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ORIGINAL PAPER

Growth performance and caecal microbiota of broilers fed Indian gooseberry and Habanero pepper powders

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ABSTRACT. This study evaluated the impact of Indian gooseberry powder and Habanero pepper powder on the ceacal microbiota and growth performance of broiler chickens. A total of 48 male Ross 308 broilers were randomly divided into four groups: a basal diet as the control group (CON), a basal diet supplemented with 0.02% chili pepper powder (CPP), a basal diet supplemented with 0.02% Indian gooseberry powder (IGP), and a basal diet supplemented with 0.02% chili pepper powder and 0.02% Indian gooseberry powder (CI200). The chickens' growth performance was monitored weekly over a period of 42 days. Feed conversion ratio (FCR) differed ($P < 0.05$) between the CI200 and control groups, with no mortalities observed. Quantitative polymerase chain reaction was used to quantify gut bacterial species, showing that supplementation with IGP increased the abundance of *Lactobacillus* sp. ($P < 0.01$) in the ileum and reduced the population of *Escherichia coli* ($P < 0.01$) in the caecum of broilers. NextGen sequencing and beta-diversity analysis revealed significant differences between the IGP, CI200 and control groups ($P < 0.05$). *Firmicutes* and *Bacteroidetes* were the dominant phyla across all groups. In the IGP group, the relative abundance of *Lactobacillaceae*, *Bacteroidaceae* and *Bacillaceae* was higher compared to the other groups ($P < 0.05$), while the counts of *Bacillaceae*, *Corynebacteriaceae* and *Erysipelatoclostridiaceae* were increased compared to the IC200 group in all treatments. No significant difference in bacterial taxa was detected in the CPP group. The increased abundance of bacteria in the IC200 group resulted in significant improvements in chicken weight gain and FCR in this study.

Introduction

Broiler chickens have been selectively bred to meet the increasing consumer demand for chicken meat, resulting in rapid growth improvements (Devatkal et al., 2019). However, modern broilers exhibit a higher metabolic activity, making them more susceptible to heat stress (Settar et al., 1999). High temperature and humidity, coupled with overcrowding or poor rearing management, can result in oxidative stress, which is one of the major causes that affect poultry growth (Akbarian et al., 2016). Therefore, substances or feed additives that reduce the effects of oxidative stress in animals may play a role in improving growth.

Habanero pepper (*Capsicum chinense*), dried to obtain chili pepper powder (CPP), contains bioactive compounds such as capsaicinoids (capsaicin, 69%)

and polyphenol (0.76%). These compounds exhibit anti-inflammatory properties and have been shown to decrease lipid peroxidation and microbial growth (Suganya et al., 2016; Liu et al., 2021). By reducing lipid peroxidation, capsaicin helps protect the cells and tissues of the intestinal tract from oxidative damage. Indian gooseberry powder (IGP), or amla (*Emblica officinalis* Gaertn.), contains polyphenols like gallic acid, ellagic acid, different tannins, and flavonoids such as rutin and quercetin (Kumar et al., 2018). It has strong antioxidant, anti-inflammatory and immunomodulatory properties (Kumar et al., 2018), and improves feed digestion, metabolism, and nutrient absorption in broilers (Kumar and Singh, 2005). Patel et al. (2016) also reported that IGP supplementation (at 0.4 and 0.8%) improved growth performance of broilers.

A study conducted by Li et al. (2022) demonstrated that CPP supplementation enhanced gut health by enhancing its oxidative status and bacterial composition. Similarly, Kim et al. (2015) reported that CPP increased antimicrobial activity in the intestine. A study by Kumar et al. (2011) found that substances in Indian gooseberry were active against pathogenic bacteria, which was consistent with the results of Saeed and Tariq (2007), who showed antimicrobial effects of amla. However, in most experiments, chickens are divided into small groups, typically 20–25 per cage with the same feeder in pen for total feed intake per pen. The objective of this study was to determine the effect of CPP and IGP supplementation, as well as their combination on the caecal microbiota of broiler chickens housed in pens on a separate floor during the grower and finisher periods.

Material and methods

Animals, experimental design, and diets

The experimental protocol was approved by the Animal Ethics Committee of Kasetsart University (ACKU66-AGR-009). A total of 48 1-day-old male Ross 308 broilers were housed in an evaporative cooling system. The chicks were randomly allocated to 48 individual pens (0.4 × 1.2 m/bird) divided into four dietary treatments, with 12 replicates per treatment. The experimental period lasted 42 days. Table 1 shows the feed formulation and calculated nutrient composition. The diet was based on the Ross 308 strain handbook (Aviagen, 2018). The treatments consisted of a basal diet as the control (CON), a basal diet + 0.2 g/kg chili pepper powder (CPP), a basal diet + 0.2 g/kg Indian gooseberry powder (IGP), and a basal diet + 0.2 g/kg chili pepper powder and 0.2 g/kg Indian gooseberry powder (CI200).

Table 1. Ingredients and nutritional composition of the basal diet used in the experiment

Ingredients	Amount, kg		
	Starter (days 0–10)	Grower (days 11–24)	Finisher (days 25–42)
Maize	55.46	58.28	62.95
Soybean meal 48%	38.60	35.17	30.14
Rice bran oil	2.11	3.15	3.86
Monocalcium phosphate 22%	0.77	0.59	0.45
Limestone	1.29	1.16	1.05
Salt	0.58	0.47	0.31
Sodium bicarbonate	–	0.15	0.30
DL-methionine	0.32	0.26	0.23
L-lysine	0.20	0.12	0.13
L-threonine	0.10	0.06	0.03
Vitamin and mineral premix[1]	0.24	0.24	0.24
Choline chloride 60%	0.08	0.08	0.08
Antioxidant and toxin binder	0.16	0.16	0.16
Anticoccidial	0.05	0.05	–
Phytase 10000, IU/g	0.01	0.01	0.01
Nutrients by calculation, %			
metabolizable energy, kcal/kg	3000	3100	3200
crude protein	23	21.5	19.50
fibre	3.59	3.45	3.26
fat	4.68	5.77	6.56
methionine	0.67	0.60	0.54
methionine + cysteine	1.08	0.99	0.91
lysine	1.44	1.29	1.16
threonine	0.97	0.88	0.78
valine	1.13	1.06	0.97
calcium	0.96	0.87	0.79
total phosphorus	0.72	0.67	0.62
available phosphorus	0.48	0.44	0.39
Na	0.23	0.23	0.21
dietary electrolyte balance, mEq/kg	246	250	244

[1] premix provided per kg of diet: IU: vit. A (transretinyl acetate) 10000, vit. D_3 (cholecalciferol) 3000, vit. E (all-rac-α-tocopherol) 30; mg: menadione 1.3, thiamine 2.2, riboflavin 8, nicotinamide 40, choline chloride 400, calcium pantothenate 10, pyridoxine HCl 4, biotin 0.04, folic acid 1, vit. B_{12} (cobalamin) 0.013, Fe (from ferrous sulphate) 80, Cu (from copper sulphate) 8.0, Mn (from manganese sulphate) 110, Zn (from zinc oxide) 60, I (from calcium iodate) 1.1, Se (from sodium selenite) 0.3

Growth performance and sample collection

Growth performance data were collected during the grower (days 11–24) and finisher (days 25–42) phases. At 24 and 42 days of age, the birds were individually weighed. These measurements were used to calculate body weight gain (BWG) and individual feed intake (IFI). Feed conversion ratio (FCR) and feed cost per gain (FCG) were also determined.

DNA Isolation, 16s rRNA V3V4 amplification, and data analysis

Caecal contents were collected from six birds per treatment group, selected based on the mean body weight in each pen. DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany). To analyse the metagenomic profile, a 16S sequencing library was constructed for amplicons sequenced using Illumina systems (Illumina, San Diego, CA, USA). The following forward primer: 5′-CCTACGGRRBGCASCAGKVRVGAAT-3′, and the reverse primer: 5′-GGACTACNVGGGTWTCTAATCC-3′ (V3 and V4) were used in the PCR assay.

The resulting sequences were analysed using Quantitative Insights Into Microbial Ecology (QIIME2 v2022.11). The sequencing of the 16S rRNA gene yielded quality-controlled raw reads ranging from 65092 to 91374 across 24 samples, with an overall frequency of 1898763. This sampling depth was selected for the analysis of α-diversity, β-diversity, relative microbial abundance, and unweighted UniFrac distances. Shannon diversity (alpha-diversity) was assessed to measure community evenness, richness, and the number of observed operational taxonomic units (OTUs). Bray Curtis and unweighted UniFrac metrics were used for the analysis of beta-diversity. The Linear Discriminant Analysis (LDA) Effect Size (LEfSe Galaxy v1.0) was utilised to identify differences in the abundance of each taxon between samples.

Real-time PCR

The DNA was used for real-time PCR amplification with specific bacterial primers (Table 2), using a Bio-Rad CFX96 real-time PCR System (Bio-Rad, Hercules, CA, USA) and the 5 × HOT FIREPol® EvaGreen® qPCR Mix Plus (Thermo Fisher Scientific, Waltham, MA, USA). Bacterial counts were determined in qPCR using a standard curve established for each run. The genomic size of the bacteria was used for calculations, and results were reported as log CFU/g of digesta.

Table 2. Real-time PCR primers

Genes	Primer sequence (5'→3')	Product size, bp
Lactobacillus sp.[1]	F-AGCAGTAGGGAATCTTCCA	341
	R-CACCGCTACACATGGAG	
Salmonella spp.[2]	F-TCATCGCACCGTCAAAGGAACC	284
	R-GTGAAATTATCGCCACGTTCGGGCAA	
Escherichia coli[3]	F-CATGCCGCGTGTATGAAGAA	585
	R-CGGGTAACGTCAATGAGCAAA	

[1] Walter et al., 2001; [2] Li et al., 2012; [3] Penders et al., 2007

Statistical analysis

The data were analysed using the general linear model (GLM) procedure in SAS (SAS Institute, Inc., Cary, NC, USA, 1996) in a completely randomised design. Results are reported as means ± standard deviation (SD) and standard error of the means (SEM). One-way ANOVA was performed to test for differences among the experimental groups, and Turky's tests was applied to determine significant differences in the means at $P < 0.05$. The statistical model employed was:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij},$$

where: Y_{ij} – observed response for the i^{th} treatment in the j^{th} replicate, μ – overall mean of the response variable, τ_i – effect of the i^{th} treatment (with i = 1, 2, ..., t, where t is the number of treatments), ε_{ij} – random error associated with the j^{th} replicate of the i^{th} treatment, assumed to be normally distributed with a mean of 0 and variance σ^2 (i.e., $\varepsilon_{ij} \sim N(0, \sigma^2)$).

Results

Growth performance

Table 3 presents the results regarding the growth performance of broiler chickens. During the grower period (days 11–24), birds from the CI200 group had a significantly improved FCR compared to the control group, and no broiler mortality was recorded during this phase. However, the experimental treatments did not lead to improvements in growth performance parameters during the finisher phase. As for the overall performance (days 10–42), broilers in the CI200 group showed a significant improvement in FCR.

Microbial community composition

Th analysis of microbiome diversity indices revealed no significant differences between the CON group and the treatment groups in terms of the number of observed OTUs, Shannon's diversity, Pielou's evenness, and Faith's diversity indices (Figure 1A–D). However, for beta-diversity of the microbiota, the unweighted UniFrac measures differed between the ICP, CI200, and control groups ($P < 0.05$; Figure 1E).

The three main phyla identified were *Firmicutes* (70–76%), *Bacteroidetes* (21–27%), and *Actinobacteria* (0.4–0.9%) (Figure 2A). The LDA effect size was employed to detect significant differences in caecal bacterial taxa from phylum to genus levels between the treatment groups. No statistically significant differences were identified between species at the phylum level. However, the LEfSe

Table 3. Effect of dietary chili pepper and Indian gooseberry powders supplementation on broiler performance

Parameter	Treatment				SEM	*P*-value
	CON	CPP	IGP	CI200		
Grower (11–24 days)						
initial body weight, g	301.73 ± 10.16	309.18 ± 13.90	301.91 ± 17.40	297.67 ± 8.85	13.09	0.23
final body weight, g	1391.36 ± 195.48	1492.18 ± 76.18	1451.09 ± 111.64	1451.82 ± 112.33	130.38	0.36
body weight gain, g	1090 ± 193.22	1183 ± 67.17	1149.18 ± 109.19	1154.18 ± 108.56	126.69	0.39
average daily gain, g/day	77.83 ± 13.80	84.50 ± 4.80	82.08 ± 7.80	82.44 ± 7.75	9.05	0.39
feed intake, g/day	1442.91 ± 102.04	1447.27 ± 121.28	1409.36 ± 114.80	1361.36 ± 125.02	115.93	0.30
feed conversion ratio	1.36 ± 0.26^{b}	1.22 ± 0.06^{ab}	1.23 ± 0.10^{ab}	1.18 ± 0.07^{a}	0.16	0.04
feed cost per gain	27.26 ± 5.28	24.63 ± 1.19	24.69 ± 1.96	23.88 ± 1.39	3.10	0.06
mortality, %	8.33	0	0	0	–	–
Finisher (25–42 days)						
initial body weight, g	1391.36 ± 195.48	1487.83 ± 74.18	1454.42 ± 107.07	1457.58 ± 108.95	130.38	0.36
final body weight, g	3326.36 ± 418.65	3443.64 ± 288.77	3359.09 ± 208.68	3540 ± 243.56	298.35	0.36
body weight gain, g	1935 ± 249.66	1951.45 ± 294.92	1908 ± 154.34	2088.18 ± 255.90	243.05	0.33
average daily gain, g/day	107.5 ± 13.87	108.41 ± 16.38	106 ± 8.57	116.01 ± 14.22	13.50	0.33
feed intake, g/day	3412.91 ± 290.46	3491.73 ± 377.89	3256.91 ± 310.28	3378.64 ± 284.87	314.81	0.39
feed conversion ratio	1.78 ± 0.14	1.81 ± 0.24	1.71 ± 0.19	1.63 ± 0.18	0.19	0.15
feed cost per gain	34.95 ± 2.75	35.92 ± 4.74	33.81 ± 3.67	32.48 ± 3.62	3.81	0.18
mortality, %	8.33	8.33	0	0	–	–
Overall (11–42 days)						
initial body weight, g	301.73 ± 10.16	309.18 ± 13.90	301.91 ± 17.40	297.64 ± 8.85	13.09	0.23
final body weight, g	3326.36 ± 418.65	3443.64 ± 288.77	3359.09 ± 208.68	3540 ± 243.56	298.35	0.36
body weight gain, g	3024.64 ± 414.83	3134.45 ± 287.27	3057.18 ± 200.95	3242.36 ± 243.99	295.97	0.36
average daily gain, g/day	94.52 ± 12.96	97.95 ± 8.98	95.54 ± 6.28	101.32 ± 7.62	9.25	0.34
feed intake, g/day	4855.82 ± 357.34	4939 ± 418.32	4666.27 ± 391.40	4740 ± 288.72	365.42	0.32
feed conversion ratio	1.62 ± 0.16^{b}	1.58 ± 0.15^{ab}	1.53 ± 0.09^{ab}	1.47 ± 0.10^{a}	0.14	0.04
feed cost per gain	32.08 ± 3.25	31.53 ± 2.94	30.27 ± 1.82	29.29 ± 2.04	2.69	0.06
mortality, %	16.67	8.33	0	0	–	–

CON – control, CPP – 0.02% chili pepper powder, IGP – 0.02% Indian gooseberry powder, CI200 – 0.02% chili pepper powder and 0.02% Indian gooseberry powder; SEM – standard error of the mean; data are presented as mean ± SD (n = 12); ^{ab} – means in the same row with different uppercase superscripts are significantly different at $P < 0.05$

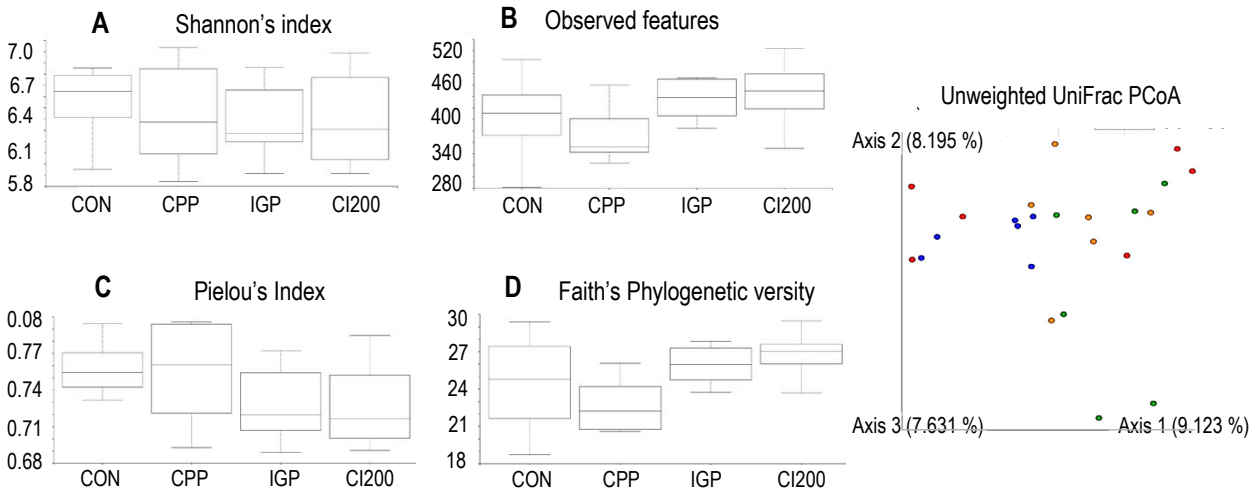

Figure 1. Alpha diversity in the caecum as measured by the Shannon diversity index (A), observed features (B), Pielou's Index (C) and Faith's phylogenetic diversity (D). Beta diversity measured by unweighted UniFrac metrics in the caecum (E). Control (CON, red), diet with chili pepper powder (CPP, blue), diet with Indian gooseberry powder (IGP, orange) and diet with chili pepper powder and Indian gooseberry powder (CI200, green). Each dot represents an individual sample

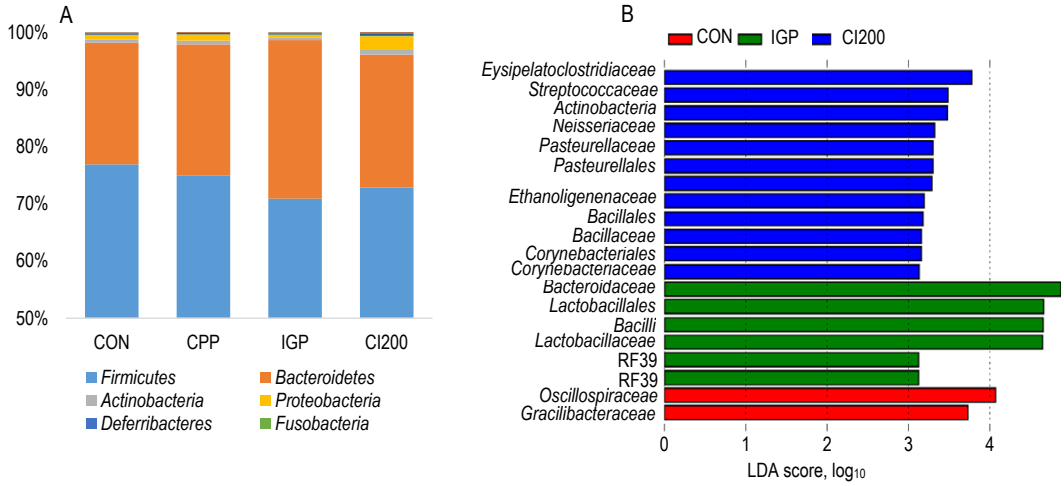

Figure 2. (A) Relative abundance of bacterial taxa at various taxonomic levels across different treatments. (B) Linear discriminant analysis (LDA) effect size (LEfSe) of the caecal microbiota in broilers fed chili pepper and Indian gooseberry powders at the family level. Horizontal bars represent the effect size for each taxon, with the length of each bar representing the $\log_{10}$ transformed LDA score, indicated by vertical dotted lines. Control is shown in red, Indian gooseberry powder (IGP) in green, and chili pepper powder and Indian gooseberry powder (CI200) in blue. The threshold for discriminative features on the logarithmic LDA score was set to 2.0. Grouped data were initially analysed using the Kruskal-Wallis test with a significance level set at 0.05 to determine differential distribution between groups. Taxa that were found to be differentially distributed were used for LDA model analysis to rank the relative difference in abundance between groups. No significant differences in taxa were detected between treatments at the phylum level and in the CPP group at the family level

analysis revealed significant differences in microbial families (LDA score threshold of 2) between the ICP, CI200, and control groups (Figure 2B). There were no significant differences in taxa between treatments in the CPP group, while in the ICP group, the relative abundance of *Lactobacillaceae, Bacteroidaceae, Bacillaceae,* and RF39 was higher compared to the remaining groups ($P < 0.05$). The families *Erysipelatoclostridiaceae, Streptococcaceae, Neisseriaceae, Ethanoligenenaceae, Bacillaceae*, and *Corynebacteriaceae* were significantly more abundant in all treatments compared to IC200, whereas higher counts of *Oscillospiraceae* and *Gracilibacteraceae* were observed in the control.

qPCR-based quantification of target bacteria

The number of bacterial species in the gastrointestinal (GI) tract of broiler chickens, as determined by qPCR from the duodenum, jejunum, ileum, and

Table 4. Determination of bacterial load by real-time PCR

Bacteria, log CFU/g	Treatment				SEM	*P*-value
	CON	CPP	IGP	IC200		
Duodenum						
Escherichia coli	7.29 ± 1.37	8.28 ± 0.45	7.32 ± 0.89	7.97 ± 0.23	0.89	0.15
Lactobacillus sp.	9.35 ± 0.80	8.77 ± 0.94	9.39 ± 0.27	8.82 ± 0.54	0.70	0.31
Salmonella spp.	ND	ND	ND	ND	–	–
Jejunum						
Escherichia coli	6.94 ± 0.98	7.14 ± 0.30	7.26 ± 0.45	7.54 ± 0.58	0.62	0.43
Lactobacillus sp.	9.78 ± 0.65^{ab}	9.30 ± 0.42^{ab}	10.17 ± 0.35^{a}	9.37 ± 0.51^{ab}	0.57	0.02
Salmonella spp.	ND	ND	ND	ND	–	–
Ileum						
Escherichia coli	8.10 ± 0.29	7.66 ± 0.72	7.68 ± 0.93	7.92 ± 0.18	0.59	0.57
Lactobacillus sp.	9.93 ± 0.54	10.31 ± 0.20	10.62 ± 0.42	9.96 ± 0.47	0.48	0.03
Salmonella spp.	ND	ND	ND	ND	–	–
Caecum						
Escherichia coli	6.74 ± 0.60^{b}	8.38 ± 1.52^{a}	6.56 ± 0.69^{b}	9.58 ± 0.67^{a}	1.52	<0.0001
Lactobacillus sp.	8.83 ± 0.10	8.92 ± 1.07	8.43 ± 1.81	8.78 ± 1.37	1.19	0.92
Salmonella spp.	ND	ND	ND	ND	–	–

CON – control, CPP – 0.02% Chili pepper powder, IGP – 0.02% Indian gooseberry powder, CI200 – 0.02% chili pepper powder and 0.02% Indian gooseberry powder; ND – not detected, SEM – standard error of the mean; data are presented as means ± SD (n = 6); ^{ab} – means in the same row with different superscripts are significantly different at $P < 0.05$

cecum, is shown in Table 4. The counts of *Lactobacillus* sp. were highest ($P < 0.05$) in the jejunum and ileum in the IGP group. In the caecum, *E. coli* abundance was significantly reduced in the CPP and IGP groups ($P < 0.01$) compared to the control group, whereas no significant differences were observed in other sections of the GI tract. *Salmonella* spp. was not detected in any segment of the GI tract.

Discussion

In the present study, supplementation with 0.02% chili pepper and 0.02% Indian gooseberry powders (CI200) improved the feed conversion ratio of broilers during the grower phase and across the entire period from 11 to 42 days of age compared to birds fed the control diet. No differences in FCR were observed between treatments with chili pepper powder (CPP), Indian gooseberry powder (IGP), or the combination of these compounds. Additionally, in the finisher phase, there were no significant differences recorded in body weight gain and FCR. Previous study indicated that feed intake (FI) can be influenced by capsaicinoids (Wang et al., 2020), yet FI in the present study was not reduced in the CPP/CI200 groups. This was consistent with the findings of Liu et al. (2021), who observed that supplementation with natural capsicum extract at 80 mg/kg reduced FCR, likely improving feed efficiency. This may be attributed to the regulatory effect of capsaicin on cholecystokinin hormones in the gastrointestinal tract (Yamamoto et al., 2003) and digestive enzyme activity (Li et al., 2022). Although polyphenol supplementation has been shown to exert an inhibitory effect on digestive enzymes (Mohamed et al., 2019), in the present study, IGP and its combination with capsaicin (CI200) improved FCR. Previous research suggests that both polyphenols and capsaicin possess antioxidant properties (Luo et al., 2018). Moreover, a study by Marić et al. (2021) indicated that supplementation with CPP at 0.5–1.0% resulted in the highest final body weight of chickens (Marić et al., 2021). The positive effects of IGP were confirmed by Naik et al. (2020), who reported that its supplementation at doses of 0.5–1.0% improved the efficiency of broiler feeding, which was consistent with findings from earlier studies (Kumar et al., 2013; Dalal et al., 2018).

Previous research on capsaicin indicated its potential benefits for the ceacal microbiota, including anti-inflammatory properties and its role in protecting against obesity (Mahalak et al., 2022). Polyphenols have been shown to modulate changes in ceacal microbial populations (Etxeberria et al., 2013; Lillehoj et al., 2018). In the present study, the unweighted UniFrac measure for beta-diversity of microbiota showed a statistically significant distancing between the IGP, CI200, and the control groups. Beta-diversity reflects the degree of biodiversity within bacterial communities. The assessment of changes in microbial diversity as a result of chili pepper and Indian gooseberry powder supplementation indicated an increase in this parameter. However, microbiota composition varied depending on the amount of powder supplementation, host physiology and the number of broilers sampled. Selma et al. (2009) reported that polyphenols and their metabolites affected microbial growth and population, thereby modulating ceacal microbiota. Additionally, grape products have been shown to modulate intestinal microbiota by increasing microbial diversity (Viveros et al., 2011), which in turn reduces the colonisation by opportunistic pathogens (Deplancke et al., 2002). Similarly, capsaicin has been associated with increased diversity of the microbial community (Xia et al., 2021; Mahalak et al., 2022). However, in the present study, supplementation with chili pepper and Indian gooseberry powders did not result in significant changes in bacterial abundance at the phylum level.

Firmicutes and *Bacteroidetes* were identified as the core microbial phyla, aligning with findings from other studies (Song et al., 2017; Quail et al., 2020; Xia et al., 2021). The relative abundance of *Bacteroidaceae* and *Lactobacillaceae* was also expected to increase with ICP supplementation, as polyphenols are known to promote the growth of beneficial bacteria (Viveros et al., 2011; Iqbal et al., 2020; Wang et al., 2022). These observations were confirmed by real-time PCR, which revealed an increased population of *Lactobacillus* sp. in the jejunum and ileum. *Lactobacillus* and *Bacteroides* have been associated with improved feed efficiency through the stimulation of gut health in broilers (Singh et al., 2012). However, no significant effects were noted for any genera in response to CPP administration. Interestingly, the combination of IGP and CPP increased the abundance of beneficial bacteria in the ceacal contents when compared to supplementation with either compound alone. The relative abundance of *Ethanoligenenaceae, Bacillaceae, Erysipelatoclostridiaceae,* and *Streptococcaceae,* all of which belong to the phylum *Firmicutes*, were found to be highest in the CI200-treated group. According to Li et al. (2021), a polyphenol diet could

reduce the relative abundance of *Streptococcaceae*. While *Streptococcus* species are part of the normal intestinal microbiota, they can also cause opportunistic infections in poultry. In this study, the number of *E. coli* was notably reduced in the ceacal contents of chickens in the CPP and IGP groups (Table 4). In contrast, a previous study found that catechin supplementation at 150 mg/l stimulated the growth of *E. coli* (Etxeberria et al., 2013). Meanwhile, Kafantaris et al. (2017) reported that grape seed extract exhibited antibacterial activity against *E. coli*, and *Enterobacteriaceae*. Moreover, *in vitro* tests have demonstrated that capsaicin exerts bactericidal effects against *E. coli* (Nascimento et al., 2014). However, in the present study, no significant differences were observed in the ileal population of *E. coli* in the IGP group. Additionally, *Bacillaceae* of the family *Erysipelatoclostridiaceae*, were reported to be abundant in the caecum of broilers treated with blueberry by-products (Das et al., 2020). This bacterial family is recognised for its antimicrobial, antioxidant, and immune-modulating activities in broilers, making it a candidate for potential probiotic applications (Elshaghabee et al., 2017).

Conclusions

The combination of chili pepper and Indian gooseberry powders at 0.2 g/kg increased the feed conversion ratio during the grower phase, as well as the abundance of *Streptococcaceae, Ethanoligenenaceae, Bacillaceae*, and *Corynebacteriaceae* in the caecum. Additionally, supplementation with Indian gooseberry powder increased the population of *Bacteroidaceae* and *Lactobacillaceae* in the caecum. Overall, the compounds tested – both individually and in combination – altered the microbiota composition of the caecal content in broiler chickens.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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