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Embryo Development and Chick Performance of Local Chicken Following *In-ovo* Injection of L-Arginine Into Local Chicken Eggs

Muhammad Taufik[1], Urfiana Sara[1], Muhammad Yunus[1], Fatma Maruddin[2], and Muhammad Azhar[1]*

[1]Department of Animal Agriculture, Politeknik Pembangunan Pertanian Gowa, Gowa, 92171, Indonesia
[2]Department of Animal Agriculture, Hasanuddin University, Makassar, 90245, Indonesia

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* Corresponding author:
E-mail:
muhammadazhar030390@gmail.com

ABSTRACT

This study aims to determine the effect of *in-ovo* injection of L-arginine into local chicken eggs on embryo development and chick performance of local chicken. A total of 160 eggs were incubated using semi-automatic incubator with temperature of 37-38ºC and relative humidity of 55-65%. The injected L-arginine solution has a concentration of 0.5% (m/v). *In-ovo* feeding treatment was divided into 4 groups, the first treatment was without injection (control), the second treatment was the injection of 0.2 mL L-arginine solution 0.5% (m/v)/egg, the third treatment was the injection of 0.4 mL L-arginine solution. 0.5% (m/v)/egg, and fourth treatment was the injection of L-arginine solution 0.6 mL 0.5% (m/v)/egg. The results showed that embryo mortality and hatchability were lower with L-arginine injection treatment than without injection. All treatments showed no effect on extraembryonic fluid absorption, but there was an increase in embryo weight in the injection treatment 0.2, 0.4, 0.6 mL L-Arginine 0.5% /eggs 2,355 g, 2,577 g, 2,705, respectively. In conclusion, an *in-ovo* injection of L-arginine in local chicken eggs has a good effect (the beneficial effect) on embryo death and embryo performance. Injection of 0.4 ml L-Arginine 0.5% /eggs improved hatchability and Newly Hatched Chick Weight (NHCW).

Keywords: Chick quality, Embryo performance, Hatchability, *In-ovo* Injection, L-arginine

Introduction

Local chickens in Indonesia have the characteristics of slow growth and low performance. Post-hatching chicken performance was reported by Abousaad *et al.* (2017a), Yu *et al.* (2018b), Yu *et al.* (2018b), Araújo *et al.* (2020), and Givisiez *et al.* (2020) that highly dependent on the growth and development of the embryo. Stimulants to increase the growth and development of the embryo can be done by providing additional nutrition to the embryo. In birds, the amount of nutrients needed for the chicken embryo is limited in the egg. Kim *et al.* (2023) implies these nutrients cannot be increased or decreased naturally depending on the expression of genes that control egg size.

Under normal conditions, larger eggs (broilers) will produce larger embryos than small eggs (local chickens). Reports of Zhao *et al.* (2017a), Zhao *et al.* (2017b) and Kim *et al.* (2020) in broilers, Rahardja *et al.* (2018) and (Rahardja *et al.* 2019) in local chickens, Syahruddin *et al.* (2018) in local ducks, Zhang *et al.* (2018) and Zhu *et al.* (2019a) in the domestic pigeon, indicating that the correlation occurs because larger eggs have more nutrients for embryo development than smaller eggs. This condition will also have an impact on the early growth performance of chickens after hatching in which larger eggs and embryos were better than the small one. Production performance (egg size, hatching weight, body weight, etc.) of local chickens can be improved by crossbreeding with better performance of chicken (Padhi, 2016; Soliman *et al.,* 2020). However, the crossbreed will have an impact on the genetic degradation of local chicken germplasm. Therefore, such method is needed to increase the nutritional status of local chicken eggs without causing genetic degradation of local chicken germplasm.

In-ovo feeding is a technique used to add exogenous nutrients to poultry eggs during the incubation period. The results of previous studies have shown that *in-ovo* feeding can increase embryo growth and development, chick quality, and performance after hatching (Abousaad *et al.,* 2017b; Zhang *et al.,* 2018; Zhu *et al.,* 2019a; Araújo *et al.,* 2019 and Araújo *et al.,* 2020). However, *in-ovo* feeding was also reported Azhar *et al.* (2016) and Rahardja *et al.* (2018) to have negative impacts such as embryo death and decreased hatchability. Exogenous nutrients are added to eggs through the injection process. The types of compounds used have been widely reported

including amino acids (Zhao *et al.,* 2017a; Zhu *et al.,* 2019b and Wang *et al.,* 2020), carbohydrate derivatives (Slawinska *et al.,* 2020; Zhang *et al.,* 2020), vitamins (Zhu *et al.,* 2020; Fatemi *et al.,* 2021a and Fatemi, *et al.,* 2021b), minerals (Peebles *et al.*, 2021), and probiotics (Li *et al.,* 2021; Castañeda *et al.,* 2021).

L-arginine is one of the amino acids that is widely used as an ingredient in *in-ovo* feeding. Fouad *et al.* (2012); Yu *et al.* (2018b) and Yu *et al.* (2018a) each reported that L-arginine has an important role in embryonic growth and development processes such as muscle cell hyperplasia, stimulates growth hormone synthesis, and a precursor to the formation of other amino acids. Other research showed that the addition of 1.0% L-arginine increased the embryo weight by 5.43 g (Azhar *et al.*, 2016). Tong *et al.* (2013) also reported that injection of L-arginine before incubation could increase hatchability and daily gain of body weight. While Fouad *et al.* (2012) reported that the administration of L-arginine in excess can cause uncontrolled cell division.

Implementation of *in-ovo* feeding using L-arginine in local poultry eggs has been reported by researchers by treating different concentrations of L-arginine solution and injection time. Injection of L-arginine 1% (m/v) into broiler eggs on day 17.5 of incubation has been reported by (Yu *et al.*, 2018b), and injection of L-arginine 0.5% (m/v), 1% (m/v), and 1.5% (m/v) in local chicken eggs on the 10th day of incubation was reported by (Azhar *et al.*, 2016). While the injection of 1.5% (m/v) L-arginine on local duck eggs on the 8th day of incubation was reported by (Syahruddin *et al.*, 2018). Based on these previous studies, the higher the concentration of L-arginine decreases the hatchability. In this study, a different amount of injection will be treated using a concentration of L-arginine 0.5% (m/v) into local chicken eggs. This study aims to determine the effect of *in-ovo* injection of L-arginine on age at embryo death, embryo performance, and chick performance of local chicken.

Materials and Methods

Bird and egg incubation

The incubation process and *in-ovo* injection were carried out at the Integrated Laboratory of the Department of Animal Agriculture, Politeknik Pembangunan Pertanian Gowa. A total of 160 eggs with 36-40 g of weight per egg were obtained from local hens type Kampung Unggul Balitbangtan (KUB) aged 9-12 months in a local commercial chicken nursery in Bantaeng Regency, South Sulawesi. Prior to incubation, eggs were disinfected using 40% alcohol and incubated in a semi-automatic incubator with a temperature range between 37-38^{o}C, and a relative humidity of 55-65%. Eggs were turned 3 times a day at 07.00 AM, 13.00 PM. 17.00 PM during period of day 3 up to day 18 of incubation. On the day-8 of incubation, egg candling was carried out, and the eggs that were not fertile were removed from the incubator. Fertile eggs were randomly divided into 4 treatment groups.

In-ovo injection

Injection of L-arginine into eggs was carried out on day-9 of incubation. L-arginine was provided by a medical supply store in Makassar, South Sulawesi. The injected L-arginine solution had a concentration of 0.5% (m/v) (0.5 g L-arginine/100 mL physiological NaCl 0.9%) according to recommendation both of (Azhar *et al.*, 2016) and (Syahruddin *et al.*, 2018) in local poultry eggs. *In-ovo* injection treatments was divided into 4 groups, the first treatment was without injection (control), the second treatment was injection of 0.2 ml L-arginine solution/egg, the third treatment was injection of 0.4 mL L-arginine solution/egg, and forth treatment was injection of 0.6 mL L-arginine solution egg. The injection procedures were carried out according to method described by (Azhar *et al.*, 2016). In detail, deposition of *in-ovo* solution in the albumen area at a depth of 10 mm from the eggshell, using an automatic injector with a needle size of 26G. The hole in the eggshell was then sealed tightly using silicone. All injection processes were carried out under aseptic conditions.

Embryo observation

On the day-17 of incubation, each egg selected as a sample taken from the incubator was weighed using an analytical balance before embryo observation. The eggs were then broken and separated based on the egg components (shell, yolk, albumen, and embryo). Each egg component was weighed after being placed in a petri dish. The embryos that have been separated from the egg components were measured for embryo length, wing length, and leg length using a caliper. While the chest circumference was measured using a measuring tape.

Age at embryo died and chick performances

All chicks hatched on day-21 of incubation were counted and weighed. Unhatched eggs are cracked to determine the age at which the embryo died. The age of embryonic death was determined based on the morphological characteristics of the embryo from the observations (Tong *et al.*, 2013). Hatchability was calculated based on method of (Zhu *et al.*, 2019a), namely number of hatchling squabs/the number of fertilized eggs x 100.

Statistics analysis

The data obtained were analyzed for variance based on Completely Randomized Design (CRD). If the treatment shows a significant effect, it is continued with Duncan's test using SPSS 16.0 software. Data analysis results are presented in the form of mean ± standard deviation based on each treatment.

Table 1. Embryo mortality and age at embryo died following *in-ovo* injection of L-Arginine

Parameters	*In-ovo*			
	Control (without injection)	Injection 0.2 mL L-arginine 0.5% /egg	Injection 0.4 mL L-arginine 0.5% /egg	Injection 0.6 mL L-arginine 0.5% /egg
Embryonic mortality (% of fertile eggs)				
- Early *	3.3±5.7	3.6±3.2	1.6±2.8	1.6±2.8
- Middle *	25.0±7.0[b]	8.6±2.3[a]	14.0±6.1[a]	9.0±3.4[a]
- Late *	21.3±1.1[b]	8.6±3.2[a]	14.0±2.6[a]	8.3±5.7[a]
Age embryo died (day)	20.0±1.0	19.5±1.5	19.5±0.5	19.0±1.0

* early (0-7 day of incubation), middle (8-14 day of incubation), and late (15-21 day of incubation).
[a,b] Means in the same rows with different superscripts differ significantly (P<0.05).

Results and Discussion

Age embryo died

The effect of *in-ovo* injection using L-Arginine on the age of embryo death is presented in Table 1. Middle embryo dead and late embryo dead were significantly (P<0.05) higher in the treatment without injection compared to the other treatments. Meanwhile, early embryo dead and age embryo dead did not show a significant effect (P>0.05) with *in-ovo* injection using L-Arginine.

Embryo died after *in-ovo* injection can occur due to microbial infection (Azhar *et al.*, 2016), damage to the embryo sac (Araújo *et al.*, 2020), and changes in egg fluid concentration (Rahardja *et al.*, 2018). In this research, the percentage of embryonic mortality was highest in the treatment without injection in the middle and late stages. These results indicated that the injection method did not have a significant impact on embryonic mortality. Zhao *et al.* (2017a) and Han *et al.* (2020) reported that, embryonic death occurred in the middle and late stages was mainly caused by excessive of temperature and humidity, microbial infection, and low egg nutrition. The report indicated that the high embryo mortality in the non-injection treatment occurred due to the low availability of nutrients for embryonic development. While in the *in-ovo* L-Arginine injection, the mortality rate was low because of the increased of nutrients availability.

L-Arginine is reported Miri *et al.* (2022) and Yu *et al.* (2018a) have an important role in the body's biological and physiological processes, such as the raw material for hormone formation. L-Arginine will stimulate IGF-1 production through (Foye *et al.*, 2007). The increase in IGF-1 causes an increase in organogenesis activities such as proliferation, differentiation, and maturation. Unlike L-Arginine, ovo feeding uses other amino acids to increase the availability of energy for the embryo (Zhang *et al.*, 2018; Firman *et al.*, 2023; Han *et al.*, 2020).

Embryo performance

The performance of 16-day-old embryos following *in-ovo* injection of L-Arginine can be seen in Table 2. Egg weight loss was significantly (P<0.05) lower in the injection treatment of 0.6 ml L-Arginine 0.5%/egg. While the embryo weight and the ratio of embryo weight: egg weight were significantly (P<0.05) heavier in the egg with *in-ovo* injection treatment compared to the control group.

Egg weight will decrease in weight as incubation time increased. Omede *et al.* (2017), Givisiez *et al.* (2020), and Fatemi *et al.* (2021a) explained that during the incubation period the embryo will consume all the nutrients in the egg. This process will lead to lower egg weight at the end of the incubation period. Injection of 0.6 mL L-Arginine 0.5%/egg resulted in the highest egg weight loss. These results indicated that L-Arginine at certain concentrations can increase the metabolic activity of the embryo. A similar report was also submitted (Foye *et al.,* 2006) that *in-ovo* feeding L-arginine was able to increase the metabolic activity of the embryo. Increased metabolic activity during incubation through (mTOR) signal pathway messenger RNA (mRNA) expressions (Yu *et al.*, 2018a).

The results showed that there was no change in extraembryonic fluid weight in all treatments, but there was an increase in embryo weight with *In-ovo* L-arginine injection treatment. This finding agrees with report (Azhar *et al.*, 2016) and differs from (Yu *et al.*, 2018a). These results indicate that *in-ovo* injection of L-arginine in local chicken eggs functions to stimulate growth hormone and metabolism or as an additional energy source for embryonic development.

In-ovo injection L-Arginine increased the weight of the embryo. Previous research reports suggested that embryo weight is strongly influenced by egg weight (Syahruddin *et al.*, 2018); Araújo *et al.*, 2019). The results showed an increase in embryo weight followed by an increase in the ratio of embryo weight: egg weight in the L-Arginine injection treatment. These results confirmed that the increase in embryo weight was caused by L-Arginine injection and not due to egg weight. During incubation, L-Arginine was reported to stimulate the production of growth hormone (Murakami *et al.*, 2012; Subraniyan *et al.*, 2019), insulin hormone (Foye *et al.*, 2007; Silva *et al.*, 2012; Yu *et al.*, 2018b), and increase the energy availability of the embryo (Fouad *et al.*, 2012; Yu *et al.*, 2018a). The increase in growth hormone causes an increase in organogenesis activities such as proliferation, differentiation, and maturation (Daneshyar *et al.*, 2010). These activities may be the cause of the increased organ mass, resulting in a heavy embryo. Increased insulin with L-Arginine injection is also an important factor in increasing organ mass. Foye *et al.* (2006) explained that increasing insulin levels in embryos with *In-ovo* feeding L-Arginine will have an impact on increasing liver and muscle glycogen. Glycogen

Table 2. Performance of 16 days old embryos following *in-ovo* injection of L-Arginine

Parameters	*In-ovo*			
	Control (without injection)	Injection 0.2 mLL-arginine 0.5% /egg	Injection 0.4 mL L-arginine 0.5% /egg	Injection 0.6 mL L-arginine 0.5% /egg
Egg weight loss (g)	5.485±2.373[a]	3.027±0.624 [a]	2.642±0.716 [a]	2.742±0.655[b]
Eggshell weight (g)	4.335±0.488	4.367±0.317	4.285±0.236	4.887±0.540
Albumen weight (g)	2.990±1.426	3.800±2.008	3.097±0.859	4.712±1.429
Yolk weight (g)	13.925±3.517	11.895±1.740	10.227±1.741	13.632±2.562
Embryo weight (g)	12.435±0.896[a]	14.790±1.332[b]	15.012±0.677[b]	15.140±1.432[b]
Embryo weight/egg Weight ratio (%)	30.509±2.637[a]	38.127±1.482[b]	41.128±3.105[b]	37.711±2.119[b]
Leg length (cm)	3.035±0.267	3.577±0.325	3.145±0.203	3.000±0.646
Bust (cm)	4.457±0.111	5.397±0.541	5.185±0.834	5.047±0.044
Wing length (cm)	2.580±0.375	2.927±0.164	3.005±0.285	2.550±0.542
Embryo length (cm)	6.670±0.811	8.185±0.200	7.370±0.333	7.140±1.507

[a,b] Means in the same rows with different superscripts differ significantly (P<0.05).

Table 3. Chick quality by *in-ovo* injection of L-Arginine

Parameters	*In-ovo*			
	Control (without injection)	Injection 0.2 mL L-arginine 0.5% /egg	Injection 0.4 mL L-arginine 0.5% /egg	Injection 0.6 mL L-arginine 0.5% /egg
Egg weight (g)	40.830±1.850	38.800±3.260	36.577±1.577	40.295±3.859
Fertility (%)	93.25±4.71	96.75±4.71	96.75±2.36	96.75±2.36
Hatchability (%)	56.75±4.71[a]	83.25±2.36[c]	73.25±2.36[b]	83.25±2.36[c]
NHCW (g)	25.000±1.688[a]	27.086±1.080[ab]	28.126±1.512[b]	27.557±1.078[b]
NHCW/egg weight Ratio (%)	61.41±6.25	70.17±6.28	76.93±3.92	69.05±9.41
Embryo growth to NHCW (g)	12.565±1.102	12.296±1.392	13.114±2.098	12.417±1.725

NHCW: Newly hatched chick weight.
[a,b,c] Means in the same rows with different superscripts differ significantly (P<0.05).

has been reported Kornasio *et al.* (2011) and Shafey *et al.* (2012) to alter muscle mass.

Chick quality

The chick quality with *in-ovo* injection using L-Arginine is shown in Table 3. Hatchability significantly (P<0.05) increased with *in-ovo* injection treatment using L-Arginine compared to treatment without injection. Injection of 0.2 ml and 0.6 ml of L-Arginine 0.5%/egg produced the best hatchability. *In-ovo* injection using L-Arginine significantly (P<0.05) increased hatching weight.

Hatchability of injection treatment 0.2, 0.4, 0.6 mL L-Arginine 0.5% /eggs increased compared to treatment without injection, respectively 26.78%, 16.5%, 26.5%. The increase in hatchability occurred because the mortality of embryos in the injection treatment was lower than without injection. The results of the study Al-Daraji *et al.* (2012) and Rahardja *et al.* (2018) showed that *In-ovo* feeding increased hatchability. However, a different report was shown by Zhang *et al.* (2018), Zhu *et al.* (2019a), and Araújo *et al.* (2020) that *In-ovo* feeding decreased hatchability. Meanwhile Silva *et al.* (2012) and Slawinska *et al.* (2020) reported that there was no effect of *In-ovo* feeding on hatchability.

The results showed an increase in NHCW in the injection treatment of 0.4 mL and 0.6 mL L-Arginine 0.5%/eggs. The NHCW value was the impact of the L-Arginine injection treatment which resulted in heavier embryos than the treatment without injection. The same results were also reported Syahruddin *et al.* (2018), Yu *et al.* (2018a), and Subramaniyan *et al.* (2019), that *in-ovo* L-arginine can increase chick weight. In this study also found the value of embryo growth to NHWC no difference between all treatments. This value illustrates that the possibility of the injected L-arginine has been absorbed by the embryo more quickly, so that there is no longer any effect of L-arginine on the growth and development of the embryo.

Conclusions

The results clearly showed that *in-ovo* injection of L-arginine in local chicken eggs has a beneficial effect on embryo death and embryo performance. Injection of 0.4 mL L-Arginine 0.5% /eggs improved Hatchability and NHCW (Newly Hatched Chick Weight).

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