Evaluation Of Caecal Microbiota Transplantation in Early Feeding and Its Impact on Growth, Intestinal Health and Immunity in Broiler Chickens

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Abstract

This study was evaluated the influence of Caecal Microbial Transplantation (CMT) on growth performance, intestinal histology and immune function of broiler chickens. The CMT was collected from contents of healthy caecal Ross 308 chickens at 42 days of age. The caecum content was processed with normal saline and glycerol in ratios of 1:5 and 1:10, then stored -18 °C until transplantation. Three hundred and seventy five Ross 308 chicks were randomly assigned into five treatments, with three replicates and 25 chicks in each replicate, as follows: (T1) control group, only normal saline by spraying feed; (T2) ratio of (1:5) caecal solution by spraying feed; (T3) ratio of (1:10) caecal solution by spraying feed; (T4) ratio of (1:5) caecal solution by drinking water; and (T5) ratio of (1:10) caecal solution by drinking water. The results showed that the addition of CMT significantly increased final body weight, weight gain and EPEF compared with control group. There was no significant effect on feed intake compared with control group. Also, The FCR of broilers improved significantly with addition of CMT compared with control group. Both administrations of CMT supplementation significantly increased number of LAB in ileum digest. While, there were no significant differences observed among treatments on total coliform bacteria in ileum and caecum digesta and on LAB in caecum digesta. The jejunum villus height was significantly increased compared with control group. Also, the lymphoid immune organs indicated that CMT significantly increased the weight of bursa of Fabricius and spleen. This investigation has found that early feeding of CMT had a significant effect on growth performance, intestinal histology and immune function of broiler chickens.

Key words :
Microbiota, Caeca microbiota transplantation, Early feeding, Broiler production.

Introduction

The term "microbiome" refers to all microorganisms associated with a specific organism [1]. In chickens, the microbial composition of the gastrointestinal tract (GIT) varies across different sections, such as the crop, gizzard, intestines, caeca, and colon. This composition is influenced by several factors, such as nutrition, genetics, age, and environmental conditions; among these, nutrition plays a crucial role in shaping the gut microbiota [2,3]. Notably, the caeca are particularly significant because they contain dense and diverse microbial populations that are essential for fermenting undigested feed components and producing short-chain fatty acids (SCFAs), which are beneficial to the host [4 .[

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Caecal microbiota transplantation (CMT) is a process in which functional and beneficial microbes from the caeca of a healthy donor are introduced into another host. This technique restores natural microbiota, enhances disease resistance, and supports overall health in the recipient [5]. In newly hatched chickens, the caeca are first colonized by members of the family Enterobacteriaceae (phylum Proteobacteria), followed by Lachnospraceae and Ruminococcaceae from the phylum Bacteroidetes, along with Veillonellaceae from Firmicutes, which establish themselves [6 .[

The potential of CMT to enhance developmental performance, regulate immune responses, and minimize gastrointestinal disorders has been demonstrated in numerous studies. For example, through modifying the intestinal microbial profile and regulating cytokine expression, CMT significantly decreased necrotic enteritis in broilers [7]. Similarly, transplanting caeca contents from donor birds of one breed into chicks of another breed significantly changed the composition of the early microbiota [8]. This suggests that donor selection can influence the gut microbiome's developmental trajectory.

Hatchery-born chicks are typically transported directly to the farm, during which they may acquire atypical microbiota from transport trucks, the environment, or handlers [9]. Chicken GIT is colonized by bacterial species starting from the day after hatching [10]. On the other hand, the microbial composition changes throughout the life cycle due to bacterial replacement with new taxa [11]. Therefore, a key focus of intestinal microbiota modulation is to increase beneficial microbes while reducing pathogenic populations. Pathogens contribute to diseases and pose significant food safety challenges, necessitating industry efforts to enhance product safety for human consumption [12,13.[

The aim of this study was to evaluate the influence of early-life cecal microbiota transplantation (CMT) from the healthy selected donor chickens on growth performance, gut microbiota, intestinal histology and immune function of broiler chickens.

Materials and Methods

Ethical approval

The Scientific Ethical Committee of the Animal Resources Department, College of Agricultural Engineering Sciences, Salahaddin University, Erbil, approved this study (No: 3462 on 25/11/2024.(

Donor Birds and Caecal Content Preparation

Eight healthy chickens (Ross 308 strain) raised without antibiotics were used as donors. Birds were humanely sacrificed at 42 days of age after feathers were removed, skin was sanitized immediately, and both caeca were excised from the gastrointestinal tract. Forty-five grams of caeca content was used and mixed with normal saline supplemented with 10% glycerol at 1:5 and 1:10 caecum content. The number of caeca bacteria colonies was 1.0×109 CFU/g. The mixture was stored at temperatures below -18 °C until the day of transplantation. As shown in Figure 1.

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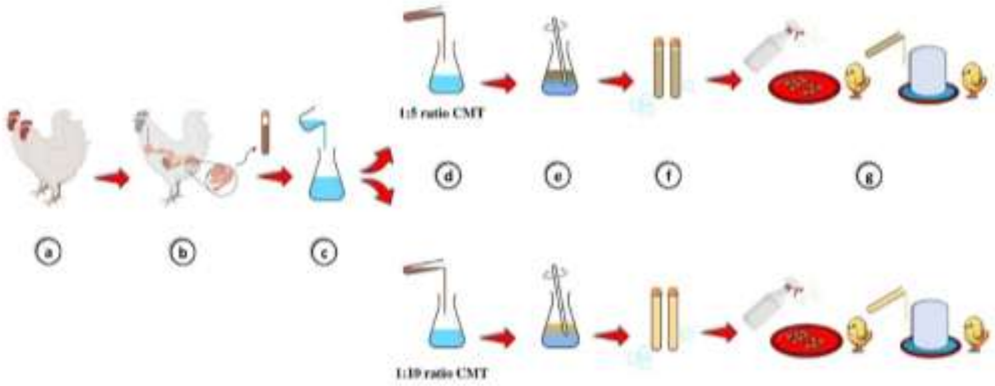

Figure 1. Preparation and administration of caecum microbiota transplantation for one-day-old Ross-308 broiler chicks.

Experimental Design

This study was conducted in local field in Erbil, Kurdistan Region of Iraq at 18th of October until 2nd of November 2024. A total of 375 broiler chicks (Ross 308 strain) were brought from the Al-Kherat hatchery, Erbil, Iraq, and randomly assigned into five treatments in a deep litter house, with each treatment consisting of 75 chicks, further divided into three replicates (n = 25). All chicks were reared in (1.2×2 m) floor pens. Feed and water were provided ad libitum throughout the experimental period. Treatments include: T1 (Control): only normal saline sprayed on feed, T2: a ratio of (1:5) caecal solution sprayed on feed, T3: received a ratio of (1:10) caecal solution sprayed on feed, T4: a ratio of (1:5) caecal solution in drinking water and T5: a ratio of (1:10) caecal solution in drinking water. Each broiler chick's CMT treatment is at a rate of 60 ml of CMT per kilogram of feed or liter of water on the first day of age. CMT was administered immediately after the chicks arrived .

Growth Performance

Growth performance was evaluated over five weeks, and 44 g is the initial weight recorded, final weight, body weight gain, feed intake, and feed conversion ratio (FCR) across three dietary phases: starter (days 1-15), grower (days 16-28), and finisher (29-35) were recorded. Additionally, the European Production Efficiency Factor (EPEF) was calculated using this formula: (EPEF = liveability (%) × live body weight (kg) × 100/ age (day) × FCR), as described by Mirza [14 .[

Immunological organs

At the end of the experiment, two birds from each replicate were killed by cervical dislocation, and the weight of the bursa of Fabricius and spleen was recorded using an electronic digital scale and then calculated relative organ weight.

Analyses of Gut Microbiota

At the end of the experiment period, two birds were randomly selected from each replicate and killed via cervical dislocation, then their ileum and caecum contents were collected to analyse the intestinal microbes (Lactobacillus spp. and total coliform bacteria). Samples were serially diluted between 10-1 and 10-7, and 0.1 ml of each dilution was plated onto sterile selective agar media. MacConkey agar (Sigma-Aldrich, UK) was used for total coliform counts, while MRS agar (Sigma-Aldrich, UK) was employed for lactic acid bacteria. The colony-forming units (CFU) were determined by counting microbial colonies, and CFU per gram was calculated and expressed logarithmically.

Histology Examination of Jejunum

One bird from each replicate was randomly selected, and the part of the jejunum was separated and washed with distilled water, then mixed in a 10% formalin solution to prevent decomposition. Tissue processing: serial dehydration, wax infiltration, and cleaning. Sections were cut into five-micron-thick sections using a microtome and fixed onto slides for examination. Standard histological staining was followed using hematoxylin and eosin stains. Hematoxylin, which exhibits a blue colour, selectively stains nucleic acids in the cell nucleus. Eosin, pink in colour, stains proteins within the cytoplasm and extracellular matrix. Stained slides were examined using a light microscope equipped with a digital camera at 10x magnification. Measurements of villus height and crypt depth (μm) in the jejunum was performed using Image J software [4.[

Statistical analysis

Data analysis was conducted using SPSS version 27 [15] to analyses a one-way ANOVA. Summary statistics included means and standard errors. Significant differences between parameters were determined using Duncan's test at a 0.05 significance level [16.[

Result

Effect of Caecum Microbiota Transplantation on Broiler Productivity

The effects of caecum microbiota transplantation (CMT) at ratios of 1:5 and 1:10 administered through feed or drinking water on broiler live body weight, weight gain, feed intake, FCR, and EPEF are summarized in Table 1. All treatments began with the same average initial body weight (44 g). The results showed that the addition of CMT significantly ($P<0.05$) increased final body weight and weight gain compared with control group except T2. There was no significant ($P>0.05$) effect on feed intake among treatments. The FCR of broilers improved significantly ($P<0.05$) with addition of CMT difference, with T1 recording the highest FCR and CMT groups compared with control group except T4. Also, the results showed that T5 had significantly ($P<0.05$) highest EPEF, while T1 and T4 recorded the lowest EPEF .

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Table 1. Effect of Caecum Microbial Transplantation at different ratios and ways on growth performance of broilers (Mean±Standard Error.(

Treatments	Growth Performance				
	Final weight (g)	Weight gain (g)	Feed intake (g)	FCR	EPEF
T1	1891.63±13.85 b	1847.64±13.85 b	3059.79±63.01 a	1.66±0.04 a	313.81±9.82 c
T2	2026.94±7.30 ab	1982.93±7.30 ab	2943.06±62.32 a	1.48±0.04 b	378.12±15.05 ab
T3	2057.64±56.07 a	2013.64±56.07 a	3077.09±34.31 a	1.53±0.03 b	366.67±9.17 b
T4	2051.79±52.58 a	2007.79±52.58 a	3146.81±52.95 a	1.57±0.04 ab	312.06±14.17 c
T5	2184.03±70.17 a	2140.03±70.17 a	3106.44±34.46 a	1.45±0.03 b	407.20±9.21 a
P. value	0.019	0.019	0.136	0.018	<0.001

abc means in the same column, different superscripts are significantly different (p<0.05 .(

Bacteria counting

Table 2 shows the influence of effects of caecum microbiota transplantation (CMT) as early feeding at ratios of 1:5 and 1:10n in diet and drinking water on the composition of microflora in the ileum and caecum digesta at 35 days of age. Both administrations of CMT supplementation significantly (P<0.01) increased number of Lactobacillus spp. in ileum digest, except T3. While, there were no significant (p>0.05) differences observed among treatments on total coliform bacteria in ileum and caecum digesta and on LAB in caecum digesta .

Table 2. Effect of Caecum Microbial Transplantation at different ratios and ways on ileum and caecum bacteria count (Log10 CFU Ml-1) of Broilers at 35 days of age (Mean±Standard Error.(

Treatments	Ileum		Caecum	
	LAB	Total coliform bacteria	LAB	Total coliform bacteria
T1	4.77±0.26 ^{b}	6.72±0.29 ^{a}	8.33±0.02 ^{a}	8.70±0.04 ^{a}
T2	6.87±0.15 ^{a}	7.01±0.17 ^{a}	8.43±0.04 ^{a}	8.79±0.05 ^{a}
T3	6.01±0.06 ^{ab}	6.66±0.29 ^{a}	8.47±0.13 ^{a}	8.64±0.11 ^{a}
T4	6.69±0.29 ^{a}	6.93±0.16 ^{a}	8.42±0.04 ^{a}	8.75±0.08 ^{a}
T5	6.37±0.02 ^{a}	7.08±0.07 ^{a}	8.17±0. 09 ^{a}	8.67±0.04 ^{a}
P. value	0.032	0.600	0.109	0.616

ab means in the same column; different superscripts are significantly different (p<0.05 .(

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Histology

Table 3 shows the effects of caecum microbiota transplantation (CMT) as early feeding at ratios of 1:5 and 1:10, administered through feed or drinking water, on the histology of the small intestine, jejunum part of the broiler at 35 days of age. The jejunum villus height was statistically ($P<0.05$) higher in treatment groups and the highest villi recorded at T2 also the higher crypt depth recorded in T2 and T4 and the lower recorded in control group. While, While, there were no significant ($p>0.05$) differences observed among treatments on villi area. Also, Figure (2) showed morphologically the differences among the groups and the control group at the end of the study.

Table 3. Effect of Caecum Microbial Transplantation at different ratios and ways on Jejunum Histology of Broilers at 35 days of age (Mean±Standard Error.(

Treatments	Villi height (um)	Crypt Depth (um)	Villi Area (um)
T1	874.64±7.44 ^{c}	131.88±10.59 ^{c}	87486.07±19495.21 ^{a}
T2	1202.50±37.59 ^{a}	224.58±11.26 ^{a}	134701.47±4070.57 ^{a}
T3	1109.80±20.60 ^{b}	150.69±11.19 ^{bc}	98777.07±3070.15 ^{a}
T4	1131.70±18.34 ^{b}	201.10±2.80 ^{a}	121638.93±7686.28 ^{a}
T5	1155.71±11.88 ^{ab}	169.30±11.05 ^{b}	100632.40±22441.45 ^{a}
P. value	<0.001	<0.001	0.153

abc means in the same column, different superscripts are significantly different (p<0.05.(

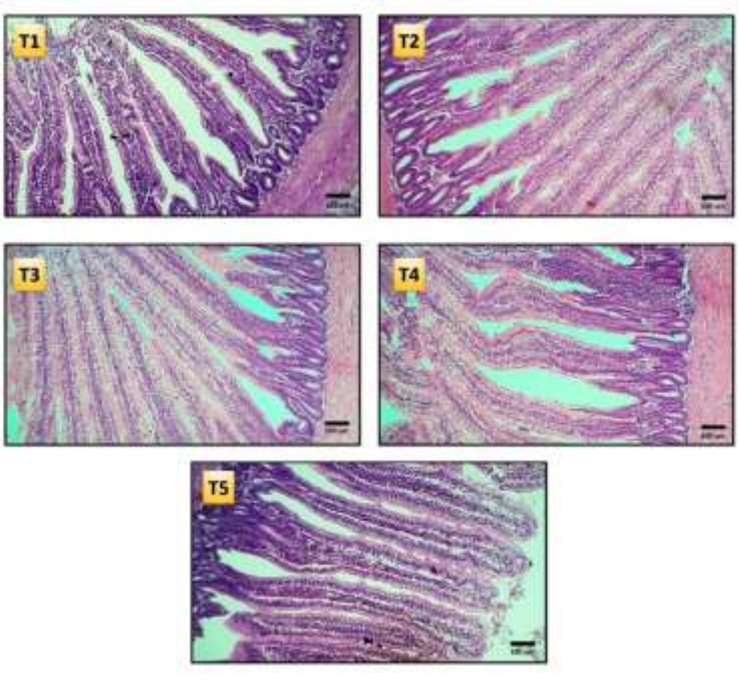

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Figure 2: Jejunum tissue stained with haematoxylin and eosin from 35-day-old broilers administered caeca microbiota transplantation. The groups include: T1: control, T2: ratio of (1:5) caecal solution by spraying feed, T3: ratio of (1:10) caecal solution by spraying feed, T4: ratio of (1:5) caecal solution by drinking water and T5: ratio of (1:10) caecal solution by drinking water (10x Magnification .(

Immunological Organs Weight (g(

The influence of caecam microbiota transplantation (CMT) at ratios of 1:5 and 1:10 administered through feed or drinking water on the immunological organ weight in the broiler is presented in Figure 3. The results from lymphoid immune organs indicated that CMT significantly (P<0.05) affects the weight of the bursa of Fabricius and spleen. A significant difference was observed, with T5 recorded as the highest bursal weight, and in the spleen, T1 and T5 showed the highest weights.

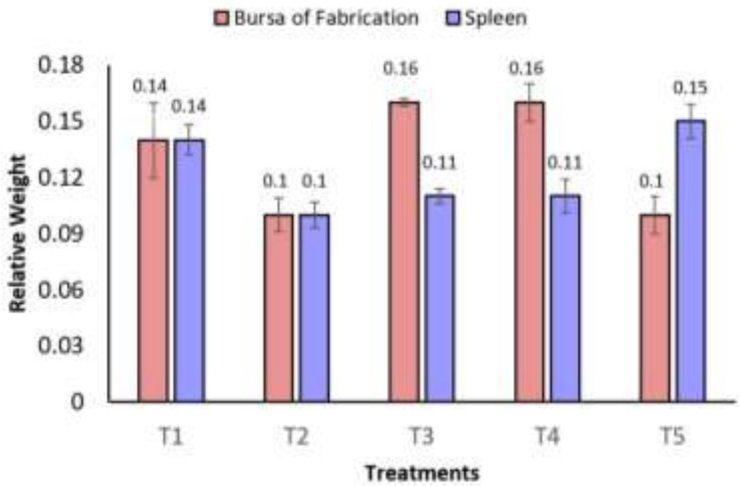

Figure 3. Effect of different caecum microbiota ratios on relative immunological organs weight (g) in broiler.

Discussion

The majority of the microbiome consists of a diverse set of bacteria that can be classified as either commensal, pathogenic, or beneficial to the host, of which a variety of factors, such as genetics, age, environment, diet, and administration of feed additives, can influence their occurrence and interactions [17,18,19]. CMT functionally resembles probiotics, promoting a balanced microbiota and supporting immunity. However, CMT provides a broader variety of microbial species compared to probiotics.

The findings of the present study are similar to Ramírez et al. [20], who found that the caeca microbiota transplantation through five generations at ratios 3:1 at 7 days of age, as oral and environmental transplantation, significantly affects the body weight of broilers at 14 days of age. Similar observations were reported by Khalid et al. [21], who found that caeca microbiota transplantation at 1:6 and 1:12 significantly improved broilers' body weight and weight gain. Hussain et al. [22] observed that the E. faecalis, B. fragilis, and Ligilactobacillus

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salivarius of the probiotic at 5× 107 CFU/chick per day in the broiler diet has no significant effect on average feed intake. Siegerstetter et al. [23] found that faecal microbiota transplantation (FMT) at a 1:100 ratio in high and low Residual has no significant effect on feed intake .

Furthermore, our study revealed that CMT significantly influenced immune organ development by increasing the relative weight of the bursa of Fabricius and spleen. This finding suggests that supplementing microbiota during early life may enhance the maturation and activity of lymphoid tissues. Mirza [4] reported that adding P. acidilactici and L. animalis to the diet has a significant effect on the weight of bursal fabrication. Similarly, supplementing 0.02% with a probiotic blend delivering 4×109 CFU/g comprising Bacillus subtilis and Bacillus licheniformis added in the diet shows significant differences in the weight of the spleen [24]. Olnood et al. (2015) reported that feeding a diet containing probiotics (L. johnsonii, L. crispatus, L. salivarius, and an unidentified L. sp.) significantly improved spleen weight at day 35.

The findings of Gérard et al. [25] suggest that Lactobacillus supplementation does not affect coliform bacteria in caeca content. Olnood et al. [26] reported that feeding a diet containing probiotics (L. johnsonii, L. crispatus, L. salivarius, and an unidentified L. sp.) does not significantly affect caecum LAB at 35 days. Similarly, Fajardo et al. [27] found that supplementation with Lactococcus lactis and Lactobacillus casei has no significant difference in caeca total coliforms. There are speculate that this disparity might result from the fact that cecal microbiota transplantation (CMT) was only administered on the first day of life and the lack of the long-term effects of CMT under large-scale conditions.

Ma et al. [28] reported that freshly FMT at a ratio of 1:6, administered orally once per day during 28 days, significantly affected villi length compared to the control group. Zhang et al., [29] used the same ratio and their findings observed that FMT can affect villus length at 30 and 60 days of age. According to Feng et al. [30] found that orally administered 1:10 FMT to broiler chickens from 5 to 11 days of age significantly affects villi length at 21 days of age compared to the control group. By using daily 1:6 oral FMT for 4 weeks, villi length and crypt depth of chickens significantly increase compared to the control group at 30, 60, and 90 days of age [31.[

Conclusion

This research indicates that early feeding of CMT positively influence a better growth rate, gut integrity, and the development of villi length in broilers. This study suggests that CMT can use a practical tool to boost overall flock productivity in broiler production.

Conflict of interests

There is no conflict of interest .

Authors contribution

The author has written, drafted, analyzed data, and finalized the manuscript .

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