0)	0 (0)	0 (0)
	Sphingobacteriia	0 (0)	0 (0)	0 (0)	1 (0.012)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	0 (0)	33 (0.387)
	Chloroplast	2170 (24.1)	192 (2.49)	16 (0.206)	768 (9.00)
Firmicutes	Bacilli	6531 (72.5)	6814 (87.0)	7674 (98.9)	7090 (83.1)
	Clostridia	26 (0.289)	471 (6.01)	57 (0.734)	398 (4.66)
	Erysipelotrichi	3 (0.033)	1 (0.013)	0 (0)	2 (0.023)
Lentisphaerae	Lentisphaeria	0 (0)	0 (0)	0 (0)	0 (0)
Proteobacteria	Alphaproteobacteria	144 (1.60)	3 (0.038)	0 (0)	70 (0.820)
	Betaproteobacteria	9 (0.100)	2 (0.026)	1 (0.013)	7 (0.082)
	Deltaproteobacteria	0 (0)	0 (0)	0 (0)	4 (0.047)
	Epsilonproteobacteria	0 (0)	0 (0)	3 (0.039)	9 (0.105)
	Gammaproteobacteria	2 (0.022)	349 (4.45)	0 (0)	5 (0.059)
Tenericutes	Mollicutes	0 (0)	0 (0)	0 (0)	0 (0)
Thermi	Verrucomicrobiae	0 (0)	0 (0)	0 (0)	0 (0)
Total		9003	7834	7763	8537

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

ples were classified into "others". The intestinal segments of duodenum, jejunum, and ileum had a higher relative abundance of Bacilli, and as the birds aged, the percentage of Bacilli decreased, whereas the cecum had a higher relative abundance of Clostridia and as the birds aged, the percentage of Clostridia increased.

Table 10. Abundance of bacterial 16S rDNA sequences (n=31337) identified from the ileum flora of local Omani chicken.

		Abundance of Sequence (No. of Sequence [%]) at Day:			
Phylum	**Class**	**Day 5**	**Day 15**	**Day 25**	**Day 35**
Actinobacteria	Actinobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Coriobacteriia	0 (0)	31 (0.397)	21 (0.336)	98 (1.36)
Bacteroidetes	Bacteroidia	0 (0)	1320 (16.9)	695 (11.1)	2187 (30.4)
	Flavobacteriia	0 (0)	0 (0)	0 (0)	0 (0)
	Sphingobacteriia	0 (0)	0 (0)	0 (0)	0 (0)
Cyanobacteria	4C0d-2	0 (0)	0 (0)	0 (0)	119 (1.65)
	Chloroplast	0 (0)	0 (0)	0 (0)	0 (0)
Firmicutes	Bacilli	657 (9.33)	29 (0.371)	17 (0.272)	471 (6.54)
	Clostridia	5417 (76.9)	6361(81.5)	4953 (79.2)	4191 (58.2)
	Erysipelotrichi	222 (3.15)	45 (0.576)	143 (2.29)	57 (0.792)
Lentisphaerae	Lentisphaeria	0 (0)	0 (0)	1 (0.016)	2 (0.028)
Proteobacteria	Alphaproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Betaproteobacteria	0 (0)	0 (0)	0 (0)	0 (0)
	Deltaproteobacteria	0 (0)	0 (0)	31 (0.496)	11 (0.153)
	Epsilonproteobacteria	0 (0)	0 (0)	7 (0.112)	0 (0)
	Gammaproteobacteria	745 (10.58)	22 (0.282)	354 (5.66)	2 (0.028)
Tenericutes	Mollicutes	0 (0)	0 (0)	0 (0)	0 (0)
Thermi	Verrucomicrobiae	0 (0)	0 (0)	30 (0.480)	60 (0.834)
Total		7041	7808	6252	7198

Note: Values in the parentheses are abundance of Sequence (No. of Sequence [%]) at Day

Table 11. P-value distribution of 16S rDNA gene sequence libraries compared the abundance differences of microbial communities among samples from different segments for cobb 500 broiler chicken.

Class	P-Value					
	Duodenum-Jejunum	Duodenum-Ileum	Jejunum-Ileum	Caecum-Duodenum	Caecum-Jejunum	Caecum-Ileum
4C0d-2	0.881	0.514	0.680	0.208	0.212	0.203
Actinobacteria	0.374	0.352	0.119	0.670	0.823	0.611
Alphaproteobacteria	0.467	0.885	0.635	0.006	0.039	0.055
Bacilli	0.485	0.900	0.767	0	0	0
Bacteroidia	0.831	0.390	0.320	0.314	0.318	0.299
Betaproteobacteria	0.918	0.048	0.318	0.038	0.218	0.027
Chloroplast	0.037	0.068	0.765	0.019	0.009	0.018
Clostridia	0.753	0.820	0.901	0.002	0.002	0.001
Coriobacteriia	0.863	0.283	0.394	0.045	0.046	0.010
Deinococci	0.564	0.646	0.851	0.268	0.174	0.324
Deltaproteobacteria	1	1		0.239	0.141	0.162
Epsilonproteobacteria	0.497	0.556	0.538	0.347	0.263	0.324
Erysipelotrichi	0.681	0.080	0.619	0.075	0.070	0.068
Gammaproteobacteria	0.228	0.606	0.505	0.566	0.464	0.768
Mollicutes	0.194	0.094		0.138	0.087	0.126
Thermoleophilia	1	0.500	0.495	0.512	1	
Unclassified				0.347	0.370	0.324

Bacterial Taxonomic Composition of the Duodenum of Local Omani Chickens across Age Periods

Bacteria classified according to their respective Phylum and Class, found in the duodenum of local Omani chickens at different ages are presented in Table 7. Eighteen bacterial microbiota at the Class level were found in duodenum. Of the 33443 reads, Bacilli were the most abundant, at 90.8% of the total sequences, while sequences related to Clostridia accounted for 4.17% of the total sequences. Actinobacteria and Chloroplast represented a very small percentage of 1.13% and 1.72%, respectively of the total sequences. Across different age periods Bacilli were the dominant group, representing 72.5 % at Day 5, 97.7 % at Day 15 and 97.0 % at Day 25 and 96.1 % at Day 35 of the sequences. Clostridia sequences fluctuated from 13.1% at Day 5, 1.83% at Day 15, 1.35% at Day 25 and 0.366% at Day 35. Both Actinobacteria and Bacteroidia - related sequences were Day 5: 2.55%, Day 15: 0.0%, Day 25: 0.235% and Day 35: 1.82% and Day 5: 0.024%, Day 15: 0.0%, Day 25: 0.022%, and Day 35: 0.038%, respectively. Proteobacteria group-related sequences were detected at smaller percentages across age periods.

Bacterial Taxonomic Composition of the Jejunum of Local Omani Chickens Across Age Periods

Bacteria classified according to their respective Phylum and Class, found in the jejunum of local Omani chickens at different ages are presented in Table 8. Eighteen bacterial microbiota at the Class level were found in jejunum. Of the 31255 reads, Bacilli were the most abundant, at 89.84% of the total sequences. Chloroplast represented 6.53% of the total sequences. Clostridia accounted for 1.76% of the total sequences. Actinobacteria and Gammaproteobacteria represented a small percentage of 1.15 % and 0.032 %, respectively of the total sequences. and Chloroplast were 6.53%. Across different age periods Bacilli were the dominant group, representing 75.5% at Day 5, 96.8% at Day 15, 93.5% at Day 25 to 95.5% at Day 35 of the sequences. Clostridia sequences fluctuated from 2.50% at Day 5, 2.86% at Day 15, 1.33% at Day 25, and 0.139% at Day 35. Chloroplast sequences were 18.4% at Day 5, 0.229% at Day 15, 3.53% at Day 25 to 2.47% at Day 35 of the sequences. Actinobacteria sequences were Day 5: 2.33%, Day 15: 0.025%, Day 25: 0.646%, and Day 35: 1.51%, while Gammaproteobacteria sequences were Day 5: 0.012%, Day 15: 0.013%, Day 25: 0.106%, and Day 35:0.0%.

Bacterial taxonomic composition of the ileum of Local Omani chickens across age periods

Bacteria classified according to their respective Phylum and Class, found in the ileum of local Omani chickens at different ages are presented in Table 9. Eighteen bacterial microbiota at the Class level were found in ileum. Of the 31337 reads, Bacilli were the most abundant, at 84.8% of the total sequences. Chloroplast accounted for 9.49% of the total sequences. Clostridia and Gammaproteobacteria represented a small percentage of 2.87% and 1.07%, respectively of the total sequences. Only a few Actinobacteria (0.88%) related sequences were detected.

Table 12. P-value distribution of 16S rDNA gene sequence libraries compared the abundance differences of microbial communities among samples from different segments for cobb 500 broiler chicken.

	P-Value					
Class	**Day 5-15**	**Day 5-25**	**Day 5-35**	**Day 15-25**	**Day 15-35**	**Day 25-35**
4C0d-2	0.315	0.442	0.688	0.277	0.021	0.057
Actinobacteria	0.343	0.875	0.481	0.271	0.872	0.494
Alphaproteobacteria	0.736	0.850	0.057	0.620	0.121	0.065
Bacilli	0.925	0.933	0.599	0.911	0.248	0.555
Bacteroidia	0.347	0.511	0.328	0.213	0.287	0.344
Betaproteobacteria	0.746	0.337	0.128	0.575	0.092	0.080
Chloroplast	0.669	0.812	0.094	0.742	0.230	0.143
Clostridia	0.839	0.929	0.868	0.779	0.285	0.860
Coriobacteriia	0.185	0.598	0.144	0.277	0.129	0.717
Deinococci	0.068	0.119	0.681		0.388	0.373
Deltaproteobacteria	1	0.494	0.485		0.188	0.201
Epsilonproteobacteria	0.159	0.264	0.163			
Erysipelotrichi	0.574	0.681	0.934	0.634	0.554	0.661
Gammaproteobacteria	0.052	0.483	0.215	0.066	0.112	0.123
Mollicutes	0.259	0.624	0.335	0.277	0.244	0.536
Thermoleophilia			0.111		0.121	0.107
Unclassified	0.347	0.376	0.386			

Proteobacteria sequences represented 1.72% of the total sequences. Across different age periods Bacilli were the dominant group, representing 72.5% at Day 5, 87.0% at Day 15, 98.8% at Day 25 to 83.1% at Day 35 of the sequences. Clostridia sequences fluctuated from 0.289% at Day 5, 6.01% at Day 15, 0.734% at Day 25, and 4.66% at Day 35. Chloroplast sequences were Day 5: 24.1%, Day 15: 2.49%, Day 25: 0.206%, and Day 35: 9.0%, while Actinobacteria sequences were Day 5: 1.29%, Day 15: 0.026%, Day 25: 0.155%, and Day 35: 1.72%. Proteobacteria group-related sequences were detected at smaller percentages across all age periods.

Bacterial Taxonomic Composition of the Cecum of Local Omani Chickens Across Age Periods

Bacteria classified according to their respective Phylum and Class, found in the cecum of local Omani chickens at different ages are presented in Table 10. Eighteen bacterial microbiota at the Class level were found in cecum. Of the 28299 reads, Clostridia were the most abundant, at 73.9% of the total sequences. Bacteroidia and Gammaproteobacteria accounted for 14.9 % and 3.97 %, respectively of the total sequences. Bacilli and Erysipelotrichi represented a small percentage of 4.15% and 1.65%, respectively of the total sequences. Across different age periods Clostridia were the dominant group, representing 76.9% at Day 5, 81.5% at Day 15, 79.2% at Day 25 to 58.2% at Day 35 of the sequences. Bacilli sequences fluctuated from 9.33% at Day 5, 0.371% at Day 15, 0.272% at Day 25, and 6.54% at Day 35. Bacteroidia sequences were Day 5: 0.0%, Day 15: 16.9%, Day 25: 11.1%, and Day 35: 30.4%, while Erysipelotrichia sequences were Day 5: 3.15%, Day 15: 0.576%, Day 25: 2.29%, and Day 35: 0.792%.

Differences of Microbial Communities among Samples from Different Intestinal Segments of Local Omani Chicken

The p-value distribution of 16S rDNA gene sequence libraries used to compare the quantitative differences of microbial communities among samples from different intestinal segments of local Omani chickens is presented in Table 11. Statistical comparisons of the libraries showed that the composition of the Duodenum-Jejunum, Duodenum-Ileum, Cecum-Duodenum, Cecum-Ileum Cecum-Jejunum bacterial microbiota differed significantly ($p < 0.05$), suggesting that each region established its own bacterial community. The number of Alphaproteobacteria, Bacilli, Betaproteobacteria, Chloroplast, Clostrdia and Coriobacteriia differed significantly across different intestinal segments ($p < 0.05$). Bacilli were the dominant 16S rDNA sequences in the duodenum, jejunum, and ileum libraries, whereas Clostridia were the dominant 16S rDNA sequences in the cecum libraries.

Differences of Microbial Communities among Samples from Local Omani Chickens of Different Age Groups

The p-value distribution of 16S rDNA gene sequence libraries comparing the quantitative differences of microbial communities among samples from local Omani chickens at different age groups are presented in Table 12. Statistical comparisons of the libraries revealed that there were no significant differences ($p > 0.05$) between

the microbial compositions at different age groups: Day 5–15, Day 5–25, Day 5–35, Day 15–25, Day 15–35, and Day 25–35. The results of the statistical evaluation at certain age groups revealed that the percentage of bacterial microbiota of 4C0d-2 varied significantly ($p < 0.05$). The average percentage of 4C0d-2 was detected at significantly higher level ($p < 0.05$) at Day 35 (0.529%) of age than at Day 15 (0.0%) of age.

Taxonomic Composition Distribution of the Bacterial Community in Intestinal Segments at the Class-Level of Local Omani Chickens

From Figure 2, it can be seen that the diversity of the bacterial community of intestinal segments of local Omani chickens changed from one age period to the next. Species of that exhibited an abundance less than 0.5% in all samples were classified into "others". The intestinal segment of duodenum, jejunum, and ileum had a higher abundance of Bacilli, and as the birds aged, the percentage of Bacilli decreased, whereas the cecum had a higher abundance of Clostridia, and as the birds aged, the percentage of Clostridia increased.

Discussion

The aim of this study was to generate a phylogenetic diversity census of bacteria identified in the intestinal segments (duodenum, jejunum, ilium and cecum) of Local Omani and Cobb 500 broiler chickens raised in open-sided house from 0 to 35 day of age using 16S rDNA-based analysis. However, little is known about the

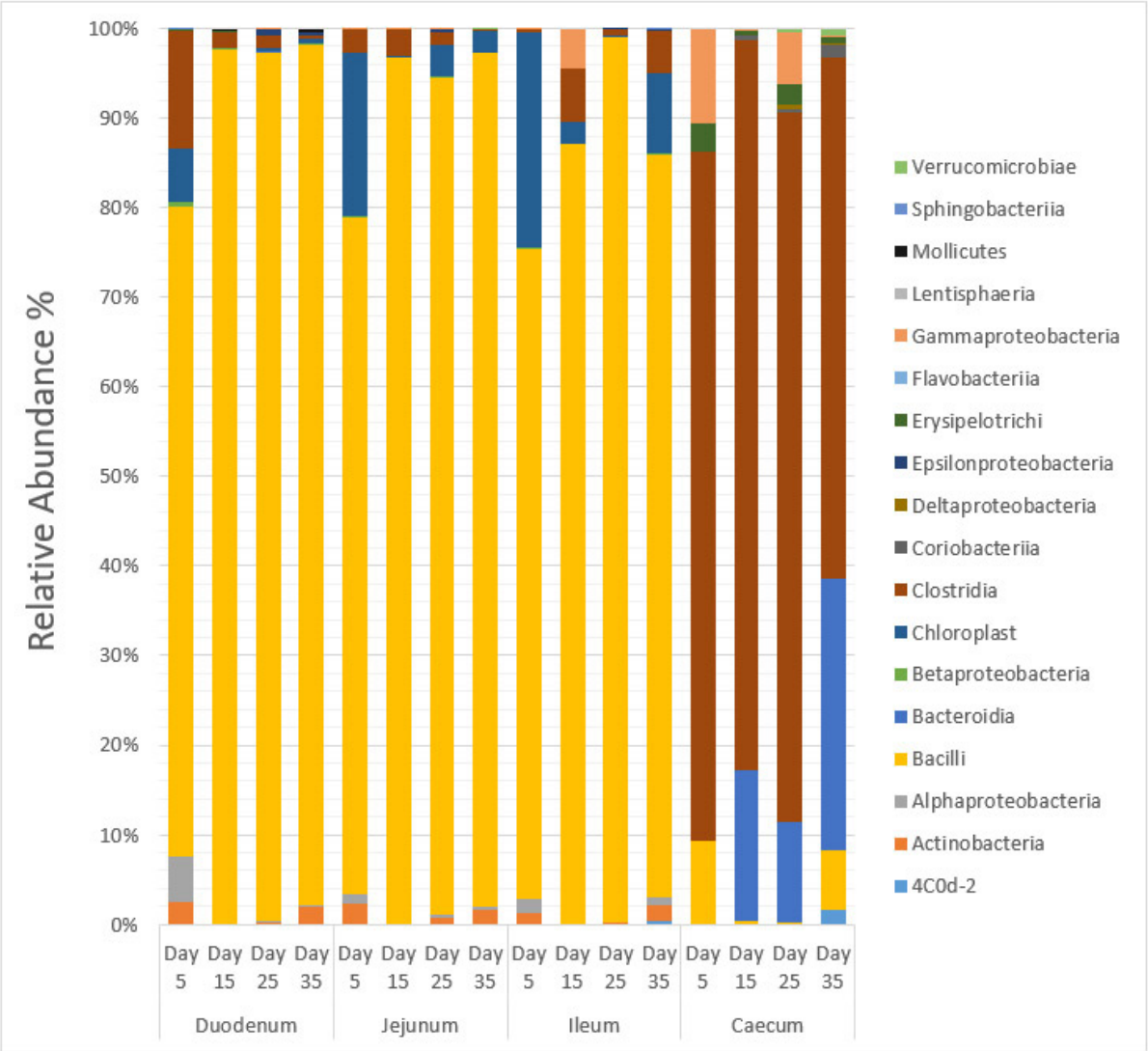

Figure 4. Percentage of relative abundance of bacterial community of local Omani chickens.and determined from different intestinal segments at different age periods from 16S rDNA libraries.

intestinal bacterial community composition and succession for birds especially those with various growth rates such as the indigenous chickens raised in naturally ventilated open-sided house system. The open-sided house is widely practiced by a majority of small and medium scale farmers in the developing countries. A greater understanding of the chicken gut function and microbiology will enhance chicken's health and productivity raised in naturally ventilated open sided house system.

The data obtained in the study revealed the heterogeneity of bacterial populations found in different intestinal segments as derived from molecular detection and bioinformatics analysis. As a result, the current study's findings have been confined to the most quantitatively significant bacterium classes. Therefore, this study focused solely on Bacilli and Clostridia, the two most common groups in the Firmicutes phylum. The statistical analyses of microbial community libraries within each breed among samples from different intestinal segments at different age groups revealed no significant differences ($p > 0.05$) in the current investigation. Quite the contrary, the bacterial microbiota of each breed differed significantly ($p<0.05$) across distinct intestinal segments, suggesting that each region established its bacterial community with different relative abundances.

It is anticipated that diverse bacteria will emerge in various intestine segments as each segment's function and environmental circumstances differ from one another (Rehman et al., 2007; Wise and Siragusa, 2007). In the current study the most reflective differences in the microbial population in the intestinal segments of the two breeds was detected between Day 5 to 25. One possible explanation is that at early age periods, immediately after hatching, is the most critical period in the life of the chick. During this early age period, there is the transition from yolk to oral nutrition associated with major physical and functional development of the digestive tract and organs (Ravindran, 2003) resulting in unstable environmental conditions of the digestive tract's microecosystem.

Different studies observed similar results that the microbial community structure varies with age and the microbial community structure was impartially stable and is replaced by a stable bacterial community once the rate of the intestinal development lessened (Mackie et al., 1999; Xu et al., 2003; Amit-Romach et al., 2004). The unique microbial community at 3-5 days of age suggests that the early bacterial community is relatively transient and is replaced by a stable bacterial community later in life (Lumpkins et al., 2010; Glendinning et al., 2019).

In the current study, our data showed that Bacilli was the most dominant Class in the duodenal flora of Cobb 500 at a younger age than Local Omani breed (Cobb 500: Day5 98.9 %; Local Omani: Day5 72.5 %). Clostridia was the second most abundant Class in the duodenum. Their levels were high in Local Omani than in Cobb 500 at an early age (Local Omani: Day5 13.1 %; Cobb 500: Day5 0.062 %, respectively). It is well documented that the chicken cecum and its mucosal tissue are dominated by Clostridia related species (Bjerrum et al., 2006; Gong et al., 2007; Lund et al., 2010). Clostridia are mainly involved in fermentation and can ferment a wide variety of substrates including monosaccharides and polysaccharides (Jones and Woods, 1986). The larger number of Clostridia in the duodenum of Local Omani chickens during early intestinal development possibly have no or limited function in nutrient absorption and subsequently the duodenum functions at a lessened level when compared to that of Cobb 500 chickens. According to Al-Marzooqi et al. (2020) the higher abundance of Clostridia that are associated with fermentation act in an inhibitory fashion for nutrient absorption in the intestine of the local Omani chickens and the villus development is slowed down.

The morphological analysis in a study by Al-Balushi (2021) showed that villus height to villus width ratio in Cobb 500 broilers was significantly higher compared to the Local Omani chickens (14.18 versus 9.74, respectively $p < 0.01$). It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes, and nutrient transport systems (Amat et al., 1996). Enterocyte enzymatic activity and structure are two of the most important features of the intestinal mucosal physiology (Caspary, 1992). Al-Marzooqi et al., (2019) concluded that villus development has a profound effect on the growth performance of the chickens. Many studies proved that variations in bacterial population between certain broiler lines might be attributed to differences in villi height, which could result in a wider distance between the crypt and the lumen with increased villus height, creating a niche for specific bacteria (Suau et al., 1999; Salzman et al., 2002; Lumpkins et al., 2010). As a result, villi height might be referred as a contributing element to the bacterial community's habitat.

Conclusion

The dynamics of the gut microbial community or microbial balance are still far from fully understood. However, the future development of the Local Omani chicken breed for rapid growth production requires a further selection of lines that should take in consideration the intestine's overall developmental rate. In addition, future studies will need to look into histological alterations related to intestinal function. It is essential to establish baseline values for production parameters of Local Omani chickens and characterize the overall performance of Local Omani chickens. Future research should be focused by identifying gut bacteria that can be associated with improved/poor chicken growth performance. Moreover, future studies need to be directed towards development of diets, such as the utilization of probiotics to increase the development of Bacilli during early intestinal development and subsequent better utilization of nutrients in the Local Omani chickens.

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