

I. INTRODUCTION

1.1. Background

Hybrid ducks are the result of a cross between peking ducks and khaki campbell ducks that produce offspring with fast body weight growth and good feed efficiency. Research shows that hybrid ducks have a shorter rearing period, which is about 45 days until they reach optimal harvest weight (Ridwan *et al.*, 2019). These advantages make hybrid ducks the main choice in the broiler duck farming industry in Indonesia. Hybrid duck meat has a more tender texture with lower fat content than local ducks, which is favoured by consumers and increases market demand for this product (Ashshofi *et al.*, 2019). Good adaptation to tropical environments and disease resistance are also important factors in the selection of hybrid ducks as potential commercial poultry (Saputra *et al.*, 2021).

The duck population in Indonesia is spread in various regions, data from the Ministry of Agriculture in 2023 recorded a duck population of 48,425,903 heads spread throughout Indonesia, with the largest concentration on the islands of Java and Sumatra. Ducks are waterfowl that have a significant contribution to the livestock sector, especially in egg and meat production. Duck eggs have high nutrient content, such as protein, fat, and many vitamins. Duck eggs are used as raw materials in various food products such as salted eggs and savour indonesian martabak

(Lestari & Nugraha, 2021). The productive egg-laying ability of ducks is the main attraction, with an average production of 200-300 eggs per year. Ducks have disease resistance and are able to adapt to different environments, such as paddy fields and open water. This rapid adaptation to the environment makes ducks an easy to cultivate and profitable poultry choice (Supriyadi, 2018).

Duck egg production has two main functions, namely as hatching eggs for hatching ducklings and as consumption eggs. Hatching eggs are selected based on size, shape and shell quality, which affect hatching success. Consumption eggs are utilised as a source of high nutrients, such as protein, vitamins and minerals, and can be processed into products such as salted eggs or pindang. While husbandry, feed and environmental management factors strongly influence productivity, good husbandry practices and selection of quality eggs are key to successful production for consumption or breeding (Novitasari dkk., 2021; Prasetyo & Wahyu, 2019).

The lack of nutrients in duck eggs in Indonesia is a major challenge in improving egg quality and hatching success. Lack of essential nutrients such as protein, vitamins and minerals in duck feed has a direct impact on the quality of eggs produced. Eggs with low nutrient content tend to have an unbalanced composition, affecting embryonic development and increasing the risk of disease in hatched ducks (Sukmawati & Hartono, 2020). This problem is caused by suboptimal

feeding practices on many farms where essential nutrient requirements are often not met. Low egg quality results in reduced hatching rates and overall duck productivity (Abd El-Hack *et al.*, 2020).

Hatchability of duck eggs is one of the important indicators in duck farming that reflects the success rate of hatching eggs into ducklings. Hatchability of duck eggs can be influenced by various factors, including egg quality, parent age, and incubation temperature and humidity. The hatchability of duck eggs ranges from 85%-90% under optimal incubation conditions, namely temperatures around 37.5°C-37.8°C with 60%-70% humidity to prevent dehydration of eggs which is a major factor in hatching (Siregar *et al.*, 2020).

Hatching egg optimisation aims to increase hatching success by ensuring ideal conditions during the incubation process. Factors such as temperature, humidity, ventilation and egg rotation play an important role in supporting embryo development. Regular egg rotation ensures even heat distribution and prevents embryo adhesion to the shell. Selection of high quality eggs, such as appropriate weight, shells without cracks, and from healthy parents, also determines success. Modern technologies such as *in-ovo* nutrient injection and digital-based monitoring have expanded optimisation approaches, providing opportunities to improve hatch efficiency and quality (Decuypere & Bruggeman, 2020; Rocha *et al.*, 2022).

Vitamin E and selenium are two essential nutrients that have important roles in maintaining cell health and function. Vitamin E is a group of fat-soluble vitamins that act as antioxidants that protect cells from oxidative damage caused by free radicals. Selenium functions as an important cofactor in antioxidant enzymes such as glutathione peroxidase, which helps reduce oxidative stress in the body. These two nutrients are not only important for adult bird health but also have a direct effect on egg quality. The addition of vitamin E and selenium to feed or through injection in eggs has been shown to improve the nutritional quality of eggs, increase embryo viability, and reduce embryo mortality during incubation (Abd El-Hack *et al.*, 2020).

Injection of vitamin E and selenium into hatching eggs is an innovative method to improve hatching success and chick quality. Their combination can enhance cellular defence capacity during embryonic development, particularly under suboptimal environmental conditions. The *in-ovo* injection technique allows the delivery of nutrients directly into the egg, usually in the late stages of incubation, which ensures high bioavailability and a positive impact on embryo metabolism. Studies have shown that vitamin E and selenium injection can improve hatchability, duckling weight and reduce the incidence of structural anomalies in chicks (Surai, 2016; Karadas *et al.*, 2018; Nowaczewski *et al.*, 2021).

Bursa fabricius in ducks is a lymphoid organ that plays an important role in the development of the body's immune system,

especially in the formation of B lymphocytes that function to produce antibodies. The bursa fabricius of ducks plays a vital role in the immune processes that occur during development. The bursa fabricius is located in the proximal part of the cloaca and has the role of regulating the humoral immune response, which involves the production of antibodies. Early in embryonic development, the bursa begins to form a small protrusion from the posterior cloaca and begins to show epithelial structures. Days 7-10, the bursa undergoes differentiation into organised follicles with epithelial folds (plicae). Colonisation by lymphocytes begins at 11-15 days of age when B-cell precursors from the liver and bone marrow migrate to the bursa and begin to differentiate. By hatching, the bursa reaches its maximum size and functions optimally as a centre for B cell formation (Smith & Beal, 2019). The development of the bursa fabricius is influenced by genetic factors, age, and environmental conditions, which can have a direct impact on the body's resistance to disease. Decreased fabricius bursa function can result in a decreased immune system, making ducks more susceptible to disease infection (Patterson *et al.*, 2015; Leshchinsky & Rattenborg, 2017).

Research on *in-ovo* injection of vitamin E and selenium in poultry eggs has grown rapidly in recent years. Vitamin E and selenium have great potential in supporting the development of the immune system, especially with regard to the structure and function of the bursa fabricius, a major lymphoid organ in poultry. Previous studies have shown that vitamin E

supplementation can help boost immune activity by preserving the structure and function of the bursa fabricius from oxidative stress damage. Selenium, as a component of the antioxidant enzyme glutathione peroxidase, also plays a role in protecting the bursa fabricius from the negative effects of free radicals, thus supporting overall immune function (Surai, 2016). Most of these studies focussed on commercial chicken breeds and were limited to different doses and adding times. Research on the synergistic effect of *in-ovo* injection of vitamin E and selenium in duck eggs is still very limited. More in-depth research on the optimal dose, time of adding, and effect of the combination of vitamin E and selenium in duck eggs is needed to obtain more specific and applicable data in the poultry industry.

1.2. Problem Formulation

Based on the background, the formulation of the problem in this study is how the effect of *in-ovo* injection of vitamin E and selenium on the microanatomy of the bursa fabricius in hybrid duck embryos?

1.3. Research Objectives

The purpose of this study was to analyse the microanatomy of the bursa fabricius of hybrid duck embryos injected with vitamin E and selenium *in-ovo*.

1.4. Research Benefit

The results of this study are expected to be a source of scientific information to the public, especially poultry farmers regarding the *in-ovo*

injection of vitamin E and selenium to improve the performance and productivity of hybrid ducks. Vitamin E and selenium have the potential to become additional nutrients for hybrid duck eggs that are easily available so that hybrid duck farmers can get quality egg products.