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Effect of Broiler Age and Breeding Region on Bacterial Population Changes of Ileum

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Abstract

Chicken gut microbiota is affected by factors such as diets, environmental ,and bird age. In the current study, the effects of age and region on the ileum bacterial population of broiler chickens were investigated. A total of 2679 chickens in four geographical regions of Iran were sacrificed in the first to eighth week of age. Stool samples were collected and DNA was extracted and analyzed for the detection of Lactobacillus, Enterococcaceae, Clostridiaceae, Streptococcaceae, and Actinobacteria, using specific primers and probes. Purified amplicons were quantified by QuantiFluor® and pooled for sequencing. Findings showed that *L. acidophilus* was the dominant bacterium during the first four weeks, and was substituted with *L. crispatus* and *L. salivarius* in the next four weeks. So, the Lactobacillus family was the most dominant bacteria at all ages showing its essential role in chicken physiology. The age of chickens significantly affected the percentage of *L. crispatus*, *L. acidophilus*, *L. salivarius*, Clostridiaceae, Enterococcaceae, Streptococcaceae, and Actinobacteria. The breeding region influenced Streptococcaceae, with the highest percentage in the hot region. Chicken weight had a significant effect on Enterococcaceae. Broiler breeder age and distance to the nearest farm had no effect on ileum bacterial populations. This study showed there are several factors during the broiler breeding period that have an impact on microbial population changes at different ages.

Introduction

The microbial population in the gut plays an essential role in determining livestock health and production due to its effect on gastrointestinal immunology, physiology, biochemistry, development, and disease resistance. It also has an important impact on the quality of animal products (Gong *et al*. 2002). The gut microbiota makes a protective barrier by attaching to the enterocyte epithelial walls, decreasing the chance for colonization of the pathogenic bacteria (Yegani and Korver, 2008). It contributes to the competitive removal of pathogens or non-indigenous microbes (Dibner and Richards, 2005). Well-working microbiota in the small intestine of poultry can influence the proper digestion and absorption (Gong *et al*. 2002; Smits *et al*. 1997). Microorganisms can interact with the gastrointestinal tract (Van Leeuwen *et al*. 2004) and may affect the bird's physiology and immunological status (Klasing *et al*. 1999). This interaction may have positive or negative effects on chicken physiology, depending on the function of the gut microbial population (Droleskey *et al*. 1994).

The gut microbial population is affected by different diets, feed additives (e.g., prebiotics, probiotics), antibiotics, , and pathogens (Pan and Yu, 2014; Yeoman *et al*. 2012; Zhao *et al*. 2013), as well as birds age (Ballou *et al*. 2016; Danzeisen *et al*. 2013). Studies on chicken gut microbial populations have shown diverse results due to different environmental factors, technical methods, and biological variations in the hosts (Brooks *et al*. 2015; Lozupone *et al*. 2013).

Modern technologies in poultry production have acted as a barrier to the natural transmission of microbial populations between generations of birds. Poultry-producing systems are dependent on the use of antibiotics in their diets to combat bacterial pathogens. Antibiotic resistance and the potential existence of

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antibiotic residuals in poultry products have led to a search for more efficient substitutions in their diets to stimulate beneficial microbiota in the chicken gut (Gong *et al.* 2002; Stanley *et al*. 2014). Factors affecting chicken intestinal microbiota have been categorized into biological and environmental factors. Biological factors include chicken age, sex, breed, gut region, and maternal factors. Among environmental factors, hygiene, medication, feed, housing, geographical location, and temperature can be mentioned (Stanley *et al.* 2014). Biological knowledge of the gut microbial population and gut-microbe interactions over different periods and in diverse climatic regions could help find alternative strategies such as probiotics (Barrow, 1992) to combat pathogens and support the poultry industry.

The most common method for the detection of poultry gut microbiota has been bacterial culture (Barnes, 1979; Mead, 1989). However, this method is selective or not applicable to some bacteria (Ricke and Pillai, 1999; Theron and Cloete, 2000). Molecular techniques have recently shown promising applications, even in the detection of non-cultivable or misclassified bacteria (Tellez *et al*. 2006). The use of bacterial 16S ribosomal RNA (rRNA) gene sequencing has provided promising results in the detection of bacterial composition and diversity in the chicken gut (Lu *et al*. 2003b; Nathiya *et al*. 2012).

In the present research, to determine the effect of different environmental/biological factors on the microbial population of chicken ileum, the PCR-sequencing technique was used to detect different bacterial species promptly (eight weeks of the breeding period) at different geographical regions; then, the effects of age and region on the bacterial population change were analyzed.

Materials and Methods

Ethical Standards-All animal procedures including the use, care, and management of broiler chickens were conducted according to the guidelines of the Iranian Council of Animal Care (1995) and approved by the Animal Care Committee of Isfahan University of Technology.

Mixed-gender broiler chicks (Ross 308), raised in four different geographical regions of Iran (1: north/Gorgan, mean temperature: 29.6ºC; 2: northeast/Mashhad, mean temperature: 27.5ºC; 3: center/Arak, mean temperature: 26.4ºC and 4: southwest/Ahvaz, mean temperature: 38.2 ºC) were considered for analysis. All farms had used standard sterile water and Ross standard ration. Erythromycin, tetracycline, and tylosin were the antibiotics administered on the farms. Broilers in all farms were raised on wooden shavings. The distance of each farm to the nearest farm was recorded. The age of their broiler breeders was in the range of 40 to 61 weeks. The samples were collected from a total of 2679 broiler chickens in four farms (Gorgan: 182, 157, 177, and 170; Mashhad: 173, 176, 179, and 184; Arak: 180, 167, 178 and 186; Ahvaz: 140, 136, 142, and 152),. The weight of chickens was recorded before sampling. Chickens were randomly selected and sacrificed in the first to eighth week of age, by intracranial injection of ketamine (0.2 mL for the first three weeks, and 0.6 mL for other weeks) and the stool samples were collected from the ileum immediately. The samples were transferred to the laboratory on ice and kept at the temperature of -70 ºC until use. Total DNA was extracted from the stool samples using QIAamp DNA Stool Mini Kit, (QIAGEN, Valencia, CA) following the manufacturer's guide. The DNA samples were analyzed for detection of Lactobacillus (L. *crispatus*, L. *salivarius* subsp. DSM 20554, and L. *acidophilus*), Enterococcaceae, Clostridiaceae (C. perfringens and C. butyricum), Streptococcaceae and Actinobacteria. All specific primers and probes matching 16s rDNA are represented in Table 1. PCR was performed in triplicate using a 20 μL reaction system with 5 μM each primer, 10 ng DNA template, 4 μL 1× FastPfu Buffer, 2.5 mM dNTPs and 0.4 μL FastPfu Polymerase (TransGen Biotech, Beijing, China). PCR condition consisted of heating at 95°C for 2 min and 25 cycles including denaturation at 95°C for 45 s, annealing at specific temperatures for 45 s and extension at 72°C for 60 s, as well as a final extension at 73°C for 3 min. PCR products from the same sample replicates were pooled together and visualized after electrophoresis on an agarose gel. The desired PCR fragments were cut out and extracted using AccuPrep® Gel Purification Kit (Bioneer, Daejeon 306-220, Korea).

For Streptococcaceae identification, the extracted DNA was digested with *Hind III*, *Hae III*, and *EcoRI* restriction enzymes, and the products were blotted on Hybond N+ nylon membrane and hybridized with 5'end 32 P labeled 16S rRNA synthetic oligo probe (5'AAGAGTTTGATCCTGGCTCAG3') (Biobasics, Canada) at 37 °C. The hybridized membrane was washed and autoradiographed according to a study conducted by Seppälä *et al*. (1994).

The purified amplicons and fragments were quantified by QuantiFluor® (Promega, Fitchburg, WI) using Fluorometer and pooled together with an appropriate proportion for sequencing using Illumina MiSeq platform obtained from Seqlab Co. (Göttingen, Germany). The sequences were compared with the available sequences in GenBank using the BLASTN tool (http://blast.ncbi.nlm.nih.gov/Blast). Sequence identities of more than 97% were regarded as belonging to the same species (Tannock, 1999).

Statistical analysis was performed using the SAS software, Proc. GLM. The following model was used to determine the effects of region and chicken age (week), on the bacterial population of the ileum. Chicken weight, breeder age, and distance to the nearest farm were considered covariates.

$$y_{ijk}= \mu + AG_i + REG_j + \beta1(AGB) + \beta2(DIS) + \beta3(WT) + \varepsilon_{ijk}$$

where y_{ijk} is the bacterial proportion, m is the overall mean, AG_i is the fixed effect of the chicken age, REG_j is the fixed effect of geographical region, $\beta1$, $\beta2$, and $\beta3$ are the regression coefficients for AGB (covariate effect of breeder age), DIS (covariate effect of distance to the nearest farm), and WT (covariate effect of chicken weight), and ε_{ijk} is the random residual effect. Comparisons of means were performed according to Student's t-test (calculated by least squares methods). The significance probability level was defined at $P \leq 0.05$.

Table 1. PCR primers and probes

Bacteria		Accession Number	Primers/ Probes	Sequences (5'-3')	Reference
Lactobacillaceae	*L.crispatus*	NZ_CP039266.1	Cri 16SI Cri 16SII	GTAATGACGTTAGGAAAGCG ACTACCAGGGTATCTAATCC	Walter *et al*. 2000
	L.salivarius	NZ_LT604074.1	LsaliF LactoR	CGAAACTTTCTTACACCGAATGC GTCCATTGTGGAAGATTCCC	Byun *et al*. 2004
	L.acidophilus	NC_021181.2	L-aci-1 L-acid-2	TGCAAAGTGGTAGCGTAAGC CCCTTTCCCTCACGGTACTG	Brolazo *et al*. 2011
Enterococcaceae		NZ_AP025635.1	338 F 806R	ACTCCTACGGGAGGCAGCA GGACTACHVGGGTWTCTAAT	Zhu *et al*. 2019
Clostridiaceae	*C.perfringens*	NZ_CP075979.1	Forward Reverse	AGATGGCATCATCATTCAAC GCAAGGGATGTCAAGTGT	Kikuchi *et al*. 2002
	C. butyricum	NZ_CP013239.1	Forward Reverse	TACCGCATGGTACAGCAATT TCGCGAGGTTGCATCTCAT	This study
Actinobacteria			243F AB1165R	GGATGAGCCCGCGGCCTA ACCTTCCTCCGAGTTGAC	Haesler, 2008
		NZ_JAGPXE010000030.1	Act283F Act1360R	GGGTAGCCGGCCUGAGAGGG CTGATCTGCGATTACTAGCGACTCC	McVeigh *et al*. 1996

Results

The sequences of Lactobacillaceae, Enterococcaceae, Clostridiaceae, Streptococcaceae, and Actinobacteria were detected in all stool samples. Statistical analysis indicated that the age of chickens had a significant effect on the percentage of *L. crispatus*, *L. acidophilus*, *L. salivarius*, Clostridiaceae, Enterococcaceae, Streptococcaceae ($P < 0.0001$), and Actinobacteria ($P < 0.01$). Changes in each bacterial species are compared between different weeks in Table 2. Lactobacillaceae was the most abundant family during the breeding preriod. *L. acidophilus* was the dominant bacteria during the first four weeks of age, and then, was substituted with *L. crispatus* and *L. salivarius* in the next 4 weeks. Clostridiaceae showed a significant decrease at early stages followed by an increase at later stages. Similar variations were observed for Enterococcaceae and Streptococcaceae with an initial increase in early weeks followed by a later decrease. Actinobacteria showed a completely different variation profile, which was stable at the initial stages with a high peak in the 4th week; followed by a significant decrease.

Table 2. Effect of age on the percentage of bacterial species in the ileum of broiler chickens [LSMeans ± SE]

Bacterial species	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
L. crispatus	0.053 ^{ab} ± 0.073	0.038 ^{b} ± 0.066	0.076 ^{bd} ± 0.075	0.285 ^{c} ± 0.065	0.670 ^{e} ± 0.073	0.521 ^{ef} ± 0.065	0.420 ^{cf} ± 0.077	0.350 ^{acdf} ± 0.128
L. acidophilus	0.257 ^{a} ± 0.078	0.568 ^{b} ± 0.071	0.572 ^{bc} ± 0.080	0.248 ^{a} ± 0.069	0.043 ^{ad} ± 0.078	0.023 ^{d} ± 0.069	0.013 ^{d} ± 0.082	0.010 ^{ad} ± 0.136
L. salivarius	0.023 ^{a} ± 0.020	0.015 ^{a} ± 0.019	0.043 ^{a} ± 0.021	0.037 ^{a} ± 0.018	0.066 ^{a} ± 0.020	0.254 ^{b} ± 0.018	0.361 ^{c} ± 0.021	0.390 ^{c} ± 0.036
Clostridiaceae	0.130 ^{a} ± 0.010	0.042 ^{b} ± 0.009	0.066 ^{bc} ± 0.011	0.060 ^{bc} ± 0.009	0.073 ^{c} ± 0.010	0.087 ^{c} ± 0.009	0.149 ^{ad} ± 0.011	0.190 ^{d} ± 0.018
Enterococcaceae	0.048 ^{ac} ± 0.008	0.082 ^{b} ± 0.007	0.057 ^{a} ± 0.008	0.025 ^{d} ± 0.007	0.023 ^{d} ± 0.008	0.018 ^{d} ± 0.007	0.020 ^{d} ± 0.008	0.020 ^{cd} ± 0.014
Streptococcaceae	0.090 ^{ac} ± 0.019	0.217 ^{b} ± 0.017	0.136 ^{a} ± 0.020	0.020 ^{d} ± 0.017	0.013 ^{d} ± 0.019	0.013 ^{d} ± 0.017	0.014 ^{d} ± 0.020	0.010 ^{cd} ± 0.034
Actinobacteria	0.032 ^{ade} ± 0.011	0.022 ^{ac} ± 0.010	0.027 ^{aeg} ± 0.012	0.081 ^{b} ± 0.010	0.060 ^{eb} ± 0.011	0.045 ^{ae} ± 0.010	0.010^{cdfg} ± 0.012	0.020 ^{aef} ± 0.020
Rest unknown	0.365 ^{ac} ± 0.080	0.015 ^{b} ± 0.072	0.023 ^{bd} ± 0.081	0.244 ^{ade} ± 0.070	0.053 ^{be} ± 0.080	0.040 ^{be} ± 0.070	0.013 ^{b} ± 0.083	0.010 ^{be} ± 0.139

$^{a-g}$ Values with different letters in each row indicate significant differences at $P \leq 0.05$.

The region of broiler chicken breeding significantly changed Streptococcaceae percentage in stool collected from the ileum ($P = 0.0220$). Chickens studied in Ahvaz had a significantly higher Streptococcaceae percentage in their ileum in comparison to other regions. Other bacterial species

were not significantly affected by the geographical regions of farms. However, some significant differences were found in the percentages of Actinobacteria and Clostridiaceae between some regions (Table 3).

Chicken weight had only a significant effect on Enterococcaceae ($P = 0.0048$), which was increased by an increase in weight (b = 0.37). Broiler breeder age and distance to the nearest farm had, however, no effect on the bacterial population of the chicken ileum through the farms ($P < 0.05$).

Table 3. Effect of the breeding region on the percentage of bacterial species in the ileum of broiler chickens [LSMeans ± SE]

Bacterial species	Ahwaz	Arak	Gorgan	Mashhad
L. crispatus	$0.343^{a} \pm 0.039$	$0.332^{a} \pm 0.040$	$0.342^{a} \pm 0.033$	$0.424^{a} \pm 0.033$
L. acidophilus	$0.363^{a} \pm 0.028$	$0.388^{a} \pm 0.028$	$0.351^{a} \pm 0.023$	$0.337^{a} \pm 0.023$
L. salivarius	$0.058^{a} \pm 0.013$	$0.068^{a} \pm 0.013$	$0.069^{a} \pm 0.011$	$0.052^{a} \pm 0.011$
Clostridiaceae	$0.072^{ab} \pm 0.012$	$0.073^{ab} \pm 0.012$	$0.096^{a} \pm 0.010$	$0.063^{b} \pm 0.010$
Enterococcaceae	$0.037^{a} \pm 0.009$	$0.0501^{a} \pm 0.009$	$0.043^{a} \pm 0.007$	$0.045^{a} \pm 0.007$
Streptococcaceae	$0.067^{a} \pm 0.007$	$0.0441^{b} \pm 0.007$	$0.036^{b} \pm 0.006$	$0.042^{b} \pm 0.006$
Actinobacteria	$0.060^{ab} \pm 0.009$	$0.0449^{abc} \pm 0.009$	$0.063^{b} \pm 0.007$	$0.035^{c} \pm 0.007$

[a-b] Values with different letters in each row indicate significant differences at $P \leq 0.05$.

Discussion

Several factors can have both short- and long-term effects on the intestinal microbiota composition; these may even originate from the hatching stage or a previous generation (Kers *et al*. 2018). Among the factors that can affect poultry gut microbial composition and poultry performance, antibiotics and feed composition can be noted. However, a stronger effect on the composition of the microbiota was shown to be related to the chicken environment (battery cages vs. floor pens) (Pedroso *et al*. 2006). In the present study, we investigated the effect of chicken age, farm geographical region (as fixed effects) and chicken weight, breeder age, and distance to the nearest farm on the ileum microbial population change in broiler chickens. It is well-known that diets, hosts, and environment can significantly affect the gut microbiota. Comprehensive studies on the intestinal microbial population will help us understand the dynamics of the microbiota function and structure, providing information about intestinal health in poultry (Kers *et al.* 2018).

Providing information about different variables such as feed composition and access, chicken age and sex, vaccinations, breeding systems, farm density, location, environmental conditions and temperature in a study can improve the repeatability and interpretation of the data related to chicken intestinal microbiota (Kers *et al.* 2018).

One of the major factors affecting the performance of chickens is the local climate of a poultry flock. The temperature in poultry houses is almost controlled; however, the geographic region may affect the climate in the poultry flock and as a result, the intestinal microbiota of chickens may be affected (Videnska *et al*. 2014; Zhou *et al*. 2016). Hot and highly humid weather may also reduce broiler chicken production (Laudadio *et al*. 2012). There are some studies available, which have evaluated the effects of heat stress on the performance and microbiota composition changes in broiler chickens, using 16S rRNA sequencing for detection (Lan *et al*. 2004; Sohail *et al*. 2015). It is demonstrated that heat stress can cause susceptibility to *E. coli* (Laudadio *et al.* 2012). Changes in the normal intestinal microbiota due to stress decrease the innate immunity and may help *Salmonella* bind to and colonize the intestinal epithelial cells (Burkholder *et al*. 2008; Soliman *et al*. 2009). Birds exposed to higher temperatures for 24 h showed greater changes in the ileal than the cecal content, thereby indicating higher sensitivity of the microbial population in the ileum compared to the cecum (Burkholder *et al*. 2008). In the present study, chickens in Ahwaz, a hot and humid region in the southwest of Iran, had a significantly higher Streptococcaceae percentage in the ileal content compared with those in Arak, Gorgan, and Mashhad. It seems that this bacterial family was more sensitive to heat stress than the other studied bacteria, and this was shown by the higher growth rate.

It has been revealed that microbial community structure changes with age (Barnes *et al*. 1972; Salanitro *et al*. 1974). Also, there is a report noting that microbial population is almost stable during rapid skeletal growth (2 to 3 weeks of age), followed by significant change at the end of the 7th week. Here, we detected sequences related to Lactobacillus, Enterococcaceae, Clostridiaceae, Streptococcaceae, and Actinobacteria using 16s rDNA-specific primers and sequencing. Lactobacillaceae was found as the most abundant family in all age groups, which was similar to the previous studies (Danzeisen *et al,* 2013; Lu *et al*. 2003a). *L. acidophilus* was the most dominant sequence during weeks 1 to 4, which was substituted with *L. crispatus* in the 5th week, and *L. crispatus and L. salivarius* in weeks 6 to 8. This developmental route was in concordance with a previous study (Lu *et al*. 2003a) on bacterial flora diversity in the chicken ileum, reporting that the dominant sequences from days 7 to 21, 28 to 49, and on day 49 of age belonged

to *L. acidophilus*, *L. crispatus L. salivarius*, respectively. It seems that the Lactobacillaceae population goes under significant changes during the studied eight weeks, i.e. different profiles of increase and decrease were observed for different species during the first and second four weeks. This may be due to the changes in the physiological condition of the growing broilers. In a previous in vitro study, it was shown that *L. acidophilus* can reduce the expression of IL-10, IFN-γ, and TGF-β in CD8+ cells, while *L. salivarius* acts inversely (Brisbin *et al*. 2012). These results can be related to the immunological changes during chicken growth. In our study, *L. crispatus* percentage was stable during the first three weeks but increased after the 4th week and was almost stable thereafter. Lu *et al*. (2003a) have shown that *L. crispatus* sequences were dominant in the ileal library from 4 to 7 weeks. Our observations showed that *L. acidophilus* increased in the 2nd week; then it was stable until the 4th week, and finally decreased. A very similar result was obtained by Lu *et al*. (2003a), who noted that the dominant sequences homologous to *L. acidophilus* existed between? 7 to 21 days of age. *L. salivarius* was stable throughout the first 5 weeks and then increased until the 7th week and remained stable thereafter. In the previously mentioned study (Lu *et al*. 2003a), *L. salivarius* had a fairly similar pattern of change, except that it showed a rising shift up on day 49 of age (after the 7th week). In another study (Ranjitkar *et al*. 2016), a sharp increase was observed in the relative abundance of *L. salivarius* from 15 to 22 days in the ileum, which remained high during the growing period (until 36 days). Clostridiaceae decreased in the 2nd week and remained almost stable until the 6th week; however, a slow rate of increment was observed; then, it dramatically increased during the 7th and 8th weeks. This result was somehow different from a previous study (Lu *et al*. 2003a), which revealed the increase in clostridia percentage as early as days 3 to 49. However, Clostridiaceae has been reported to increase from 5% on day 8 to 19% on day 36 (end of the 5th week) (Ranjitkar *et al*, 2016). Enterococcaceae and Streptococcaceae showed a similar variation pattern, which significantly increased in the 2nd week; then a significant reduction occurred in the 3rd and 4th weeks, and this remained stable thereafter. Both Enterococcus and Streptococcus were the dominant 16S rDNA sequences in the ileal libraries for 7 to 21 days of age, according to Lu *et al*. (2003a). In another study, Enterococcaceae has shown some moderate abundance on day 7, but decreased sharply on day 15 and remained low throughout the growing period (studied until 36 days). Actinobacteria was stable throughout the first three weeks, increased significantly in the 4th week, decreased significantly in the 6th and 7th weeks, and then, remained stable. However, no Actinomyces were detected in the study of Lu *et al*. (2003a). Altogether, it shows that the 4th and 7th weeks are the critical ages for broiler chickens since some dramatic changes occur in the microbial community structure of the ileum at these times. Our results, therefore, confirmed the conclusion drawn by Lu *et al*. (2003a), revealing that the microbiota was fairly stable during 14 to 28 days of age (the time of rapid skeletal growth) and then had a big change at the end of the 7th week (during the period of significant weight gain). Gut microbial changes at different ages during the eight weeks of growth are in interaction with immunological responses needed at each stage and are needed for bird health and productivity (Brisbin *et al*. 2012; Oakley and Kogut, 2016; Zhang *et al*. 2022)

Different studies on humans or mice have shown that obesity could be associated with some changes in the bacterial phyla proportions, leading to fewer Bacteroidetes and more Firmicutes (Hildebrandt *et al*. 2009; Ley *et al*. 2005; Ley *et al*. 2006). However, a study on the relationship between the microbiota in the chicken gut and body weight has shown that there is no significant correlation between Firmicutes/Bacteroidetes in the ileum, and body weight (Han *et al*. 2016). In the present research, two phyla of Firmicutes and Actinobacteria were studied and only one bacterium from the phylum Firmicutes (Enterococcaceae) was positively related to weight gain. In a study on *Enterococcus faecium* supplementation of the broiler diets, it was reported that this probiotic affected the diversity of the broiler gut microbiota and increased the bacterial population in jejunal and ileal chymes. *Enterococcus faecium*-like species increased in ileum upon the probiotic treatment. Although daily weight gain and feed intake were not affected, the feed conversion ratio has slightly enhanced (Luo *et al*. 2013). In a complement study, broilers fed with *Enterococcus faecium* showed improved carcass composition with relatively more pectoral and leg muscles and less abdominal fat on day 42 (Zheng *et al*. 2014). The weight gain in our study, which was related to the Enterococcaceae population change, should be studied at both muscle and fat levels to find the positive or negative effects.

Our research showed that the age of breeders does not affect bacterial population change in the broilers' ileum. There is no report on the effect of breeder age on the changes in the intestinal microbial population of broiler chickens. However, a recent study on the origin of chicken embryo gut microbiota conducted on 23- and 34-week-old breeders have shown that the bacterial population diversity of oviduct increases with maturity in the breeders. Specific bacterial species detected in the maternal oviduct microbiota consisted of most of the cecal bacterial community in the chicken embryo. This demonstrates that the maternal oviduct bacteria may be partially transferred to the progeny during egg formation (Lee *et al*. 2019). We had supposed that the breeder age at the egg formation time

may affect the intestinal bacterial population of the progeny due to the changes occurring in microbiota in the breeder reproductive tract. However, no change in bacterial community was observed in chickens with breeders at the age of 40 to 61 weeks; hence, this hypothesis should be further investigated by analyzing the bacterial community of the chickens with breeders at younger ages.

The gut microbial community has long been studied due to its significance in host immunity and pathogen control. In this study, the effect of age and breeding region on the broiler chicken ileum microbiota was investigated, demonstrating that there are a lot of factors that can greatly influence the microbial population, and therefore, may change the host immunity, physiological indices, or productive performance, all of which should be further studied. The Lactobacillus family was the most dominant bacteria at all ages, indicating its indispensable role in chicken physiology. Farms in hot areas showed changes in the Streptococcaceae percentage, which may influence their production efficiency. However, there are other bacterial species not included in the present research that need to be studied. This information will help better understand the dynamics of chicken microbiota and intestinal health, as well as control the microbial structure and function according to environmental factors. Whether the alteration in ileal bacterial diversity affects the production in the broiler industry needs to be further investigated.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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