Effect of *In-ovo* Injection of L-Arginine on Hatchability, Chick Quality, Performances and Muscle Histology of Native Chicken

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ABSTRAK

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Penelitian ini bertujuan untuk mengetahui pengaruh *in-ovo* injeksi menggunakan *L-arginine* terhadap daya tetas, kualitas *day old chick*, performan dan histologi otot ayam lokal. *In-ovo injeksi* dilakukan pada hari ke-10. Sebanyak 375 butir telur fertil dengan ranges bobot rata-rata 39-43 g dikelompokkan menjadi 5 perlakuan. Perlakuan pertama tanpa injeksi (kontrol negatif), Perlakuan ke-2 injeksi larutan NaCl fisiologis 0,9% (kontrol positif), Perlakuan ke-3 injeksi larutan *L-arginine* 0,5 g per 100 ml NaCl fisiologis 0,9% (0,5%, m/v), Perlakuan ke-4 injeksi larutan *L-arginine* 1,0 g per 100 ml NaCl fisiologis 0,9% (1,0%, m/v), dan Perlakuan ke-5 injeksi larutan *L-arginine* 1,5 g per 100 ml NaCl fisiologis 0,9% (1,5%, m/v). Injeksi dilakukan pada area runcing telur dengan posisi vertikal (runcing dibawah, tumpul diatas). Injeksi dilakukan dengan kedalaman 10 mm dari cangkang telur menggunakan injektor otomatis. Hasil penelitian menunjukkan bahwa semua perlakuan menghasilkan daya tetas yang sama. *In-ovo* injeksi *L-arginine* memberikan pengaruh yang menguntungkan terhadap kualitas anak ayam dan perfoma pasca tetas, konsentrasi larutan *L-arginine* yang digunakan tidak menyebabkan pengaruh negatif terhadap kematian embryo. *In-ovo* injeksi of 0,5% *L-arginine* meningkatkan bobot tetas, bobot badan mingguan, massa otot, dan ukuran *myofiber*.

Kata Kunci: *In-ovo*, *L-arginine*, Perfoman, *Myofiber*

ABSTRACT

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This study aimed to determine the effect of *in-ovo* injection of L-arginine on hatchability, chick quality, performances, and muscle histology of native chicken. *In-ovo* injection was carried out on the 10th day. A total of 375 fertile eggs with an average weight ranged 39-43 g were grouped into 5 treatments. The first treatment was without injection (negative control), the second treatment was injection of NaCl solution 0.9% (positive control), the third treatment was injection of L-arginine solution 0.5 g per 100 ml of NaCl 0.9% (0.5%, m/v) The fourth treatment was injection of 1.0 g L-arginine solution per 100 ml of NaCl 0.9% (1.0%, m/v), and the fifth treatment was injection of 1.5 g L-arginine solution per 100 ml of NaCl 0.9% (1.5%, m/v). The injection was carried out at the pointed area of the egg in a vertical position (pointed below, blunt above). The Injection was carried out with a depth of 10 mm from the eggshell using an automatic injector. The results showed that all treatments produced the same hatchability. *In-ovo* injection of L-arginine has a beneficial effect on chick quality and post-hatch performance, the concentration of L-arginine solution used did not cause embryo death. *In-ovo* injection of 0.5% L-arginine increased hatching weight, weekly body weight, muscle mass, and myofiber size.

Key Words: *In-ovo*, L-arginine, Performances, Myofiber

INTRODUCTION

In-ovo injection technology is a technique of adding nutritional substances directly into eggs during the incubation period. *In-ovo* feeding aims to stimulate the growth and development of the embryo and the performance of the lifelong effect after hatching. The results of previous studies showed that *in-ovo* injection can increase embryo weight and hatching weight (Zhao et al. 2017; Abousaad et al. 2017; Araújo et al. 2019), hatchability (Zhu et al. 2020; Oke et al. 2021), post-hatch performance (Zhu et al. 2019; Wang et al. 2020; Slawinska et al. 2020), and tissue histology (Zhao et al. 2017b; Fatemi et al. 2021). However, *in-ovo* injection technology has also been reported (Zhang et al. 2018; Araújo et al. 2020) to have a negative impact on embryo mortality. The report showed that *in-ovo* injection method can be a solution to increase the productivity of native chickens.

Embryo death caused by the *in-ovo* injection method can occur due to the concentration of the solution injected into the hatching eggs (Zhang et al.

2018; Kop-Bozbay and Ocak 2019). The *in-ovo* concentration of the solution that does not match the embryonic fluid causes differences in osmolarity. This condition causes embryonic fluid cannot be absorbed by the embryo and results in the death of the embryo. The results of research Omede et al. (2017) showed the osmolarity of the amniotic fluid from late-incubation (day 17) broiler eggs was 278.8 mmol/kg. Furthermore Vassallo et al. (2019) reported that differences in osmolarity would impair absorption of extraembryonic fluid nutrients.

L-arginine has been widely reported as an *in-ovo* substance that has an important role in stimulating embryo growth and development (Fouad et al. 2012; Yu et al. 2018; Omidi et al. 2020). L-arginine can stimulate IGF-1 which plays an important role in embryonic muscle cell division (Chen et al. 2013). It is assumed that muscles with a greater number of cells in the embryonic phase will grow and develop more rapidly after hatching. It was observed Subramaniyan et al. (2019) that L-arginine was also able to increase the activity of muscle cell division. In addition, Azhar et al (2016) also reported that *in-ovo* injection of L-arginine was able to improve post-hatch performance.

Investigation of the optimum concentration of L-arginine in a solution injected by the *in-ovo* method has become one of the goals of many types of research. The best L-arginine concentrations reported by previous researchers vary widely. 0.5% or 5 mg L-arginine per ml sterile water in the broiler (Omidi et al. 2020), 2% or 2 g L-arginine per 100 mL sterile distilled water in quail (Al-Daraji et al. 2012). 10 g L-arginine per 1 L solvent in the broiler (L.L. Yu et al. 2018b). L-arginine 100 g/µL/egg (Subramaniyan et al. 2019). 1.% L-arginine solution per broiler embryo (Gao et al. 2018). *In-ovo* injection of L-arginine has been carried out by Azhar et al (2016) on native chickens. This study refers to that research with refines the incubation management and more complex observation parameters. Reports on the concentration of L-arginine through *in-ovo* injection technology on native chickens are still very limited, so it is necessary to make observations to determine the effect of *in-ovo* injection of L-arginine on hatchability, chick quality, performances, and muscle histology of native chicken.

MATERIALS AND METHODS

Bird and incubation

The eggs used came from native chicken breeding in Pinrang Regency, South Sulawesi. Parent chickens from eggs have an age ranged of 55-70 weeks which were reared with an intensive system. Collected eggs have been stored for 2-4 days before incubation period. All incubation, *in-ovo* injection, and rearing activities were carried out at the Poultry Production Technology Laboratory, Hasanuddin University.

The incubator used has a semi-automatic type with separate setter and hatcher models. Before use, the incubator was sterilized with 40% formalin and 70% alcohol. The egg incubation process lasts for 21.5 days. The incubator from the first to the 17th day was maintained at a temperature of 37-38 ^{o}C with a humidity of 55-60%. The temperature from day 18 to 21 was raised to 38-39 ^{o}C with a humidity of 65-70%. Egg turning was done 3 times a day (7 am, 3 pm, and 11 pm) from the 4th to the 18th day. On the 7th day, the observation was carried out to determine the fertile eggs. Meanwhile, eggs that were not fertile or experience death of the embryo will be removed. The hatching vent was gradually opened on the 4th day of the incubation period and fully opened on the 7th day.

In-ovo procedure

In-ovo injection was carried out on the day tenth. A total of 375 fertile eggs with an average weight ranged of 39-43 g were grouped into 5 treatments and arranged according to a completely randomized design (CRD). The first treatment was without injection (negative control), the second treatment was an injection of NaCl solution 0.9% (positive control), the third treatment was an injection of L-arginine solution 0.5 g per 100 ml of NaCl 0.9% (0.5%, m/v), the fourth treatment was injection of 1.0 g L-arginine solution per 100 ml of NaCl 0.9% (1.0%, m/v), and the fifth treatment was injection of 1.5 g L-arginine solution per 100 ml of NaCl 0.9% (1.5%, m/v). The injection was carried out in the pointed area of the egg in a vertical position (pointed below, blunt above). The injection was carried out with a depth of 10 mm from the eggshell using an automatic injector with a needle size of 26 G x 0.5 (0.45 x 13 mm). The target of *in-ovo* solution deposition is albumen. Each egg was injected 0.5 ml of *in-ovo* injection solution. The hole in the eggshell after the injection process is sealed using silicone. All *in-ovo* injection processes were carried out under aseptic conditions.

Hatching and animal husbandry

On day 21.5 of incubation, all hatched chicks were weighed. Meanwhile, eggs that did not hatch were observed to determine the age of embryonic death based on the morphology of embryonic development (Tong et al. 2013) regarding the stages of development of chicken embryos during incubation. The calculation of hatchability is based on method Zhu et al. (2019), namely the number of chick hatch/number of fertilized eggs x 100.

After hatching, 45 DOCs were placed randomly based on each treatment on a pen measuring height x length x width (0.5 x 1 x 0.5 m). The pen has walls made of bamboo insulated and lined with sawdust litter with a thickness of 3 cm. Before use, the pens were sterilized with a disinfectant. Each pen was filled with 3 chicks, 1 male and 2 female. The rearing process was carried out based on commercial chicken management standards. The feed used was commercial feed (crumble BP 11) with nutritional content of 13% water content, 21-23% protein, 5% fat, 5% fiber, 7% ash, 0.9% calcium, and 0.6% phosphorus. Feed and drinking water were provided without restrictions.

Sample collection and muscle histology

Collection of muscle samples begins with slaughtering chickens at the age of 42 days, the slaughtering process is carried out in an Islamic way. Muscle samples collected were from 2 chickens (1 male and 1 female) per treatment unit. The muscle samples collected were right pectoralis major and pectoralis minor muscles which had been separated from other bones, organs, and muscles using a scalpel. The muscle samples obtained were immediately weighed. After weighing the Pectoralis minor muscle was separated from the Pectoralis major muscle. The pectoralis major muscle was then put into a sample pot containing 10% formalin solution for further histological preparations.

Histology samples were made based on Chen et al. (2012), muscle samples from formalin solution were hydrated through a series of alcohols with increasing concentrations. The samples were transferred one by one into each alcohol concentration and allowed to submerge in each alcohol concentration for approximately 15 seconds. The sample was then put into xytol and finally immersed in paraffin. Using a microtome, the sample was thinly sliced and then stained with haemotoxylin-eosin on a glass object and covered with a cover glass.

Observation of histological images

Histological images were obtained from the results of shooting using a microscope with an OptiLab device connected to a computer. The results of the histology images obtained were trnsfered into the Axio Visio Rail program. 4.8.2. Observation of histology images program Axio Visio Rel. 4.8.2. was adjusted to the magnification scale of the microscope used when taking pictures. Observation of the number of myofibers was carried out at 10x magnification, while the diameter and surface area of myofiber were measured using 40x magnification.

Observation parameters

Observations on the incubation process were egg weight, fertility, embryonic mortality (early, middle, late), albumen weight, yolk weight, and Ratio hatching weight/Egg weight. The post-hatching parameters were hatchability, age embryo died, hatching weight, performance (feed intake, body weight gain, body weight, feed conversion ratio), muscle mass, and muscle histology (number of myofiber, myofiber surface area) of male and female native chickens.

Statistical analysis

Analysis of variance for data collected was done based on Completely Randomized Design (CRD). The treatment which showed a significant effect was then continued with Duncan's test using IBM SPSS 20.0 software. Data from the analysis of each treatment is presented in the form of mean ± standard deviation.

RESULTS AND DISCUSSION

Hatchability, embryonic mortality, and age embryo died

Table 1 shows that there is no effect of treatment ($P>0.05$) on hatchability, embryonic age died, and embryonic mortality (early, middle, and late embryo died). All treatments produced the same hatchability. Similar results were also reported by (Al-Daraji et al. 2012) in quail, (Gao et al. 2018) in broilers, and (Zhu et al. 2019) in domestic pigeons. However, in contrast to reports (Araújo et al. 2020) on broilers, (Abousaad et al. 2017b) on turkeys, (Zhang et al. 2018) on domestic pigeons, and (Silva et al. 2012) on broiler breeders. The absence of differences in hatchability indicated that the *in-ovo* injection technique did not damage the extraembryonic sac and the concentration used was following the osmolarity of the egg albumen, so it did not cause embryo death. Embryo mortality data support this argument, with no differences in embryo mortality across all treatments. However, further observations need to be made regarding the osmolarity value of each treatment to ensure that the *in-ovo* solution osmolarity is following the albumen osmolarity on the 10th day of incubation.

Embryo mortality is the main factor that determines high or low hatchability. The more embryos died, the lower the hatchability value and vice versa. In general, embryonic death during incubation can be caused by many factors such as outside epigenetic temperature (Loyau et al. 2014), low humidity (El-Hanoun et al. 2012), egg nutrition (Oladokun & Adewole 2020),

Table 1. Effect of *in-ovo* injection of L-arginine on hatchability, embryonic mortality, and age embryo died of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Fertility (%)	74.29±4.95	75.24±6.60	73.33±3.30	74.29±2.86	65.71±9.90
Hatchability (%)	52.38±4.58	51.91±4.00	52.05±3.93	51.95±4.00	50.88±2.51
Embryonic mortality (% of fertile eggs)					
Early *	0.09±0.03	0.08±0.04	0.06±0.02	0.06±0.04	0.08±0.04
Middle *	0.08±0.03	0.09±0.05	0.08±0.04	0.06±0.02	0.05±0.01
Late *	0.31±0.06	0.33±0.04	0.35±0.06	0.36±0.03	0.38±0.02
Age embryo died (day)	19.55±1.26	18.84±1.31	19.42±1.34	19.26±1.35	18.78±1.22

*early (0-7 days of incubation), middle (8-14 days of incubation), late (15-21 days of incubation)

Table 2. Effect of *in-ovo* injection of L-arginine on extraembryonic weight and chick quality of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Egg weight (g)	42.37±0.45	42.82±0.74	42.59±0.31	42.69±0.40	42.20±0.10
Albumen weight (g)	5.21±0.31	5.25±0.31	5.04±0.65	5.06±0.48	5.13±0.77
Yolk weight (g)	7.36±0.48^{b}	7.83±0.50^{b}	6.27±0.32^{a}	6.38±0.31^{a}	6.29±0.39^{a}
Hatching weight (g)	26.68±0.34^{a}	27.66±1.11^{a}	29.39±0.79^{b}	29.83±1.31^{b}	29.22±0.23^{b}
Ratio hatching weight / Egg weight (%)	62.97±0.15^{a}	64.57±1.48^{a}	69.00±1.36^{b}	69.89±3.25^{b}	69.25±0.71^{b}

Means ± SD in the same rows with different superscripts (a,b) differ significantly (P<0.05). Albumen weight and yolk weight were collected on the day 17 of incubation

eggabnormalities (Onbaşilar et al. 2014). The age of death of chicken embryos was divided into 3 main groups (Al-Shamery and Al-Shuhaib 2015). The first group, namely early-embryo dead, are embryos that die in the first stage of embryogenesis (1-7 days). The second group, namely middle-embryo dead, are embryos that die in the second stage of embryogenesis (8-14 days). The third group, namely late-embryo dead, is embryos that die in the last stage of embryogenesis (15-21 days). The average age of embryonic death occurs at the late stage of embryogenesis (late-embryo dead). This indicates that the embryonic death that occurs is assumed not to be caused by the *in-ovo* injection technique and the concentration of L-arginine because *in-ovo* injection was carried out on the 10th day of the incubation period (second stage of embryogenesis).

Extraembryonic and chick quality

The effect of *in-ovo* injection of L-arginine on extraembryonic and hatchability characteristics of native chicken is presented in Table 2. *In-ovo* injection of L-arginine significantly affected (P<0.05) yolk weight, hatching weight, and hatching weight ratio/ egg weight, while egg weight and albumen weight did not show any effect (P>0.05). Yolk weight decreased with *in-ovo* injection of L-arginine, but there was no difference between *in-ovo* treatments. All *in-ovo* treatments of L-arginine increased hatching weight and hatching weight/egg weight ratio.

Albumen weight did not show any difference for all treatments. This indicates that the concentration of the *in-ovo* injection solution does not interfere with albumen absorption activity. Changes in the osmolarity of the embryonic environment with *in-ovo* injection, as a cause of impaired nutrient absorption by the embryo. Nutrient absorption can occur because the osmolarity of the injected solution is different from the osmolarity of the embryonic environment (Oladokun & Adewole 2020). The albumen weight that did not differ also indicated that the embryos treated with *in-ovo* injection absorbed more L-Arginine than the control. Absorption of albumen by the embryo through the *sero-amniotic connection* pathway, albumen then enters the amniotic fluid and is utilized by the embryo. The egg turning process is the main factor determining albumen content changes. Willems et al. (2014) reported that the turning process would assist albumen through the sero-amniotic connection.

In-ovo injection of L-arginine produced lighter yolks than the control treatment. This may be due to *in-ovo* injection of L-Arginine increase the metabolic rate of the embryo, so that absorption of the yolk is more than the control treatment. A high metabolic rate will require more energy. The increased energy requirements of the embryo are required to support organogenesis activities (Hu et al. 2013). During incubation, the yolk is the most important source of energy for the embryo. The limited amount of egg carbohydrates (1%) causes fat to become the main energy source for the embryo through the process of gluconeogenesis (Nangsuay et al. 2015). Alshamy et al. (2018) reported that the transport of yolk fat to the embryo via blood vessels is endocytosis, after hatching absorption of nutrients from the yolk through *Meckel's diverticulum*.

L-arginine injection via the *in-ovo* technique resulted in a higher hatching weight than control. The increase in hatching weight may be due to the increase in embryo weight produced by the administration of L-arginine. Onbaşilar et al. (2014) showed that heavier embryos would produce higher hatching weights than lighter embryos. Increased liver and muscle glycogen concentrations may also be responsible for the increased hatching weight (Hu et al. 2013). The increase in glycogen is believed to occur due to the low weight of the yolk with the administration of L-arginine.

At the end of the incubation period, the energy needs of the embryo will increase to carry out the hatching process. During this process, most of the embryo's energy is obtained from the breakdown of liver and muscle glycogen (Yang et al. 2019). The greater the number of energy reserves (glycogen) needed to support hatchery activities. When the energy reserve status of the embryo is low during the hatching process, there will be an overhaul of protein tissue, especially muscle (Dong et al. 2013). The reshuffle of muscle protein tissue causes a decrease in muscle mass so that it will have an impact on low hatching weight. The results of the study Shafey et al. (2012) showed that an increase in glycogen of 1.5 mg/g of organs would increase the hatching weight of 1.9 g. Meanwhile Al-Daraji et al. (2012) reported that the increase in hatching weight with *in-ovo* injection of L-arginine occurred due to the increase in muscle mass during the myogenesis process.

Parameter of hatching weight to egg weight ratio was observed to avoid the effect of egg weight on hatching weight. The eggs that were sampled had relatively the same weight. Heavier eggs were reported to produce higher hatching weights than lighter eggs (Dymond et al. 2013). The higher the ratio of hatching weight to egg weight, the higher the growth rate of the embryo, even though it comes from eggs with smaller sizes. The ratio of hatching weight to egg weight was higher in the L-arginine treatment than in the control. This happened because the hatching weight obtained in the treatment with L-arginine was also higher. Results obtained indicated that the embryonic growth rate was higher with *in-ovo* injection of L-arginine.

Post-hatch performance

As shown in Table 3, feed consumption, weight gain, body weight, and feed conversion were significantly affected ($P<0.05$) by *in-ovo* injection treatment on the 7th week of rearing day. On day 14, *in-ovo* injection of L-arginine showed an effect ($P<0.05$) on feed consumption, body weight, and feed conversion, but not on weight gain. Bodyweight gain, body weight, and feed conversion on day 21 were significantly affected ($P<0.05$) by *in-ovo* treatment, while feed consumption did not show any difference between treatments. Effect of treatment on post-hatch performance observation on day 28 was significant ($P<0.05$) on weight gain and body weight, but feed consumption and feed conversion did not show any different ($P>0.05$).

Feed consumption on the 7th day of observation showed a decrease with the injection of L-arginine 0.5% and 1.5%, while on the 14th day the lowest feed consumption was in the control + treatment. Observations on the 21st and 28th days did not show any difference in feed consumption between treatments. Weight gain on the 7th day increased with the injection of 0.5% and 1.5%, the same results also occurred on the 21st day of observation. However, on day 28, the highest weight gain was with the injection of 1.5% L-arginine. Observations on the 7th, 14th, and 21st days showed that all the treatments could increase body weight, while at week 28, the highest body weight was with the injection of 1.5%. *In-ovo* injection of L-arginine 0.5% and 1.5% showed lower feed conversion values on the 7th and 21st-day observations. However, the feed conversion did not show any difference between the treatments on the 14th and 28th-day observations.

Results of the analysis showed that there was no difference in feed consumption from all treatments on the 14th, 21st, and 28th days of observation. However, on the 7th day of observation, showed that the injection of 0.5% and 1.5% resulted in lower feed consumption compared to the other treatments. These results indicated that the metabolic energy requirements of chickens are the same whether injected with L-arginine or not on days 8 to 28. However, before the 8th day, the energy requirements of *in-ovo* injection chickens were higher than control. In the growth phase, metabolic energy was focused on supporting hypertrophiccell activity (Chen et al. 2013). The more cells that

Table 3. Effect of *in-ovo* injection of L-arginine on post-hatch performances of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
day 0 to day 7th					
Feed intake (g/bird)	47.88±0.52^{c}	47.69±0.53^{bc}	46.85±0.39^{a}	46.93±0.26^{bc}	46.71±0.70^{a}
Body weight gain (g/bird)	39.46±0.10^{b}	37.37±1.56^{a}	41.52±2.12^{c}	40.45±0.28^{bc}	41.80±0.14^{c}
Bodyweight (g/bird)	66.17±0.34^{b}	64.98±0.76^{a}	70.45±1.06^{c}	70.47±0.83^{c}	70.99±0.06^{c}
Feed conversion ratio	1.21±0.01^{b}	1.27±0.06^{c}	1.13±0.05^{a}	1.16±0.01^{ab}	1.11±0.02^{a}
day 7st to day 14th					
Feed intake (g/bird)	91.80±1.75^{b}	86.63±4.38^{a}	92.98±1.67^{b}	92.43±1.74^{b}	91.08±1.35^{b}
Body weight gain (g/bird)	58.88±3.90	58.09±3.27	60.52±3.47	64.18±3.56	63.87±2.05
Bodyweight (g/bird)	125.05±3.87^{a}	123.07±3.92^{a}	130.97±4.22^{b}	134.65±3.83^{b}	134.87±2.10^{b}
Feed conversion ratio	1.56±0.12	1.50±0.14	1.54±0.06	1.44±0.06	1.43±0.07
day 14st to day 21th					
Feed intake (g/bird)	156.26±5.04	153.42±7.37	155.84±7.08	155.43±4.07	155.47±4.19
Body weight gain (g/bird)	77.05±2.78^{a}	76.96±6.36^{a}	88.99±0.97^{c}	81.52±3.83^{ab}	87.26±5.57^{bc}
Bodyweight (g/bird)	202.11±2.26^{a}	200.04±2.78^{a}	219.96±5.11^{b}	216.18±7.07^{b}	222.13±5.15^{b}
Feed conversion ratio	2.03±0.04^{b}	2.02±0.26^{b}	1.75±0.06^{a}	1.91±0.10^{ab}	1.78±0.06^{a}
day 21st to day 28th					
Feed intake (g/bird)	212.80±7.75	232.49±1.48	232.68±2.01	232.69±1.22	231.51±1.51
Body weight gain (g/bird)	84.73±3.09^{a}	90.64±5.20^{ab}	89.30±5.89^{a}	97.12±6.07^{bc}	103.09±3.42^{c}
Bodyweight (g/bird)	286.84±2.87^{a}	290.69±3.37^{a}	309.27±10.85^{b}	313.32±1.59^{b}	325.26±6.88^{c}
Feed conversion ratio	2.52±0.36	2.57±0.12	2.61±0.14	2.40±0.16	2.24±0.06

Means ± SD in the same rows with different superscripts (a,b,c) differ significantly (P<0.05). Bodyweight was measured on day 7, 14, 21, and 28

Table 4. Effect of *in-ovo* injection of L-arginine on muscle histology of native chicken

Variables	*In-ovo* Treatment				
	Control (-)	Control (+)	L-Arginine 0.5%	L-Arginine 1.0%	L-Arginine 1.5%
Male					
Muscle mass (g)	29.026±3.172^{a}	29.748±2.654^{a}	35.385±2.945^{b}	37.490±3.530^{b}	38.195±3.085^{b}
Bodyweight Ratio (%)	5.396±0.256^{a}	5.407±0.427^{a}	5.408±0.075^{a}	6.147±0.304^{b}	6.195±0.403^{b}
Number of Myofiber (n/mm^2)	91.00±4.00	81.67±10.50	97.67±1.52	95.00±6.55	92.00±7.93
Myofiber (µm)	47.73±3.02^{a}	48.36±1.42^{a}	60.11±4.05^{b}	62.49±3.95^{b}	62.19±3.92^{b}
Myofiber Surface Area (µm^2)	1874.8±323.9^{a}	1627.6±233.8^{a}	2488.8±81.7^{b}	3118.7±130.1^{c}	2820.9±194.7^{bc}
Female					
Muscle mass (g)	24.281±2.379^{a}	24.571±2.378^{a}	29.490±2.950^{b}	30.758±3.048^{b}	30.686±1.587^{b}
Bodyweight Ratio (%)	5.170±0.133	5.030±0.674	5.230±0.103	5.729±0.701	5.783±0.299
Number of Myofiber (n/mm^2)	91.33±6.50	77.00±4.58	88.67±6.65	93.00±7.21	93.33±8.08
Myofiber (µm)	38.07±4.76^{a}	45.66±2.30^{a}	59.68±5.31^{b}	57.60±6.81^{b}	58.31±9.24^{b}
Myofiber Surface Area (µm^2)	1227.0±118.8^{a}	1735.8±321.1^{a}	2813.5±445.3^{b}	2343.9±145.6^{b}	2591.3±417.8^{b}

Note: Means ± SD in the same rows with different superscripts (a,b,c) differ significantly (P<0.05)

experience hypertrophy, the higher the amount of energy needed (Yu et al. 2018). Based on this, it is assumed that chickens that received L-arginine treatment had more cell numbers than other treatments because they had a high metabolic energy requirement. Although, it is still necessary to do further observations to prove this.

L-arginine injection resulted in higher final body weight than without L-arginine administration at all measurement times. The same results were also shown in weight gain. Optimal final body weight was obtained from injection of L-arginine with a concentration of 0.5%. The final weight gain occurs because L-arginine injection can increase muscle mass. Muscle mass was reported by Yang et al. (2019) as an important component in the chicken body that will determine body weight. Muscle weight has a positive correlation with body weight (Chen et al. 2013). The report explains that giving L-arginine can increase muscle mass, resulting in high body weight. It was also reported by Al-Daraji et al. (2012) that administration of L-arginine could increase muscle mass.

The injection of L-arginine 1.0% and 1.5% on the 7th and 21st-day of observations resulted in lower feed conversion values compared to the other treatments. The low feed conversion value may occur due to the increased performance of the digestive tract with the administration of L-arginine. Similar results were obtained by Al-Daraji et al. (2012) in quail. In general, the digestive tract (small intestine) that can work optimally has a large surface (Proszkowiec-Weglarz et al. 2020). It also can release digestive enzymes at maximum levels (Castro et al. 2020). Fouad et al. (2012) reported that L-arginine stimulate small intestinal cell proliferation. Thus, an increase in L-arginine levels in the embryonic phase will cause an increase in the number of small intestinal cells (Castro et al. 2020). Proszkowiec-Weglarz et al. (2020) explained that the increase in the size of the villi and crypts was the result of the high proliferative activity of small intestinal cells at the beginning of growth. This causes the amount of nutrients absorbed is also increasing.

Massa and histology of muscle

In the male sex, muscle mass, muscle mass/body weight ratio, myofiber diameter, and myofiber surface area were significantly affected ($P<0.05$) by the treatment, but the number of myofibers did not show any change (Table 4). The ratio of muscle mass/body weight and the number of muscles myofibers with female sex showed no difference between treatments ($P>0.05$). Meanwhile, muscle mass, the diameter of myofiber, and surface area of myofiber were significantly affected by treatment ($P<0.05$) (Table 4). Muscle mass with the injection was higher than control, both male and female. The ratio of muscle mass/bodyweight of male chickens increased with *in-ovo* injection of L-arginine 1.0% and 1.5%, respectively. *In-ovo* injection of L-arginine caused an increase in myofiber diameter, in both male and female sexes and optimally at a concentration of 0.5%. Maximum myofiber surface area occurred with the injection of 1.0% in the male sex, whereas in female sex optimal myofiber surface area with the injection of 0.5%.

L-arginine injection resulted in higher muscle mass than without L-arginine injection, in both male and female sexes. The increase in muscle mass was caused by an increase in myofiber diameter with the administration of L-Arginine compared to the control. Muscle size is determined by the size of myofibers (Chen et al. 2013). The wider the size of the myofibers, the higher the muscle mass. The larger myofiber size with L-arginine treatment may also be due to the higher number of myoblast cells during myogenesis as reported Xiao et al. (2017) in broiler. The increase in myofiber size was due to L-arginine being an IGF-1 stimulator. Increased concentrations of IGF-1 using L-arginine were also reported by (Yu et al. 2018) in layer chickens and (Castro et al. 2020) in broiler. Chen et al. (2013) suggested that L-arginine can increase IGF-1 gene expression by increasing IGF-1 RNA transcription activity. The L-arginine stimulant process on IGF-1 production is believed to occur through the L-arginine/IGF-1R mechanism (Chen et al. 2013). Another report explains that the increase in IGF-1 concentration occurs because L-arginine and amino acids produced by L-arginine (proline and glutamine) are important amino acids in the chemical structure of IGF-1 (Fouad et al. 2012). In this study, IGF-1 levels were not measured. Thus, the assumption of an increase in IGF-1 levels with *in-ovo* injection of L-arginine needs to be investigated further.

IGF-1 has a very important role during the myogenesis process. In the early phase of myogenesis, IGF-1 plays a role in stimulating the proliferative activity of myoblast cells (Musumeci et al. 2015). The results of the study Xiao et al. (2017) showed that an increase in the concentration of IGF-1 at the beginning of the myogenesis phase caused an increase in the number of myoblast cells. The effect of IGF-1 on myoblast proliferation activity begins with a signal from IGF-1 that activates Mitogen-Activated Protein Kinase (MAPK) pathway (Endo 2015). MAPK pathway is an activity that occurs in the cell nucleus with the IGFr-Ras-Raf-MEK-ERK mechanism (Hu et al. 2016).

CONCLUSION

In-ovo injection of L-arginine has a beneficial effect on chick quality and post-hatch performance, the concentration of L-arginine solution used does not

cause a negative effect on embryo death. *In-ovo* injection of 0.5% L-arginine increased hatching weight, weekly body weight, muscle mass, and myofiber size.

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