Efficacy of Early Nutrition Programming for Improving The Performance of Kampung Chicken

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ABSTRACT

A Research of Early Life Nutritional Programming (ELPN) was a two consecutive experiment to elucidate the efficacy of in ovo supplementation (IOS) and neonatal nutrition (NN) on the hatching and post-hatching performances of Kampung chicken (KC). There were 960 hatching eggs were used and randomly divided into 4 experimental groups with 4 hatching periods as replication, using 60 hatching eggs per treatment unit: P0 (negative control), P1 (isotonic saline), P2 (5%Glucose), and P3 (0.5% Glutamine +5% Glucose). On d7 incubation, a 0.5 ml nutrient solution was injected into the albumen of P1, P2, and P3. A 20 newly hatched chicken (NHC) were selected from each group and treated as NN0 (without) and NN1 (with) neonatal nutrition (NN) composed of 0.1 commercial probiotics, 5% inulin and 10% lysine dissolved in 1L of tap water and provided as drinking water for 4 of 8 week-experiment. The results showed IOS significantly lowered the hatchability compared to the control eggs, but the weight of NHC was not affected (P<0.05). IOS and NN were significantly increased final LBW, from the lightest, 779.42±34.39 g (negative control) compared to the heaviest, 1147.54±81.48 g (IO Gln+Gluk, and NN), which also indicated by a significant better FCR. Structurally, intestinal gross and histomorphometry examinations indicated an increased capacity of digestion and absorption, which indicated an increase the ratio of length/weight of each small intestine segment, and a wider absorption surface area of villus. In conclusion, ELNP resulted in markedly heavier final life bodyweight which is facilitated by digestive structure and function development.

Keywords: Early life nutrition programming, Native Chicken, in ovo feeding, neonatal nutrition.

I. INTRODUCTION

Based on molecular characterization studies, Indonesian native chickens (INC) [1] and many exotic commercial breeds [2] have been recognized as the results of domestication and breeding program of red jungle fowl (Gallus gallus). In Indonesia there are currently known at least 50 clumps [3], which can be grouped into 2 groups, namely identifiable with specific characteristic (as Pelung chicken), and nonidentifiable clump with no specific characteristic [4], and commonly called as Kampung chicken, and it is believed to be the most abundant of INC [5,6]. Compared to exotic breeds of broiler or layer, the productivity of Kampung chicken is very much lower, but the appreciation price of the consumer to Kampung chicken products, either egg or meat is very much better.

Various aspects have been investigated to improve the performance of Kampung chicken [7], which basically resulted from the cross-breeding program with an exotic breed in addition to better management in nutrition and health. This approach has resulted in a significant better performance but has diluted of Kampung chicken characteristics as a INC.

Application of IOS and NN techniques and briefly termed as ELNP are common in the broiler to improve the performance, even though it is known that the broiler has a superior genetic background as meat producer.

The present study reported herein is the application of the Early Live Nutrition Programming [8] on Kampung chicken, which is IOS of glucose and mixed glucose–glutamine, and NN of mixed pre– probiotic–lysine, as a strategy to provide specific additional nutrients earlier during the incubation period, then continued soon after hatching to complete the growth and development of digestive system faster, in particular.

II. MATERIALS AND METHOD

This study was conducted at the Laboratory of Animal Physiology, and the Laboratory of Poultry Science and Technology, Faculty of Animal Science Hasanuddin University. The experimental procedures were performed in accordance with the ethical principles for experimental protocol (IRB Protocol No. UH 21110720). which was approved by the Local Ethics Committee of MedicalResearch, Faculty of Medicine–Hasanuddin.
University (No.783/UN4.6. 4.5.31/PP36/2021), and duration approval from 10 December 2021 to 10 December 2022.

The research was conducted in 2 stages.

1) First Stage

The first stage was arranged as a Randomized Block Design of 4 treatment groups with 4 blocks of the incubation period (totaling 16 experimental units): P0 – no IOF (negative control), P1 – IOF 0.9% NaCl (positive control), P2 (5% Glucose), P3 (5% Glucose + 0.5% Glutamine). P2 and P3 were diluted in 0.9% NaCl isotonic-sterile solution.

There were 60 eggs/unit/period, or 240 eggs/period used, produced from 4 day collection from a population of skc, stored at a room temperature (24-28 °C) till placing time into the incubator which has been set at 37.4 °C min – 37.8 °C max.

At d7 incubation, the eggs were candled to discard unfertilized and dead embryo eggs, and live embryonated eggs were selected, prepared and divided randomly into 4 treatment groups of IOS (P0, P1, P2, and P3). A 0.5 ml nutrient solution of IOS was infused into the albumen of live embryonated eggs.

From d18 to hatching days (d21–d22 incubation), the eggs were placed in hatching, in which each newly hatched chicken (NHC) would be coded with the same code as the egg. At d22 incubation, NHC and unhatched eggs were collected and the hatching ability of fertile eggs could be calculated as: (number of NHC/number of fertile eggs) × 100%; Embryonic mortality was determined macroscopically as early mortality (from 1 to 7 days), middle mortality (from 8 to 17 days) and late mortality (from 17 to 21 days) and expressed as percentage of fertile eggs.

2) The Second Stage

The second stage was carried out for 8 weeks (56 d), aimed to elucidate the main effect of in ovo supplementation, neonatal nutrition and their interaction on the post-hatching performance, gross and histo-morphometry of the intestine. After hatching, all NHC were evaluated, cleaned, weighed, and fitted wing tags coding the same code as the egg.

They were placed in brooding boxes (40 cm × 70 cm, for 7 – 8 NHC) equipped with a 15 W lamp. There were 2 subgroups of each treatment group of the first stage, which are with (N1) and without (N0) neonatal nutrition. Accordingly, the second stage was a factorial experiment 4×2 with 3 blocks of incubation period as replication, totalling 24 treatment units, with 15 NHC being used per treatment unit. The neonatal nutrition consisted of 1% L-lysine + 1% Inulin (Prebiotic) + 0.1% Commercial Probiotic diluted in 1 l of tap water, and given as drinking water ad libitum for 28 d.

The standard commercial feed (Table 1) and tap water were used and provided ad libitum, and their intakes were monitored daily (g/d), and body weights were monitored two weekly. Cumulative daily feed and water intakes were calculated on the treatment group basis of each replication.

B. Small Intestine Gross Morphometry

At the end of the second stage, two chickens (male and female) with body weights close to the mean were selected from each treatment unit. They were slaughtered by bleeding the left-right jugular veins, and all visceral organs were carefully removed. For the gross morphometry examination of the small intestine, the entire small intestine from the gizzard to the large intestine was removed.

| TABLE I: NUTRIENT COMPOSITION OF THE COMMERCIAL DIET USED DURING THE SECOND STAGE EXPERIMENT (1–56 D) |
|---------------------------------------------------------------|
| Composition | 1-56 day |
| Energy – ME (kcal/kg) | - |
| Protein (%) | 21.00 |
| Fat (%) | 4.08 |
| Fiber (%) | 5.05 |
| Calcium (%) | 1.10 |
| Phosphor (%) | 0.30 |
| Alfa toxin (µg/kg) | 50.00 |
| L-lysine (%) | 1.20 |
| L-methionine (%) | 0.45 |
| Met + Cys (%) | 0.89 |

The small intestine was removed, and the total length (cm) and the weight (g) were measured. The small intestine was divided into 3 segments of the duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from the entry of the ducts to Meckel’s diverticulum), and ileum (from Meckel’s diverticulum to the ileocecal junction).

C. Small Intestine Histo-morphometry [9], [10]

Approximately 2-3 cm of middle parts of the duodenum, jejunum and ileum were cut as intestinal samples for histo-morphometry examination. The samples of each segment were fixed in 10% formalin buffered solution and soaked for 24 h. Following fixation, the samples were processed through a series of alcohol which concentration is increasing (70, 80, 90 and 95%). The samples were transferred one by one to each alcohol concentration and allowed to soak for about 10 s. Then, the sample is inserted into xylol and finally immersed in paraffin wax. After paraffin moulding, 4 slices of 4 µm thick were prepared from each sample using a microtome, and fixed on a glass slide, then stained with hematoxylin-eosin.

The histological preparations that were ready in the glass slide were observed and measured using a computer microscope, Zeiss Primo Star, equipped with an OptiLab Projector (camera) and histological picture appears on the monitor screen Optilab viewer 2.2. Histomorphometry measures were determined using an image processing and analyzing system of Axio vs 40V4.8.2.0.3.

The histo-morphometry measures comprised villi height (VH), apical width (AW), basal width (BW), and crypt depth (CD). For measurement, 3 villi were selected randomly from each slice. For each, the villus height was measured from tip to base, excluding the intestinal CD. The villus width was measured at the basal and apical parts. A total of 12 villi from 4 slices were measured and regarded as the mean for each chicken. The villus surface area (VSA) was calculated from the VH, basal width BW and AW [9]. The 12 calculations of villus surface area were expressed as the mean for each chicken.
D. Statistical Analysis

Data resulting from the first stage experiment comprised hatchability, live weight of the newly hatched chicken and its ratio to egg weight, embryo mortality distribution. These data were analyzed using one way of analysis of variance (ANOVA) with a general linear model (GLM) based on a randomized block design of 4 replications as blocks of the incubation period for analyzing 4 treatments of IOS.

Data resulting from the second stage experiment comprised post-hatching performance (body weight, feed intake and water intakes, feed conversion rate (FCR), gross morphometry of the small intestine (weight and length) and histo-morphometry of light microscopic examination of each segment of the small intestine (VH, AW, BW, CD, and VSA).

These data were analyzed two ways ANOVA with GLM of a factorial experiment 4 x 2 based on randomized block design of 4 replication blocks. All data from the first and the second stage experiments were analyzed using the statistical package of SYSTAT vs 13.2 [11]. The significant differences between mean values are stated at a level of 5% maximum.

III. RESULTS

A. Hatching Performance

In TABLE II, IOS of 5% Glucose (P2) or mixed with 0.5% Glutamine (P3) diluted in isotonic NaCl did a significant influence on reducing the hatchability compared to those of negative (P0) and positive controls (P1) (P<0.05). There was no significant effect of IOS on the live weight of the newly hatched chicken (P>0.05), but the ratio of live weight/hatching egg weight was significantly higher P1 and P3 than that of P0-negative control. Embryo mortality of the fertile hatching eggs treated IOS including those of positive control eggs (P1) mostly occurred during d8 to d17 incubation period (P<0.05), while in the negative control (P0) the mortality mostly occurred in the last 3 days.

B. Post Hatching Performance

Us Live weights, feed intakes and FCR are shown in Table 3. Feed intakes during 1–4, 5–8 weeks and overall phase were not affected (P>0.05) either by IOS, NN or their interaction. FCR indicated a positive significant response (P<0.05) on IOS, but it was not affected by NN (P>0.05).

C. Gross Morphometry of Small Intestine

The effects of IO supplementation and neonatal nutrition on the Gross morphometric of the intestine (duodenum, jejunum, ileum, caecum, and colon) are shown in Table III and presented as the length-weight ratio. Except on the duodenum, the effects of IO supplementation of P2 and P3, and positive control (P1) on the other segments resulted in significant higher ratio values compared to those of negative control (P0), while there was no significant effect of neonatal nutrition on the sizes of the gross morphometric of the intestine.

| TABLE II: EFFECTS OF IN OVO GLUTAMINE (GLN)AND GLUCOSE (GLUC) ON HATCHING PERFORMANCES |
|---------------------------------|---------|---------|---------|---------|---------|
| Parameter                        | P0      | P1      | P2      | P3      | P<value |
| Σ egg x block                    | 60±4    | 60±4    | 60±4    | 60±4    | P>0.05  |
| Egg W (g)                       | 46.50±5.71 | 45.93±3.89 | 46.47±4.45 | 46.40±1.04 | P>0.05  |
| Fertility (% d7)                | 96.6±0.72 | 96.8±1.91 | 95.0±3.97 | 94.5±1.91 | P>0.05  |
| Hatchability (%)                | 75.8±2.14 a | 70.91±3.69 b | 58.4±3.45 c | 66.8±2.66 bc | P>0.05  |
| DOC BW (g)                      | 31.8±3.32 | 32.6±3.04 | 31.9±3.24 | 32.8±1.85 | P>0.05  |
| DOCBW/EW (%)                    | 68.6±3.07 a | 71.42±1.92 b | 69.42±0.43 a | 70.42±0.66 b | P>0.05  |
| Mortality0-7d (% fertile egg)   | 4.32±1.52 a | 4.35±0.74 a | 5.71±1.78 a | 3.67±1.71 a | P>0.05  |
| Mortality8-17d (% fertile egg)  | 4.75±0.78 a | 18.23±3.12 b | 27.10±3.94 c | 21.51±2.04 b | P>0.05  |
| Mortality18-22d (% fertile egg) | 15.08±0.70 a | 6.52±3.26 b | 8.78±2.85 b | 6.73±2.14 b | P>0.05  |

Mean values within the same row followed with the different letters below are significantly different (P<0.05).

| TABLE III: EFFECTS OF IN OVO GLN AND GLUC, AND EARLY NUTRITION OF PRE-, PROBIOTIC AND LYSINE ON POST HATCHING PERFORMANCES |
|-------------------------------|---------|---------|---------|---------|---------|
| 0                             | P0      | P1      | P2      | P3      | P<value |
| Body weight x block (g)       | 31.8±2.32 a | 32.8±2.89 a | 32.7±2.46 a | 33.27±1.76 a | P>0.05  |
| BW 4 weeks (g)                | 386.7±22.21a | 384.17±20.64a | 404.28±38.16ab | 421.47±33.89bc | P>0.05  |
| BW 8 weeks (g)                | 799.42±34.39a | 799.53±46.21a | 836.61±32.72ab | 870.37±37.83b | P>0.05  |
| Feed intake 0-4 w (g/a/d)     | 42.83±2.61a | 42.53±1.83a | 44.57±1.69a | 43.44±1.17a | P>0.05  |
| FCR 0-4 w Feed intake 0-8 w   | 3.38±0.23 a | 3.38±0.28 a | 3.36±0.17 a | 3.31±0.24 a | P>0.05  |
| FCR 0-8 w Feed intake 5-8 w   | 46.85±5.74a | 46.88±5.32a | 48.22±5.83a | 48.01±6.39a | P>0.05  |
| FCR 5-8 w Feed intake 5-8 w   | 3.51±0.26a | 3.42±0.17a | 3.36±0.18a | 3.21±0.22a | P>0.05  |
| FCR 5-8 w Feed intake 5-8 w   | 4.74±10.36a | 47.11±6.75a | 48.65±4.15a | 47.25±5.88a | P>0.05  |
| FCR 5-8 w Feed intake 5-8 w   | 3.47±0.28a | 3.44±0.19a | 3.41±0.13a | 3.18±0.16a | P>0.05  |

Mean values within the same row followed with the different letters below are significantly different (P<0.05).
TABLE IV. EFFECTS OF IN OVO GLN AND GLUC, AND EARLY NUTRITION OF PRE-, PROBIOTIC AND LYs ON GROSS-MORPHOMETRY OF SMALL INTESTINE

| Parameter                      | Duodenum cm/g | Jejunum cm/g | Ileum cm/g | Caecum cm/g | Colon cm/g |
|--------------------------------|---------------|--------------|------------|-------------|------------|
|                               | P0            | P1           | P2         | P3          |            |
| Mean values within the same row followed by the different letters below are significantly different (P<0.05). |


d. Histo Morphometry of Small Intestine

Histo-morphometry examination of the duodenum (Table IV) indicated that in IOS of glucose or along with glutamine did a significant increase of both the width of the villus area (VA) and villusbasalis (VB) but did not affect the villus height (VH) and crypt depth (CD). On the jejunum, IO supplementation increased significantly the CD, VA and VB, but it did not affect VH. All sizes of ileum were increased significantly as a result of IOS. However, the effects of NN and its interaction with IOS were not significant on all sizes of each segment of the small intestine. Concurrently, IOS resulted in significant increase in the villus surface area of all intestinal segments.

IV. DISCUSSION

Based on positive preliminary studies in this laboratory with Glutamine and Glucose diluted in NaCl isotonic sterile solution as the IOS solution, and with pre-biotic and Lys diluted in tap water as NN formula was developed to increase nutrients availability for better growth and development during embryonal and neonatal – postnatal periods [12]-[16]. Overall, IOS glucose alone or along with glutamine, and neonatal nutrition of mixed pre-, probiotic and lysine significantly improve the performance of KC.

One of the possible reasons affecting hatchability of the eggs treated IOS of Glucose (P2) and Glucose-Glutamine (P3) lower than the controls (P0 and P1) is the osmolarity of IOS solution. Indicated that the nutrient solution of the IOS composition of the solution is preferably from 50, 100, 200, 300, to about 600 or 700 mOsm, not higher than 800 mOsm, and the optimum hatchability observed at about 400-600 mOsm. The calculated osmolarity of the IOS solution used in the present experiment was around 285 to 300 mOsm, which is lower than the optimum level. The results obtained in this study might primarily be caused by osmotic balance alteration, and consequently affected embryo live and development, which is indicated by lower hatchability, and resulted in the embryo mortality that mostly occurred after IOS (d7-d17 incubation). The most negative control mortality occurred between d18-d21 incubation. This may be an indication of insufficient energy availability until the end of the hatching process.

The second stage of the experiment aimed to elucidate the main effect of IO supplementation, the main effect of neonatal nutrition, and their interaction on some attributes of post-natal performances (feed intake, live weight, feed conversion rate) including gross- and histomorphometry of intestine. The results of this study showed daily feed intake during 1-4, 5-8 weeks, and overall phase 1-8 weeks were not significantly affected either by IOS alone or along with neonatal nutrition treatment, while live body weights of the chicken of P2 and P3 were heavier significantly, which relate with improving feed conversion rate (FCR) compared to those in negative and positive controls. Live body weights of positive control (P1) at 4 and 8 weeks of age were significantly heavier than those of negative control (P0). Interestingly, under control conditions (P0 and P1), neonatal nutrition treatment (S1 vs S0) did not significantly affect the live body weights of the chicken. There is an interaction effect between IOS and NN on live body weight. Under IOS of P2 and P3, NN S1 is seen to contribute to make significant heavier live body weights compared to those of non-NN S0. The contribution of NN during 30 d of the second step experiment is suspected as an indication of the efficacy of neonatal nutrition on increasing the cell size of the organs, in which the skeletal muscle fibre in particular (hypertrophy). An increase of myofiber size (hypertrophy) is as a typical characteristic of postnatal myofiber growth rather than an increase the number of myofibers (hyperplasia), because in

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most species, the total number of myofiber is fixed at birth [18]-[20].

Data of the present study showed that IO supplementation did not influence the live weights of newly hatched chick, but it was suspected to influence prenatal myogenesis. This is a complex process including the proliferation of myoblast precursors, differentiation, alignment, and fusion to form multinucleated myotubes then finally forming mature muscle fibres, consisting of primary and secondary myofibers [21]-[23]. Primary myofibers form during the initial stage of myogenesis and continued with secondary myofibers form during the second wave of myogenesis, as the majority of muscle fibres [24]. In chicken, development and maturation in structure and function of the skeletal muscle are during the incubation period. The primary myofibers form from about d 6 of incubation [25], [26] Secondary, or type II myofibres are derived from a separate population of myotubes and begin differentiation between d 12 to 16 of incubation [27], [28]. Differentiation of both primary and secondary myofibres is complete by 75% of the incubation period, and the total number of myofibers is therefore determined before the final stage of avian embryonic development [20]. The number of primary myofibers is mostly dependent on genetic potency, while secondary myofibers are mostly dependent on the environment.

Moreover, the effects of IO supplementation and neonatal nutrition on gross morphometric of the intestine (duodenum, jejunum, ileum, caecum, and colon) is shown in Table III. The values are presented as the length to weight ratio of each segment. It is an approach to indicate the specific structure and function of each intestinal segment, which varies among the three segments along the small intestine [29]. For instance, the intestinal mucosa decreases in thickness as the villi and crypt decrease in depth from the duodenum to the ileum.

Except on the duodenum, the effects of IO supplementation of P2 and P3, and positive control (P1) on the other segments resulted in significantly higher values of the ratio compared to that of negative control (P0), intestine. A higher value of the ratio means that one gram of the segment is having a longer and thinner segment. Continued with histo-morphometry analysis, the present study indicates that the measures of intestinal morphology were significantly affected and changed by IO supplementation, which includes an increased villus high (VH) of the duodenum, jejunum and ileum, decreased crypt depth (CD) of duodenum and jejunum but not ileum, increasing of the VH/CD ratio and villus surface area (VSA) of each intestinal segment. However, the effect of neonatal nutrition on those parameters was not significant.

Several previous studies have assessed the effects of IOS on structural and functional development of the intestinal mucosa, showing that IO supplementation efficiently improves post-hatch intestinal development in broilers, turkeys, and quails. Studies have focused mostly on IO supplementation of amino acids and carbohydrates [30]-[34]. An increase in length/weight ratio of intestinal gross morphometry and intestinal villus of histo-morphometry may enhance digestive and absorptive functions of the intestine, which is as resulted from an increased absorptive area and increased activity among intestinal brush border enzymes. A higher value of VH/CD also indicates a higher rate of maturity and functional capacity of enterocytes. A decrease in the villus height/crypt depth ratio is considered to be deleterious to digestion and absorption, and vice versa. Additionally, it is also associated with increased rates of crypt-cell proliferation and lead to a faster enterocyte turnover [35].

V. CONCLUSION

In conclusion, the efficacy of IO supplementation of mixed glucose - glutamine, and continued with neonatal nutrition as an alternative approach that improves the post-hatch performance of local chicken is through the better efficiency of feed conversion rate, which is resulted from the higher capacity of digestion and absorption of the intestine.

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CONFLICT OF INTEREST

Authors declare that we do not have any conflict of interest.

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