Embryo development of hatching eggs of superior local broiler (ALPU) with Arab native chickens (Kamaras)

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Abstract. Efforts to improve the quality of local chickens, especially free-range chickens, are carried out by improving genetic traits, namely through a strict selection method to produce broilers with the name of Superior Local Broilers (ALPU) and laying purposes with Arab Kampung Chickens (Kamaras). This study was conducted to obtain information on the comparison of ALPU and Kamaras embryo development for genetic improvement as the basis for further research. This study used 105 ALPU hatching eggs and 105 Kamaras hatching eggs, the observations were carried out for 21 days. Eggs were collected for 7 days and then put into the incubator, then observed every day by breaking each 5 ALPU and Kamaras eggs to see 7 parameters of embryo development in the form of: body weight, body length, head circumference, neck length, beak length, wing length and leg length. The results showed that there was no significant developmental difference between ALPU and Kamaras embryos. However, the embryo weight and beak length of Kamaras tended to be larger than those of ALPU, while the head circumference and wing length of ALPU tended to be larger than that of Kamaras. In conclusion, the embryonic development between ALPU and Kamaras showed relatively similar embryo development during the hatching period.

1. Introduction

Indonesian people tend to like native chicken compared to purebred chicken because the meat is chewy, and the fat content is low [1]. Domestic chicken needs have not been met by native chicken farms in Indonesia because of its maintenance on a small scale business, limited environmental conditions, low productivity, slow growth, and the nature (incubating and nurturing) that has not been lost [2;3;4].

Efforts to improve the quality and productivity of local poultry, especially native chickens, are still in the development stage. The development is by conducting a strict selection whose offspring are superior broilers called Superior Local Broilers (ALPU) and crossing with two other breeds of chickens, namely broilers and arabic chickens whose offspring, among others, are called Kamaras chickens as laying type chickens. In general, Arabic chicken eggs are more like native chicken eggs, but the level of egg production is higher than that of native chicken eggs. The results of this cross were expected to eliminate unwanted traits, increase productivity, and accelerate the growth of local chickens. The purpose and objective of this cross between chicken breeds is to improve the quality and productivity of local chickens (village chickens).

Research on the growth and development of embryos in local chickens from selection and crosses has not been done much. Therefore, it is necessary to conduct this study to find out how to compare the
growth and development of embryos in hatching eggs of ALPU and Kamaras chickens in an effort to complete the data as information for further research.

2. Materials and methods

2.1. Place and time of research
The research was conducted at the Laboratory of Science and Technology of Poultry Production and the Laboratory of Animal Breeding and Reproduction, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh. The study was conducted for 21 days of observation.

2.2. Research tools and materials
The equipment used is an automatic incubator of the Oxtagon brand made in Japan with a capacity of 200 grains, stereoscopic electric microscope, AND GR202 digital electric scale, digital caliper, measuring ruler with a capacity of 30 cm, tweezers, scissors, petri dishes, measuring cups, surgical equipment, and digital camera. The hatching eggs collected from breeders at the Animal Husbandry Field Laboratory (LLP) for 7 days amounted to 105 eggs each, ALPU and KAMARAS. The materials used are formalin 40%, KMnO₄, aquadest, alcohol, cotton, and marker materials to clarify the measurement sample, namely: neutral red dye 0.5%, hematoxylin eosin (HE), neutral buffer formalin 5% and neutral buffer formalin 10%.

2.3. Sampling technique
The chicken eggs collected were selected based on the criteria for hatching eggs, then the shells were cleaned, the eggs were weighed, then incubated at 37-380 Celsius with a humidity of 60-70%. Samples of chicken embryos were collected based on the stages of embryo development starting at 12 hours of age until the eggs hatched at the age of 21 days. At the early age of 0 to 4 days of embryo development, albumin was removed from the egg and then the embryo was stained with 0.5% neutral red to provide visuals of the somites for analysis and to provide color contrast to the embryo's body tissues.

Local chicken embryos from the age of 4-21 days during the hatching period were removed from the eggshell then cleaned and separated from the extra-embryonic membrane, then washed with physiological NaCl solution and stained with hematoxylin eosin (HE) to provide a contrasting picture of the development of embryonic organs. The washed embryos aged 4-15 days were then fixed with 5% neutral buffer formalin. After 15 days of hatching age, 16-21 days of age, they were fixed in 10% neutral buffer formalin to minimize shrinkage and then analysed. Body size analysis of embryos over the age of 4 days was carried out by weighing and measuring body length, from the cranium following the vertebral groove to the tip of the tailbone, head circumference and neck length, beak size from nostril to anterior end of beak, wing length and leg length. Measurements were made at the age of 6 days.

2.4. Research variable
Embryo body weight, embryo body length, embryo head circumference, embryo neck length, embryo beak length, embryo wing length and embryo leg length. The size of embryo development was obtained by weighing the weight of the embryo using a digital scale and the unit of embryo weight was grams. Others, namely head circumference, neck, beak, body length, wings, legs were measured using a calliper with units of millimetres. Measurements using a thread tool so that it can follow the curves of the embryo's body easily, after that it is measured using a calliper, but before that the thread is stretched on paper and marked to make it easier to measure.

2.5. Data analysis
The data obtained were tabulated and the mean, covariance value and standard deviation (SD) were determined using SPSS software for quantitative data. Qualitative data were analysed descriptively.
3. Results and discussion

3.1. Embryo weight development
Embryo body weight and development can be seen in Figure 1. The figure showed that Kamaras and ALPU chickens based on statistics achieved significantly different weights ($P<0.05$) on several days of observation. On observation after 12 days Kamaras had a significantly higher embryo weight than ALPU.

![Figure 1. Embryo weight development (grams).](image)

Embryo weight is a measure used to determine the growth rate of embryo development during the incubation period by weighing the total weight of the embryo during the incubation period for 21 days. In this study, the observation of embryo weight was carried out on day 4 to day 21 of the incubation period. Observation of embryo weight showed that Kamaras chicken had superior embryo growth based on its weight. This can be seen in Figure 1, the growth of Kamaras chickens began to look superior on the incubation period of 13-21 days with an average weight of $8.30 \pm 0.501$ g and the weight of ALPU chicken embryos of $7.01 \pm 1.022$ g then on day 21 the weight of Kamaras chicken embryo was still superior to alpu chicken, which was $31.86 \pm 1.795$ g.

The avian embryo develops outside the mother’s body. Nutrient intake for the embryo is also only obtained from albumin in the egg to develop [5]. The embryonic period to develop is 21 days. At this time, it is necessary to pay attention to the state of the egg environment. There are three important factors that influence the development of the embryo during incubation, namely temperature, humidity, and the constituent substances of the egg itself [6]. Therefore, in this study an egg incubator was used to replace the broodstock. In addition to the environment, breed also influences the development of the embryo. For example, White Leghorn chickens develop faster than Barred Plymouth Rock chickens [7].

3.2. Development of embryo body length
Body size analysis of embryos over the age of 4 days was carried out by measuring the length of the body from the cranium following the vertebral groove to the tip of the coccyx. The early development of the embryo starts from the blastulation process where at the end of the process of cleavage the balstomeric mass will form the basis of a prospective body called a blastula [7].
The results showed that the embryo of Kamaras chicken grew faster based on length, seen the difference from the beginning of the incubation period to the end of the incubation period, the body size of Kamaras chicken was 75.22 ± 1.565 mm and ALPU 74.25 ± 1.321 mm. The development of the Alpu and Kamaras chicken embryos in this study appeared to be larger than the body length of the super Java chicken embryo. At the age of 15 days, the body length of the Alpu and Kamaras chicken embryos respectively were 56.75 ± 2.651 mm and 61.45 ± 2.121 mm, the Java super chicken embryo was smaller at the age of 15 days of incubation 48 mm [8], but the Arab chicken embryo looks bigger, reaching 67.20 mm [9].

3.3. Development of embryonic head circumference

Entering the age of 18 hours of incubation the head folds began to be clearly visible, the embryo had begun to enter the transition stage at the age of 23 hours of incubation, when the anterior head folds and somites began to be clearly seen. In embryos aged 25 hours, the head folds are very clear [10]. Measurement of head circumference of chicken embryos can be measured on the 4th day of the incubation period.

The development of the head circumference of the Kamaras chicken embryo at the beginning of the incubation period until the 19th day was faster than that of the ALPU chicken embryo. Then on the next day until the end of the incubation period, the average head circumference of ALPU chickens increased rapidly. Where there was a very clear difference in size between the two samples in this study, namely the 20th and 21st days. In sequence, Kamaras chickens have a smaller average head circumference,
namely $58.46 \pm 1.143$ mm and $59.87 \pm 1.481$ mm, while ALPU chickens had a larger head circumference, namely $59.32 \pm 1.658$ mm and $62.78 \pm 2.259$ mm.

3.4. Embryonic neck length development

The average neck length of the embryos of the two chickens, namely ALPU and Kamaras chickens, in this study began to be measured at the incubation period of days 4-21.

![Figure 4. Development of embryonic neck length (mm).](image)

The development of the neck length of the two types of chickens was relatively the same from the beginning of the incubation period until the 14\textsuperscript{th} day. After that, there was an unequal increase from days 15-17, Kamaras chickens had a longer neck size ($16.46 \pm 0.444$ mm, $17.37 \pm 0.307$ mm and $18.31 \pm 0.850$ mm, respectively) than ALPU chickens (namely $14.22 \pm 1.534$ mm, $15.03 \pm 1.894$ mm and $16.75 \pm 1.198$ mm). Then at 18\textsuperscript{th} day, the development of the embryonic beak of Kamaras chicken began to slow down until the end of the incubation period, the beak size of the embryos of ALPU and Kamaras chickens became relatively the same.

3.5. Embryonic beak length development

The next parameter was the length of the embryo's beak. In Figure 5, the beak length of the ALPU chicken embryo was smaller and its growth was slower than that of the Kamaras chicken embryo.

![Figure 5. Development of embryonic beak length (mm).](image)

The growth of the beak of the ALPU chicken embryo appears to have started to increase during the incubation period of 13-15 days, then the embryonic beak of the Kamaras chicken appears to start to match on the 16th day which gradually remains the same until the end of the incubation period, which
is the 21st day. On day 20th, the beak size of the embryo decreased. In this phase, the beak of the chicken embryo decreases in size due to the exfoliation of the periderm layer [11].

The development of the embryonic beaks of the two chickens in this study was in accordance with previous studies on super-Javanese chickens aged 12-14 days, which stated that a 12-day-old chicken embryo had a beak length of 3.1 mm, an age of 13 days of 3.5 mm, and an age of 14 days the beak length increases to 4 mm.

3.6. Embryo wing length development

Embryo development can be observed through changes in the length of the wings at the beginning of its development. Wing development can be used as a marker to estimate the age of the embryo in chickens. The stages of development on the wings are elongated, curved and then clearly visible parts [11].

![Figure 6. Development of embryonic wing length (mm).](image)

The results showed that Kamaras chicken embryos had faster wing growth than ALPU chicken embryos from the beginning of the incubation period until the 19th day, which was 37.78±1.118 mm compared to 35.78±0.712 mm. However, when entering the end of the incubation period on day 20th, the growth of the wing size of the ALPU chicken embryo increased rapidly by 43.06±1.488 mm and the Kamaras chicken slowed down to 39.61±0.724 mm. At the age of 15 days of incubation, the wing length of the ALPU chicken embryo was 24.73 mm and the Kamaras was 31.13 mm, while in another study it was reported that the wing length of the Java super chicken embryo was only 17 mm [8], and the Arabic chicken was 31.5 mm higher [9].

3.7. Embryonic leg length development

The measurement of the leg length of the ALPU and Kamaras chicken embryos was carried out on the 4th day of incubation, this was because the embryonic development of limb buds had begun to be seen and had a size that was longer than the width of the body and soles of the feet. After 5 days of incubation, chicken embryos already have three fingers and limbs that continue to elongate [11].

![Figure 7. Development of embryonic leg length (mm).](image)
The embryo leg length parameter showed the same results at the end of the incubation period, namely on the 21st day 47.82 mm in ALPU chickens and 47.73 mm in Kamaras chickens. However, it can be seen that the difference in the length of the embryo's legs occurred during the 9th, 12th and 13th day of incubation. Then, compared with previous studies on super-Javanese chickens [8] on the 15th day of incubation, the embryonic leg length was 29 mm, this result was smaller than the leg length of the ALPU and Kamaras chicken embryos, which were 32.95 mm and 35.57 mm, respectively. However, the results in this study were still smaller with the leg length of the Arab chicken embryo, which was 48.5 mm [9].

The percentage of embryonic development can be seen in Table 1. In several parameters measured, Kamaras chicken were superior for embryo weight, body length, head circumference and neck length. While the ALPU chicken was superior in beak length, wing length and leg length. However the difference was not too big.

Table 1. Percentage of embryonic development of ALPU and Kamaras chickens.

| Parameters         | ALPU chickens | Kamaras chickens |
|--------------------|---------------|------------------|
| Embryo weight      | 83.23%        | 83.74%           |
| Body length        | 50.17%        | 52.11%           |
| Head circumference | 31.42%        | 31.98%           |
| Neck length        | 55.17%        | 57.07%           |
| Beak length        | 75.61%        | 73.65%           |
| Wing length        | 56.85%        | 51.72%           |
| Leg length         | 57.92%        | 53.65%           |

ALPU chickens and Kamaras chickens showed the same development, this was because the feed treatment was not different, so the percentage growth rate did not differ. In another study that used these two types of chicken, it also did not produce differences in egg fertility quality due to differences in egg weight [12].

Figure 8. Graph of percentage (%) of embryo development between ALPU and Kamaras chickens.

Different egg quality will cause different embryo development, there is a linear relationship between chicken weight and egg weight [13]. The treatment of feed technology during embryogenesis will also affect the development of the embryo [14]. Meanwhile, in this study, the egg weights of both ALPU and Kamaras chickens did not differ, resulting in relatively the same embryo weight.
4. Conclusion
The embryonic development of Alpu and Kamaras chickens was relatively similar.

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