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Research Article
Microbial Community Dynamics in the Gastrointestinal Tract of Indigenous Omani Chickens

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Abstract

Background and Objective: The microflora in the gastrointestinal tract influences the digestion, health and wellbeing of chickens. This study was conducted to assess the relative abundance of bacterial microflora in different segments of the gastrointestinal tract (duodenum, jejunum, ilium and cecum) of indigenous local Omani chickens. **Materials and Methods:** One hundred fifty one-day-old chicks of indigenous (local Omani) chickens were raised under an intensive management system and fed a nonmedicated corn-soybean meal diet from 0-35 days of age. **Results:** Using 16S rDNA-based analysis, the study showed that each intestinal segment developed its own bacterial community and the diversity of the bacterial community changed from one age period to the next. In addition, the 16S rDNA sequences of Lactobacillales were dominant in the duodenum, jejunum and ileum libraries, whereas the 16S rDNA sequences of Clostridiales were dominant in the cecum libraries. The relative abundance of the bacterial microbiota differed significantly ($p<0.05$) across different intestinal segments. **Conclusion:** Each region developed its own bacterial community and the relative abundances of the bacterial community were quite different.

Key words: 16S rDNA, Omani chickens, gastrointestinal tract, bacterial microflora, gut microbiota

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The study of the gut microbiota has become a topic of interest. It is well recognized that the gut microbiota plays an important role in the metabolism, nutrient utilization, growth performance and health of the host[1,2]. The diversity and composition of the intestinal microbiome in chickens are intensely influenced by different factors, such as diet and age of birds and the complexity and biodiversity increase as chickens grow[3,4]. It has been shown that each region of the gastrointestinal tract (GIT) develops its own distinctive microbial profile and the structure of the microflora becomes more complex and changes in relation to the age of chickens, location in the digestive tract, feed, breed and environment[5-8].

With the expansion of livestock husbandry practices in Oman, local "indigenous" chicken production is becoming a widespread activity. The economic and social values of local chicken production in developing countries of the Middle East, including Oman, especially in rural areas, are well recognized; therefore, it is essential to explore opportunities for improving the production of local chickens. Local chicken production is among the farming activities in the rural communities of Oman that provide opportunities for food security and income for many rural families[9]. Several studies have shown the beneficial effects of gut microbiota on the physiological functions, metabolism, immune system, digestion and nutrient absorption of the host[6]. Evaluation of the bacterial community and intestinal development of different genetic lines of chickens has become a recent point of interest[10]. However, little is known about how the composition of the microbiome differs among different strains of chickens, especially those with different growth rates, including indigenous chickens. A better understanding of chicken gut function and microbiome will provide a new opportunity for the improvement of local chicken health and production.

The use of molecular techniques that involve analyzing the structure of bacterial communities by determining the characteristic features of microbial DNA extracted from community samples has overcome the difficulties encountered in culturing microbes[11,12]. Using such techniques, it was found that 90% of the bacteria in the chicken GIT were previously unknown species[13]. In addition, a metagenomics technique (a nonculture-based approach) was developed and enabled researchers to comprehensively study microbial communities in different ecosystems[14]. Metagenomic analysis has provided significant information on the changes/succession in the microbial community[14].

Understanding the taxonomic composition of the bacterial community, diversity and succession in the gastrointestinal tract will permit the detection of disruptions in the microbiota. This information is essential and may enable manipulation of the intestinal flora to enhance intestinal health and bird performance in general. The objective of this study was to assess the relative abundance, diversity and changes with age in the microbial population identified in different parts of the intestine of local Omani chickens using 16S rDNA-based analysis.

MATERIALS AND METHODS

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. The study was approved by the Animal Research Ethics Board at Sultan Qaboos University.

Bird husbandry: A total of one hundred fifty one-day-old chicks of indigenous chickens (local Omani) were obtained from reputable commercial hatcheries at Barka in Muscat. Chicks were inspected on arrival to ensure that all chicks were free from deformities and signs of disease. Standard operating procedures of broiler house management[15] were followed throughout the experiment. The closed house unit, cages, feeders and drinkers were cleaned and disinfected through fumigation before the experiment. In addition, strict hygiene and biosecurity measurements were implemented. On the day of arrival, the chicks were individually weighed and placed into narrow weight classes. Birds of relatively low or high body weight were excluded. Six birds were randomly assigned to each of 15 suspended wire cages (62×62×37 cm) such that all cages had nearly a similar average initial weight. Feed was available *ad libitum*. The cages were in an environmentally controlled shed (closed house) maintained at 33°C on day 1 and reduced by 3°C each week until a constant temperature of 22°C was reached. The lighting program was 23L:1D.

Dietary treatment: Chicks were fed a nonmedicated conventional corn-soybean meal diet, i.e., devoid of other dietary supplements that may influence the composition of the microflora, from 0-35 days of age. The composition of the experimental diet is presented in Table 1. There were 15 replicates with each replicate cage containing six birds (a total of 90 birds). Birds per replicate combinations were randomly allocated.

Table 1: Composition of the experimental diet (g kg^{-1} dry matter) used in the experiment

Ingredients	Amount (g kg^{-1} dry matter)
Corn	516.90
Soybean meal (46%)	396.30
Vegetable oil	41.10
Monocalcium phosphate	19.60
Limestone	15.00
Salt	3.10
Vitamin and mineral premix[1]	2.00
DL-methionine	3.00
Calculated analysis (per kg)	
ME (kcal kg^{-1})	3035.00
Crude protein (g)	225.00
Lysine (g)	12.90
Methionine (g)	6.30

[1]The vitamin and mineral premix provides the following quantities per kilogram of diet; Vitamin A (retinol): 10,300 IU, Vitamin D_3 (cholecalciferol): 2,500 IU, Vitamin E (DL-α-tocopheryl): 40.00 mg, Vitamin K_3 (menadione): 3.75 mg, Vitamin B_1 (thiamin): 1.00 mg, Vitamin B_2 (riboflavin): 6.50 mg, Vitamin B_6 (pyridoxine): 6.00 mg, Vitamin B_{12} (cyanocobalamin): 0.01 mg, Calcium pantothenate: 18.00 mg, Niacin: 30.00 mg, Folic acid: 2.00 mg, Biotin: 0.06 mg, Flavomycin: 50.00 mg, Ethoxyquin: 125.00 mg, Choline: 650.00 mg, Molybdenum: 2.00 mg, Manganese: 120.00 mg, Iron: 7.00 mg, Cobalt: 1.00 mg, Zinc: 90.00 mg, Iodine: 1.50 mg, Selenium: 0.15 mg

Sample collection: At 5, 15, 25 and 35 days of age, one bird per cage was randomly selected. Selection was based on the body weight of the birds and the 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 birds received a xylazine-ketamine combination at a dose of 5 mg of xylazine [Ilium Xylazil-20-Xylazine 20 mg mL^{-1} (as hydrochloride) Troy Laboratories Pty Limited, Glendenning, Australia] and 25 mg of ketamine [Ketamil Injection-Ketamine 100 mg mL^{-1} (as hydrochloride) Troy Laboratories Pty Limited, Glendenning, Australia] intramuscularly to put the bird under deep sedation and anesthesia. When the bird was completely immobilized, it was euthanized by cervical dislocation. Subsequently, an incision was made at the bottom of the breastbone and a large V shape was cut towards the head. At the apex of the V shape, the abdominal cavity was opened, taking care not to rupture the intestine below. Once a sufficiently large opening had been made, the small intestine was carefully removed from the abdominal cavity until the ileal-cecal-colonic junction was observed. The duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to yolk stalk), ileum (from yolk stalk to ileocecal junction) and cecum (two horns) were differentiated, separated by serial bowel clamps and cleaned using 70% alcohol wipes. Sections approximately 4 cm long (including digesta) were cut from the mid regions of the duodenum, jejunum, ileum and ceca. Dissecting instruments were cleaned with 70% ethanol after use on each bird. The entire process of collecting intestinal contents was performed on a thoroughly cleaned workbench and required less than 30 min.

The contents collected from the four parts of the intestine were placed in sterile, labeled 15 mL conical tubes. Samples were placed on ice, immediately transported to the laboratory and stored in a -80°C freezer until analysis. Analysis of all samples was started two weeks after the end of the experiment on day 35. The conditions were the same for all collected samples.

DNA extraction: Total DNA was extracted from the contents of each luminal content sample (duodenum, jejunum, ileum and cecum) using a QIAamp DNA Stool Mini Kit (QIAGEN, CA, Hamburg, Germany) according to the manufacturer's instructions. The integrity of the 16 DNA samples was evaluated by measuring the optical density using a NanoDrop 2000 (Thermo Electron Corporation, Waltham, MA, USA) at wavelengths of 260 and 280 nm. The integrity of the DNA extracts was assessed visually using 1.0% agarose gel (containing ethidium bromide) electrophoresis.

Polymerase chain reaction amplicon and high-throughput sequencing: 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 each primer, 10 ng of DNA template, 4 μL of 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 at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec and 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 from 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 by the Illumina MiSeq platform.

Sequencing analysis: All the raw sequences obtained from Illumina MiSeq were first filtered for quality control to obtain operational sequences. The quality control and analysis of the sequences were performed using the software Quantitative Insights into Microbial Ecology (QIIME, v1.8.0)[16]. The paired-end reads from the DNA fragments were merged using FLASH[17]. Sequence data were treated by read trimming and the V3-V4 sequences were identified; a set of sequences

Table 2: The average alpha-diversity indices (chao 1, simpson and shannon indices) of the data distribution

	Chao 1		Simpson		Shannon	
Intestinal Segment	Mean	STD	Mean	STD	Mean	STD
Duodenum	412.8817	70.12375	0.29095	0.182177	2.31758	0.77987
Jejunum	385.5347	33.66046	0.35272	0.241770	1.92108	0.68161
Ileum	411.7906	59.19865	0.18317	0.051630	2.29453	0.25445
Cecum	220.8237	45.37880	0.06827	0.022040	3.51811	0.23431
p-value	0.0327		0.03506		0.02520	

The differences were considered to be significant at $p<0.05$

with ≥97% identity was defined as an operational taxonomic unit (OTU). The UCLUST[18] clustering method was used to cluster operational taxonomic units. The defined OTUs were assigned to different taxonomic levels (phylum, class, genus and family) at a cutoff of 97%. The clustered OTUs were also used to construct 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 data analyses: Bioinformatics and statistical analyses were performed using the QIIME and R packages (v3.1.1). The alpha-diversity indices (Chaol, ACE, Shannon diversity index and Simpson indices) 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 the R package (v3.1.1)[19] 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

Data generated by molecular detection and bioinformatics analysis revealed the diverseness of bacterial populations existing in different intestinal segments. The 16S rDNA analysis generated a huge database that is beyond the scope of this manuscript. Therefore, the findings of the present study have been limited to the classes/orders of bacteria with the most quantitative significance.

Sequencing overview: A total of 1,354,560 sequences were obtained; the number of sequences ranged from 58,498-12,785 and the sequences were clustered into 155-485 OTUs for each sample, resulting in a total of 829 OTUs for all samples at the 97% sequence similarity value. The microbial complexity, similarity in microbial community composition, microbial community composition and abundance are summarized in their respective sections below.

The microbial complexity: The microbial complexity in the duodenum, jejunum, ileum and cecum was estimated on the basis of alpha-diversity indices (Chao1 index, Simpson index and Shannon indices) (Table 2). Chao1 was used to estimate species richness; Simpson's and Shannon's indices were used to indicate species diversity. There was a significant difference between intestinal segments across different age periods when comparing index means.

Similarity in microbial community composition: The similarity and difference of the microbial community composition in intestinal content samples taken from local Omani chickens (4 gut sections) are shown in the PCoA plot, with PC1 accounting for 46.55% of the total variation and PC2 accounting for 21.30%. As a result, microbial communities of the duodenum, jejunum, ileum and cecum formed clusters. There were overlaps among the 4 clusters, indicating that microbial communities of the duodenum, jejunum and ileum were more similar. The microbial communities of the cecum formed a distinct cluster separate from those of other gut sections (Fig. 1a). The heat map analysis associated with the similarity of microbial community composition was performed at the order level and revealed the richness and diversity of bacterial communities in the gut content of each sample (Fig. 1b).

Microbial community composition and abundance: The rarefaction curves tended to reach the saturation plateau, with

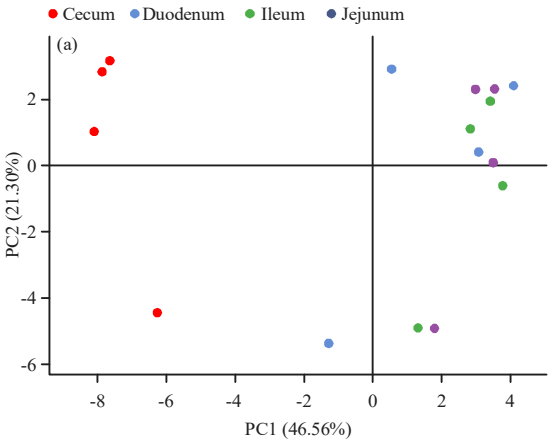

Fig. 1a: Principal coordinate analysis (PCoA) of the dissimilarity between the microbial samples: PCoA plotted against the PC1 vs. PC2 axes. The percentages indicate the relative contribution of the two principal coordinates (PC1-PC2)

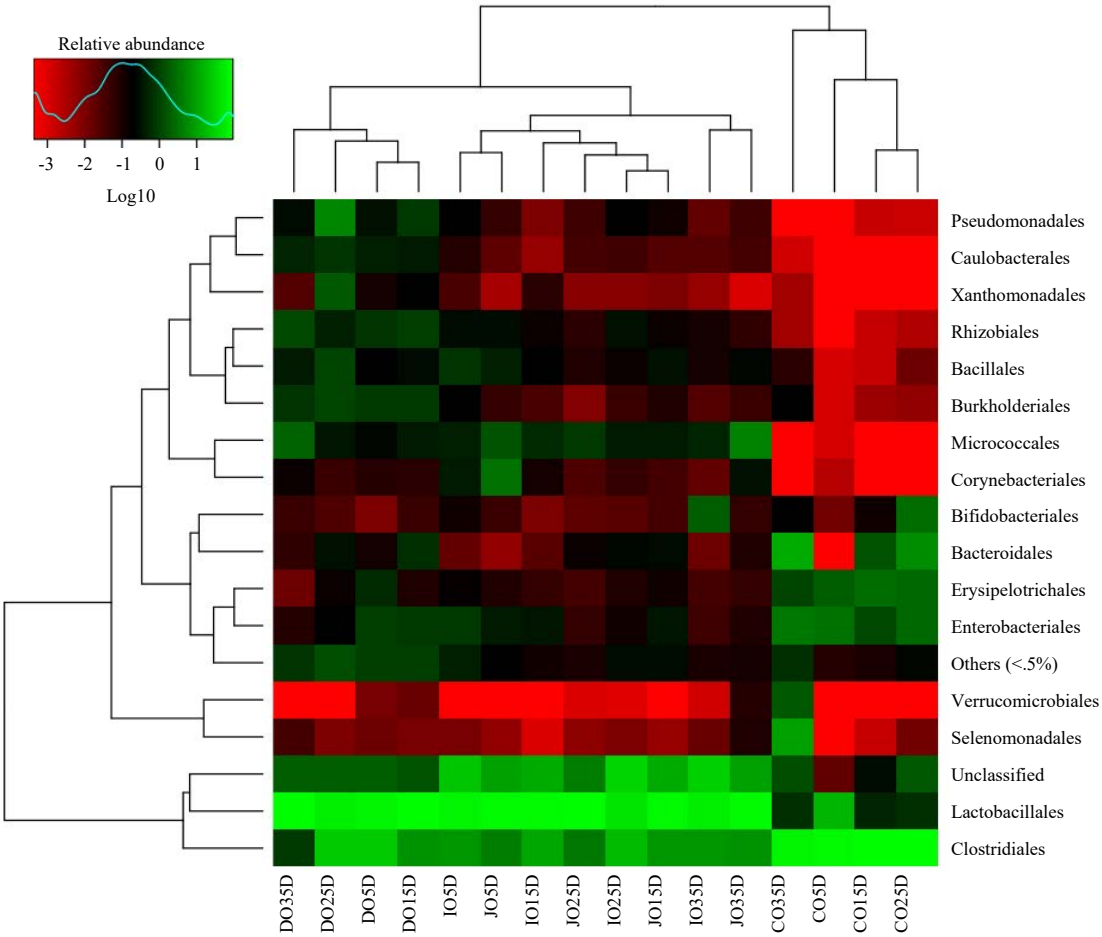

Fig. 1b: Heat map showing the orders with significant differences in relative abundance among the intestinal segments at different age periods

the sampled sequence number increasing, indicating that the number of sequences sampled was large enough to estimate the phenotype richness and microbial community diversity at the 97% similarity threshold (Fig. 2). The microbial community

Table 3: Abundance of bacterial 16S rDNA sequences (n = 387091) isolated from the duodenal microbiota of local Omani chickens

		Abundance of sequence (No. of sequence [%]) on day			
Class	Order	Day 5	Day15	Day25	Day35
Actinobacteria	Bifidobacteriales	9(0.010)	44(0.048)	35(0.032)	51(0.053)
	Corynebacteriales	74(0.082)	68(0.074)	57(0.052)	142(0.147)
	Micrococcales	215(0.239)	358(0.392)	410(0.376)	2128(2.20)
Bacteroidia	Bacteroidales	115(0.128)	587(0.643)	334(0.306)	65(0.067)
Bacilli	Bacillales	178(0.198)	258(0.282)	1229(1.13)	373(0.385)
	Lactobacillales	62076(69.07)	79935(87.52)	66531(60.98)	90255(93.26)
Clostridia	Clostridiales	23473(26.12)	6131(6.71)	29622(27.15)	871(0.900)
Erysipelotrichia	Erysipelotrichales	511(0.569)	87(0.095)	171(0.157)	15(0.015)
Negativicutes	Selenomonadales	13(0.014)	11(0.012)	12(0.011)	38(0.039)
Alphaproteobacteria	Caulobacterales	408(0.454)	368(0.403)	792(0.726)	485(0.501)
	Rhizobiales	698(0.777)	873(0.956)	481(0.441)	1204(1.24)
Betaproteobacteria	Burkholderiales	772(0.859)	803(0.879)	1239(1.14)	729(0.753)
Gammaproteobacteria	Enterobacteriales	911(1.014)	782(0.856)	215(0.197)	76(0.079)
	Pseudomonadales	274(0.305)	793(0.868)	6020(5.52)	274(0.283)
	Xanthomonadales	105(0.117)	190(0.208)	1936(1.78)	28(0.029)
Verrucomicrobia	Verrucomicrobiales	10(0.011)	15(0.016)	0(0)	0(0)
Unclassified	Unclassified	33(0.037)	34(0.037)	14(0.013)	47(0.049)
Total		89875	91337	109098	96781

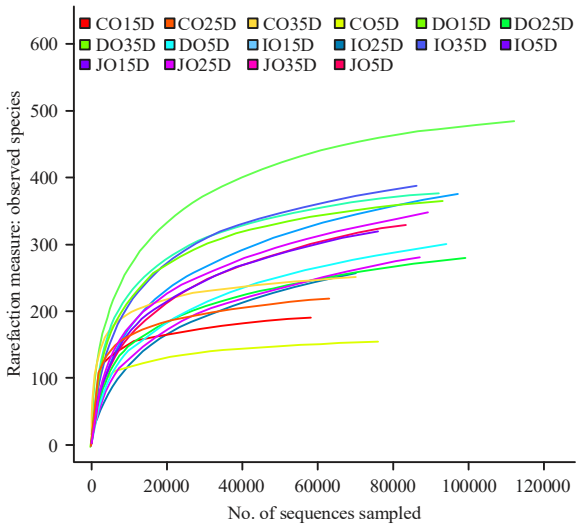

Fig. 2: Rarefaction curve sequences showing the microbial community complexities in the intestinal segments of local Omani chickens at different age periods

composition and abundance of each intestinal segment at different ages are summarized in their respective sections below.

Microbial composition of the duodenum: Bacteria found in the duodenum of local Omani chickens at different ages were classified according to their respective class and order, as presented in Table 3. Sixteen bacteria in the duodenum were identified at the order level. Of the 387091 reads, Lactobacillales, from the class Bacilli, was the most abundant order at 77.2% of the total sequences. Clostridiales, a representative order from the class Clostridia, was the second most abundant order, accounting for 15.5% of the total sequences. At the class level, Actinobacteria, Alphaproteo bacteria and Gammaproteo bacteria represented small percentages of 0.93, 1.37 and 2.99% of the total sequences, respectively. Across different age periods, Lactobacillales was the most dominant group, representing 69.07% of the total sequences on day 5, 87.52% on day 15, 60.98% on day 25and 93.26% on day 35. Clostridiales was the second most abundant order, with relative abundances of 26.12% on day 5, 6.71% on day 15, 27.15% on day 25 and 0.90% on day 35. Comparatively, Corynebacteriales, (member of the class Actinobacteria), Bacteroidales, (class, Bacteroidia), Erysipelotrichales (class, Erysipelotrichia), Selenomonadales (class, Negativicutes), Rhizobiales (class, Alphaproteobacteria), Burkholderiales (class, Betaproteobacteria) and Enterobacteriales (class, Gammaproteobacteria) group-related sequences were detected at smaller percentages across all age periods.

Microbial composition of the jejunum: Bacteria found in the jejunum of local Omani chickens at different ages were classified according to their respective class and order, as presented in Table 4. Sixteen bacterial microbiota at the order level were found in the jejunum. Of the 307154 reads, Lactobacillales, from the class Bacilli, was the most abundant order at 90.10% of the total sequences. Clostridiales, a representative order from the class Clostridia, was the second most abundant order, accounting for 6.02% of the total sequences. At the class level, Actinobacteria, Alphaproteobacteria and Gammaproteobacteria represented a small percentage of 3.05, 0.18 and 0.32% of the total sequences, respectively. Across different age periods,

Table 4: Abundance of bacterial 16S rDNA sequences (n=307154) isolated from the jejunum microbiota of local Omani chickens

		Abundance of sequence (No. of sequence [%]) on day			
Class	Order	Day 5	Day15	Day25	Day35
Actinobacteria	Bifidobacteriales	40(0.053)	30(0.045)	18(0.021)	48(0.060)
	Corynebacteriales	2643(3.50)	30(0.045)	26(0.031)	270(0.336)
	Micrococcales	1235(1.64)	313(0.466)	692(0.822)	4029(5.01)
Bacteroidia	Bacteroidales	5(0.007)	212(0.316)	128(0.152)	77(0.096)
Bacilli	Bacillales	360(0.477)	232(0.346)	80(0.095)	228(0.284)
	Lactobacillales	66979(88.80)	59819(89.13)	79938(94.94)	69101(85.93)
Clostridia	Clostridiales	3393(4.50)	5796(8.64)	3081(3.66)	6208(7.20)
Erysipelotrichia	Erysipelotrichales	81(0.107)	98(0.146)	33(0.039)	50(0.062)
Negativicutes	Selenomonadales	5(0.007)	4(0.006)	6(0.007)	81(0.101)
Alphaproteobacteria	Caulobacterales	18(0.024)	21(0.031)	33(0.039)	33(0.041)
	Rhizobiales	235(0.312)	110(0.164)	60(0.071)	54(0.067)
Betaproteobacteria	Burkholderiales	47(0.062)	71(0.106)	8(0.010)	43(0.053)
Gammaproteobacteria	Enterobacteriales	330(0.438)	265(0.395)	47(0.056)	80(0.099)
	Pseudomonadales	48(0.064)	107(0.159)	38(0.045)	38(0.047)
	Xanthomonadales	3(0.004)	8(0.012)	7(0.008)	1(0.001)
Verrucomicrobia	Verrucomicrobiales	0(0)	0(0)	1(0.001)	74(0.092)
Unclassified	Unclassified	2(0.003)	1(0.001)	1(0.001)	1(0.001)
Total		75424	67117	84197	80416

Table 5: Abundance of bacterial 16S rDNA sequences (n = 267869) isolated from the ileum microbiota of local Omani chickens

		Abundance of sequence (No. of sequence [%]) on day			
Class	Order	Day 5	Day15	Day25	Day35
Actinobacteria	Bifidobacteriales	118(0.174)	9(0.011)	22(0.033)	1377(2.74)
	Corynebacteriales	340(0.503)	109(0.131)	56(0.084)	14(0.028)
	Micrococcales	412(0.609)	535(0.644)	407(0.610)	341(0.678)
Bacteroidia	Bacteroidales	17(0.025)	23(0.028)	231(0.346)	11(0.022)
Bacilli	Bacillales	665(0.983)	201(0.242)	155(0.232)	81(0.161)
	Lactobacillales	58201(86.03)	70757(85.12)	47547(71.22)	43102(85.64)
Clostridia	Clostridiales	6286(9.29)	10840(13.04)	17497(26.21)	5187(10.31)
Erysipelotrichia	Erysipelotrichales	143(0.211)	56(0.067)	93(0.139)	26(0.052)
Negativicutes	Selenomonadales	10(0.015)	1(0.001)	10(0.015)	12(0.024)
Alphaproteobacteria	Caulobacterales	74(0.109)	5(0.006)	42(0.063)	20(0.040)
	Rhizobiales	247(0.365)	135(0.162)	302(0.452)	84(0.167)
Betaproteobacteria	Burkholderiales	173(0.256)	33(0.040)	49(0.073)	20(0.040)
Gammaproteobacteria	Enterobacteriales	749(1.11)	351(0.422)	135(0.202)	32(0.064)
	Pseudomonadales	186(0.275)	10(0.012)	200(0.300)	14(0.028)
	Xanthomonadales	29(0.043)	66(0.079)	8(0.012)	4(0.008)
Verrucomicrobia	Verrucomicrobiales	0(0)	0(0)	1(0.001)	1(0.002)
Unclassified	Unclassified	2(0.003)	0(0)	3(0.004)	2(0.004)
Total		67652	83131	66758	50328

Lactobacillales was the most dominant group, representing 88.80% of the sequences on day 5, 89.13% on day 15, 94.94% on day 25 and 85.93% on day 35. Clostridiales was the second most abundant order, with relative abundances of 4.50% on day 5, 8.64% on day 15, 3.66% on day 25 and 7.20% on day 35. Both Corynebacteriales and Micrococcales (members from the class Actinobacteria)-related sequences had relative abundance of 3.50% on day 5, 0.045% on day 15, 0.031% on day 25 and 0.336% on day 35 and 1.64% on day 5, 0.466% on day 15, 0.822% on day 25 and 5.01% on day 35, respectively. Relatively, Bacteroidales, (class, Bacteroidia), Erysipelotrichales (class, Erysipelotrichia), Selenomonadales (class, Negativicutes), Rhizobiales (class, Alphaproteobacteria), Burkholderiales (class, Betaproteobacteria) and Enterobacteriales (class, Gammaproteobacteria) group-related sequences were detected at lower levels across all age periods.

Microbial composition of the ileum: Bacteria found in the ileum of local Omani chickens at different ages were classified according to their respective class and order, as presented in Table 5. Sixteen bacterial microbiota at the order level were found in the ileum. Of the 267869 reads, Lactobacillales, from the class Bacilli, was the most abundant order at 82.39% of the total sequences. Clostridiales, a representative order from the class Clostridia, was the second most common order, accounting for 14.86% of the total sequences. At the class level, Actinobacteria, Alphaproteobacteria and

Table 6: Abundance of bacterial 16S rDNA sequences (n = 266173) isolated from the cecum microbiota of local Omani chickens

		Abundance of sequence (No. of sequence [%]) on day			
Class	Order	Day 5	Day 15	Day 25	Day 35
Actinobacteria	Bifidobacteriales	10(0.013)	73(0.125)	1718(2.76)	142(0.204)
Corynebacteriales	2(0.003)	0(0)	0(0)	0(0)	
Micrococcales	1(0.001)	0(0)	0(0)	0(0)	
Bacteroidia	Bacteroidales	0(0)	916(1.57)	3573(5.74)	8685(12.51)
Bacilli	Bacillales	1(0.001)	1(0.002)	9(0.014)	51(0.073)
Lactobacillales	11772(15.46)	305(0.523)	401(0.644)	467(0.672)	
Clostridia	Clostridiales	60256(79.14)	54535(93.58)	53432(85.76)	48354(69.62)
Erysipelotrichia	Erysipelotrichales	1534(2.02)	1753(3.01)	1508(2.42)	780(1.12)
Negativicutes	Selenomonadales	0(0)	1(0.002)	8(0.013)	7015(10.10)
Alphaproteobacteria	Caulobacterales	0(0)	0(0)	0(0)	1(0.001)
Rhizobiales	0(0)	1(0.002)	2(0.003)	3(0.004)	
Betaproteobacteria	Burkholderiales	1(0.001)	3(0.005)	4(0.006)	159(0.229)
Gammaproteobacteria	Enterobacteriales	2564(3.37)	686(1.18)	1648(2.65)	2627(3.78)
Pseudomonadales	0(0)	1(0.002)	1(0.002)	0(0)	
Xanthomonadales	0(0)	0(0)	0(0)	3(0.004)	
Verrucomicrobia	Verrucomicrobiales	0(0)	0(0)	0(0)	1166(1.68)
Unclassified	Unclassified	0(0)	0(0)	0(0)	0(0)
Total		76141	58275	62304	69453

Gammaproteobacteria represented small percentages of 1.40, 0.34 and 0.67% of the total sequences, respectively. Across different age periods, Lactobacillales was the most dominant group, with relative abundances of 86.03% on day 5, 85.12% on day 15, 71.22% on day 25 and 85.64% on day 35. Clostridiales was the second most abundant order, with relative abundances of 9.29% on day 5, 13.04% on day 15, 26.21% on day 25 and 10.31% on day 35. Moderately, Corynebacteriales, (class, Actinobacteria), Bacteroidales, (class, Bacteroidia), Erysipelotrichales (class, Erysipelotrichia), Selenomonadales (class, Negativicutes), Rhizobiales (class, Alphaproteobacteria), Burkholderiales (class, Betaproteobacteria) and Enterobacteriales (class, Gammaproteobacteria) group-related sequences were detected at smaller percentages across all age periods.

Microbial composition of the cecum: Bacteria found in the cecum of local Omani chickens at different ages were classified according to their respective class and order, as presented in Table 6. Sixteen bacterial microbiota at the order level were found in the cecum. Of the 266173 reads, Clostridiales, a representative order from the class Clostridia, was the most abundant order at 81.37% of the total sequences. Lactobacillales was the second most common order of the class Bacilli, accounting for 4.89% of the total sequences. At the class level, Actinobacteria, Alphaproteobacteria and Gammaproteobacteria represented small percentages of 0.73, 0.003 and 2.83% of the total sequences, respectively. Across different age periods, Lactobacillales was the most dominant group, with relative abundances of 15.46% on day 5, 0.523% on day 15, 0.644% on day 25 and 0.672% on day 35. Clostridiales was the second most abundant order, with relative abundances of 79.14% on day 5, 93.58% on day 15, 85.76% on day 25 and 69.62% on day 35. Modest percentages of sequences for Bacteroidales (class, Bacteroidia), Erysipelotrichales (class, Erysipelotrichia) and Enterobacteriales (class, Gammaproteobacteria) were observed with relative abundances of 0.0% on day 5, 1.57% on day 15, 5.74% on day 25 and 12.51% on day 35; 2.02% on day 5, 3.01% on day 15, 2.42% on day 25 and 1.12% on day 35 and 3.37% on day 5, 1.18% on day 15, 2.65% on day 25 and 3.78% on day 35, respectively. Relatively, Corynebacteriales (class, Actinobacteria), Rhizobiales (class, Alphaproteobacteria) and Burkholderiales (class, Betaproteobacteria) group-related sequences were detected at lower levels across all age periods except for Selenomonadales (class, Negativicutes), which was detected at 10.10% on day 35.

Differences in microbial communities among samples from different intestinal segments of local omani chickens: The p value distribution of the 16S rDNA gene sequence libraries comparing the relative abundance differences of microbial communities among samples from different intestinal segments of local Omani chickens is presented in Table 7. Statistical comparisons of the libraries revealed that the bacterial microbiota composition of the duodenum-jejunum, duodenum-ileum, cecum-duodenum, cecum-ileum and cecum-jejunum differed significantly ($p<0.05$), suggesting that each region developed its own bacterial community. The relative abundance of Bacillales, Burkholderiales, Caulobacterales, Clostridiales, Corynebacteriales, Enterobacteriales, Erysipelotrichales, Lactobacillales, Micrococcales, Pseudomonadales, Rhizobiales and Xanthomonadales differed significantly across different intestinal segments ($p<0.05$). 16S rDNA

Table 7: p-value distribution of 16S rDNA gene sequence libraries comparing the abundance differences of microbial communities among samples from different age periods of local Omani chickens

	p-value					
Order	Day 5-15	Day 5-25	Day 5-35	Day 15-25	Day 15-35	Day 25-35
Bacillales	0.54	0.97	0.60	0.79	0.97	0.77
Bacteroidales	0.08	0.27	0.37	0.71	0.56	0.79
Bifidobacteriales	0.94	0.50	0.28	0.49	0.31	0.93
Burkholderiales	0.94	0.96	0.94	0.95	0.97	0.95
Caulobacterales	0.84	0.84	0.97	0.82	0.88	0.87
Clostridiales	0.97	0.91	0.81	0.95	0.82	0.77
Corynebacteriales	0.32	0.26	0.32	0.61	0.56	0.26
Enterobacteriales	0.35	0.68	0.79	0.96	0.83	0.91
Erysipelotrichales	0.91	0.96	0.61	0.93	0.66	0.74
Lactobacillales	0.94	0.83	0.98	0.86	0.96	0.87
Micrococcales	0.64	0.79	0.26	0.90	0.18	0.19
Pseudomonadales	0.69	0.51	0.70	0.57	0.57	0.49
Rhizobiales	0.94	0.69	0.94	0.82	0.94	0.79
Selenomonadales	0.58	0.70	0.36	0.04	0.35	0.34
Verrucomicrobiales	0.81	0.62	0.30	0.57	0.33	0.30
Xanthomonadales	0.63	0.53	0.33	0.60	0.23	0.50
Unclassified	0.78	0.91	0.83	0.85	0.72	0.96

Table 8: P-value distribution of 16S rDNA gene sequence libraries comparing the abundance differences of microbial communities among samples from different intestinal segments for local Omani chickens

	p-value					
Order	Duodenum-Jejunum	Duodenum-Ileum	Ileum-Jejunum	Cecum-Duodenum	Cecum-Jejunum	Cecum-Ileum
Bacillales	0.465	0.65	0.85	0.02	0.01	0.05
Bacteroidales	0.327	0.15	0.60	0.09	0.06	0.07
Bifidobacteriales	0.767	0.35	0.35	0.30	0.29	0.84
Burkholderiales	0.000	0.00	0.65	0.00	0.93	0.84
Caulobacterales	0.001	0.00	0.68	0.00	0.00	0.01
Clostridiales	0.172	0.72	0.06	0.00	0.00	0.00
Corynebacteriales	0.350	0.63	0.44	0.00	0.27	0.08
Enterobacteriales	0.253	0.70	0.61	0.01	0.00	0.00
Erysipelotrichales	0.369	0.47	0.85	0.00	0.00	0.00
Lactobacillales	0.610	0.19	0.01	0.00	0.00	0.00
Micrococcales	0.466	0.66	0.19	0.07	0.04	0.00
Pseudomonadales	0.206	0.23	0.60	0.16	0.01	0.03
Rhizobiales	0.002	0.00	0.33	0.00	0.01	0.00
Selenomonadales	0.809	0.28	0.54	0.46	0.44	0.47
Verrucomicrobiales	0.662	0.13	0.42	0.47	0.48	0.46
Xanthomonadales	0.225	0.27	0.12	0.21	0.03	0.05
Unclassified	0.002	0.00	0.01	0.02	0.00	0.00

sequences of Lactobacillales were dominant in the duodenum, jejunum and ileum libraries, whereas 16S rDNA sequences of Clostridiales were dominant in the cecum libraries.

Differences in microbial communities among samples 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 of different age groups are presented in Table 8. Statistical comparisons of the libraries revealed that there were no significant differences ($p>0.05$) among the microbial composition of different age groups: day 5-day 15, day 5-day 25, day 5-day 35, day 15-day 25, day 15-day 35 and day 25-day 35.

The taxonomic composition distribution of the bacterial community in intestinal segments at the order level: Figure 3 shows that the diversity of the bacterial community in the intestinal segments of local Omani chickens changed from one age period to the next. Species with abundances less than 0.5% in all samples were categorized as 'Unclassified'. The intestinal segments of the duodenum, jejunum and ileum had a higher abundance of Lactobacillales and as the birds aged,

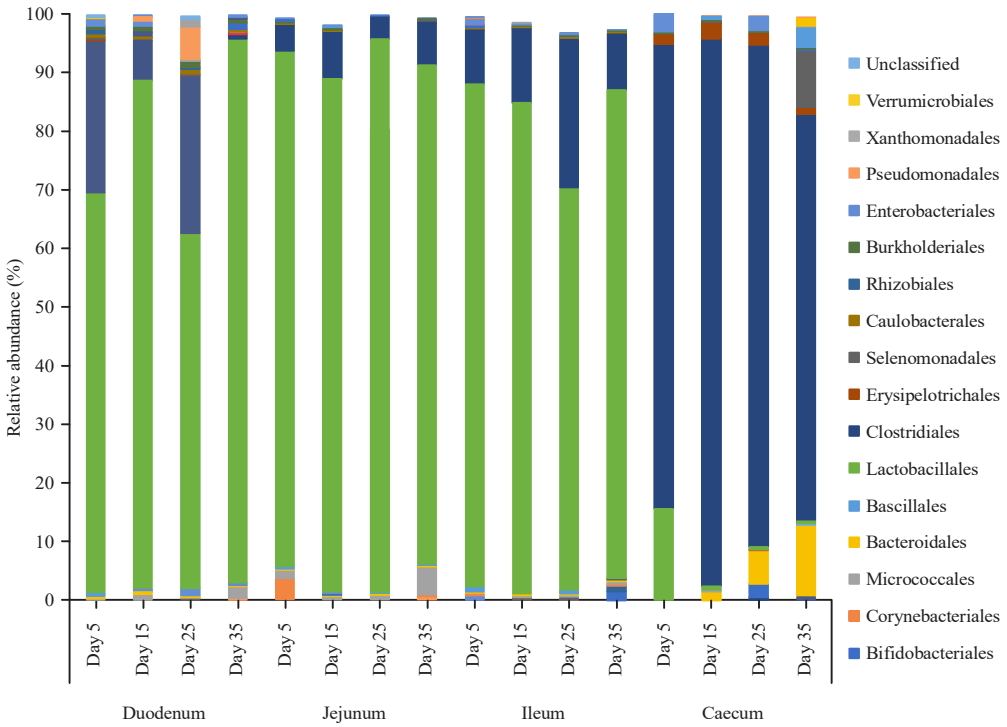

Fig. 3: Relative abundance of bacterial communities of local Omani chickens determined from different intestinal segments at different age periods from 16S rDNA libraries

the percentage of Lactobacillales decreased, whereas the cecum had a higher abundance of Clostridiales and as the birds aged, the percentage of Clostridiales increased.

DISCUSSION

A healthy gut is often considered an indicator of a healthy host, which in turn digests nutrients more efficiently for optimum animal production[20]. The most important characteristic of a healthy gut is the balance of its microflora. However, it is well recognized that many bacteria have not yet been cultured in the laboratory because their growth requirements are still unknown. Several molecular studies using 16S ribosomal DNA for phylogenetic analysis have yielded more detailed insight into the composition of the microbial community of this ecosystem[7,21-23]. In this study, the dynamics of the microbiota in the duodenum, jejunum, ileum and cecum of local Omani chickens supplied with a commercial diet were examined through 16S rDNA gene sequencing. In the present study, the statistical comparisons of the compositions of different 16S rDNA libraries of microbial communities revealed that each region from different intestinal segments developed its own bacterial community and the relative abundances of the bacteria in these communities were quite heterogeneous.

Our data showed that Lactobacillales was the dominant order of bacteria in the duodenum, jejunum and ileum throughout all age periods. In contrast, Clostridiales was the most abundant order detected in the cecum at different ages. Our results agree with the previous study of Lu *et al.*[22], who reported that nearly 70% of sequences from the ileum were related to those of Lactobacillus, whereas Clostridiaceae-related sequences at 65% were the most abundant group detected in the cecum. An interesting observation across the intestinal segments of the Omani chickens was that members from the class Actinobacteria-related sequences were detected at smaller percentages (0.93-3.01%) at different age periods. This very low number of Actinobacteria was not in agreement with reports of Actinobacteria being important in the upper digestive tract of other high-producing broilers (10-13%), such as Ross 308 chickens[24]. This observation suggests that smaller numbers of Actinobacteria in Omani chickens are not in keeping with optimal development of these intestinal segments.

Our study showed that the microbial community structure was moderately transient at an early age (day 5) and replaced by a relatively stable bacterial community during the period of rapid growth (15-35 days of age). Similar observations were reported by other studies which showed

that different regions of the chicken bowel also harbor different microorganisms and that the microbial community structure varies with age[7,11,22].

It is well known that the chicken GIT undergoes developmental changes that affect the microbiome as the different segments of the GIT become differentiated[25]. The findings in the current study showed that microbial composition and abundance in the four intestinal segments are different from each other, suggesting that different intestinal microbial compositions could influence intestinal function. The alpha-diversity indices (Chao1 index, Simpson index and Shannon index) showed that the microbial abundance was significantly different among different intestinal sections. Differences in microbial composition between the four intestinal segments/locations might be a cause and consequence of gut functional differences[10,26,27].

The results of PCoA showed that only the microbial community in the cecum formed a distinct cluster from those of the other three intestinal segments, signifying that the microbial composition of the duodenum, jejunum and ileum appeared to share some resemblance. In addition, the hierarchically clustered heat map analysis showed that the intestinal microbial composition of the duodenum, jejunum and ileum appeared to be observably comparable but was different from that of the cecum. Both analyses suggested that the microbial composition and abundance were different among the four intestinal segment locations, which might be because each intestinal segment has its own function with different microbial requirements. These findings were in accordance with those of Lu *et al.*[22], Xiao *et al.*[24] and Al-Marzooqi *et al.*[23], which provide evidence that gut microbiota compositions differed in different intestinal sections.

CONCLUSION

Our study shows that each region of different intestinal segments developed its own bacterial community and the relative abundance was quite diverse. Further study should be directed to evaluate the effect of dietary probiotics on the bacterial populations of Omani chicken using molecular techniques with 16S rDNA and the effect of these probiotics on growth performance and intestinal development. In addition, future studies will need to examine histological alterations related to intestinal function.

SIGNIFICANCE STATEMENT

This study revealed the heterogeneity of bacterial populations existing in different intestinal segments of local Omani chickens. This study will help researchers understand the diversity and succession of the bacterial community and consequently will permit the detection of disruptions in the microbiota. Thus, this information may enable manipulation of the intestinal flora to enhance intestinal health and bird performance in general.

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