

## Embryo Development and Post-Hatch Performances of Kampung Chicken by *in Ovo* Feeding of L-Arginine

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### ABSTRACT

The research was conducted to evaluate embryo development, post-hatch performances, and growth rate of kampung chicken treated *in-ovo* feeding of L-Arginine. A total of 135 kampung chicken fertile eggs (weight 42-43 g) were used and divided into 5 treatment groups of three replications. They were placed in the semi-automatic incubator. The first group was without *in-ovo* feeding (negative control); the second group was *in-ovo* feeding of saline 0.9% (positive control); the 3, 4, and 5 groups were *in-ovo* feeding of 0.5, 1.0, and 1.5% L-Arginine, respectively. *In-ovo* feeding of L-Arginine were injected into albumen on day 10 of incubation period using automatic syringe in the narrow end side of egg by inserting needle through a small hole at 10 mm depth. After hatching, all day old chicks were placed in floor pens (1 x 0.5 x 0.5 m) accordance with the previous egg groups. The results showed that *in-ovo* feeding of L-Arginine increased weight and circumference of the embryo, but did not affect the length of embryo. *In-ovo* feeding of L-Arginine resulted in a higher body weight gain and a lower feed conversion even though feed intake was not significantly different compared to the control groups. The growth rate performance up to 6 weeks rearing increased significantly by increasing L-Arginine administration to 1.0%. It can be concluded that embryo development and post-hatch performances of kampung chicken were markedly increased by *in-ovo* feeding of L-arginine.

*Key words: embryo, in-ovo feeding, amino acid, productivity, kampung chicken*

### ABSTRAK

Penelitian ini bertujuan untuk mengetahui perkembangan embrio, performa dan tingkat pertumbuhan ayam kampung setelah menetas dengan perlakuan *in-ovo feeding* menggunakan L-Arginine. Sebanyak 135 butir telur ayam kampung fertil (bobot 42-43 g) dimasukkan ke dalam mesin tetas semi-otomatis dan dibagi menjadi 5 kelompok perlakuan 3 ulangan. Perlakuan pertama ialah tanpa injeksi (kontrol negatif); perlakuan ke-2 diinjeksi dengan saline 0,9% (kontrol positif); perlakuan ke-3, ke-4, dan ke-5 masing-masing diinjeksi dengan L-Arginine 0,5%; 1,0%; dan 1,5%. *In-ovo feeding* dilakukan pada hari ke-10 inkubasi dengan injeksi ke dalam albumen menggunakan syringe otomatis dengan memasukkan jarum melalui lubang kecil dengan kedalaman 10 mm. Setelah menetas, semua *day old chick* dipindahkan ke dalam pen (1 x 0,5 x 0,5 m) dengan alas litter sesuai dengan perlakuan telur sebelumnya. Hasil penelitian menunjukkan bahwa *in-ovo feeding L-Arginine* dapat meningkatkan bobot dan lingkar dada embrio, tapi tidak berpengaruh terhadap panjang embrio. *In-ovo feeding L-Arginine* menghasilkan pertambahan bobot badan yang lebih tinggi dan konversi pakan yang lebih rendah, serta konsumsi pakan yang tidak berbeda dibandingkan dengan kontrol. Performa tingkat pertumbuhan pada pemeliharaan di atas 6 minggu nyata meningkat dengan meningkatnya pemberian L-Arginine sampai 1,0%. Dapat disimpulkan bahwa perkembangan embrio dan performa ayam kampung setelah menetas nyata meningkat dengan *in-ovo feeding L-Arginine*.

*Kata kunci: embrio, in-ovo feeding, asam amino, produktivitas, ayam kampung*

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## INTRODUCTION

Kampung chicken is one of native chicken breeds in Indonesia. It is mostly found in rural areas reared in conventional, semi intensive and full intensive systems. Compared with commercial breed, kampung chicken in their original habitat showed a better adaptability and disease resistance (Sulistyoningsih *et al.*, 2013; Azahan *et al.*, 2014; Tamzil *et al.*, 2015), but lower performance as an animal-protein producer (meat and egg).

Muscle mass is an important indicator in determining body weight. Increased muscle mass has a very close correlation with the higher final body weight (Fernandes *et al.*, 2009; Fouad *et al.*, 2012). On the optimum environmental conditions such as nutrition, management, and climate, the number of muscle cells will be the determinant of muscle mass. Grodzik *et al.* (2013) reported that more muscle number is formed in the embryonic phase, and then more muscle cells will hypertrophy. These conditions will have an impact on the increasing of growth rate, so higher body weight can be achieved in a short time.

Change in the number of muscle cells in poultry is only occurred during the incubation period (embryonic development) (Guo-song *et al.*, 2012; Grodzik *et al.*, 2013). The number of muscle cells in this period will not be changed and only experienced magnification (hypertrophy) after passing through the embryonic phase. The level of growth hormone in the embryonic phase determines the activity of the cell proliferation (Fernandes *et al.*, 2009; De Carvalho *et al.*, 2012). Manipulation of growth hormone levels can be done by increasing the availability of nutrients for embryos, especially protein (Coskun *et al.*, 2014). However, the chicken embryo development occurs outside the body and nutritional needs of embryos have been determined in the process of egg formation, so we need the right method to add exogenous nutrients into the egg.

*In-ovo* feeding is a technique of adding exogenous nutrition into eggs in the incubation period. *In-ovo* feeding was reported to increase the performance of chickens after hatching (Daneshyar *et al.*, 2010; Kornasio *et al.*, 2011; Chen *et al.*, 2013; Shafey *et al.*, 2014; Al-Shamery & Al-Suhaib, 2015). This condition is believed to occur because of the optimal development of the embryo during embryogenesis.

L-Arginine is an amino acid that is classified as a semi-essential amino acid (Al-Daraji *et al.*, 2012). Foye (2005) reported that the administration of L-Arginine in the embryonic phase could increase the growth rate of turkey after hatching, while Murakami *et al.* (2012) suggested that L-Arginine was an important stimulator of growth hormone release. Another study report that L-Arginine can increase muscle mass (Fernandes *et al.*, 2009). In addition, L-Arginine is also a basic amino acid which has a major role as a stimulator of other amino acids like proline, ornithine, glutamine, and creatine (Al-Daraji *et al.*, 2012); as well as compounds that play important roles in the physiological activities of chicken such as nitric oxide, polyamines, and dimethylarginine-sa (Fouad *et al.*, 2012).

Administration of L-Arginine at a dose of 0.7% in turkeys (Foye *et al.*, 2006) and 1.0% in quail (Al-Daraji *et al.*, 2012) by *in-ovo* feeding could increase body weight and post-hatch performance. The present study was conducted to evaluate the effect of *in-ovo* feeding of L-Arginine on embryo development and post-hatch performance of kampung chicken.

## MATERIALS AND METHODS

This research used 135 fertile eggs with an average weight range of 42-43 g which were obtained from 50 weeks of age hens kept in an intensive rearing system. All eggs were placed into a semi-automatic incubator with the temperature of 37.5-38.5 °C and relative humidity of ±65%. The experimental eggs were arranged in a completely randomized design (CRD) with 5 treatments and 3 replications. Each replication consisted of nine eggs. From day 3 until 17 of the incubation period, the eggs were turned 3 times a day (at 07:00, 15:00, and 22:00). On day 7 of the incubation period, the eggs were checked for fertility. The infertile eggs were then replaced with fresh prepared fertile eggs.

*In-ovo* feeding was applied according to the design of 5 treatment groups. The first group was without injection (negative control), the 2<sup>nd</sup> group was injected with saline solution of 0.9% (positive control), the 3<sup>rd</sup> group was injected with 0.5% L-arginine (0.5 g per 100 mL saline m/v), the 4<sup>th</sup> group was injected with 1.0% L-arginine (1.0 g per 100 mL saline m/v), and the 5<sup>th</sup> group was injected with 1.5% L-arginine (1.5 g per 100 mL saline m/v). The material was injected into albumen (0.5 mL/egg) on day 10 of the incubation period. Before being injected, the broad end of eggs was on the top position. The injection was carried out using an automatic syringe into the narrow end of the egg with the depth of 10 mm of the needle length. The injected area was cleaned with alcohol and covered using paraffin and nail paint. Then all eggs were returned back into the incubator.

After hatching, all day old chick (DOC) were placed in arranged floor pens (length x width x height/1 x 0.5 x 0.5 m) in accordance with the treatment arrangement. Each pen consists of 3 unsexed chickens. Each pen was equipped with 60 watt light bulb, a feeder, and a drinker. Feed (Table 1) and drinking water were provided *ad libitum*. Vitamin was given *via* the drinking water at the

Table 1. Nutrient content of feed (Crumble BP 11)

Nutrient	(%)*
Water	13.0
Protein	21.0-23.0
Fat	5.0
Fiber	5.0
Ash	7.0
Calcium	0.9
Phosphors	0.6

\* Note: Analysis by PT. Charoen Pokphand Indonesia.

ages of 1, 2, 3, 7, 8, and 9 d, while antibiotics were given at the age of 4, 5, 6, 15, 16, and 17 d. At the age of 4<sup>th</sup> day, the vaccination (ND live) was administered *via* eye drops.

The observation of embryo development begins with the breaking of eggs on day 18 of the incubation period (3 eggs per treatment in each replication). Variables measured were body weight, embryo length, and pectoral circumference of the embryo that were measured after the embryo was separated from the other components such as albumen, allantoin, amnion, and yolk sac. Post-hatch performance was evaluated by measuring variables of feed intake, body weight gain, and feed conversion efficiency. The growth rate was obtained by measuring the body weight of chicken at the ages of 1, 2, 3, 4, 5, and 6 weeks. Data were analyzed following the analysis of variance. If the treatment showed significant effect it was then continued to be tested with Duncan's multiple range test (Gaspersz, 1991).

## RESULTS

*In-ovo* feeding of L-Arginine into kampung chicken eggs on day 10<sup>th</sup> of incubation period significantly af-

fected ( $P < 0.05$ ) embryo weight, pectoral circumference, body weight gain, feed conversion, and weekly body weight, but did not affect embryo length, and feed intake (Tables 2, 3, and 4). The results showed that *in-ovo* feeding of L-Arginine increased embryo weight, pectoral circumference, body weight gain, and feed conversion, and weekly body weight compared to control group. Negative and positive control treatments showed no significant effect on the variables measured.

## DISCUSSION

*In-ovo* feeding of L-Arginine resulted in a higher embryo weight. The increasing of embryo weight may be due to the increase in muscle mass as it is affected by administration of *in-ovo* feeding. L-Arginine was reported as one of the amino acids that could stimulate muscle development (Zhao *et al.*, 2011; Fu *et al.*, 2016). Based on this report, we assumed that *in-ovo* feeding of L-Arginine increased L-Arginine content in the muscle and resulting the increased muscle mass and heavy embryo.

L-Arginine has been reported to increase growth hormone (Sahin *et al.*, 2006; McKnight *et al.*,

Table 2. Embryo development of *in-ovo* feeding of L-Arginine kampung chickens on day 18 of incubation period

Variables	<i>In-ovo</i> feeding				
	Negative control	Positive control	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Embryo weight (g)	14.13±0.35 <sup>a</sup>	15.03±2.75 <sup>a</sup>	19.56±0.43 <sup>b</sup>	18.58±2.49 <sup>b</sup>	18.78±0.87 <sup>b</sup>
Embryo length (cm)	7.42±0.02	7.65±0.45	8.22±0.15	8.23±0.38	7.83±0.54
Pectoral circumference (cm)	5.00±0.30 <sup>a</sup>	5.20±0.20 <sup>ab</sup>	5.64±0.05 <sup>c</sup>	5.53±0.09 <sup>bc</sup>	5.52±0.06 <sup>bc</sup>

Note: Means in the same rows with different superscripts differ significantly ( $P < 0.05$ ).

Table 3. Post-hatch performance of *in-ovo* feeding of L-Arginine kampung chickens at the age of 42 days

Variables	<i>In-ovo</i> feeding				
	Negative control	Positive control	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Feed intake (g/bird)	1127.16±37.40	1118.73± 7.80	1140.98±14.20	1137.52± 2.10	1131.80± 7.20
Body weight gain (g/bird)	515.46±16.40 <sup>a</sup>	511.47±17.20 <sup>a</sup>	565.15±15.50 <sup>b</sup>	617.44±11.50 <sup>c</sup>	604.51±12.60 <sup>c</sup>
Feed conversion	2.19± 0.08 <sup>c</sup>	2.17± 0.07 <sup>c</sup>	2.02± 0.08 <sup>b</sup>	1.84± 0.04 <sup>a</sup>	1.87± 0.02 <sup>a</sup>

Note: Means in the same rows with different superscripts differ significantly ( $P < 0.05$ ).

Table 4. The average weekly body weight of kampung chicken post *in-ovo* feeding of L-Arginine (g/bird)

Week	<i>In-ovo</i> feeding				
	Negative control	Positive control	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
I	66.15± 0.42 <sup>a</sup>	64.87± 0.89 <sup>a</sup>	70.40± 1.31 <sup>b</sup>	70.33± 0.97 <sup>b</sup>	71.00± 0.08 <sup>b</sup>
II	124.48± 4.52 <sup>ab</sup>	122.72± 4.73 <sup>a</sup>	131.27± 5.12 <sup>bc</sup>	134.52± 4.69 <sup>c</sup>	135.18± 2.56 <sup>c</sup>
III	201.75± 2.64 <sup>a</sup>	200.00± 3.42 <sup>a</sup>	220.40± 6.17 <sup>b</sup>	215.50± 8.51 <sup>b</sup>	221.75± 6.24 <sup>b</sup>
IV	286.86± 3.52 <sup>a</sup>	290.20± 3.96 <sup>a</sup>	310.45± 12.98 <sup>b</sup>	322.05± 7.35 <sup>b</sup>	315.32±11.20 <sup>b</sup>
V	396.88± 9.66 <sup>a</sup>	405.91±13.32 <sup>a</sup>	427.53± 5.52 <sup>b</sup>	459.48± 9.86 <sup>c</sup>	450.65±10.35 <sup>c</sup>
VI	542.14±16.34 <sup>a</sup>	542.13±17.40 <sup>a</sup>	594.21±14.82 <sup>b</sup>	647.27±12.07 <sup>c</sup>	633.73±12.39 <sup>c</sup>

Note: Means in the same rows with different superscripts differ significantly ( $P < 0.05$ ).

2010; Murakami *et al.*, 2012) and insulin (Foye, 2005; Fernandes *et al.*, 2009; Silva *et al.*, 2012). L-Arginine could increase growth hormone by GH/IGF signaling pathway. The increase in growth hormone caused the increased organogenesis activity such as proliferation, differentiation, and maturation of cells (Deprem & Gulmez, 2007; Daneshyar *et al.*, 2010).

Averages feed intakes of all treatments were not significantly different. This may occur due to the type and form of feed used in all treatments were similar, therefore the feed palatability was not different. This level of palatability proved that the feed intake did not differ.

The value of feed conversion showed a decrease with higher concentrations of L-arginine. Injection of L-arginine about 1.0% and 1.5% resulted in lower feed conversion ratio than the other treatments. Group of chicken with a lower feed conversion ratio showed an improvement of digestive tract performance as might be influenced by the L-Arginine administration. Therefore, the absorption of nutrients was more effective and impact on the derivation of feed conversion. Similar results were also reported by Foye *et al.* (2009) on turkeys, Al-Daraji *et al.* (2012) on quail, and De Carvalho *et al.* (2012) on laying hens.

As reported by Fouad *et al.* (2012), L-Arginine stimulated cell proliferation of small intestine. Increasing L-arginine levels in the embryonic phase would lead to an increase in the number of small intestine cells (Lin *et al.*, 2011). Tako *et al.* (2004) and Cheled-Shoval *et al.* (2011) explained that an increase in the size of the villi and crypts were the result of the increase in the number of cells because proliferation activities of the small intestine were high at the beginning of growth. Increasing the size of the villi and crypts will lead to an increase in the surface area of the small intestine (Foye, 2005). This increase caused an increase in nutrient absorption. While Smirnov *et al.* (2006) and Ospina-Rojas *et al.* (2013) explained that the increasing of the surface area of small intestine was the result of the increased mucin gene expression by goblet cells.

Although the injection of L-Arginine resulted in a higher body weight, at the early week of rearing period, different concentrations of L-Arginine that were injected did not affect body weight. However, at the late weeks of the rearing period, chicken injected with the higher dose of L-Arginine had the higher body weight. This condition indicated that the higher concentration of L-Arginine injected resulted the higher number of muscle cells.

Changes in the number of muscle cells is actually not directly influenced by L-Arginine. L-Arginine is a stimulator of IGFs which will work as a stimulator of proliferation activity of muscle cells in the embryonic phase. Therefore, L-Arginine stimulates the production of IGFs (IGF-1 and IGF-2) through the mechanism of GH/IGFs axis (Fernandes *et al.*, 2009; Hazim *et al.*, 2012). The increasing levels of IGFs is caused by the increased sensitivity of hormone receptors in the mechanism of GH/IGFs axis by L-Arginine (Foye *et al.*, 2009; De Carvalho *et al.*, 2012). While Fouad *et al.* (2012) reported that Arginine was an essential raw material in

the chemical structure of IGFs. Increasing levels of IGFs on embryonic phase would inhibit the activity of myostatin and activate the MAP kinase pathway (Kollias & McDermott, 2008). MAP kinase pathway activity causes cell proliferation process for a long time.

## CONCLUSION

*In-ovo* feeding of L-Arginine increased embryo development of kampung chicken. Injection of 1.0% concentration of L-Arginine *in-ovo* increased post-hatch performances and growth rate.

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