



Histomorphometry Thymus Of Hybrid Duck (*Anas Platyrrhynchos Domesticus* L.) After Nd Vaccination And Inclusion Of Moringa Leaf (*Moringa Oleifera* Lam.) Flour As Feed Additive

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Abstract

The health status of poultry is one of the factors that determine productivity. Administration of Newcastle Disease (ND) vaccine and improved nutritional quality can improve thymus health status. This study was designed to determine the histomorphometric of hybrid duck thymus after the ND vaccine and the inclusion of Moringa leaf flour as a feed additive. This study used a complete randomized design

with 32 hybrid ducks aged seven days, divided into four treatments and each treatment consisted of eight replication. The treatment was 0%, 2.5%, 5%, and 7.5% Moringa leaf flour, given for 6 weeks. The removal of the thymus organ was carried out at the end of the study, with ducks being sacrificed by using the halal method, followed by thymus isolation. The thymus organ that has been obtained is then weighed, and stored in a BNF solution. Histological preparation using the hematoxylin-eosin (HE) method. The results showed that Moringa leaf flour with exposure to the ND vaccine had no significant effect ($P > 0.05$) on thymus weight, the ratio of the thymus: body weight, lobular diameter, cortex width, medulla width, and corpuscular diameter. This study concluded that ND vaccination and Moringa leaf flour feed additives inclusion in hybrid ducks could maintain the morphometry of the thymus. This research can be applied, but it is necessary to do immunohistochemistry staining to see cellular changes.

Keywords: Leaf flour, ND vaccine, morphometry of thymus.

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Introduction

Ducks are one of the livestock commodities that act as a source of animal protein for the community. In general, ducks are farmed for egg and meat production. In Indonesia, the demand for duck meat continues to increase along with the development of the agroalimentair industry. However, most duck meat still comes from unproductive female ducks or male ducks raised for 10-12 weeks (Rukmiasih *et al.*, 2015). One of the breeds of ducks that has the potential to be developed as meat ducks is hybrid ducks. Hybridization in ducks is common to produce offspring that have better quality than their parents (Ottenburghs *et al.*, 2016). Hybridization of ducks in Indonesia can result from crossing between local ducks or crossing introduced ducks (Pekin ducks) with local ducks (Agriflo, 2012). The increase in poultry products, including meat production, is determined by various factors, namely nutrition, health status, maintenance management, genetics, and metabolic potential (Starcevic *et al.*, 2021).

The health status in poultry is almost the same as in mammals, it can be judged by the immune response. Ducks have a better immune response than chickens (Budiman *et al.*, 2019). Understanding the work of the immune response in ducks can be done by studying the basic structure of lymphoid tissue. The thymus and bursa Fabricius are primary lymphoid organs derived from the in situ colonization of hematopoietic stem cells, becoming competent producers of T cells and B cells, respectively. The poultry thymus is located in the neck section, parallel to the vagus nerve and the internal jugular vein, and consists of 7-8 separate lobes on each side of the neck, reaching a maximum size of 10-12 mm at the age of 3-4 months (Kaiser and Balic, 2015). Each thymus lobe is divided into the cortex and medulla. The cortex looks darker than the medulla. Young T cells in the cortex will be transferred to the medulla to mature and function actively in detecting various infectious agents that attack the body (Budiman *et al.*, 2019). The size of the thymus is influenced by the lifespan of the poultry. The size of the thymus in newly hatched birds is relatively large and reaches its maximum size in the adult age of the sexes. Thymus involution generally occurs after 4 months of age, depending on the type of poultry (Kaiser and Balic, 2015; Dahariya *et al.*, 2020). The thymus in ducks is involution in adulthood. Thymus involution can also be caused by stress, so animals that die from a long illness have a relatively small thymus size (Amirtaghavi and Hamed, 2019; Schat *et al.*, 2014).

Hybrid ducks have a good immune response like local ducks in general, even so, ducks can still develop diseases at various maintenance ages. To prevent the transmission of the disease, breeders administer vaccines. The type of vaccine that is often given to ducks is the ND (*Newcastle Diseases*) vaccine. ND cases in Indonesia are still high and there is no effective treatment to prevent ND (Pratama *et al.*, 2016; Yuliani and Sakan, 2018). Recognizable signs of ND are



disorders of the respiratory, digestive, and central nervous systems (Agustin and Ningtyas, 2021). Another effort that can be made to prevent ND disease in ducks is to modulate the immune system of ducks by improving feed quality. Sharing plant types contain phytochemicals that have the potential to be immunomodulators (Amir *et al.*, 2014). The Moringa plant (*Moringa oleifera* Lam.) contains various phytochemical components that have benefits for animal and human health. Moringa leaves contain vitamin A, vitamin C, vitamin D, vitamin E, calcium, potassium, and iron (Gopalakrishnan *et al.*, 2016). Moringa leaf flavonoids have hypoglycemic, antibacterial, antifungal, antioxidant, and anti-inflammatory potential (Fidrianny *et al.*, 2021). Moringa leaf flour supplementation can enhance the humoral immune response mediated by the ND vaccine in broilers aged 42 days in broilers (Su and Chen, 2020). The use of Moringa leaves as an additive to poultry feed is still being explored today. Information on Moringa leaf flour as a feed additive for hybrid ducks is still very limited so this study was designed to study the histomorphometry of the thymus of hybrid ducks post-vaccination ND and the inclusion of Moringa leaf flour feed additives.

Methods



Time and place of research, as well as ethical clearance

The research took place from February to April 2022, located in a poultry housing in Banyumanik, Semarang, Central Java, Indonesia. This research has been approved by the Health Research Ethics Commission of the Faculty of Medicine Universitas Diponegoro (UNDIP), No. 04/EC/H/FK/-UNDIP/I/2022.

Experimental animals and research design

The hybrid ducks used are a cross between Pekin ducks (females) and Magelang (males), the amount was 32 ducks, seven days old, obtained from hybrid duck breeders in Pringapus village, Karangjati, Semarang Regency. Research ducks are acclimatized for 1 week in a box cage, after acclimation is completed, ducklings are transferred to a battery cage measuring 45×35×65 cm³. Feed and drinking are provided ad libitum, the feeding times were two times a day in the morning (07.00 AM) and afternoon (03.00 PM), and drinking water is changed daily. Cage sanitation and measurement of cage temperature and humidity are carried out daily. This study used a Complete Randomized Design with four treatments and eight tests each. The concentration of Moringa leaf flour used is 0% (feed without moringa leaf flour), 2.5%, 5%, and 7.5%. The additive application of Moringa leaf flour in the feed is given for six weeks, starting with ducks aged 3 weeks to 8 weeks.



Feed preparation

The feed used in this study was standard commercial feed BR-1 (broiler -1) for ducks aged 1-3 weeks and BR-2 (broiler-2) feed for ducks aged 4-8 weeks. Feedstocks are made once every 1 week. Moringa leaf flour is obtained from the local market, and produced by Flozindo Purbalingga Central Java. Moringa leaf flour with a predetermined concentration is mixed with standard commercial feed BR-1 or BR-2 until homogeneous. The composition of feed that has been mixed with Moringa leaf flour can be seen in Table 1.

Table 1. The Composition and Nutrient Content of Feed for Hybrid Ducks During the Study. 2022

Feed Ingredients	Concentration of Moringa leaf flour in feed (%)							
	Starter period (1-3 weeks)				Finisher period (4-8 weeks)			
	0	2.5	5	7.5	0	2.5	5	7.5
Standard commercial feed	100	97.5	95	92.5	100	97.5	95	92.5
Moringa leaf flour	0	2.5	5	7.5	0	2.5	5	7.5
Total (%)	100	100	100	100	100	100	100	100
Nutrient content								
Metabolic energy (kcal/kg)	2970.20	2947.14	2924.08	2901.01	3040	3016.94	3000.88	2949.81
Crude protein (%)	21.94	22.29	22.64	22.99	18.63	18.98	19.31	19.74
Crude fat (%)	3.55	3.49	3.42	3.35	7.26	7.19	7.11	7.07
Crude fiber (%)	3.56	3.52	3.47	3.43	4.26	4.22	4.12	4.00
Calcium (%)	0.78	0.79	0.79	0.80	0.87	0.87	0.88	0.88
Phosphor (%)	0.51	0.50	0.48	0.47	0.50	0.49	0.47	0.47

The standard commercial feed in this study contains yellow corn, rice bran, meat bone meal (MBM), crude palm oil (CPO), pollard, corn gluten meal (CGM), vitamins, and minerals.

ND (Newcastle Disease) Vaccination

The ND vaccine used is the Medivac ND La Sota vaccine. Hybrid ducks aged 7 days are vaccinated with ND 1 and at 14 days old are vaccinated with ND 2. Administration of the vaccine through eye drops (ocular route) by the protocol listed by the vaccine manufacturer.

Thymus histomorphometry

The organ to which histomorphometric preparations are made is the thymus. The thymus is obtained after the duck is terminated at week 8, that is, by cutting the jugular vein and artery, esophagus and trachea. The thymus was isolated, then weighed to determine the weight of the organ, followed by fixation into 10% BNF (buffer neutral formalin), then preparations were made using the paraffin method and hematoxylin-eosin (HE) staining. The finished histological preparations were then observed using photomicrographs to obtain a histological picture of the thymus. Next, measurements of the diameter of the lobular with the formula (long axis + short



axis)/2, the width of the cortex, the width of the medulla, and the diameter of the corpuscular with the formula (long axis + short axis)/2) were carried out.

Data Analysis

The data obtained were analyzed using a one-way ANOVA at a 95% confidence level. Further tests using the LSD test, and all data analysis was done with the SPSS version 24 program.

Results and Discussion

The morphometry of the hybrid duck thymus after ND1 and ND2 vaccinations, as well as given feed additives of Moringa leaf flour did not change. Thymus weight, ratio of thymus: body weight, lobular diameter, cortex width, medulla width, and thymus corpuscular diameter showed no significant difference ($P > 0.05$; Table 2) in all treatment groups. The thymus of hybrid ducks in this study was relatively the same in all treatment groups, this condition indicated that the administration of ND1 and ND2 vaccines accompanied by Moringa leaf flour feed additives did not interfere with the development of the thymus, and did not cause involution (atrophy) of thymus size.

Table 2. The Thymus Morphometry of Hybrid Duck Vaccinated with ND1-2 and Inclusion of Moringa Leaf Flour as Feed Additive, 2022

Parameters	Moringa Leaf Flour Concentration (%)			
	0 ($\bar{x} \pm SD$)	2.5 ($\bar{x} \pm SD$)	5 ($\bar{x} \pm SD$)	7.5 ($\bar{x} \pm SD$)
Thymus Weight (%)	1.05 $\pm$ 0.19	1.01 $\pm$ 0.18	1.02 $\pm$ 0.14	1.16 $\pm$ 0.07
Ratio of thymus: Body Weight (gr)	1.72 $\pm$ 0.26	1.66 $\pm$ 0.26	1.69 $\pm$ 0.21	1.87 $\pm$ 0.07
Lobular Diameter(mm)	1.64 $\pm$ 0.09	1.41 $\pm$ 0.24	1.67 $\pm$ 0.05	1.59 $\pm$ 0.02
Cortex Width (mm)	0.44 $\pm$ 0.03	0.43 $\pm$ 0.05	0.44 $\pm$ 0.04	0.45 $\pm$ 0.04
Medulla Width (mm)	0.49 $\pm$ 0.01	0.42 $\pm$ 0.07	0.39 $\pm$ 0.04	0.44 $\pm$ 0.01
Corpusculum diameter (mm)	0.86 $\pm$ 0.02	0.87 $\pm$ 0.04	0.84 $\pm$ 0.00	0.87 $\pm$ 0.02

Description: The data obtained did not differ markedly ($P > 0.05$) on all treatments; the data displayed is an average ($\bar{x}$) $\pm$ standard deviation (SD)

The relatively same size of the thymus indicates that the health status of ducks is at the same level. Nutrition plays an important role in maintaining the health status of animals. Moringa leaf flour feed additives contain phytonutrient components, namely beta-carotene, vitamin C, and vitamin E which have the potential to be antioxidants. In addition, moringa leaf flour also contains polyphenols which also act as natural antioxidants that capture free radicals and reduce oxidative enzymes in the thymus. Phytonutrients in Moringa leaves apart from being antioxidants also play



a role in increasing the immune response through mRNA regulation in immune response mediators IL2 (interleukin 2) and IL6 (interleukin 6) (Jacques *et al.*, 2020). IL2 is produced by CD4+, important in improving the proliferation of T cells and B cells, as well as the regulation of cell communication to activate the immune response (Erniati and ezraneti, 2020). Rachmawati and Rifa'i (2014) found Moringa leaf extract at various doses can increase the production of CD4+ (T-helper cells) and CD8+ (T-cytotoxic cells). CD4+ and CD8+ are marker molecules that are present on the surface of activated T cells. T-cell activation can improve antigen elimination.

A histomorphometric overview of the thymus of hybrid ducks exposed to the ND1-ND2 vaccine and given moringa leaf flour feed can be seen in Figure 1. The administration of vaccines and the addition of moringa leaf flour in the feed did not provide a noticeable histological difference in all treatments. The thymus is divided into several lobules bordered by trabeculae shaped like connective tissue. The lobular has a part of the cortex and medulla, in the HE coloring of the cortex, it appears to have a darker color and is located at the edges, while the medulla has a lighter color and is in the deeper part of the cortex. Budiman *et al.* (2019) suggest that the poultry thymus has a characteristic that mammals do not have, namely in the medulla the poultry thymus has Hassal's body (Hassal corpusculum).

Hassal's body plays an important role in producing the antigenic properties of surrounding cells. As a result, the thymus that does not have Hassal's body cannot function normally (Abdian, 2017). Budiman *et al.* (2019), show that the Hassal body is a multicellular compensatory of nonlimpotics and participates in prenatal physiological activities that have unique properties and active functionalities.

The age of ducklings also affects the histology of the thymus. The peak thickness of both the cortex and the medulla is at a certain age. This is to the opinion of Budiman *et al.* (2019) that the thymus cortex of ducks will thicken at the age of 1 month to 2 months, and will decrease at the age of 2 months to 3 months, at the age of 2 months is the peak period of proliferation of cells in the cortex, a decrease that occurs at the age of 3 months is caused by T cells that migrate a lot to the medulla.

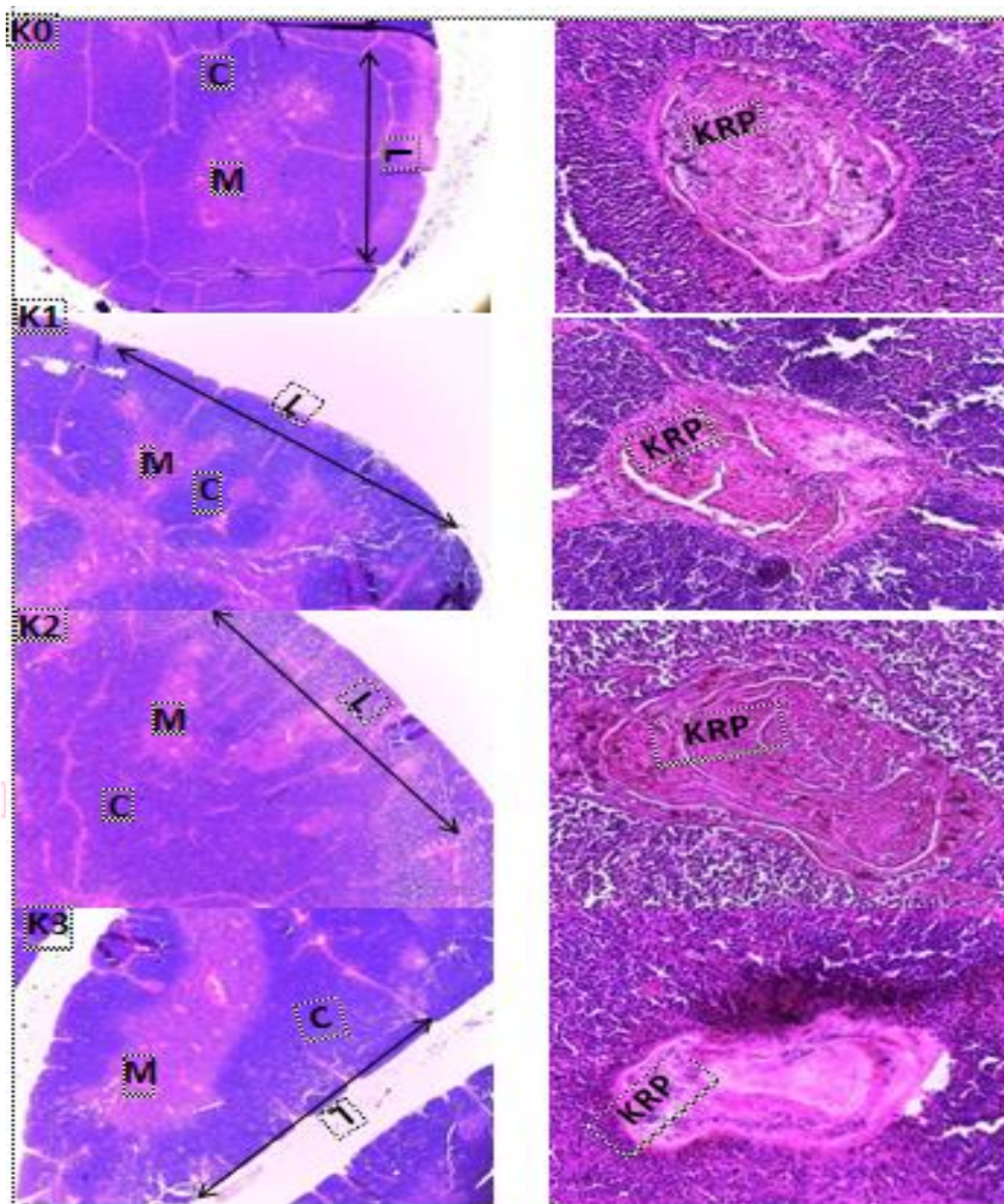


Figure 1. Histology of the Thymus of Hybrid Ducks Vaccinated with ND1-2 and Given Moringa Leaf Flour. K0 (control), K1 (2.5% Moringa Leaf Flour Affix), K2 (5% Moringa Leaf Flour Affix), and K3 (7.5% Moringa Leaf Flour Affix). L: Lobule, M: Medulla, C: Cortex and KRP: Corpusculum. HE Coloring Magnification 40×10 (Thymus, Left Image) and Magnification 100× (Corpusculum, Right Image). 2022



Conclusion and Recommendation

The conclusion of this study was that ND vaccination and Moringa leaf flour feed additives inclusion in hybrid ducks could maintain the morphometry of the thymus. This research can be applied, but it is necessary to do immunohistochemistry staining to see cellular changes.

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