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NATIVE CHICKEN EMBRYO QUALITY IMPROVEMENT THROUGH IN OVO FEEDING

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Key words : *Lysine. methionine, in ovo feeding, native chicken, and embryo*

Abstract–The aim of this research were to produce a higher embryonic growth and a higher hatching weight, as well as to find out the most effective time for amino acids injection into incubated eggs. The study was carried out in a factorial (4 x 2) arrangement according to completely randomized block design. The first factor was different levels of amino acid injections i.e. A_0 = (control), A_1 = injection of lysine at a dose of 3.78 mg/0,5 ml of sterile distilled water, A_2 = injection of methionine at a dose of 1,91 mg / 0,5 ml of sterile distilled water, A_3 = injection of lysine + methionine at dose of 3,78 lysine+ 1,91 mg methionine/0,5 ml of sterile distilled water. The second factor was different injection time, namely T_1 = injection on day 7 of incubation and T_2 = injection on day 14 of incubation. Total number of treatment combination was 8. The incubation of eggs was run three times in wich each period served as a blok/replication for each treatment combination. Therefore total number of eggs used for the entire experiment was 900. Parameters measured were yolk weight, albumen weight, embryo weight and hatching weight. no acid that the amino acid injection into eggs significantly affected ($P < 0,05$) the weight of the embryo and hatching weight. The timing of amino acid injection significantly affected ($P < 0,05$) the weight of the yolk but the interaction between amino acids and time of injection had no significant effects ($P > 0,05$) on all parameters. We conclude that amino acid injection (in ovo feeding) with either lysine, methionine, or combination of lysine and methionine can increase the weight of the embryo and hatching and the moust effective time to inject amino acid on day 14 of incubation.

INTRODUCTION

Native chickens are known to have several shortcomings namely variable productivity levels among individuals, limited breed availability, high mortality rate of over 40% (1), low embryo growth and low hatching weight. The low productivity of native chicken is suspected to be due to a deficiency in essential amino acids, especially lysine and methionine. Both lysine and methionine are critically essential amino acids. (2) revealed that deficiencies or imbalances in protein and amino acids could result in abnormalities and even a mortality. In chickens, the embryo does not have a direct relationship with its mother during the embryonic development and the nutrients necessary for embryos are derived from the eggs themselves. For normal embryonic development to occur, there needs to be sufficient supply of nutrients within the egg.

Research on lysine and methionine supplementation in the diet has been carried out extensively. Research from (3) revealed that a low protein feed containing 12% protein but offset by supplementation of amino acids lysine and methionine can result in normal production, even though the smaller egg weight. Furthermore (4) revealed that quantitively, the average chicken egg weight increased after supplementation with lysine or methionine. Research on the injection of external nutrients through in ovo feeding of carbohydrates (5), vitamins (6), amino acids (7), and (8), towards broiler poultry eggs have been done in Japan. The results of these studies showed that amino acid injection in ova resulted in hatchability of 90.9% compared to 84.4% in controls or injection of amino acids increases hatchability by 7.7%.

So far, amino acid injection study has never been done in native chicken in Indonesia in general and especially in South Sulawesi. Therefore we

conducted this research by injecting amino acids, i.e. lysine, methionine, or combination of lysine and methionine into the eggs of native chicken eggs (in ovo feeding). The goal of this in ovo feeding was to stimulate an increase in metabolic activity through increased availability of nutrients, the amino acid lysine and methionine, in the egg. The use of nutrients, specially lysine and methionine, in the egg. The use of nutrients in the yolk is expected to result in an increase of embryo growth and hatching weight.

MATERIAL AND METHODS

This research was carried out from January to April 2013 in the Laboratory of the Poultry Production, the Faculty of Animal Science, Hasanuddin University, Makassar. The experiment was conducted in factorial arrangement (4 x 2) according to completely randomised block design. The first factor was different levels of amino acid injection, i.e. A_0 = (control), A_1 = injection of lysine at a dose of 3.78 mg/0.5 ml of sterile distilled water, A2 = injection of methionine amino at a dose of 1.91 mg/0.5 ml of sterile distilledwater, A3 = injection of lysine +methionine at a dose of 3.78 mg lysine + 1.91 mg methionine / 0.5 ml of sterile distilled water. The second factor was different injection time, namely T1 = injection on day 7 of incubation,T2 = injection on day 14 of incubation. Amino acids used were crystalline amino acids with a high degree of purity, diluted with sterile distilled water. Each treatment combination was repeated three times giving total number of experimental units was 24.

Nine hundreds of native chicken eggs were randomly divided into 3 hatching groups/blocks. Each group consisted of 300 hatching eggs. Before placing into the incubator, the eggs were weighed to obtain initial weight of the egg. On the 6th day of incubation, an observation (candling) was performed to determine the fertile eggs. On Day 7 and day 14 of incubation, appropriate doses of amino acids were administered towards the fertile eggs, using a modified injection method (8). Prior to injection of the amino acids, the eggs first drilled using a special tool. After the injection was done the eggs were covered with solid paraffin to prevent the entry of bacteria into the egg. On the 18th day of incubation two eggs from each treatment were broken to determine the weight of the yolk, albumen weight, embryo weight. On day 21 the hatching weight was measured.

The parameters measured in this study were yolk weight, albumen weight, embryo weight and hatching weight. Experimental data were subject to analysis of variance (ANOVA). Significant effect of treatments were further determined using the LSD test. All data analysis was carried out using SPSS version 16.

RESULTS AND DISCUSSION

Weight of Yolk

Weight of yolk was measured on day 18 of incubation time. Analysis of variance indicated that time of injection significantly (P<0,05) affected the yolk weight. However, amino acid injection and interaction betwen amino acid and time of injection had not significant effects (P>0,05) the weight of yolk. The weight of yolk injected on the day 7 of incubation time was higher (P<0,05) compared to that injected on the day 14 of incubation time (Fig.1). This means that injection on the day 14 was far more effective than injection did on the day 7 as indicated by less weight on yolk weight from egg injected on day 14.The low weight of the yolk from egg injected on day 14 of incubation compared with that injected on day 7 of incubation is due to the higher metabolic rate in day 14 resulting in a more rapid absorption of amino acid within the body, consequently producing a lower weighing yolk. Yolk is source of nutrients for the formation and growth of the embryo (9),(10), and (11).

Weight of Albumen

The albumen weight according to the experimental

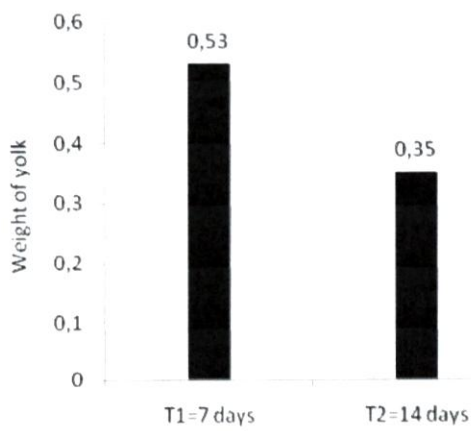

Fig. 1. Weight of yolk (g) injected amino acids with different time

design is presented (Fig.2). Analysis of variance indicated that type of amino acid, time injection, and interaction between amino acid injection and time of injection did not affect significant (P>0,05) the albumen weight. This means amino acid injection injection time and interaction between amino acid and injection time gave have the same response in terms of the size of the weight of albumen on day 18 of incubation. Thereby improving the quality of nutrition through in ovo feeding has not been proven to have any impact on the size of the weight of the albumen. The weight of the egg albumen is determined by nutrient supply of the parent.

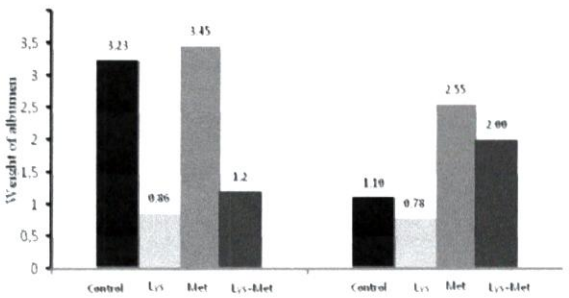

Fig. 2. Weight of albumen (g)

The result of this study is not in agreement with that reported by (8), who found that there was no more yolk and albumen on day 19 of incubation. This is might be due to the different incubation time and different breed chicken used. The breed of chicken has been reported to have different proportion of egg white and yolk (12). The proportion of egg white and yolk are also influenced by the age of the chicken (13). Albumen weight, wich is about 65% of the total weight of the egg, is mainly composed of water and proteins functioning not only in providing suitable environment for the embryo but also in providing additional nutrients necessary for embryonic development (14).

Weight of Embryos

In this particular study, the embryo growth was determined by measuring the weight of the embryo on day 18 of incubation. Accross the time of injection, the average embryo weight for each treatment was 17,18 g, 25,27 g, 23,58 g, and 26,45 g, respectively for treatment A0, A1, A2, and A3 (Fig.2). Accross amino acid injection, the embryo weight for time of incubation was 22,71g and 23,53g respectively for treatment T1 and T2. Analysis of variance indicated that amino acid injection significantly affected (P<0,05) the embryo weight, while time of injection as well as interaction between amino acid injection and injection time did not have a significant effect (P>0,05) on weight of embryo. The average weight of embryo obtained from the eggs injected with amino acids was higher than that obtained from control egg (25,7 g vs 17,2 g). The results of this study is in agreement with that reported by (8) who stated that the weight of embryo which was obtained from the injected eggs, measured on the day 19 of incubation, was significantly higher compared to that of control eggs.

The heavier weight of embryos injected with amino acids is suspected to be due to the

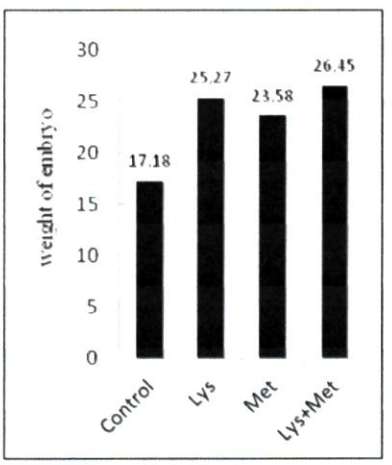

Fig. 3. Weight of embryo (g)

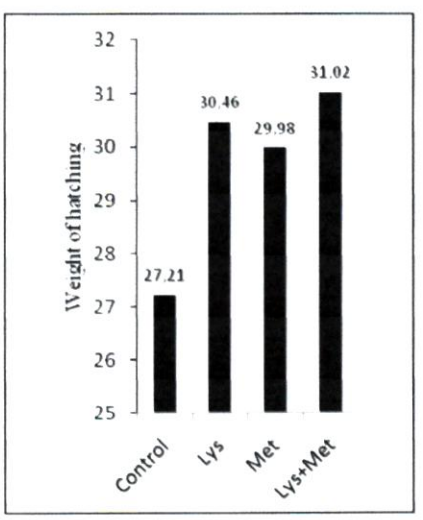

Fig. 4. Weight of hatching (g)

improvement of the nutritional quality through in ovo feeding, which helps to increase the thickness and length of the muscle fibers. Thickening of the muscle fibers are caused by the division and multiplication of myofibril, whereas addition of sarcomere at the ends of elongated muscle fibers causes myofibril, thereby increasing the weight of the embryo. The weight of embryos injected amino acids 31,53% higher than without amino acid injection (control). This suggests that increasing the nutritional quality of eggs during incubation before hatching can affect the growth of the embryo. This is presumably due to fulfilled the needs of the amino acid lysine and methionine for growth of the embryo during incubation.

Amino acids lysine and methionine are critically essential amino acids. Lysine and methionine play an important role in the biochemical processes required as enzyme cofactors. Lysine and methionin plays a role in the incorporation and cell growth. Role of lysine and methionine is very important in the early development of chicken embryos (15). Amino acid deficiency will result in an abnormal embryo growth, and can lead to death of the embryo. However there is no definitive data regarding the standard requirements of lysine and methionine amino acids on the growth of the embryo, especially for natural chicken. Suryani et.al. (10) reported that the function of amino acids and the formation of the network is to replace the damaged cells. Martoharsono (16) stated that the protein has 5 functions, namely as biocatalyst (enzymes), protein reserve, protein transport, structural proteins and protection, but generally protein known as replacement of damaged tissues or cells. (17) stated that protein plays a role in the incorporation and cell growth. This phenomenon has been demonstrated by (8) that the embryo weighted obtained from eggs injected with the amino acids in the egg on the 19th day of incubation increased higher than that of eggs without amino acid injection.

Embryo growth can be determined by measuring the weight of the embryo. Fig. 3 shows that the weight of the embryos on day 18 of incubation. Analysis of variance indicated that amino acid injection significantly (P<0,05) affected the embryo weight, injection time and interaction between amino acid injection and injection time did not affect the yolk weight. Fig.3 shows that the weight of the embryos on day 18 of incubation.The figure reveals that the weight of embryo of those injected with amino acids was significantly (P<0,05) higher than that without amino acid injection regardless of injection time.The heavier weight of embryos are suspected to be due to the improvement of the nutritional quality through in ovo feeding, which helps to increase the thickness and length of the muscle fibers. Thickening of the muscle fibers are caused by the division and multiplication of myofibril, whereas addition of sarcomere at the ends of elongated muscle fibers causes myofibril, thereby increasing the weight of the embryo. The weight of embryos injected.

Amino acids lysine and methionine are critically essential amino acids. Lysine and methionine play an important role in the biochemical processes required as enzyme cofactors. Amino acid deficiency will result in an abnormal embryo growth, and can lead to death of the embryo. Analysis of variance showed that there is a significant effect (P <0.05) of amino acid injection towards the weight of the embryo. This suggests that increasing the nutritional quality of eggs during incubation before hatching can affect the growth of the embryo. The results of this study showed that the amino acid lysine injection at a dose of 3,78 mg/ 0.5 ml of distilled water, methionine 1,91 mg/0,5 ml of distilled water and a mixture of 3.78 mg of lysine and methionine 1,91 mg/0,5 ml of distilled water can increase the weight of the embryo by day 18 of incubation. This is presumably due to fulfilled the needs of the amino acid lysine and methionine for growth of the embryo during incubation. However there is no definitive data regarding the standard requirements of lysine and methionine amino acids on the growth of the embryo, especially domestic poultry and chicken in general. (10) wrote that the function of amino acids and the formation of the network is to replace the damaged cells. (16) stated that the protein has 5 functions proliferation, namely as biocatalyst (enzymes), protein reserve, protein transport, structural proteins and protective, but generally a protein known as replacement of damaged tissues or cells. (17), the protein plays a role in the incorporation and cell growth.

This phenomenon has been demonstrated by (8) that the injection of the amino acids in the egg measured on the 19th day of incubation was higher than the weight control embryos. The main influence factors of injection time and the interaction between amino acid injection and injection time on the eggs had no effect (P>0,05) on the weight of the embryo. This indicates equal effectiveness between

the injection time of 7 days and 14 days incubation towards the weight of the embryo. Interaction between the injection of amino acids and injection time do not affect of responding to the increased weight of the embryo.

Weights of Hatching

Weights were obtained after the chicken eggs hatched using the incubator. Analysis of variance indicated that amino acid injection significantly (P<0,05) affected the hatcing weight. However, injection time and interaction between amino acid injection time did not affect the hatching weight.

Out of the four kinds of amino acid injections on either the 7th or the 14th day of incubation, eggs injected with the amino acid lysine produced eggs with the heaviest hatching weights, in which injection with amino acid lysine of 3,78 mg /0,5 ml of sterile distilled water on day 7 resulted in 30,90 ± 1,04 g and 33,20 ± 5,17 g on day 14. The mean hatching weight observed inthis experiment was higher than those reported by (18). Injection with the amino acid resulted in heavier weights of hatching eggs compared to without amino acid injections.This is due to the sufficient lysine and methionine requirement fulfillment in embryonic growth therefore resulting in heavy hatching weights in these injections. Besides that, weight difference is also determined by differences in the content of hatching egg white and content of yolk, which are used as nutrients for embryonic development (19). Another thing that affects the hatching weight is the metabolic rate and the inclusion of egg yolk in the body which can be used for cell growth and therefore affecting the weight of hatch.Fig.4 shows the influence of amino acid injections as the main factor in increasing the weight of embryos. The results of this study showed that injection of the amino acid leads to the heaviest weight. This could be because lysine and methionine supplementation through eggs can meet the needs of growing embryonic cells, therefore increasing embryo growth and results in a higher weight of hatching .The hatching weights of eggs injected with amino acids were 10,73% higher compared to untreated eggs (control).

CONCLUSIONS

Based on the results and discussion, it can be concluded that:

1. Amino acid injection (in ovo feeding) can increase on weight of embryo and hatching eggs.
2. Timing of amino acid injection on day 14 of incubation is more effective compared to day 7.
3. Type of amino acid injection, injection time and interaction between amino acid type with the time of injection had no significant effect (P> 0,05) on the weight of albumen

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