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Morphoanatomy and size of male Alabio ducks (*Anas platyrhynchos*) reproductive organs of starter period

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ABSTRACT. The testes are vital for spermatogenesis and steroid hormone production, thus serving as a critical biomarker for monitoring testicular function through organ development analysis. Until now, there has been no information on the morphoanatomical development of testicular organs in day-old Alabio ducks (DOD). This knowledge is crucial for optimizing reproductive strategies and manipulations. This study aimed to determine the growth and morphoanatomical development of testes in Alabio ducks during the starter period. A purposive sample of forty DOD male Alabio ducks was observed and measured weekly for eight weeks. Variables included testicular morphoanatomy, weight, liver weight, gonadal-somatic index (GSI), and hepatic-somatic index (HSI). Results indicate that bean-shaped, creamy-white testes are located in the abdominal cavity near the spine, attached dorsally and anterior to the kidneys. Testicular weight, length, reproductive tract weight and length, and GSI peaked at eight weeks (0.768 ± 0.06 g, 1.73 ± 0.20 cm, 0.79 ± 0.02 g, and 13.32 ± 0.65, respectively; $p < 0.01$). Testicular weight, liver weight, GSI, and HSI differed significantly weekly ($p < 0.01$). In conclusion, testicular weight in Alabio ducks increases steadily during the first eight weeks, with bean-shaped testes developing bilaterally in the abdominal cavity, attached dorsally and constrained by the mesorchial ligament.

Keywords: Alabio duck; gonadal-somatic index; morphoanatomy; South Kalimantan; testicular weight

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INTRODUCTION

Ducks have the potential to be developed and are useful as a source of protein in human diet (Rirgiyensi *et al.*, 2014; Yuriwati *et al.*, 2016; Fouad *et al.*, 2018) both as producers of meat or eggs (Kuru *et al.*, 2023). National duck egg production has been steadily climbing in recent years. Output figures indicate a consistent upward trend, with production reaching 0.344 million tons in 2021, increasing to 0.349 million tons the following year, and projected to hit 0.358 million tons in 2023 (BPS, 2024). The Alabio duck (*Anas platyrhynchos*), a local duck whose natural habitat found in Mamar Village, South Kalimantan, is a significant contributor to national duck egg production, with reported annual egg yields reaching 287 per bird (Prasetyo *et al*., 2016).

Efforts to develop duck breeds can be achieved through selection programs and effective reproductive management (Asiamah *et al*., 2020; Arias-Sosa & Rojas, 2021). Proper reproductive management can address the challenge of poor-quality breeding stock prevalent in Indonesia (Brata *et al*., 2020; Widyas *et al*., 2022). Good reproduction will lead to a significant increase in the number of livestock. Investigating the reproductive organ development of male ducks is essential given their substantial influence on offspring quality (Sheppard *et al*., 2013; Qomar *et al*., 2017). The reproductive quality of drakes is determined by the process of spermatogenesis in the testes. Spermatogenic activity can be evaluated using several approaches, including testicular organ development, gonad somatic index (GSI) values, and testicular histology.

The testes are responsible for producing spermatozoa, seminal plasma, and testosterone (Santiago-Moreno & Blesbois, 2020; Estermann *et al*., 2021). Testicular development can be monitored through morphoanatomical studies and size changes, which correlate with spermatogenic activity. Research on testicular development, body weight, morphometrics, and histology has been conducted on Muscovy ducks (Gerzilov *et al*., 2016; Bai *et al*., 2020; Li *et al*., 2020), turkeys (Parvez *et al*., 2023; Baş *et al*., 2024), Bali ducks (Adiari *et al*., 2024), Mule ducks (Pradipta *et al*., 2014), and Mojosari ducks (Adikara *et al*., 2017; Qomar *et al*., 2017). However, until now there has been no research that examines the morphoanatomy and testis size of Alabio ducks during the stater period.

The starter period represents a critical stage for ducks, influencing subsequent growth and reproductive performance. Optimal early growth is essential for achieving desired body weights in the grower and adult phases, ultimately impacting reproductive output. It can be monitored through testicular morphoanatomy development. However, information on the development of testicular morphoanatomy in Alabio ducks has not been studied. Thus, our study aims to explore the morphoanatomy, size, and development of testes in Alabio ducks during the starter period. Such knowledge will contribute to early identification of superior male candidates and provide foundational data for enhancing reproductive management strategies in this breed.

MATERIALS AND METHODS

Sample preparation. The samples used in this study were 40 male Alabio ducks of DOD age. Ducks were kept in cages equipped with electric heaters for eight weeks. The feed provided includes commercial starter-period chicken feed with a nutritional composition of 13% water, 19-21% protein, 7% fat, 4% crude fiber, 7% ash, 0.9-1.1% calcium, and 0.6-0.9% phosphorus. The research method used is an observational survey method with a purposive sampling technique. Samples were taken from 5 ducks every week. All ducks are maintained with the same system and type of feed. Lighting during the study is given in the same portion. Feed is placed on the edge of the tray then a drink is placed in the middle of the tray with ad-libitum feeding. Rations are given twice a day in the morning and evening. The feed given to ducks refers to Prasetyo (2006). Lighting during the study is given in the same portion.

GSI and HSI value measurement. Body weight was measured by weighing using a digital scale with an accuracy of 0.01 g. Then the testicles and liver were isolated by first cutting the duck using the Kosher method, namely cutting the carotid artery, jugular vein, and esophagus without anesthesia. The ducks were then placed in a paraffin bath for surgery and organ samples were taken. The ventral surface of the animal was opened and the topography of the inside was examined, Then the reproductive organs were isolated to measure the gonad weight. The male gonads that have been weighed are then used to determine the GSI, with the formula (Guettaf & San Martin, 1995):

$$\text{GSI}=\frac{Weight\ of\ gonad\ (\text{g})}{Weight\ of\ body\ (\text{g})} \times 100\%$$

The liver taken is used to calculate the HSI value, with the following formula (Guettaf & San Martin, 1995):

$$\text{HSI}=\frac{Weight\ of\ liver\ (\text{g})}{Weight\ of\ body\ (\text{g})} \times 100\%$$

Data analysis. Data in the form of GSI, HSI and testicular weight and tract, testicular length and tract, were analyzed by one-way analysis of variance (ANOVA) at a 95% confidence level using SPPS ver. 23, if significantly different then an LSD follow-up test would be carried out. The relationship between parameters was analyzed using a correlation test.

RESULTS AND DISCUSSION

Macroscopic observations of male Alabio ducks include shape, size, weight of testicles, and reproductive tract. The testicles are shaped like beans, creamy white in color, and are located in the body cavity near the spine, attached to the dorsal part of the abdominal cavity or just behind the lungs and in front of the kidneys (Fig. 1). Data on body weight, testicular weight, and length, reproductive tract weight, and length, GSI, and HSI of Alabio ducks are presented in Fig. 2.

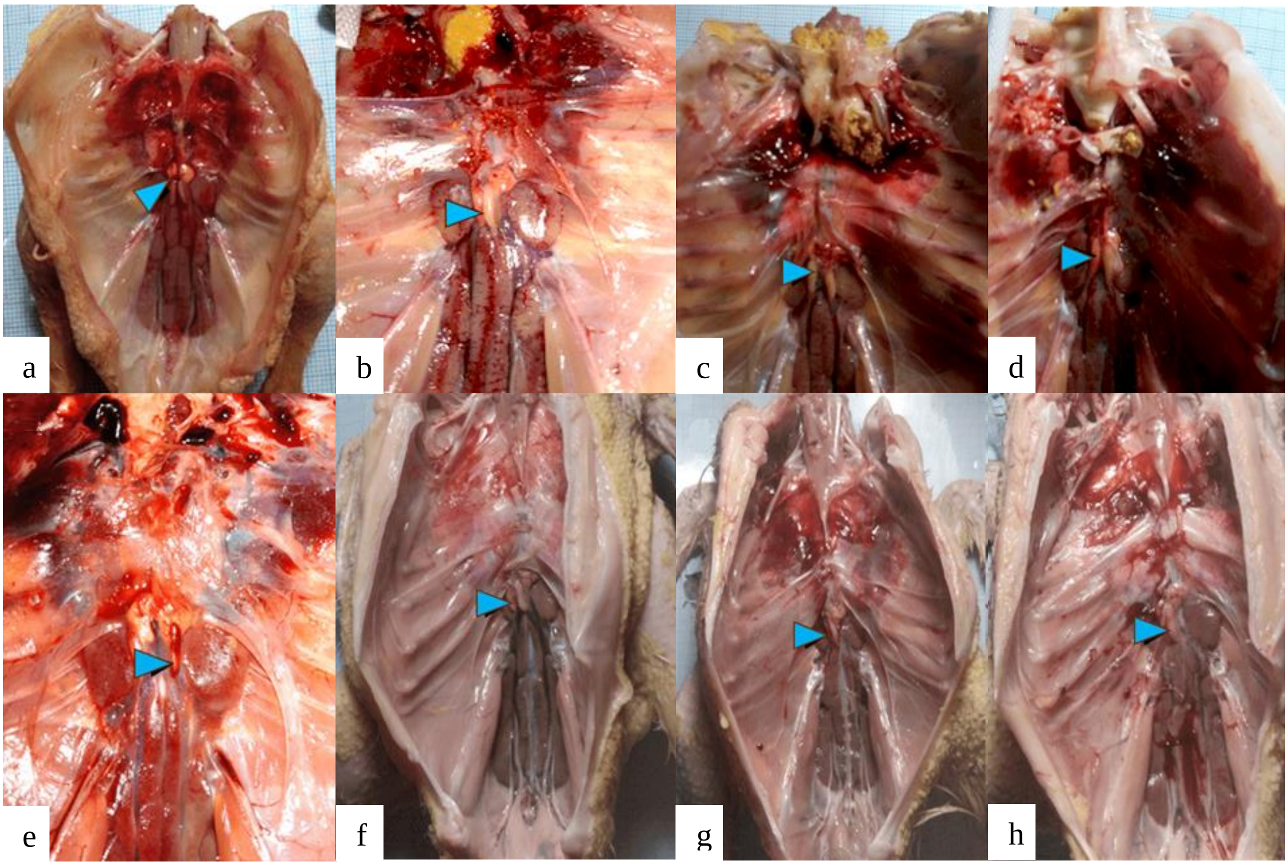

Fig. 1. Topography of the testes in the abdominal cavity of various ages: a. Testicular topography and size at 1 week old; b. Testicular topography and size at 2 weeks old; c. Testicular topography and size at 3 weeks of age; d. Topography and size of the testes at 4 weeks; e. Testicular topography and size at 5 weeks; f. Topography and size of the testes at 6 weeks; g. Testicular topography and size 7 weeks; h. Testicular topography and size at 8 weeks. Blue arrows indicate the position of the testicles

The location of the testicular organs during the starter period was visualized in Fig. 1. Topography of the testes of Alabio ducks is located in the abdominal cavity near the spine attached to the dorsal side, limited by the mesorchium ligament and just behind the lungs and in front of the kidneys. Judging from the topography, the testes are in a normal location and this topography is also found in other local ducks (Kadhem, 2014). The position of the testicles is just posterior to the lungs in front of the kidneys and close to the aorta and vena cava. The morphoanatomy of the testis is shaped like beans and creamy white in color. Even though the testicles are shaped like beans, the shape of the right and left testicles is asymmetrical. This asymmetric shape causes the difference in weight between the right testicle and the left testicle. Asymmetrical shapes on the right and left testicles are found in birds (Mizia *et al.*, 2023), Mallards ducks (Petnehazy *et al.*, 2024), turkeys (*Meleagridis gallopavo*) (Noirault *et al*., 2006), local ducks (*Anas platyrhynchos*) (Kadhem, 2014), Muscovy duck (*Cairina moschata*) (Gerzilov *et al*., 2016), and Bali duck (Adiari *et al.*, 2024). The provision of commercial feed did not show any differences in testicular location, testicular position, color and testicular shape of the samples observed.

The results of calculating the body weight of male Alabio ducks at 8 weeks varied (Fig. 2). The average body weight of male ducks at the beginning of rearing (M1) was 85.6 ± 11.44. During maintenance, the average body weight of male ducks increased until the eighth week, namely 1047.25 ± 88.61. The results of statistical analysis showed that the body weight of male Alabio ducks was very significantly different during the starter period ($p<0.01$). The average body weight of ducks increased with rearing time during the starter period.

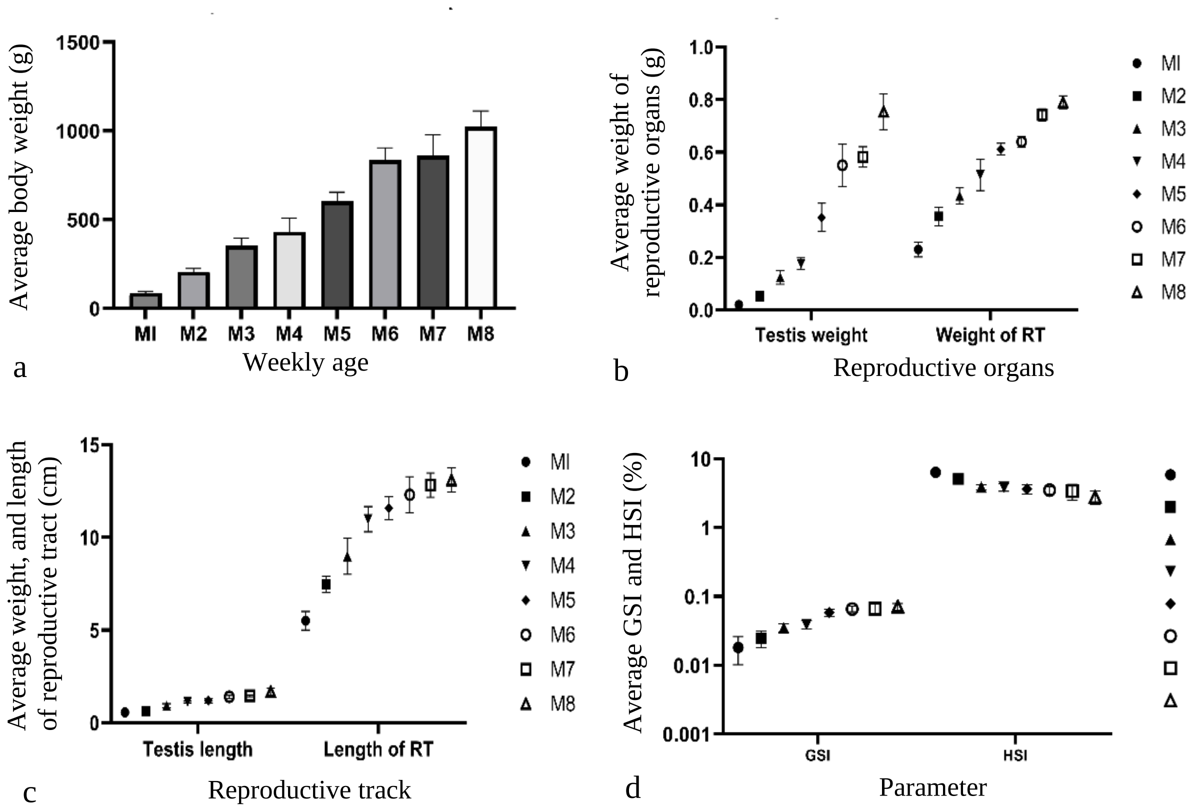

Fig. 2. The parameter measurement of male Alabio ducks during the starter period: a. body weight; b. weight of reproductive organs; c. length of reproductive organs; d. GSI and HSI value

The average weight and standard deviation of male Alabio ducks in our study (Fig. 2a) were higher than those of male local ducks aged 55-60 days (1088 g) (Ariana *et al*., 2023) and male Magelang ducks at 8 weeks (876.70 ± 43.28 g) (Purwantini *et al*., 2016). However, at relatively the same age, the body weight of Alabio ducks is lower than that of Peking ducks, about 1303.8 ± 28.4 at 7 weeks of age (Kuru *et al*., 2023) and 2796.9 until 2930 g at 12 weeks of age (Arias-Sosa *et al*., 2023). The difference in weight between Alabio ducks with other ducks is influenced by genetics (Xi *et al.*, 2023), feed (Gadde *et al*., 2017), and environmental factors (Goo *et al.*, 2019). Genetically, the same gene insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1) has been identified in ducks as the gene responsible for influencing duck body size (Zhou *et al*., 2018). Subsequent research also conducted the genome-wide association study (GWAS) on Peking ducks and identified quantitative trait loci (QTL) related to growth and feeding efficiency (Zhu *et al*., 2019). A subsequent study reported that 41 single nucleotide polymorphisms (SNPs) significantly related to growth rate were identified. In addition, development and differentiation-enhancing factor 1 (ASAP1) and LYN proto-oncogene, Src family tyrosine kinase (LYN) genes are essential candidate genes affecting the duck growth rate (Xi *et al.*, 2023). Feed that is equipped with appropriate probiotics, prebiotics, synbiotics, organic acids, enzymes, phytogenics, antimicrobial peptides, hyperimmune egg antibodies, bacteriophages, clay, and metals can improve growth performance and feed efficiency (Gadde *et al.*, 2017). Next, heat stress and stocking density are key environmental factors decreasing poultry performance (Goo *et al.*, 2019). The housing system (deep litter) or deep litter with swimming pond (DLSP) influences the productive performance of ducks, and ducks in The DLSP birds have greater live weight, weight gain, and feed conversion ratio (Krunt *et al.*, 2023). By providing feed that is adjusted to needs, the weight performance of Alabio ducks is better than other ducks. The growth performance of male Alabio ducks can be optimized by providing the energy/protein ratio at the ratio of 17.1 gives the best result on the performance of male Alabio ducks including the achievement of body weight, body weight gain, feed intake, feed conversion ratio, and carcass quality through the achievement of increasing carcass percentage, decreasing carcass fat and abdominal fat

(Biyatmoko *et al.,* 2021). Thus, monitoring the average weight of male Alabio ducks is sufficient to provide opportunities for intensive cultivation.

The size of the testicles in Alabio ducks increases during the starter period. This can be seen from its weight and length. The results of calculating the testes weight and length of Alabio ducks for 8 weeks varied (Fig. 2b). The average testicular weight and testicular length were lowest in week 1 (0.02 ± 0.01 g and 0.56 ± 011 g) then increased and were highest in week 8 (0.768 ± 0.06 g and 1.73 ± 0,20). The results of statistical analysis showed that the testis's weight and length of Alabio ducks were highly significant during the starter period ($p<0.01$). Changes in testicular weight from the pre-starter stage (1-4 weeks of age) to the starter stage (5-8 weeks of age) (Firwan *et al*., 2020) show that age influences changes in testicular morphology (Gerzilov *et al*., 2015).

The weight and length of the reproductive tract of Alabio ducks increased with time. The results of calculating the weight and length of the male Alabio duck reproductive tract for 8 weeks varied (Fig. 2c). The average weight and reproductive tract length were lowest in the first week (0.23 ± 0.02 g and 5.56 ± 0.5 g) then increased and were highest in the 8th week (0.79 ± 0.02 g and 13 .32 ± 0.65). The results of statistical analysis showed that the weight and length of the reproductive tract of male Alabio ducks were very significantly different during the starter period ($p<0.01$). Over time, the maintenance of the weight and size of the testes continues to increase, this indicates that the cell mass and volume of cells that make up the testes are increasing (Fig. 2). Duck body weight was positively correlated with testicular length, testicular weight, testicular diameter, testicular volume, and sperm production (Sun *et al.,* 2019; Mfoundou *et al.,* 2022; Chang *et al.,* 2023). Testicular weight is an indicator of the developmental activity of the seminiferous tubular cells. Which are the main components of the testis consisting of spermatogenic cells, Sertoli cells, and Leydig cells (Chu *et al*., 2023). Increasing testicular size, and testicular volume causes increased spermatogenic activity (Pradipta *et al*., 2014).

The GSI increased with the 8 weeks of maintenance and was followed by a decrease in the HSI values and the mean of the two for 8 weeks varied (Fig. 2d). The lowest GSI mean was at week 1 (0.02 ± 0.007%) and highest at week 8 (0.08 ± 0.004%). The average GSI is inversely proportional to the HSI where the HSI is lowest at week 8 (2.737 ± 0.5%) and highest at week 1 (6.335 ± 0.9%). The results of statistical analysis showed that the GSI and HSI of male Alabio ducks were highly significant during 8 weeks of rearing ($p<0.01$), and the GSI and HSI of male Alabio ducks were significantly different during the grower and early maturity periods ($p<0.05$). Kalwar *et al*. (2019) stated that the development of testicular anatomy is positively correlated with the condition of the seminiferous tubules, thus testicular anatomy can be utilized as an indicator of an individual's reproductive activity. An increase in the GSI value is associated with the process of spermatogenesis and an increase in the volume of the seminiferous tubules (Uno *et al.,* 2014; Golpour *et al.,* 2018). The higher GSI value with increasing age indicates that the testicular weight is getting heavier followed by an increase in testicular volume (Table 1). In this way, it is also possible that the volume of the seminiferous tubules will increase and spermatogenesis activity will increase towards the production of spermatozoa. The number of spermatozoa produced is influenced by testicular weight (Sharma *et al.,* 2015; Rahban & Nef, 2020, Madian *et al.,* 2023).

HSI is a comparison between the weight of the liver and the weight of the whole duck. A smaller HSI over time indicates the activity of the liver tissue in transferring cholesterol to the testicular tissue, which will be used for steroid hormone synthesis. Testosterone is a steroid hormone that plays a role in sexual development in male individuals (Ramya *et al.,* 2023), which functions in the process of spermatogenesis, total production of spermatozoa, normal sperm morphology percentage (Grande *et al.*,2022; Madian *et al.,* 2023), and development of external reproductive organs and secondary sex characteristics of males (Murawska *el al.,* 2019). The correlation between body weight and reproductive organ performance is presented in Table 1.

Table 1. Correlation of body weight with the testicular performance of Alabio ducks in the starter period

Parameter	Correlation coefficient (r)
body weight to testis weight	0.964
body weight to testis length	0.924
body weight to GSI	0.817
body weight to HSI	-0.858
testicular weight to weight of reproductive tract	0.920
testicular weight to length of reproductive tract	0.868
weight of the reproductive tract to the length of the reproductive tract	0.926
GSI to HSI	-0.678

The strong correlation between body weight and reproductive performance (Table 1) suggests a synergistic relationship between somatic cell growth and gamete development in Alabio ducks. Several studies on poultry have reported that increased body weight is followed by testicular weight such as Japanese quail (Foromo *et al*., 2023), chickens (Fragoso *et al*., 2013), and domesticated ducks (Khatun *et al*., 2019). By providing a foundation for future research and applications, our study can contribute to understanding reproductive challenges faced by native duck populations, leading to increased reproductive efficiency and productivity.

CONCLUSION

Our findings indicate that testicular weight in Alabio ducks increases steadily throughout the starter period, peaking at eight weeks. The morphoanatomy of the testes is bean-shaped, numbering two, located on the right and left sides, and develops in the abdominal cavity near the spine attached to the back side and limited by the mesorchial ligament. Further research is recommended to evaluate testicular histology at each period and to assess testicular histomorphometric and male reproductive hormone concentrations to support the results of testicular morphology observations.

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