The influence of protein levels on body weight, body dimensions, and reproductive characteristics of local chickens treated in-ovo feeding L-Arginine for two generations

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Abstract. In ovo feeding is a method to stimulate metabolic activity and nutrient availability in eggs during the incubation period with the aim of increasing embryo growth, hatching weight, development and growth after hatching. The nutritional composition, especially protein levels, in the ration given during the growth period is expected to optimize the growth and development of body components and improve reproductive characteristics of local chickens that has been treated in ovo L-arginine. The objective of the study was to determine the influence of different protein levels on body weight, body dimensions and reproductive characteristics of local chickens treated in-ovo feeding L-arginine for two generations (F2). Twelve 10-week-old female local chickens were used in this study. The chickens had received in ovo L-arginine treatment for two generations which was carried out on the 7th day of the incubation period. The chickens were randomly assigned to 3 treatments, and each treatment was represented by 4 birds. The treatments were feed of different protein levels of 16, 18 and 20% with a metabolic energy of 3000 kcal/kg. The measurement of body weight and dimensions was carried out for 9 weeks and it continued until the chickens reached the reproductive phase for observation of age, body weight and egg weight at first laying. The results indicated that the application of different protein levels during the grower period did not significantly affect the increase in body weight and body dimensions. Regression analysis between body weight gain and body dimension gain showed that there was a significant relationship between chest circumference, tibial length and metatarsus length on body weight gain in native chickens showing chest circumference as the best predictor of body weight. The reproductive characteristics of age, body weight and egg weight at the first laying was not affected by the level of protein in the ration.

1. Introduction
Local chickens are mostly developed by rural communities and widely spread in Indonesia. This chicken has a good environmental adaptation but low in productivity, such as low growth and egg production [1,3]. A lot of attention has been made to improve productivity through crossbreed, selecting and feed quality. However, these efforts are not improving significantly [3,4]. Moreover, there are indications that crossbreed can cause a decrease in adaptability and resistance to disease [5].

Amino acid injection during the incubation period known as in-ovo feeding is an alternative method being developed. The aim is to stimulate the metabolic activity by increasing the availability of nutrients in the eggs during the incubation period and to increase embryo growth, hatching weight, and the development of small intestine tissue after hatching [6]. Amino acids, such as arginine are the main
nutrient used to inject into eggs during the incubation period. Arginine is a protein substance used as a source of energy and cell proliferate [7]. The increase in the number of cells due to the injection of amino acids in hatching eggs is thought to stimulate body weight gain that is positively correlated with the body dimensions of native chickens. Quantitative characters (body weight and body size) are closely related to production in an animal [8]. There are several characteristics related to poultry productivity, namely body weight, chest circumference, chest width, and shank length. Furthermore, it was also stated that body weight has a significant relationship with body size and reproductive characteristics [9].

Growth potential that has been stimulated during the incubation period through in-ovo feeding can be optimized during the growth period by improving feed nutrition, particularly protein content. Diets that contain adequate levels of protein will lead to good bone growth because protein plays a very important role in increasing the stability of mineral deposition in bones [10,11]. The achievement of ideal body weight at the end of the growth period is closely related to the age of laying and the weight of eggs obtained at the first laying of eggs. For this reason, controlling body weight during the growth period through the provision of different protein levels needs to be done to the prospective parent from the in-ovo feeding of L-arginine. The purpose of the present study was to evaluate the influence of different protein levels on body weight, body dimensions, and reproductive characteristics of local chickens treated in-ovo feeding L-arginine for two generations (F2).

2. Materials and method

Twelve 10-week-old female local chickens were individually weighed and randomly divided into three groups with four replicates in each group and randomly allocated in floored pens. According to treatment, the birds were arranged in three treatments in a completely randomized design. The treatments were protein level of feed consisting of 16, 18, and 20% with metabolic energy of 3000 kcal/kg. The feed was prepared based on the recommendation of NRC [12] with relatively the same metabolic energy, namely around 3000 kcal/kg of feed. The composition of the feed ingredients and the nutritional content of the feed used in the study can be seen in table 1.

| Parameters        | Protein levels (%) | 16  | 18  | 20  |
|-------------------|--------------------|-----|-----|-----|
| Ingredients       |                    |     |     |     |
| Corn              | 55.75              | 48.00 | 40.00 |
| Concentrate       | 24.25              | 32.00 | 40.00 |
| Bran              | 20.00              | 20.00 | 20.00 |
| Compositions      |                    |     |     |     |
| Protein (%)       | 16.00              | 18.00 | 20.00 |
| ME(kcal/kg)       | 3011.00            | 3007.60 | 3004.00 |

The local chickens used in this study were the result of in-ovo L-Arginine amino acid for two generations (F2) where local chickens were usually selected and their eggs were taken (F1) then injected with L-Arginine 0.7 g/ml dissolved in 100 ml of NaCl solution. In each injection, 0.5 ml solution was taken and the injection was carried out on the 7th day of incubation. The results of this injection were then maintained and reselected, at the time of laying and the eggs are injected again with the same material in F2.

The birds were placed in 3 plots of 1x1x1 m, consisting of 4 birds/plots. The placement of each bird was adjusted to the feed treatment. The plots were sprayed with disinfectant and each plot was equipped with a hanging feeder, drinker, and lighting at night. The feed and water were given ad libitum. Vitamins, vaccinations, and antibiotics were applied as needed.

The measurement of body weight and dimensions was carried out for 9 weeks. Measurements were made by weighing the chickens and measuring several body dimensions of 12 hens at the start and end of maintenance. The body dimension measured were (1) Body length; extends from the base of the
collarbone to the base of the tail. (2) Wing length; extends from the base of the wing to the end of the wing or phalanges. (3) Chest circumference; measured by wrapping a measuring on the chest/scapula. (4) Beak length; extends from the base to the tip. (5) Tibia length; from the patella to the tip of the tibia. (6) Metatarsus length; from the tip of the tibia to the base of the lower metatarsus. (7) Metatarsus diameter; measuring by wrapping tape on the metatarsus. (8) Body height; from the tip of the foot to the top of the back. The experiment was continued until the chickens enter the production phase, indicated by the appearance of the egg for the first time. To avoid misidentification in the observation, the chickens were moved into individual cages. Observations of reproductive characteristics were carried out on age, weight, and weight of eggs at the time of the first production.

Data were analyzed by 1-way ANOVA. Linear regression analysis was applied to estimate body weight gain using procedure excel software, according to the following model: \( y = a + bx \), where: \( y \) = body weight gain; \( b \) is the coefficient equation; \( a \) is intercept; \( x \) is body dimensions.

3. Results and Discussion

3.1. Body weight gain and body dimensions gain

Body weight gain and body dimensions (body length, height, beak length, wing length, tibia length, breast circumference, tarsometatarsus length, and diameter) of local chickens rearing for 9 weeks are shown in table 2. The analysis of variance indicates that treatment of different protein levels did not show a significant effect on weight gain. This is probably due to the fact that the chicken used has received additional nutrients of amino acid L-arginine which can stimulate cell proliferation during the embryonal period and it was continued with the provision of high levels of feed protein (21%) during the starter period so that protein received before the onset of treatment was high. The effect of treatment in the growing period did not have a significant effect probably because the growth potential has been achieved in the previous growth period. Providing a ration with a high protein level will not be utilized optimally because it is limited by the genetic potential to utilize the protein content. In addition, if the intake of protein is excessive, a bird will release excess protein [13]. The results of previous studies reported that the performance of local chickens treated in-ovo glutamine and fed a commercial feed with a protein content of 21-23% until the age of 8 weeks showed a higher growth rate compared with untreated amino acid glutamine in the embryonal period [2] indicates that chickens that receive high protein early in life can stimulate their growth more optimally.

| Parameters                  | Protein levels (%) | 16       | 18       | 20       |
|-----------------------------|--------------------|----------|----------|----------|
| Body weight                 |                    | 511.25±130.59 | 423.75±156.01 | 480.00±275.16 |
| Body length                 |                    | 2.75±0.74  | 2.65±1.58 | 3.70±1.98 |
| Wing length                 |                    | 1.90±0.88  | 2.30±1.90 | 1.90±0.54 |
| Chest circumference         |                    | 3.12±1.10  | 3.10±0.57 | 2.90±1.75 |
| Beak length                 |                    | 0.51±0.05  | 0.15±0.05 | 0.12±0.05 |
| Tibia length                |                    | 2.10±0.48  | 2.00±0.35 | 1.95±0.51 |
| Metatarsus length           |                    | 0.70±0.33  | 0.67±0.45 | 0.77±0.51 |
| Metatarsus diameter         |                    | 0.42±0.22  | 0.40±0.08 | 0.42±0.23 |
| Body high                   |                    | 3.00±0.66  | 3.70±1.06 | 4.70±2.48 |

There were variations of body dimensions gain between individual birds appear in the study (table 2). However, the analysis of variance showed that the protein level treatments did not show a significant effect on the observed body dimensions gain. This was likely due to genetic variation. The differences in phenotypic appearance in chickens are strongly caused by genetic factors [14,15]. The phenotypic
variations of body dimension are a reflection of the large mix of groups between different breeds that occurs both as a result of selection by breeders or cross-breeding that occurs naturally [16].

Regression coefficient (b), correlation coefficient (r), and significance value (P) on the correlation between body dimensions gain and body weight gain of the local chickens are shown in Table 3. In this study, the correlation between several body dimensions gain and body weight gain was only shown by chest circumference, tibial length, and metatarsus length and the results of variance (P) showed a significant correlation (P<0.05). This indicates that among several body dimensions observed, only chest circumference, tibial length, and metatarsus length can be used to estimate the body weight of local chickens. The chest circumference and the length of the tibia have a very significant effect on body weight because these two parts contain a larger portion of the muscle. This is in accordance with the previous results which states that the morphological characteristics that have the greatest correlation with body weight are chest circumference and tibia length because they are part of the attachment of muscles with a large proportion [17]. The length of the tibia also correlates with body weight because the tibia is elongated until the chicken enters sexual maturity. This is in accordance with early report that the chicken shank reaches its maximum length at the age of 16-20 weeks [18]. In addition, shank length, beak length, chest width, back length, and chest circumference were the estimators of body weight, with shank length as the best estimator of body weight [19].

| Body dimensions          | b    | r    | P    |
|--------------------------|------|------|------|
| Body length              | 15.136 | 0.121 | 0.707 |
| Wing length              | 43.425 | 0.273 | 0.390 |
| Chest circumference      | 133.773 | 0.823** | 0.001 |
| Beak length              | 1651.429 | 0.465 | 0.127 |
| Tibia length             | 308.773 | 0.713** | 0.009 |
| Metatarsus length        | 293.340 | 0.645* | 0.023 |
| Metatarsus diameter      | 436.713 | 0.498 | 0.100 |
| Body high                | 61.253 | 0.545 | 0.066 |

Description: * and ** indicate a significant in P<0.05 and P<0.001, respectively.

3.2. Reproductive characteristics

The age, body weight, egg weight at first laying are shown in Table 4. The results indicated that feeding with different protein levels had no significant effect (P>0.05) on the age at first laying eggs. The absence of treatment effect on the age at first laying was probably due to the fact that the body weight attained at the time of laying eggs was also not influenced by the level of protein. The age at first laying is strongly influenced by body weight. Chickens will reach sexual maturity when they reach their optimal body weight [18]. High body weight can accelerate the first egg laying time (sexual maturity), while light chickens cause slower sexual maturity [20]. Furthermore, high initial body weight before 20 weeks of age can affect reproductive development [21].

| Parameters                  | Protein levels (%) |
|-----------------------------|--------------------|
|                            | 16                | 18                | 20                |
| Age at first laying (weeks) | 24.50±5.06        | 23.00±1.63        | 23.75±3.20        |
| Body weight at first laying (g/bird) | 1677±46.21 | 1453±101.68 | 1483±212.30 |
| Egg weight at first laying (g/egg)   | 38.90±2.87        | 41.22±1.64        | 41.23±1.61        |
In the present study, although body weight at first laying was high in the treatment of 16% protein level, there was no effect of protein levels on body weight at first laying. This finding was, in contrast, reported previously that nutritional content, especially protein levels in feed, was a major element that played an important role in the growth [22]. The absence of treatment effect on body weight at the time of first laying was probably due to the standard requirements of protein. The protein levels applied in this study were higher than the standard protein requirement for local chicken in the growth period. The requirements of protein and energy level of local chicken during the growth period were 14% protein and 2600 kcal/kg metabolic energy, respectively [23]. The low protein level of feed required in the growth period was due to the fact that the growth potential of local chickens is relatively lower compared to the laying period, so the improvement of feed nutrition through increasing protein content cannot affect growth significantly.

The levels of protein did not affect significantly the weight of eggs at the first laying, indicating both age and body weight did not show a significant effect. Several researchers reported association results reported previously. The weight of the egg produced at the first laying was related to the age at first laying. The hen who produces eggs faster will produce eggs with a smaller size. On the other hand, larger eggs are produced by the hen who produces the first egg at an older age [24]. Furthermore, the first laying of eggs usually results in a lighter average egg weight with more egg production but a shorter production life [25]. Egg weight was also influenced by nutritional intake during the growth period. Chickens that lack protein and amino acid intake at their growing age, will be slowing down their sexual maturity and reducing the size of the eggs produced [26]. Giving rations during growth will affect the development of the reproductive organs and sexual maturity, thus impacting the ability to produce eggs during the production period [27].

4. Conclusion
Feeding with different protein levels had no effect on weight gain, body dimensions, and reproductive characteristics (age, body weight, and egg weight at the first laying) of local chickens treated in-ovo L-arginine, but there was a significant correlation between chest circumference, tibial length and metatarsus length on body weight gain, showing chest circumference as the best predictor of body weight.

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