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Effect of faecal microbiota transplantation on growth, haematological and intestinal indices of Kedu chickens

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ABSTRACT

The study examined the effect of lyophilized fecal microbiota transplantation (FMT) from healthy chicken donors on growth performance, haematological indices and intestinal morphology of Kedu chickens during the starter phase. A number of 120 Kedu chickens (0-6 weeks old) were randomly assigned to T0 (orally inoculated with 1 mL PBS), T1 (inoculated with 0.5 mL FMT) and T2 (1.0 mL FMT). Body weight and feed intake were weekly recorded, while blood and intestinal segments were collected at week 6. Results showed that live body weight and body weight gain were highest ($p<0.001$) in T1, and the lowest in T0 group. Feed conversion ratio (FCR) was better ($p=0.001$) in T1 and T2 compared to T0 group. Feed intake was lower ($p=0.014$) in T2 than in T0 and T1 groups. The FMT administration tended to increase erythrocytes ($p=0.054$) and haemoglobin levels ($p=0.056$) of Kedu chickens. The relative weight of duodenum, jejunum, ileum, caecum and colon were lower ($p<0.05$) in T1 and T2 compared to those in T0 group. The FMT administration had no effect ($p>0.05$) on duodenal and jejunal villi height, crypt depth and the ratio of villi height to crypt depth of Kedu chickens. In conclusion, daily low-dose lyophilized FMT was associated with improved growth performance and feed efficiency in starter Kedu chickens, while haematological and intestinal morphological responses were limited.

Keywords: blood profile, Growth, Intestinal health, Microbiota, Native chickens.

INTRODUCTION

Poultry is acknowledged as a crucial sector of global food security, offering a relatively affordable and nutritious source of protein. Local chicken breeds play a vital role in the rural economy and contribute to the conservation of genetic diversity in many countries such as Indonesia. Among these local breeds, Kedu chicken holds particular importance, having been introduced to Central Java Indonesia. This breed is renowned for its remarkable ability to adapt to local envi-

ronmental challenges, including elevated atmospheric temperatures and prevalent pathogenic strains (Mustofa *et al*., 2021). Owing to its significant disease resistance and adaptability to scavenging and smallholder systems, the Kedu breed constitutes a valuable asset for numerous rural families. However, the Kedu chicken exhibits a significant limitation in its growth rate, achieving an average body weight of only 0.64 kg at two months of age (Setiaji *et al.,* 2026). Furthermore, the egg production rate is only 58%, with an average egg weight of 45 g per egg (Zamhariroh *et al*., 2019; Sutopo *et al*., 2022), reflecting low productivity that does not satisfy the increasing demand for protein. This insufficient productivity places economic strain on smallholder farmers who rely significantly on poultry for their livelihoods. Additionally, the growing preference for commercial breeds threatens local genetic resources, potentially resulting in a reduction in biodiversity and resilience within poultry systems.

In the past, the poultry industry, including local poultry sectors, frequently utilized antibiotics as growth promoters in feed to maximize growth and maintain health (Sugiharto, 2016). However, a number of countries, have enacted regulations that prohibit the use of antibiotics for growth promotion in feed. This shift necessitates the exploration of safe and effective feed supplements that can improve growth performance and sustain the health of native livestock, thereby ensuring high productivity. The gastrointestinal microbiota is crucial in regulating growth and immune responses in poultry. The gut microbiome aids in nutrient digestion, the development of immune defences, and resistance to pathogenic bacterial infections, collectively impacting overall health and performance (Gong *et al*., 2019). An imbalance or dysbiosis in the gut microbiome, which may arise from stress, disease, or an unbalanced diet, can result in reduced growth rates and heightened susceptibility to infections (Aruwa *et al*., 2021). As a result, there is increasing interest in strategies to manipulate gut microbiota as innovative antibiotic-free approaches to enhance the productivity and welfare of chickens.

Fecal microbiota transplantation (FMT) involves the transfer of gut microorganisms from a donor to a recipient, presenting a novel therapeutic strategy for restoring the gut microbiota ecosystem (Xu *et al*., 2025). In poultry species, FMT inoculation has demonstrated improvements in growth performance and disease resistance by enhancing feed efficiency, boosting immune responses, mitigating inflammatory reactions, and modulating gut morphology (Yan *et al*., 2021; Wang *et al*., 2022). However, the majority of existing research focuses on commercial broilers and layers, highlighting a significant knowledge gap concerning FMT in locally adapted chickens, such as the Kedu chicken. This gap is particularly noteworthy given the unique genetic composition and environmental interactions of indigenous chickens. Investigating the effects of FMT on Kedu chickens could provide valuable insights into optimizing gut health and functions and potentially improving growth performance and physiological conditions, thereby contributing to sustainable production systems while preserving the local gene pool (Sood *et al*., 2019). This study aimed to address this gap by examining the impact of lyophilized FMT from healthy chicken donors on growth performance during the starter phase, haematological indices and intestinal morphology of Kedu chickens.

MATERIALS AND METHODS

Preparation of FMT

Five healthy Kedu chickens, maintained on an antibiotic-free diet, were utilized as excreta donors. These chickens were selected based on the absence of clinical signs of illness, consistent feed intake, regular weight gain, alertness, vibrant plumage, ocular cleanliness and clarity (absence of discharge), clear eyes, and the absence of rough scales on legs, feet, beak, or nostrils, as well as the absence of parasites or disease. As outlined by Matsuoka *et al*. (2014), fresh faecal samples were collected each morning (20 g in 50 mL tubes) and preserved under sterile conditions. The white substance, primarily

composed of starch and uric acid from the excreta, was meticulously removed using sterile instruments to ensure purity. The remaining chocolate-brown suspension was thoroughly mixed with chilled sterile phosphate-buffered saline (PBS) at 4°C in a 1:6 ratio (6 mL of PBS per g of excreta). This mixture was subjected to centrifugation for 3 minutes at 800 ×*g* at 4°C, resulting in the retention of gut cells and beneficial bacteria in the supernatant, while denser materials settled at the bottom. The suspension was maintained on ice to facilitate the sedimentation of clear particles, after which the supernatant was filtered under vacuum through three layers of sterile medical gauze to remove any residual large particulates.

The faecal suspension was subjected to filtration followed by freeze-drying to remove water content while preserving the integrity of the microbial community. This procedure was employed to prolong storage duration and maintain the viability of the microorganisms. The lyophilized product was subsequently dispensed into sterile vials under clean room conditions and stored at -20°C for future use as needed. To assess the viability of the lyophilized product, subsamples were inoculated onto both selective and non-selective media following rehydration with a sterile diluent. Nutrient and tryptic soy agars were utilized as non-selective media to evaluate total microbial survival, while selective media were used for each bacterial group. Rapid laboratory-based screening was also conducted to confirm the absence of common poultry pathogens, including but not limited to *Salmonella* spp., *Clostridium perfringens*, Avian Pathogenic *Escherichia coli* (APEC) and avian influenza virus (AIV). The pathogen-free excreta from the donor chickens were collected and homogenized into a single faecal sample.

In vivo Trial and Data collection

The *in vivo* was approved by the Animal Ethic Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 61-06/A-11/KEP-FPP). The study included 120 healthy Kedu chickens (males and females in equal proportions) in the starting phase (0-6 weeks), which received oral FMT. The chickens

ble 1. Composition and nutritional contents of Kedu chicken feed (day 8 to 42)

	%, unless otherwise noted
Ingredients	
Yellow corn	57.4
Palm oil	1.82
Soybean meal	36.0
DL-methionine	0.20
Bentonite	1.00
Limestone	1.20
Calcium monophosphate	1.65
Premix[1]	0.27
Choline chloride	0.07
Salt	0.40
Nutrient compositions	
ME[2] (kcal/kg)	3110
Crude protein	19.8
Crude fat	6.20
Crude fiber	6.49
Ca	1.00
P (available)	0.50

[1]Mineral and vitamin per kg premix: 50,000 IU vitamin D_3 , 0.5 mg vit B_{12} , 32.5% Ca, 1% P, 6 g Fe, 4 g Mn, 0.075 g I, 0.3 g Cu and 3.75 g Zn.

[2]Metabolizable energy (ME) was calculated according to the formula: 40.81 {0.87 (crude protein + 2.25 crude fat + nitrogen- free extract) + 2.5}

were randomly assigned to three equal groups of 40 (five pens per treatment group, with eight chickens in each pen), including T0 (control group) which were orally inoculated with 1 mL PBS *via* a syringe (behind the chicken's tongue) every morning for the entire 42 days of the *in vivo* experiment. The oral gavage was chosen due to the control on doses being delivered consistently into the gastrointestinal tract because it ensures homogeneity between all experimental units (Zhang *et al*., 2022). The other treatment groups were T1 and T2, which were orally inoculated with 0.5 and 1.0 mL FMT suspension, respectively. Upon arrival to day 7, the chicks were offered commercial pre-starter feed containing 22-24% crude protein, >5% crude fibre, 5% crude fat and 7% ash (based on feed label from the feed company). From day 8 to 42, the chickens were fed corn-soybean meal-based diets (Table 1) without antibiotics with *ad libitum* access. Rice husk was used as bedding material. The pen measured 80×80 cm and had manual feeder and drinker devices. Lighting was kept on throughout experimental period. The temperature and humidity in the house were controlled by light bulb and plastic curtains during experiment.

The chickens were weighed individually once a week, and their feed intake were recorded. At the end of the study period (6 weeks-old), two male Kedu chickens, which had body weights close to the average body weight for each pen, were selected for dissection (10 chicks per treatment group). Male chicks were selected for sampling to eliminate any possible physiological variations due to gender differences. The intestine of chickens was obtained and weighed by using analytical balance. For the purpose of evaluating gut morphology, the duodenal and jejunal segments were obtained (about 2 cm) and put in a 10% neutral formalin buffer solution. Prior to dissection, blood was drawn from the wing veins of chicks and then transferred to tubes containing anticoagulant (ethylenediaminetetraacetic acid; EDTA) to assess the whole blood profile. The complete blood count analysis was conducted using a Prima Fully-Auto Haematology Analyser (PT. Prima Alkesindo Nusantara, Jakarta, Indonesia) following the manufacturer's procedures.

For the histological analysis, these intestinal segments (5 μm each) were stained using haematoxylin and eosin. Intestinal morphometric analysis was performed using ImageJ (National Institutes of Health, USA). Histological sections were examined under a light microscope, and digital images were captured at appropriate magnification. The scale bar that is included into every image was used for calibration. Crypt depth was measured from the base of the crypt to the villus-crypt junction, whereas villus height was defined as the distance between the villus tip and the junction. To assure accuracy, only entire, well-oriented villi were chosen. Each sample had at least ten to fifteen villi and matching crypts measured, and the average values were computed for statistical analysis. All measurements were performed in a blinded manner to reduce observer bias. Statistical analysis of results was performed based on analysis of variance (ANOVA; SPSS version 16.0). When the treatments significantly affected ($p<0.05$) the measured parameters, Duncan's new multiple range test was then performed.

RESULTS AND DISCUSSION

Growth Performance of Kedu Chickens

Data from the current study showed that FMT administration significantly affected growth performance of Kedu chickens (Table 2). Final body weight and body weight gain were highest ($p<0.001$) in T1, intermediate in T2, and the lowest in T0 group. Feed conversion ratio (FCR) was better ($p=0.001$) in T1 and T2 compared to T0 group. Accumulative feed intake was lower ($p=0.014$) in T2 than in T0 and T1 groups.

The observed improvement in weight gain of Kedu chickens with the administration of low-dose FMT (T1) aligned with previous research conducted in commercial broiler strains, where FMT from high-performance donors facilitated increased production performances of the birds (Ma *et al*., 2023; Xu *et al*., 2025). Indeed, the phenomenon arise in the present study was likely

Table 2. Growth performance of Kedu chickens administered different doses of FMT

Variables	T0	T1	T2	p value
Accumulative FI (g/bird)	1220 ± 7.94[a]	1228 ± 2.88[a]	1208 ± 4.92[b]	0.014
Final body weight (g/bird)	448 ± 5.96[c]	489 ± 3.80[a]	465 ± 4.33[b]	<0.001
Body weight gain (g/bird)	412 ± 5.39[c]	453 ± 3.80[a]	429 ± 3.99[b]	<0.001
FCR	2.95 ± 0.06[a]	2.71 ± 0.02[c]	2.81 ± 0.02[b]	0.001

[a,b,c]Means within a row with different superscripts differ significantly ($p<0.05$)
T0: chicks orally inoculated with 1 mL PBS, T1: chicks orally inoculated with 0.5 mL FMT, T2: chicks orally inoculated with 1.0 mL FMT, FI: feed intake, FCR: feed conversion ratio

attributable to the advantageous modulation of the gut microbiota, which subsequently improves intestinal functions in terms of nutrient digestion and absorption of Kedu chickens (Ma *et al*., 2023). The notable improvement in FCR in the T1 and T2 group further corroborates the enhancement in feed efficiency of these chickens. Xu *et al*. (2025) further reported that in addition to increasing the number of *Lactobacillus* in the intestine, FMT administration increased glutamine levels in the blood, thereby increasing myoblast proliferation and differentiation, thus improving muscle development and growth performance. In addition, FMT stimulated the expression of genes responsible for muscle development, such as MyoG, MYF5, and IGF1 in chest muscles and MyoD, MyoG, MYF6, and IGF1 in leg muscles (Xu *et al*., 2025). High-dose FMT administration (T2) exhibited inferior weight gain compared to T1 group, indicating a limited impact of FMT administration due to excessive microbial inoculation. This finding underscores a critical non-linear dose-response relationship, suggesting a threshold beyond which the gut ecosystem is unable to effectively assimilate the introduced microbes, potentially resulting in metabolic competition or localized dysbiosis (Schmidt *et al*., 2022). Similar observations have been reported in broiler strain, where the efficacy of FMT is contingent upon both the donor and the dosage (Fathima *et al*., 2022). The findings of our study emphasize the necessity of optimizing dosage, particularly in native breeds with distinct gut microbiomes.

Hematological Indices of Kedu Chickens

The data of haematological indices of Kedu chickens are shown in Table 3. Administration of FMT typically had no ($p>0.05$) effect on the haematological parameters of Kedu chickens. However, there were trend that FMT administration increased erythrocytes ($p=0.054$) and haemoglobin levels ($p=0.056$) of Kedu chickens.

The observed increases in erythrocytes and haemoglobin in the T1 group suggested that low-dose FMT may had positive effects on erythropoiesis. Indeed, the higher erythrocyte and haemoglobin levels in T1 chickens may be attributed to FMT-mediated improvement in gut health (increasing the relative abundances of butyrate-producing bacteria), which reduces systemic inflammation that can suppresses erythrocytes production. Besides enhancing iron absorption, FMT administration seemed also to be able to regulate iron homeostasis through improving hepcidin (a peptide hormone produced in the liver that has a crucial role in iron metabolism) expression, thus increasing erythrocytes and haemoglobin levels (Zhong *et al*., 2023). Additionally, microbial metabolites such as short-chain fatty acids can directly stimulate hematopoietic stem cells to support erythrocytes production in chickens (Fernandez Sanchez *et al*., 2024; Sun *et al*., 2024). The increase in erythrocyte and haemoglobin counts in the T1 group was associated with increased body weight gain in that respective group of chickens. Islam *et al*. (2025) demonstrated a positive correlation between erythrocyte counts and body weight gain in broiler chickens. In this case, erythrocytes play a crucial role in oxygen transport, which is directly essential for energy metabolism, thus supporting the physiological processes underlying growth in chickens.

Table 3. Haematological parameters of Kedu chickens administered different doses of FMT

Variables	T0	T1	T2	p value
Leukocytes (10^9/L)	75.6 ± 10.8	77.2 ± 10.8	67.1 ± 16.6	0.439
Lymphocyte (10^9/L)	59.1 ± 8.88	61.9 ± 4.55	55.2 ± 11.9	0.513
MID counts (10^9/L)	3.48 ± 0.72	3.29 ± 1.03	2.70 ± 1.00	0.404
Granulocytes (10^9/L)	12.9 ± 2.31	12.1 ± 5.67	9.18 ± 4.36	0.387
Lymphocyte %	78.2 ± 2.80	80.9 ± 6.89	83.1 ± 4.75	0.350
MID %	4.58 ± 0.29	4.16 ± 0.89	3.88 ± 0.77	0.315
Granulocyte %	17.2 ± 2.83	15.0 ± 6.05	13.0 ± 4.07	0.372
Erythrocytes (10^{12}/L)	1.99 ± 0.23	2.42 ± 0.20	2.22 ± 0.31	0.054
Hemoglobin (g/dL)	6.02 ± 0.83	7.22 ± 0.31	6.10 ± 1.02	0.056
MCHC (g/dL)	24.8 ± 3.13	23.9 ± 1.04	22.2 ± 0.44	0.141
MCH (pg)	29.6 ± 3.52	30.2 ± 1.63	27.7 ± 1.06	0.250
MCV (fL)	108 ± 43.0	125 ± 2.00	123 ± 2.60	0.534
RDW-CV (%)	34.2 ± 51.6	10.7 ± 1.26	11.8 ± 1.25	0.398
RDW-SD (fL)	48.4 ± 21.4	56.9 ± 5.81	60.9 ± 7.14	0.353
Hematocrit (%)	30.1 ± 13.0	30.1 ± 2.15	27.5 ± 3.84	0.838
Platelet (10^9/L)	19.1 ± 7.32	15.2 ± 2.28	14.6 ± 2.41	0.293
Mean platelet volume (fL)	7.26 ± 2.68	5.92 ± 0.19	6.06 ± 0.17	0.356
Platelet distribution width (%)	10.1 ± 3.00	11.0 ± 1.79	11.0 ± 1.39	0.751
Plateletcrit (%)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.552
Platelet large cell ratio (%)	6.06 ± 1.28	5.98 ± 0.39	5.86 ± 1.03	0.948

MID: represent monocytes, eosinophils and basophils, MCHC: mean corpuscular haemoglobin concentration; MCH: mean corpuscular haemoglobin, MCV: mean corpuscular volume, RDW-CV: red cell distribution width (coefficient of variation), RDW-SD: red cell distribution width (standard deviation), T0: chicks orally inoculated with 1 mL PBS, T1: chicks orally inoculated with 0.5 mL FMT, T2: chicks orally inoculated with 1.0 mL FMT

Table 4. Intestinal segment weights of Kedu chickens administered different doses of FMT

Variables (% live BW)	T0	T1	T2	p value
Duodenum	1.22 ± 0.26^a	0.93 ± 0.10^b	0.94 ± 0.17^b	0.002
Jejunum	1.98 ± 0.40^a	1.52 ± 0.17^b	1.54 ± 0.39^b	0.007
Ileum	1.47 ± 0.23^a	1.15 ± 0.21^b	1.15 ± 0.22^b	0.004
Caecum	0.76 ± 0.13^a	0.60 ± 0.11^b	0.60 ± 0.13^b	0.007
Colon	0.25 ± 0.07^a	0.19 ± 0.03^b	0.20 ± 0.06^b	0.031

[a,b]Means within a row with different superscripts differ significantly ($p<0.05$)
BW: body weight, T0: chicks orally inoculated with 1 mL PBS, T1: chicks orally inoculated with 0.5 mL FMT, T2: chicks orally inoculated with 1.0 mL FMT

Intestinal Segment Relative Weight and Morphology

FMT administration showed significant influences on the relative weight of intestinal segments of Kedu chickens (Table 4). The relative weight of duodenum, jejunum, ileum, caecum and colon were lower ($p<0.05$) in T1 and T2 compared to those in T0 group. Data on the duodenal and jejunal morphology of Kedu chickens are presented in Table 5. Both in duodenum and jejunum, the villi height, crypt depth and the ratio of villi height to crypt depth were not different ($p>0.05$) among the treatment groups during the present study.

In this study, the relative weights of the duodenum, jejunum, ileum, cecum, and colon in Kedu chickens receiving FMT were significantly lower than those receiving PBS. Recent study has shown a positive correlation between intestinal weight, villus height, and body weight in

Table 5. Small intestinal morphology of Kedu chickens administered different doses of FMT

Variables	T0	T1	T2	p value
Duodenum				
Villi height (µm)	925 ± 92.5	967 ± 259	1082 ± 67.8	0.435
Crypt depth (µm)	311 ± 70.8	310 ± 21.3	308 ± 59.8	0.996
VH/CD ratio	3.08 ± 0.63	3.13 ± 0.87	3.62 ± 1.00	0.549
Jejunum				
Villi height (µm)	843 ± 189	816 ± 137	970 ± 158	0.314
Crypt depth (µm)	286 ± 36.9	303 ± 107	265 ± 59	0.733
VH/CD ratio	2.97 ± 0.65	3.07 ± 1.46	3.78 ± 1.02	0.461

T0: chicks orally inoculated with 1 mL PBS, T1: chicks orally inoculated with 0.5 mL FMT, T2: chicks orally inoculated with 1.0 mL FMT, VH/CD ratio: villi height to crypt depth ratio

broiler strain (Ebrahimi *et al*., 2026). However, this study differed from our present findings, where the three variables were not positively correlated. Consistent with our current findings, Sugiharto *et al*. (2019) also reported no positive correlation between intestinal weight, duodenal, and jejunal villus height, and body weight in broilers. The relative weight of small intestinal segments is not solely determined by the height of the intestinal villi, but rather by the final body weight of the chicken, which serves as the denominator in the calculation (Febrianti *et al*., 2024). In this study, the final body weight was higher in Kedu chickens in groups T1 and T2 compared to T0. Therefore, the denominator in the calculation of the relatively small intestinal segment was greater in T1 and T2 compared to T0 so that the relative weights of the duodenum, jejunum, ileum, cecum and colon were smaller in these groups.

This study did not show any effect of FMT on the duodenal or jejunal morphology of Kedu chickens. Study has shown that intestinal morphology of chickens is strongly influenced by the balance of microbes in the intestine (Sugiharto, 2016). Assuming that FMT administration has a positive effect on the microbial population in the intestine (Xu *et al*., 2025), the intestinal morphology of Kedu chickens was expected to be better in chickens given FMT. Apart from the influence of the microbial population in the intestine, intestinal morphology of chickens is also significantly affected by stress conditions (e.g., heat stress) experienced by the chickens during rearing (Nanto-Hara *et al*., 2020). During the experiment, the Kedu chickens were not subjected to any stress treatment, and the temperature and humidity in the chicken house were maintained at a comfortable level for Kedu chickens (ambient temperature not more than 30°C and humidity less than 60%). Note that Kedu chickens can still survive and grow well at temperatures of 35°C (Tiya et al., 2026). Based on these conditions, it was highly likely that the bacterial population and intestinal morphology of Kedu chickens were not compromised, thus the role of FMT administration in improving intestinal morphology was not apparent.

CONCLUSION

Daily low-dose lyophilized FMT was associated with improved growth performance and feed efficiency in starter Kedu chickens, while haematological and intestinal morphological responses were limited.

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