

Application of in ovo injection of L-Glutamine for improving productivity of Indonesian native chicken: hatchability and hatching time

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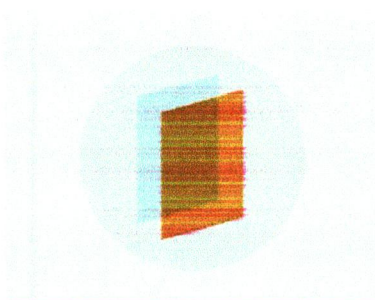
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Application of *in ovo* injection of L-Glutamine for improving productivity of Indonesian native chicken: hatchability and hatching time

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Abstract. A study was aimed to improve productivity of the Indonesian Native Chicken (INC) by the technique of *in ovo* injection (IOI) of L-Glutamine (Gln), which hatchability and hatching time were measured. A 300 fertile egg (45.26±3.65 g) of INC were used and arranged as Randomized Block Design within 3 different incubators as blocks, and 5 treatment groups of 20 eggs: negative control (P0), positive control (P1-0.5 ml sterilized saline), 2.50 (P2), 5.0 (P3) and 7.5 (P4) mg Gln in 0.5 ml sterilized saline. The injections were conducted into the albumen at d 7 of incubation. IOI of Gln resulted in a significantly heavier weight of the newly hatched chickens (NHC). Hatchability of the P1 (60.83 ± 7.52%) and P2 (62.50 ± 2.89 %) was significantly higher (P<0.01) compared to those of the other groups; On the other hand, the embryo mortality in P1 and P2 were significantly lower compared with the other three groups, which mostly occurred before d 17 of incubation. It is concluded that IOI of 2.5 mg Gln of INC increased hatchability, decreased the duration of incubation time and the heavier weight of NHC would apparently have a better performance compared to that of the untreated eggs.

1. Introduction

In Indonesian, chicken is the most widely distributed livestock. In addition to exotic breed farmed commercially, at least 31 unidentified breeds of local chicken were reported [1]. These local chickens usually called "Ayam Kampung" or "Ayam Buras" or Indonesian native chicken having different morphologic characteristics. In spite of having the potency to be developed, native chickens have a major constraint in production and reproduction. In meat production, they are growing very slow and having bad feed conversion. In reproduction, they are having low egg productions. In addition to their genetic background, low performance of the native chicken may be attributed with low management input, free scavenge for a feed around the farmer's house during the day, as mostly raised extensive traditional by the farmers for their additional income.

In ovo injection of exogenous materials may apparently be an alternative approach in order to improve the performance of native chicken. Previous investigations mostly on exotic breeds indicated that *in ovo* injection of nutrient into the hatching eggs is to supply adequate nutrient which resulted in improving the hatchability of the egg and subsequent post-hatch performance, body weight at marketing age and carcass quality of the chicken [2, 3, 4, 5, 6, 7, 8]. Moreover, post-hatch growth of the chick is determined by the nutrients present in the yolk remaining in the peritoneal cavity. Egg



contains an excess of fat and moisture but not protein [9, 10]. Previous research in this laboratory [10] indicated that IOI of Arginine (2.5, 5.0 and 7.5 mg in 0.5 ml sterilize saline) into the *Kampung* chicken resulted in a better performance of pre- up to 42 d post-hatching, including embryo development and body weight gain.

L-Glutamine (Gln) has usually been considered as a non-essential amino acid since most animal cells can synthesize it, and primary function is to store nitrogen in the muscle and to transport it between organ; however during period of rapid growth, the cellular demand for Gln outstrip its supply, and Gln become essential, hence its named as a 'conditionally' essential amino acid. As an illustration, during the first two weeks of chicken embryo growth and development, proliferating cells shows an intense appetite for Gln, reflecting its flexibility as a nutrient and mediator of other processes. Although it contributes only 4% of the amino acid in muscle protein, Gln accounts for more than 20% of the free amino acid pool in plasma and more than 40% in muscle [12, 20]. Attributed to the low input management for Indonesian native chickens, application of in ovo injection of Gln in this research aimed to elucidate the effects on hatchability and hatching time.

2. Material and Method

A total of 400 native chicken eggs were collected from traditional farmers around Bantaeng Regency – South Sulawesi. Eggs were numbered and weighed individually. Eggs were set in a semi-automatic incubator at 37.5 °C (99.5 °F) and 55% relative humidity. The eggs were turned every 3 h. Eggs were examined by candling at d 7 of incubation; the infertile eggs and the eggs containing dead embryos were removed.

After examination, 300 eggs were selected and arranged into a Randomized Block Design consisted of 3 different incubators as the block and 5 treatment groups of 20 eggs having equal weight. In accordance with the treatment group, the eggs were injected with Gln (2.5, 5.0 or 7.5 mg dissolved in 0.5 sterilized saline) as P2, P3, and P4, respectively. Whereas P0 was a control group which without injection (negative control) and P1 was a positive control which treated only with sterilized saline injection. Before injection, each egg was candled to identify the point of the inserted needle, which then cleaned the area with ethyl alcohol (70%) and a piece plastic type of 1 x 1 cm covered the point; a pointed tip of a small scissor was used to make a small hole in the middle of the type. A 0.5 ml solution was injected into the albumen of each egg using automatic syringe equipped with 22 gauge needle to a depth of 10 -13mm. At the end, the hole was sealed with nail paint and placed back into the incubator. The amino acid was provided by Merck, Darmstadt, Germany.

Hatchability percentage was calculated based on the number of hatched chicks as a percentage of the incubated eggs per replication of every treatment. Hatching time was recorded every 12 h. Chicks were observed at 12-h intervals from d 19 to d 21 (456 to 528 h) of incubation, and the weight of newly hatched chicken was recorded using a digital balance to the nearest 0.01 g. Resulted data were analysed by using Statistical Packages of the 13.1. version of Systat.

3. Results and Discussion

The results are summarized in table 1 and table 2. The initial weights of all incubated eggs were not significantly different. However, IOI of Gln increased ($P < 0.05$) the body weights (g and % of egg) of newly hatched chick significantly; while the hatchability (%) of positive control (P1) and 0.25 mg Gln (P2) groups were significantly higher compared to those of negative control (P0), 5.0 mg Gln (P3) and 7.5 mg Gln (P4). The Embryo mortality indicated a reverse result and the most mortality occurred before d 17 of incubation. The length of the incubation period of the eggs treated Gln and the positive control group was significantly faster compared with that of the negative control eggs, and hatching times of all egg groups were less than 21 days.

Previous research of IOI nutrients resulted in decreasing [6, 13, 14], increasing [15], or un-affecting [5, 13, 14, 16] the hatchability. These all previous research of IOI were conducted on the eggs of the exotic chicken breeds which have superior genetic traits of the eggs. These contradictive results about

hatchability might be attributed to the different breed, nutrition and injection techniques, depth and position of injection, nutrient and osmolality of injected solution, the day of incubation, etc.

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Table 1. Effect of in ovo injection of L-Gln on hatchability and newly hatched body weight of Indonesian Native Chicken

Parameter	In ovo group				
	P0 = Control (-)	P1 = Control (+)	P2 = 2.5 mg Gln	P3 = 5.0 mg Gln	P4 = 7.5 mg Gln
Egg weight (g)	45.57 ± 5.14 ^a	46.21 ± 3.65 ^a	45.14 ± 3.28 ^a	45.51 ± 3.30 ^a	46.51 ± 2.66 ^a
NHCW (g)	31.86 ± 0.41 ^a	33.32 ± 0.99 ^b	33.78 ± 1.31 ^b	33.57 ± 1.49 ^b	33.73 ± 1.21 ^b
NHCW (% egg)	69.92 ± 1.42 ^a	72.11 ± 0.73 ^b	74.83 ± 2.08 ^b	73.76 ± 1.76 ^b	72.54 ± 1.14 ^b
Hatchability (%)	34.80 ± 4.58 ^a	60.83 ± 7.52 ^b	62.50 ± 2.89 ^b	32.22 ± 7.81 ^a	26.11 ± 12.73 ^a
Mortality < d17 (%)	46.00 ± 4.73 ^a	20.00 ± 8.66 ^b	19.92 ± 5.54 ^b	41.67 ± 7.55 ^a	48.33 ± 5.77 ^a
Mortality > d17 (%)	19.20 ± 4.57 ^a	19.17 ± 5.23 ^a	17.58 ± 3.58 ^a	26.11 ± 5.00 ^b	25.56 ± 2.88 ^b

^{a,b}Means in the same row with different superscript differ significantly (P < 0.05).

NHCW : New Hatched Chick Weight

Table 2. Length of Incubation period and hatching distribution over the incubation period of the Indonesian native Chicken treated in ovo injection of L-Gln

Parameter	In ovo group					
	P0 = Control (-)	P1 = Control (+)	P2 = 2.5 mg Gln	P3 = 5.0 mg Gln	P4 = 7.5 mg Gln	
Incubation period (hours)	496.14 ± 4.39 ^a	482.80 ± 0.65 ^b	484.88 ± 3.18 ^b	485.33 ± 3.65 ^b	484.28 ± 6.53 ^b	
Hatching time	465-480	6.25 ± 2.87 ^a	26.47 ± 4.11 ^b	18.52 ± 4.76 ^c	18.18 ± 4.77 ^c	7.69 ± 0.86 ^a
Distrib. (%)	481-504	81.25 ± 4.65 ^b	73.53 ± 3.76 ^c	81.48 ± 6.83 ^b	72.73 ± 7.88 ^c	92.31 ± 6.73 ^a
	505-528	12.50 ± 2.46	0	0	9.09 ± 3.74	0

^{a,b}Means in the same row with different superscript differ significantly (P < 0.05)

The present study was conducted on eggs of unknown breeds of local chicken, there were some interesting results to be discussed. Firstly, the hatchability of all egg groups in this experiment was lower than those resulted from eggs of the exotic breeds. In addition to genetic background, these differences might be attributed with different managements, particularly for daily feed (quality, quantity, and continuity) intake in the condition of the traditional farming system. Candling data (unpublished) at d 7 of incubation soon before IOI indicated that the fertility of the eggs was about 75%.

Secondly, different levels of IOI of Gln resulted in different hatchability responses of eggs; hatchability of the positive control and IOI of 2.5 mg Gln was significantly higher compared with that of the negative control. When the level of IOI of Gln increased to 5.0 and 7.5 mg, hatchability was to decrease significantly to the levels as that of the negative control. In these levels, IOI of Gln, as "conditionally essential amino acid", may result in amino acid imbalance as the rate of its metabolism is lower than its supply. However, a search of the literature provided no information to support this explanation. Thirdly, the egg size of the local chickens was about 10-20 g smaller than that of the exotic breeds, it appears that IOI of 2.5 mg Gln in 0.5 ml sterilized saline is an optimal dose for the local chicken eggs. Fourthly, the weights of the newly hatched chickens (g or % of egg) of the treated egg groups (including positive control) were significantly higher than that of the negative control. In

general, IOI of amino acids is primarily aimed to provide amino acid for gluconeogenesis and then to reduce albumin catabolism and sparing for muscle and tissue protein synthesis, and consequently improving body weight at hatch [17, 18]. IOI of a mixed solution of some amino acids at early incubation resulted in increasing the weights of newly hatched chickens by 2-3.4% [15]. Moreover, in overfeeding to the late term embryos also increased hatching weight by 5-6% over controls [18]. IOI of all 20 AA increased newly chick weight 2.1% [19]. In the present study, IOI of 2.5 to 7.5 mg Gln in 0.5 ml increased the weight of the newly hatched chicken by 2.62 – 4.91% over the negative control.

Overall, the results of the present study suggested that IOI of 2.5 mg Gln in 0.5 ml sterilized saline to the eggs of Indonesian native chicken increased hatchability, the weights of the newly hatched chicken and shorten the length of incubation period.

Acknowledgment

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