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RESEARCH ARTICLE
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Effects of Early Nutrition Programming on Post-Hatching Performance and Small Intestine Characteristics of Kampung Chicken

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ABSTRACT

This study was conducted to elucidate the efficacy of early nutrition programming on the performance of Kampung chicken. The study consisted of two stages: pre-hatching (*in ovo* feeding [IOF]) and post-hatching (neonatal nutrition [NN]). In total 180 hatching eggs were used in the pre-hatching stage, which were distributed into five groups: IOF-0 (negative control), IOF-1 (positive control), IOF-2 (0.5% Arginine), IOF-3 (1% Arginine), and IOF-4 (2% Arginine). Twenty newly hatched chickens (NHCs) from each IOF group were selected and divided into NN0 (0.2% saline) and NN1 (a mix of commercial probiotics, inulin, and lysine diluted in 0.2% saline) which were provided in drinking water. In addition, 100 NHCs were used arranged as a 5×2 factorial based on a completely randomized design. A commercial diet was used in the second stage. Performance, daily feed and water intake, body weight (BW), gross- morphometric indices of small intestine segments, and histo-morphometric indices of the ileum were evaluated. The final BW improved significantly ($P<0.05$) at IOF-3 and IOF-4, and NN effectively supported further increases. IOF and NN did not significantly affect feed intake during the experiment. Examinations of gross- and histo-morphometric showed an increased capacity for digestion and absorption of small intestinal segments, which was indicated by increasing the length/weight ratio and expanding the absorption villus surface area of the ileum. In conclusion, the best beneficial synergistic effect of early nutrition programming on Kampung chickens was achieved by the combination of IOF 1% L-Arginine and NN, which indicated by BW gain and feed conversion ratio.

Keywords: Early Nutrition Programming, Kampung Chicken, Small Intestine Morphometry, Performance

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INTRODUCTION

Kampung chicken is a general name given to all local or native chickens in Indonesia, or "buras" chicken without any specific exterior characteristics, and reared as egg, or meat producers (dual purposes). At least 30 clumps of Kampung chickens have been identified which have various morphological characteristics and ability to produce meat or eggs. These variabilities may be associated with genetic background. Generally, the ability and efficiency of meat or egg producers are markedly lower than those of exotic commercial breeds of chicken (Muladno et al., 2013).

Many studies have examined the application of *in ovo* feeding (IOF) in poultry chickens of exotic commercial breeds, particularly broilers. In this technique, embryos are supplied with additional nutrients before hatching, and those nutrients will continue to be utilized by the post-hatch chicks. Over the past 20 years or more, various bioactive compounds have been applied in IOF, such as vaccines, drugs, hormones, vitamins, minerals, prebiotics and probiotics, carbohydrates, proteins, and amino acids

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(AAs, such as L-Arginine [Arg], L-Lysine [Lys], L-Glutamine) used alone or in combination, which have shown some beneficial effects on embryonic growth and development, and this is followed by the post-hatch performance (Kucharska-Gaca et al., 2017; Peebles, 2018; Alagawany, et al., 2021; Arevan et al., 2023). Moreover, numerous scientific studies have also indicated that IOF modulates the intestinal environment, which affects the regulation of epigenetic mechanisms in animals as a key role in the proliferation, differentiation, and development of body cells (Dunislawska et al., 2022; Dunislawska et al., 2023).

IOF of arginine (IOF-Arg) in broiler chicken eggs demonstrates improved breast muscle growth, which may be associated with the enhancement of protein deposition (Lu et al., 2022) and promotion of immune response (Gao et al., 2017a). IOF-Arg is also reported to increase the antioxidant capacity of the breast muscle, which has a positive effect on the health and growth of the chickens (Azhar et al., 2016; Lu et al., 2022).

The provision of neonatal nutrition (NN) immediately after hatching has various beneficial effects, such as faster maturation of digestive structures and functions to effectively stimulate the absorption of residual yolk, immunity development, and increasing muscle mass, which is conducive for growth and development, and thus the overall performance of the animals (Govinthasamy et al., 2016; Lingens et al., 2021; Gawel et al., 2022). In turkey, (Yael & David, 1999) NN results in a heavier body weight (BW) and greater breast meat yield, which is caused by myoblast hyperplasia to form new nuclei from muscle progenitor satellite cells (adult myoblasts) in muscle fibers. However, in early life, the time needed to activate satellite cells to donate their nuclei to existing cells that increase muscle mass by hyperplasia is limited. Once the donation time ends, the muscle mass is mainly increased by increasing the cytoplasm (hypertrophy) (Moore et al., 2005).

In light of various results from previous studies, IOF and NN or post-hatch feeding are applied in combination with in early nutrition programming (ENP), which is an effective strategy to modulate early growth and development of the gastrointestinal tract (GIT), which is crucial for optimum performance, better feed efficiency, and improvement overall health and production (Rahardja, 2021). However, information regarding the application of ENP even for commercial exotic breeds of chickens (broilers or layers) is limited.

Naturally, the growth and development of avian embryos depend on the nutrients contained in fertile eggs, which are sufficient for normal hatchability and subsequent post-hatch life. Although improving the productivity of Kampung chickens is simple, the nutrients eggs contain are insufficient; thus, a additional nutrients are needed to achieve maximum growth and development of embryos and post-hatch chickens. To increase the productivity of Kampung chickens, this study aimed to elucidate the interaction effects between IOF-Arg of different levels and NN containing selected bioactive nutrients, namely L-Lys, inulin, and commercial probiotics.

MATERIALS & METHODS

Ethical Approval

The experiments were performed according to the ethical principles of the experimental protocol (IRB Protocol No. UH 21110720), which was approved by the Local Ethics Committee of Medical Research, Faculty of Medicine, Hasanuddin University. All experiments performed in this study were approved by the Research Ethics Committee of Hasanuddin University.

Experimental Design

This study was conducted at the Laboratory of Animal Physiology, and the Laboratory of Poultry Science and Technology, in the Faculty of Animal Science of Hasanuddin University.

In this study, experiments were performed in two sequential treatment stages: pre- and post-hatching.

1. Pre-hatching involved IOF on 180 hatching eggs of the same weight collected -and selected from eggs produced from a flock of Kampung chickens reared on the university farm. The eggs were randomly divided into five treatment groups of 36 eggs, namely, IOF-0 (negative control), IOF-1 (0.9% sterile saline, positive control), IOF-2 (0.5% arginine [Arg]), IOF-3 (1% Arg) and IOF-4 (2% Arg), As an IOF solution, Arg was diluted in 0.9% sterile sodium chloride NaCl. The IOF solutions were infused into the albumen on day 7 incubation sequentially after candling to cull eggs with dead embryos. A standard operating procedure of egg incubation related to egg preparation, full automatic incubator, fumigation, and sterilization was followed consistently. The treated hatchlings were collected at day 20, 21, and 22 incubations, then cleaned, and placed separately for each treatment group. A day later, 20 newly hatched chickens (NHCs) were selected from each group for NN treatment
2. For post-hatching treatment, each group of selected NHCs was divided into 2 of 10 NHCs for post-hatching treatment, i.e., NN. Accordingly, 100 NHCs received NN, which was given through drinking water for 2 weeks of an 8-week rearing period. The NN regimens included NN0 (0.2% NaCl in tap water), and NN1 (a mix of commercial 0.2% probiotics + 0.5% inulin + 0.5% Lys, dissolved in 0.2 % saline). Overall, the study was a factorial experiment of 5×2 or 10 treatment units, with 10 NHCs as individual replications.

Performances of the chickens, including daily feed, water intake, and BW were measured. A commercial feed was used during the experiment, and its nutrient content is presented in Table 1. Small intestine indices were examined from samples of three segments of the small intestine (duodenum, jejunum, and ileum) of four chickens (two lightest and two heaviest) from each treatment unit.

Table 1: Ingredients and composition of feed

Content	Percentage (%)
Water content	13.00
Proteins	23.00
Fat	5.00
Fiber	5.00
Ash	7.00
Calcium	0.90
Phosphor	0.50

Source: Commercial Company.

The gross morphometric indices were the weights and lengths of the three segment samples of each animal. The weights and lengths of each segment were measured after flushing with 0.9% saline. The histo-morphometric (Lisnahan et al., 2023) were examined from the 2–3 cm of the middle part of the ileum of each animal. The ileum samples were fixed in 10% neutral-buffered formalin and soaked for 24 h. After fixation, the samples were processed through a series of alcohol in increasing concentrations (70, 80, 90, and 95%). The samples were soaked with each alcohol concentration for approximately 10 s. Then, the sample was added with xylol and finally immersed in paraffin wax. After paraffin molding, four slices of 4 µm thick were prepared from each sample using a microtome, fixed on a glass slide, and then stained with hematoxylin-eosin.

The histological preparations in the glass slides were observed and measured under Zeiss Primo Star, a digital microscope equipped with an OptiLab Projector (camera), and the histological picture appeared on the monitor screen OptiLab viewer 2.2. Histomorphometry measures were determined using Axio vs 40V4.8.2.0, an image processing and analyzing system.

The histo-morphometric indices of small intestine characteristics (Fig. 1) included villus height (VH), crypt depth (CD), villus apical width (VAW), villus basal width (VBW), and the VH/CD ratio. The apparent villus surface area (VSA) was calculated using the following formula: [((VAW + VBW)/2) × VH] (Iji et al., 2001).

Statistical Analysis

Data collected for performance and small intestine characteristics (gross- and histo-morphometrics indices) affected by IOF and neonatal treatments were analyzed using SPSS version 16 (Wilkinson, 2009) with a two-way analysis of variance and a general linear model of a factorial experiment 5×2 based on completely randomized design of 10 replications of individual chickens.

RESULTS

Production Performance

The effects of IOF on the performance of Kampung chickens are presented in Table 2. In chicken up to 4 weeks old IOF and NN did not exert significant effects on the BW. The final BW of 8-week-old chickens indicated that IOF-3 and IOF-4 were associated with significantly heavier BW compared with IOF-0, IOF-1, and IOF-2, and a additional significant effects of NN1 were observed on IOF-2, IOF-3 and IOF-4. Average feed intakes of 1–4 weeks and age of

Table 2: Performance (body weight, feed intake, and feed conversion ratio) of Kampung chickens treated with *in ovo* feeding (IOF)-Arg and neonatal nutrition (NN)

Group ↓		Parameters						
		Hatching weight (g)	4 week body weight (g)	8 week body weight (g)	Feed intake 1-4 w (g/h/d)	Feed intake 1-8 w (g/h/d)	Feed conversion ratio (1-4 w)	Feed conversion ratio (1-8 w)
P0	N0	29,73±2.93a	176.72±12.92a	443.36±24.07a	17.25±0.27a	54.45±3.19a	3.28±0.31a	6.67±0.41a
	N1		177.88±14.39a	449.41±26.02a	17.18±0.29a	55.19±0.83a	3.19±0.27a	6.66±0.20a
P1	N0	29.19±2.25a	176.93±13.95a	453.96±14.50a	17.23±0.31a	55.23±0.98a	3.27±0.31a	6.66±0.20a
	N1		177.43±9.65a	457.57±13.60a	17.27±0.17a	55.69±1.69a	3.26±0.22a	6.68±0.14a
P2	N0	29.71±2.28a	177.13±13.37a	455.34±11.86a	17.28±0.13a	55.71±1.36a	3.28±0.13a	6.63±0.19a
	N1		177.39±12.61a	465.57±9.85b	17.31±0.37a	55.66±1.44a	3.28±0.19a	6.59±0.19a
P3	N0	30.27±2.41a	173.96±13.90a	479.79±15.02b	17.43±0.12a	55.67±1.42a	3.40±0.26a	6.24±0.22b
	N1		193.58±12.64a	509.20±13.32c	17.55±0.32a	55.73±1.46a	3.30±0.26a	6.21±0.32b
P4	N0	31.45±5.11a	177.87±12.38a	505.77±13.78c	17.36±0.24a	55.81±1.64a	3.32±0.22a	5.52±0.30c
	N1		195.69±13.16a	535.88±17.29d	17.45±0.14a	56.79±2.40a	3.07±0.17a	5.30±0.37c

Notes : P0 (negative control, no IOF), P1 (positive control (IOF physiological NaCl 0.9%), P2 (IOF arginine 0.5% + physiological NaCl 0.9%), P3 (IOF arginine 1% + physiological NaCl 0.9%), P4 (IOF arginine 2% + physiological NaCl 0.9%), N0 (NaCl 0.2%), N1 (0.2% probiotics + 0.9% inulin + 0.5% lysine in NaCl 0.2%). Values (mean+SD) in a row appended with different letters indicate significant (P<0.05) differences.

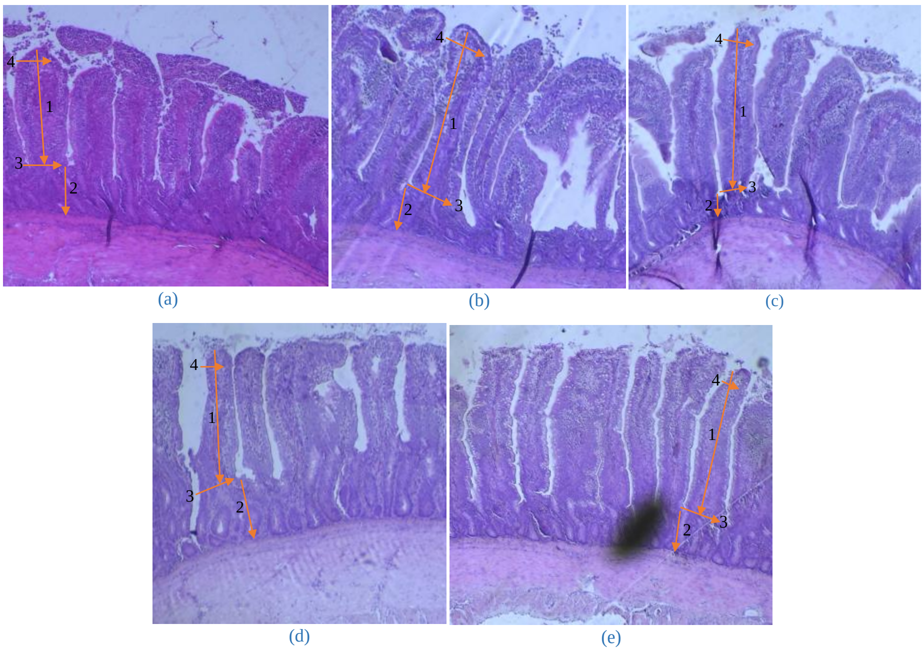

Fig. 1: Histomorfometrics of small intestine (ileum): a) P0 (IOF-0), b) P1 (IOF-1), c) P2 (IOF-2), d) P3 (IOF-3), and e) P4 (IOF-4). The numbers indicate the morphometric measurements taken: 1) the villus height, 2) the crypt depth, 3) the basal width, and 4) the apical width.

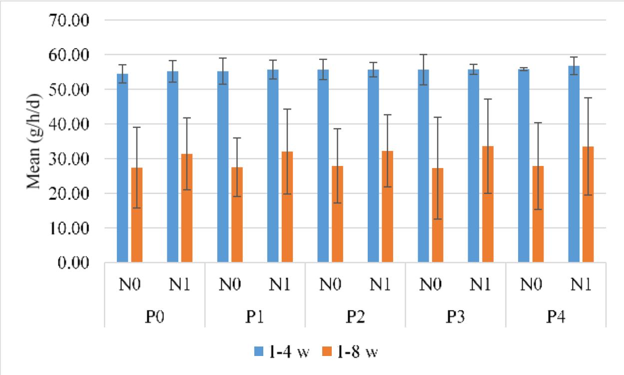

Fig. 2: Water intake of Kampung chickens treated with *in ovo* feeding (IOF)-Arg (P) and neonatal nutrition (NN); Notes: P0 (negative control, no IOF), P1 (positive control (IOF physiological NaCl 0.9%), P2 (IOF arginine 0.5% + physiological NaCl 0.9%), P3 (IOF arginine 1% + physiological NaCl 0.9%), P4 (IOF arginine 2% + physiological NaCl 0.9%), N0 (NaCl 0.2%), N1 (0.2% probiotics + 0.9% inulin + 0.5% lysine in NaCl 0.2%).

Table 3: Gross- morphometric indices in Kampung chickens treated with n ovo feeding (IOF)-Arg (P) and neonatal nutrition (NN): Ratio of the length/weight (cm/g) of each segment of the small intestine

Group		Parameters		
↓		Duodenum	Jejenum	Ileum
P0	N0	3.82±0.33a	5.41±0.18a	6.22±0.28a
	N1	4.62±0.28b	6.39±0.15b	7.39±0.13b
P1	N0	3.86±0.30a	5.45±0.18a	6.27±0.64a
	N1	4.78±0.27b	6.33±0.27b	7.64±0.38b
P2	N0	4.21±0.17a	5.81±0.37a	6.33±0.10a
	N1	4.79±0.25b	6.61±0.35b	7.56±0.39b
P3	N0	5.42±0.42c	6.37±0.45b	6.43±0.43a
	N1	5.95±0.35d	6.92±0.25c	7.93±0.47b
P4	N0	5.87±0.32d	7.66±0.16d	6.37±0.22a
	N1	5.87±0.37d	7.67±0.23d	7.89±0.29b

Values (mean+SD) in a row appended with different letters indicate significant (P<0.05) differences. P0 (negative control, no IOF), P1 (positive control (IOF physiological NaCl 0.9%), P2 (IOF arginine 0.5% + physiological NaCl 0.9%), P3 (IOF arginine 1% + physiological NaCl 0.9%), P4 (IOF arginine 2% + physiological NaCl 0.9%), N0 (NaCl 0.2%), N1 (0.2% probiotics + 0.9% inulin + 0.5% lysine in NaCl 0.2%).

Table 4: Histo-morphometric indices of the ileum of Kampung chickens treated with *in ovo* feeding (IOF)-Arg (P) and neonatal nutrition (NN): villus height (VH), crypt depth (CD), VH/CD, and villus surface area (VSA)

Groups		Parameters			
↓		VH	CD	VH/CD	VSA
P0	N0	950.49±86.35a	138.85±9.447a	6.89±0.53a	0.087±0.02a
	N1	955.50±84.64a	140.19±15.89a	6.91±0.91a	0.086±0.01a
P1	N0	955.83±88.97a	140.81±15.30a	6.98±1.23a	0.086±0.02a
	N1	965.42±12.75a	146.08±20.80a	6.88±1.41a	0.079±0.01a
P2	N0	965.12±41.11a	145.94±28.48a	6.83±1.26a	0.087±0.02a
	N1	973.93±58.48a	146.59±17.67a	6.86±1.31a	0.097±0.01a
P3	N0	1065.86±58.48a	163.68±19.08ab	7.10±2.19a	0.11±0.01b
	N1	1115.87±46.27a	175.74±24.64b	6.72±1.66a	0.12±0.01b
P4	N0	1097.98±51.29b	176.65±16.35b	6.34±1.05a	0.12±0.02b
	N1	1119.56±54.14b	188.42±20.87b	6.01±0.71a	0.11±0.02b

Values (mean+SD) in a row appended with different letters indicate significant (P<0.05) differences. P0 (negative control, no IOF), P1 (positive control (IOF physiological NaCl 0.9%), P2 (IOF arginine 0.5% + physiological NaCl 0.9%), P3 (IOF arginine 1% + physiological NaCl 0.9%), P4 (IOF arginine 2% + physiological NaCl 0.9%), N0 (NaCl 0.2%), N1 (0.2% probiotics + 0.9% inulin + 0.5% lysine in NaCl 0.2%).

1–8 weeks were not affected either by IOF or NN. In relation to the BW, the average feed conversion rate (FCR) in 1–4 weeks was not significantly affected by IOF or NN. Furthermore, IOF and NN in chickens aged 5–8 weeks showed significant improvements in IOF-3 and IOF-4 with or without additional effects of NN1. IOF did not significantly affect the water intake of chickens aged 1–4 or 1–8 weeks (Fig. 2). On the contrary, the NN1 group had significantly increased water intake at the same levels as that of the IOF group.

Gross Morphometric Indices of the Small Intestines

The effects of IOF and NN on the gross-morphometric indices of the small intestinal segments (duodenum, jejunum, and ileum) are shown as the length/weight ratio of each segment (Table 3). The ratio of each segment was used by considering that each segment has different functions. Except for the ileum, the other segments had increased ratios resulting from IOF, and these IOF effects interacted with those of NN to achieve optimal responses in each segment.

Histo-morphometric Indices of the Ileum

Histo-morphometric indices of the ileum of Kampung chickens are presented in Table 4. IOF and NN influenced significantly the histo-morphometric indices of the ileum. In this segment, IOF-3 and IOF-4 have a higher and deeper VH and CD, without additional effect of NN.

DISCUSSION

In this study, pre-hatch administration of Arg by IOF, and continued with post-hatching NN treatment (containing 0.5% Lys, 0.2% probiotics and 0.5% prebiotic - inulin diluted in 0.2% NaCl) in drinking water, provided an interaction effect and improve the post-hatching performance and development of the small intestines. Nutrient supplementation into embryonated eggs immediately after hatching influences embryo development and growth during incubation and the post-hatch performance of chicks.

The potential advantages of IOF-Arg in chickens have been reported, such as improving growth performance and development of digestive organs after hatching (Lu et al., 2022), enhancement of protein deposition, and improved breast muscle growth (Lu et al., 2022), modulation of the release of gastrointestinal hormones (Gao et al., 2017b), increased the activities of intestinal digestive enzymes (Gao et al., 2017a), and intestinal microbial diversity and community. In this study, IOF-Arg 1% (P3) and 2% (P4) demonstrated beneficial effects on the BW gain of chickens aged 8 weeks, and NN1 interacted with IOF-Arg to increase BW compared with IOF-Arg as the main factor. Up to 8 weeks old IOF-Arg did not affect the feed intake, and if it is attributed to the BW gain, this response also resulted in a better FCR. These positive interaction effects were also indicated in chickens aged 4 weeks. This positive effect of IOF-Arg in the present study is consistent with those reported in a previous study (Azhar et al., 2016). Studies on broilers (Lu et al., 2022) and ducks (Tangara et al., 2010) revealed that IOF-Arg improved hatchability, post-hatch performance, and BW.

Arg is one of the essential AAs in poultry diet, which has many biological functions and serves as a precursor in protein synthesis (Fathima et al., 2024). Arg was attributed to improving poultry performance. First, Arg is a primary component of proteins. Sufficient Arg can enhance the translation of protein synthesis (Fathima et al., 2024). Second, Arg has a secretagogue activity by which it stimulates the release of pituitary and gastrointestinal hormones, including glucagon, insulin, ghrelin, and growth hormones. This induced hormone production which can increase protein synthesis and intestinal enzyme activities, which support the structure and functions (digestion and absorption) of the intestines and, subsequently, growth performance (Fathima et al., 2024). Third, an Arg effect might be realized through the formation of ornithine, a polyamine precursor by which ornithine could increase DNA synthesis and cell proliferation (Ruan et al., 2020; Hassan et al., 2021). Another possible mechanism is that Arg could promote creatine synthesis. Arg, glycine, and methionine are the three AAs involved in creatine synthesis. Creatine was suggested to increase muscular growth (Fathima et al., 2024), thereby increasing the growth and development of the whole body.

Attributed to providing NN immediately after hatching, a positive interaction effect between NN1 (containing 0.5% Lys + 0.2% probiotic + 0.5% prebiotic-inulin diluted in 0.2% NaCl) and IOF-3 and IOF-4 on BW was observed at 8 weeks old. Regarding this positive response in BW, the interaction effect between NN1 with IOF-3 and NN1 with IOF-4 may be a better alternative compared with other chicken groups (IOF-Arg 0.5%, negative control or positive control).

Lys is one of the essential AAs and is the third of the six limiting AAs in corn-soybean meal-based diets (Fernandes et al., 1994). Lys greatly contributes to protein synthesis, muscle growth, cytokine synthesis, and lymphocyte proliferation, and thus maximizes the immune response against infection (Soglia et al., 2021). Some studies have reported that dietary Lys has beneficial effects on BW gain, feed intake, feed efficiency, and growth performance (Mousa et al., 2023). In addition, a specific relationship was found between Arg and Lys, i.e., any deficiency or excess could have negative effects on plasma and muscle AA metabolism, and thereby growth performance as a whole. This antagonism is more pronounced in Lys excess (low Arg/Lys ratio) than in Arg excess (high Arg/Lys ratio) (Lisnahan et al., 2023; Fathima et al., 2024). The result of this study showed that the parameters in the IOF-3-NN1 group are mostly in peak values. This may be an indication that IOF-Arg and NN are in the balance condition.

This study used the commercial prebiotic, inulin and a commercial probiotic which are mainly based on live *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Saccharomyces cerevisiae* and *Lactobacillus bulgaricus.*

Inulin is naturally present in many plants and is considered to have strong prebiotic properties and a valuable alternative to antibiotic growth promoter (AGP), because inulin is not digested by the enzymes of monogastric animals. Thus, inulin is fully available for th fermentation and stimulation of the growth and activity of one or a few intestinal microbiota/bacteria, mainly *Bifidobacterium spp*. (Kozlowska et al., 2016). In laying hens, inulin improved egg performance, mineral absorption and utilization, eggshell percentage, eggshell - breaking strength, and some bone quality indicators (Shang et al., 2020). In broiler chickens, dietary inulin increased the antioxidant profile, length of the intestinal villi, and nutritional quality of meat (tights and breasts) (Untea et al., 2023).

A previous study on broiler chickens (Wu et al., 2021), fed a diet supplemented with *Lactobacillus acidophilus* results that it not only improved growth performance, immune response, and intestinal barrier function of chickens but also reduced the mortality caused by *Escherichia coli*. The addition of *Bifidobacterium spp*. and *Lactobacillus spp*. in the diet of laying hens was also reported to reduce the feed intake (FI) and FCR, increasing egg weight, feed efficiency, and hen-day production (Kumalasari et al., 2023). *Lactobacillus spp*. in the diet of dual-purpose 1 week old chickens (Fesseha et al., 2021) improves the growth performance of these chickens without significant differences in feed intake. Yaqoob et al. (2022) found that a mixture of probiotics containing several microorganism strains, including *Lactobacillus spp., Bifidobacterium spp.,* and *Streptococcus spp*. on broiler chicken diet, resulted in outperformance in terms of BW, FCR, and carcass chemistry compared with the control group. Moreover, Sugiharto (2022) reported that probiotic supplementation in broiler diets ameliorates unfavorable environmental effects, such as overcrowding stress.

A combination of prebiotic and probiotic supplementation may lead to a more enhanced functionality than their supplementation, which particularly contributes to a healthy GIT by populating microbiota with a stable and diverse community of beneficial bacteria, which strengthen the gut microbiota to improve host performance and colonization resistance to gut pathogens. By enhancing the numbers of these symbiotic bacteria and promoting a dynamic equilibrium of the microbiota, probiotics prevent the outbreak of harmful bacteria, mainly through competitive exclusion, a process by which potential pathogens are excluded through competition for nutrients (prebiotic). In addition, the mechanisms of action of prebiotics and probiotics were also attributed to the production of organic acids, activation of the host immune system, and production of antimicrobial agents (Shehata et al., 2022).

The post-hatch growth and development of chickens are directly linked to the function and development of the small intestines in the digestion and absorption of nutrients. Several days before and after hatching, Kampung chickens, as well as the exotic commercial breed, experienced a critical period, in which the digestive system is still immature. During this critical period, chickens are transitioning from the metabolic and physiological conditions of nutrients used, from a yolk nutrient-based diet (mainly fat) to a solid feed diet (mainly carbohydrates and proteins). To a certain extent, the growth and development of the embryos and hatching chickens, depend on the digestion and absorption capacity of nutrients, and this capacity is associated with the growth and development of the digestive system (structural and functional), and the small intestines are one of the most important digestive organs. The results of this study indicated that IOF-Arg facilitates the growth and development of the small intestines, which were evidenced by improving gross- and histo-morphometric indices of the small intestines. The gross-morphometric indices were presented with the length/weight ratio of each segment.

The ratios of the duodenum, jejunum, and ileum optimally resulted from the interaction effect between IOF-3 and NN1, particularly for the ratios of the duodenum and jejunum. These results mean that the higher the length ratio, the better opportunity for better digestion and absorption. This result appears consistent with those of a previous study also conducted in our laboratory (Rahardja et al., 2022). The present findings showed that the responses of the length of the duodenum and jejunum on NN are positively up to IOF-2 (P3) as the results of the interaction effect, whereas the positive responses of the length of the ileum are consistently up to IOF-Arg 2% without interaction effect. This may be an appropriate combination of NN with IOF-Arg 1% and fulfill the nutrient requirement of chickens.

In line with the results of this study, a comparative study of three BW grades of the same strain of broiler chickens having the same age and rearing conditions (Yang et al., 2013) showed that heavier chickens have longer and heavier intestines, and better digestibility (energy and proteins) than lighter chickens.

Another study of the effect of different levels of dietary fibers (Sittiya et al., 2020) also indicated that up to 5% fiber in the ratio resulted in heavier BW which was associated with better digestibility and the length/weight ratio of each intestinal segment. Moreover, a comparison study of the GIT between dual-purpose and broiler chickens (Alshamy et al., 2018) showed that at a 35 days old, broiler chickens were 2.6 times heavier than the dual-purpose chickens (2013.16 vs 791.66 g), whereas the entire intestinal length of broiler chickens was growing faster, i.e., 1.53 times in length, than that of the dual-purpose chickens. In addition, during long-term feeding of low-protein or low-energy diets, chickens receiving a low-protein diet show a significant reduction not only in performance and carcass characteristics, such as breasts and wings, but also in the histo-morphometric indices of the small intestines (Incharoen et al., 2010). The results of this study indicate that the histo-morphometric indices of the ileum, VH and CD, increased with IOF-3, and no further significant increase resulted from IOF-4 and NN. This is an indication of the cellular activities of the ileal epithelium. IOF-Arg is expected as an early stimulation to initiate and modulate cellular division/proliferation and differentiation of the epithelial multipotent cells of the ileum which continue with NN and its interaction effects with IOF-Arg immediately after hatching, to attain full maturation of the ileum and the digestive tract as a whole. An increase in VH was associated with a larger surface area to absorb nutrients into the blood vessel, and an increase in CD indicates faster turnover of villous epithelial cells. The ileum as the last segment of the small intestine in Kampung chickens responded positively to the early nutrition programming, in which the VH and CD are attributed to increasing VSA for the absorption of nutrients into portal blood vessels. In broilers, villus size is not determined only by the rate of cell division/proliferation, but also by the relationship between the rates of cell division/proliferation (in the crypt) and cell death (apoptosis) followed by cell extrusion (at the apex of the villi) (Gilani et al., 2021). Therefore, to maintain the function and structure of villous epithelial cells, the proliferation rate should balance the extrusion. The results of this study may be an indication that the fluctuation (up and down) in VH and CD values of the ileum appear at the same rate among the chickens treatment groups. Thus, the VH/CD ratios were not different among the groups.

In relation to the performance of the Kampung chickens, IOF-3, and NN as mentioned previously, the gross and histo-morphometric indices of the small intestines are increasing proportionately with BW gain. This is possibly an indication of a balance between the level of IOF-Arg and NN.

Conclusion

IOF of 1% L-Arg to Kampung chickens increases growth performance, particularly in terms of BW and improves feed efficiency and promotes earlier small intestinal growth and development, which then contributes to subsequent improvement resulting from the beneficial interaction effect with NN immediately after hatching for better performance of Kampung chickens. The absorption of nutrients in the digestive system can be optimized by expanding the absorption area in the intestine.

Conflict of Interest

The authors declare that we do not have any conflict of interest. We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Author Contribution

EA, Acquisition of data, DPR and SP, conception and design of study, EA, DPR, and SP, analysis and/or interpretation of data, and drafting the manuscript.

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