Original ResearchArticle

Thymus Gland Development in White Leghorn Chickens: A Gross and Biometrical Evaluation from Hatching to 24 Weeks

**ABSTRACT**

**Aims:**To study the gross morphology of thymus gland in White Leghorn chicken from hatching to 24 weeks of age

**Study Design**: The study was conducted over an 8-month period in the White Leghorn chicken irrespective of the sex obtained from ILFC, CVSc& AH, CAU, Aizawl,Mizoram.

**Place and Duration of Study**: The study was conducted in the Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram between March 2024 to November 2024.

**Methodology**: Total 21 number of experimental birds were divided into 7 age-groups from 0 day to 24 weeks of age, at 4 weeks interval, consisting of 3 numbers in each group. The birds were sacrificed at their respective age by cervical dislocation method (Jaksch, 1981). After the bird was humanely sacrificed, a skin incision was made on mid-ventral aspect of the neck up to the thoracic cavity in order to expose the cervical and thoracic part of thymus respectively. Prior to starting the experiment, approval was secured from the Institutional Animal Ethical Committee (IAEC) at the College of Veterinary Sciences, Central Agricultural University, Selesih, Aizawl, Mizoram

**Results**: Across all age groups, the left thymus was observed to be located deeper as compared to right one. The weight of both the right and left thymus increased gradually from day 0 to 12 weeks of age, followed by a gradual decline up to 24 weeks, with a highly significant (p<0.01) difference observed between different age groups.The weight, width, and thickness of the thymus increased up to 12 weeks of age, after which a decrease began. No. of lobes highest at 8 weeks and 16 weeks.

**Conclusion**:At 24weeks of age, thymic involution was completed.

***Keywords****: Thymus, Gross, White Leghorn, Mizoram.*

1. **INTRODUCTION**

White leghorn chickens are a well-liked breed for their versatility, productivity, and adaptability to various climates. They are ideal for commercial egg production as well as for small-scale farming or backyard flocks due to their high egg productivity and low maintenance needs. The primary benefits of White Leghorn chickens is their ability to adjust to any type of environmental conditions (Kostaman *et al*., 2016).It holds the title of the top egg-producing bird in the Mediterranean category worldwide. It was distinguished by its white coloured skin, fairly long neck, noticeable chest, and elongated leg (Sapcota *et al*., 2014). Average weight of cock and hen are 2.7 kg and 1.8 kg, respectively (Sapcota *et al*., 2014). They are excellent laying hens that produce an average of 280 eggs per year, occasionally reaching as high as 300 to 320 eggs. Poultry farming significantly contribute to the rapid economic growth in India. According to the 20th livestock census, India is ranked 3rd in egg production and 6th in poultry meat production. Despite these advancements, poultry farmers encounter various challenges such as diseases and abiotic stresses that impact the immune system, causing disarray in the histological structure of organs, immune suppression, reduced production, and increased mortality (Hussan *et al*., 2009).

Poultry farming has been a traditional practice in Mizoram for many years, with numerous women participating for generations (Rahman, 2017). Most of the rural population in Mizoram consists of small and marginal farmers as well as landless labourers (Census, 2011).The majority of farmers and landless labourers in Mizoram lack sufficient land for large-scale poultry production, so they raise poultry in small quantities in their own backyard. The significant population of rural farmers are depending on the egg production which make them economically viable for the day today life.

As per the statistics of Economic survey of Mizoram (2021-2022), Mizoram state was deficient of meat and egg production in relation to its population. It has to import meat and egg to fulfil its demand from other states of India and neighboring country like Myanmar. Thereby rearing White Leghorn chicken in Mizoram is an ideal choice of breed because of its high egg production quality which can fulfil the demand of egg as well as it can also improve the economy of rural people of Mizoram.

Lymphatic system of avian species play a crucial role in providing immunity against diseases (Ayman *et al*., 2020). The thymus gland has been immunologically classified as a primary lymphoid organ responsible for the lymphocytes production, involved in cell-mediated immune responses and also modulate B cells for the production of antibodies (Leena *et al*., 2009).From thymus lymphocytes migrate to peripheral lymphoid organs such as the spleen, the caecal tonsil and the lymph nodes as the thymus involutes at adult stage and the secondary lymphoid organs provide immunity for the rest of the life (Treesh *et al*., 2014).

Due to the limited literature available on the age-wise development of the thymus gland in White Leghorn chickens, the present study aims to examine its gross anatomy and biometrical characteristics.

1. **MATERIALS AND METHOD**

The eggs of White Leghorn chicken were incubated in the hatchery for 21 days. The 21 numbers of day-old chicks were collected and divided into 7 different age-groups. All the experimental birds were reared in proper hygienic conditions, food and ad libitum water. Airflow/ventilation of the poultry shed was sufficient and biosecurity of the poultry shed as well as the farm was maintained strictly. Feeding history, vaccination schedule, and management practices of the chicken were taken into consideration. The apparently healthy chickens without any developmental disorder or detectable diseases were selected for the study.

The present research was conducted on 21 numbers of White Leghorn chicken, irrespective of sex at different stages of development. They were collected from Instructional Livestock Farm Complex (ILFC), College of Veterinary Sciences and A.H., CAU, Selesih, Aizawl, Mizoram, 796015, India. The experimental birds were divided into 7 age-groups from 0 day to 24 weeks of age, at 4 weeks interval, consisting of 3 numbers in each group (Table no. 1). The birds were sacrificed at their respective age by cervical dislocation method (Jaksch, 1981). After the bird was humanely sacrificed, a skin incision was made on mid-ventral aspect of the neck up to the thoracic cavity in order to expose the cervical and thoracic part of thymus respectively. Prior to starting the experiment, approval was secured from the Institutional Animal Ethical Committee (IAEC) at the College of Veterinary Sciences, Central Agricultural University, Selesih, Aizawl, Mizoram.

The birds were collected and their weight was recorded using a weighing scale. After that, the collected birds were sacrificed by cervical dislocation method as per the method described by Jaksch, 1981. The skin was incised by the ventral neck dissection method in order to expose the cervical and thoracic part of thymus. The collected thymus was washed in normal saline and mopped with blotting paper for the study of gross morphological features like location(Fig: 1, Fig: 2), size, shape, and colour, number of lobes and age-related changes of the same parameters. Different biometrical parameters (Fig: 5, Fig: 6, Fig: 7, Fig: 8) of the organ were recorded with the help of weighing machine, graduated tape, scale and digital Vernier calliper.

**Table 1: Experimental design of White Leghorn birds from hatching to 24 weeks of age (N=21)**

|  |  |  |
| --- | --- | --- |
|  **Age group No.** |  **Age** |  **No. of Birds** |
| **I** | **0 day** | **3** |
| **II** | **4 weeks** | **3** |
| **III** | **8 weeks** | **3** |
| **IV** | **12 weeks** | **3** |
| **V** | **16 weeks** | **3** |
| **VI** | **20 weeks** | **3** |
| **VII** | **24 weeks** | **3** |

* 1. **Statistical Analysis:**All the collected data were analysed using Statistical Package for the Social Sciences (SPSS; version 20.0) software and the results were depicted in tabular forms.
1. **RESULTS AND DISCUSSION**
	1. **Body weight of the birds**

Throughout the current study, the body weight of the White Leghorn chicken increased progressively from day 0 to 24 weeks of age. A highly significant difference (p<0.01) was observed in relation to age progression (Table 2). The present results were similar with the findings of Mahanta in (2018) in local hill fowl of Uttarakhand and Rhode Island Red birds. He stated that the body weight of the bird increased gradually from day old to 6 months of age. Contrary to this finding Hashimoto and Sugimura (1976) recorded the highest body weight at 11 weeks of age in White Pekin Duck. The variance in the current results could be attributed to the differences in breed, environmental effect and dietary supplements.

* 1. **Location of the thymus**

In the present study, thymus was found to be located in ventral aspect on either side of the neck in the form of a chain under the sub dermal adipose tissue extending from third cervical vertebra to upper thoracal segments. The similar findings were opined by Kendall (1980) and Mešťanová*et al.* (2016) in birds, Ali *et al*. (2016) in turkey, Nasser (2019) in geese and Davison *et al.* (2008) in avian species. Romanoff (1960) recorded that the thymus stretched from the bottom of the heart to the third cervical vertebra in avian species. This finding was in similar trend with the present study. However, Koch *et al.* (1973) in avian species, Haseeb *et al*. (2014) in Aseel chicken, Ali (2016) in turkey, El- Zoghby and Attia (2007) in ostrich, Gilmore and Bridges (1974) in fowl, Sultana *et al.* (2011) in ducklings, Khan *et al*. (2014) in chicken and Gulmez and Aslan (1999) in Geese observed that the thymus was extended from anterior cervical region to the anterior thoracic region. In current study, left thymus was found to be situated deeply and in close proximity to the esophagus, trachea, blood vessels, and nerves in the neck, whereas, the right thymus was positioned near the surface of the skin. The same observation was also reported by Dahariya*et al.* (2020) in Hansli chicks and Vencobb broiler chicks. In contrast, Pereira *et al*. (2017) documented that the chain of the thymic lobes shifted away from the midline as it moved towards the upper third of the neck, whereas, in middle and lower third of the neck it was located near the midline. However, Jayachitra *et al.* (2022) stated that the cervical region of the thymus started at the ninth cervical vertebra and was connected to the trachea, jugular vein, common carotid artery, and vagus nerve on both sides in turkey. The right thymus was nearer to the esophagus in the neck region and extended to the thoracic inlet at the level of the crop. The terminal part of the thoracic thymus was associated with the thyroid gland and brachiocephalic artery on both sides. In commercial broiler chicken, Islam *et al.* (2019) found that the thymus gland in day-old chicks extended from the base of the skull to the thoracic cavity, whereas, in adults it was absent in the upper one fifth of the neck. The dissimilarities observed in the present study might be due to the variations in breed.

**3.3 Thymic weight**

In the present study, the weight of both right and left thymus gradually increased from 0 day to 12 weeks of age. After that the weight gradually declined to 24 weeks of age. Between the age-groups, a highly significant difference (p<0.01) was noticed. The highest thymic weight was observed 1.21 ± 0.08 gm and 1.07 ± 0.12 gm for the left and right sides, respectively at 12 weeks of age(Table 3). The present finding was closely similar to the observations obtained by Dahariya*et al.* (2020) in Hansli chicks and Vencobb broiler chicks. The current observations were in contrary to the findings of Fadhilah *et al.* (2022) in ALPU chicken where he found the maximum weight of thymus at 15 weeks of age. In broiler chicken, maximum thymic weight was recorded on 28 days by Khan *et al. (*2014) and on 2 weeks by Tarek *et al*. (2012). Whereas in turkey, Muthukumaran *et al.* (2011) found that the thymus reached its maximum weight at the age of 6 month. Yamada *et al. (*1971) documented that the male Japanese quails reached their peak thymus weight at 5 weeks, while females reached it at 4 weeks of age. Hashimoto and Sugimura (1976) observed the maximum thymic weight at the age of 11 weeks in White Pekin Duck. Bellamy *et al.* (1982) observed the maximum thymic weight at 58 days old bird.The dissimilarities revealed in the present investigation might be due to the variations in breed.

* 1. **Shape of the thymus**

In all experimental birds, irrespective of age-groups, the thymus appeared in the shape of a bean or button like. But McLelland (1990) in avian species, Kanasiya *et al.* (2017) in Kadaknath chicken, Ayman *et al. (*2020) in Sonali chicken, Haseeb *et al*. (2014) in Aseel chicken, Akter *et al*. (2006) in broiler chicken found flat, irregularly elongated shaped thymus. Muthukumaran *et al*. (2011) and Nnadozie*et al.* (2019) documented irregularly elliptical shaped thymus in turkey. These findings were in contrary to the reports of current study that might be due to the variations in breed.

* 1. **Colour of the thymus**

The thymus of all experimental birds appeared light pink to creamy white in colour, regardless of their age. Similar findings were also reported by Sawad (2019) in broiler chick embryo. Closely similar observations were also noted by Romanoff (1960) in bird and Ali (2016) in turkey that the colour of thymus was yellowish-pink; Haseeb *et al*. (2014) in Aseel chicken, Khan *et al*. (2014) in broiler chicken, Akter *et al*. (2006) and Sultana *et al.* (2011) in Indigenous ducklings where they found pale white to yellowish white thymus. Opposing the current results Kanasiya *et al.* (2017) reported gray to black coloured thymus in Kadaknath chicken. Mahanta *et al.* (2018) found reddish black coloured thymus in local hill fowl of Uttrakhand and RIR birds. Muthukumaran *et al*. (2011) and Nnadozie*et al.* (2019) documented pale red pink thymus in turkey. Variations in breeds could account for the differences in the current results.

* 1. **Consistency of thymus**

In the present research, thymus exhibited a soft consistency in birds of all age-groups. Sultana *et al.* (2011) also found comparable results in ducklings, while Ayman *et al.* (2020) discovered similar findings in Sonali chicken.

**3.7 Thymic length**

In the present study, length of thymus showed a variable pattern among the left and right thymus with the advancement of age. The length of the left thymus increased gradually from 0 day to 20 weeks of age. The highest length of left thymus was found at the age of 20 weeks. But the pattern was different in case of right thymus. At first the right thymic length increased from 0 day to 12 weeks of age. After that it declined as involution proceed. Throughout the study, the left thymic length was more than the right one. But only at 12 weeks of age the right thymic length was more than the left. A considerable difference was observed (p<0.01) in relation to age progression from birth to 20 weeks of age (Table 4). Similar type of findings was also obtained by Dahariya*et al*. (2020) in Hansli and Vencobb broiler chickens. Opposing the current results, Mahanta (2018) in RIR birds found the maximum length at 3 months of age and in local fowl of Uttarakhand at 6 months of age. Variations in breeds could account for the differences in the current results.

* 1. **Thymic width**

In current study, thymic width increased from 0 day to 12 weeks in both the right and left thymus, followed by a decrease after 12 weeks. The thymus reached its greatest width at 12 weeks old in both the left and right sides. A notable disparity (p<0.01) was observed in age progression from birth to 20 weeks old (Table 5). These results were similar with the reports of Mahanta (2018) in local fowl of Uttarakhand but differed from the finding of Mahanta (2018) in RIR birds where he reported the maximum thymic width at the age of 6-month-old birds in both right and left thymus. Variations in breeds could account for the differences in the current results.

* 1. **Thymic thickness**

In the current research, it was noted that the thymus increased in thickness from 0 day to 12 weeks, reaching its maximum thickness on both sides. Beginning at 12 weeks, a considerable reduction with a highly significant fluctuation (p<0.01) was witnessed in both the right and left thymus. A notable disparity (p<0.05) was detected in the thymus of 12-week-old bird in between its right and left sides (Table 6). The outcomes were comparable to Mahanta's (2018) study on local hill fowl of Uttarakhand but contrasted with Mahanta's (2018) research on RIR birds, where the maximum thickness of thymus was observed in both right and left thymus of 6-month-old birds. Breed differences might be the cause of difference in the present findings.

**3.10 Number of lobes in the thymus**

In the present study, the numbers of lobes in both left and right thymus followed a fluctuating pattern. Highest number of lobes was found at the age of 8 weeks and 16 weeks old White Leghorn birds. In between the groups the biometrical values were highly significant. However, within the group only at the age of 12 weeks a significant difference (p<0.05) was observed in between the right and left thymus(Table 7). Closely related findings were also obtained by Kanasiya *et al.* (2017) in Kadaknath chicken, Muthukumaran*et al*. (2011) in turkey, Khan *et al*. (2014) in broiler chicken and Akter *et al*. (2006) in broiler chicken. In the present study, the involution of thymus was found to be completed at 24 weeks of age and the lobes were replaced by adipose tissue. This finding was in contrary with the findings of Ayman *et al.* (2020) in Sonali chicken where they reported the involution at day 56 or 8 weeks of the post-natal stage. However, in White Leghorn bird Aire *et al. (*1973) observed involution at 26 weeks of age. The variance in the current results could be attributed to the differences in breeds, environmental effect and dietary supplements.

**Table 2: Weight of the White Leghorn chickens from 0 day to 24 weeks of age (gm)**

|  |  |
| --- | --- |
| **Age of the birds/ groups** | **Weight of the White Leghorn chicken (gm)** |
| **0 day** | **43.6667 ± 1.33f** |
| **4 weeks** | **265.0 ± 18.61e** |
| **8 weeks** | **451.33 ± 10.03d** |
| **12 weeks** | **619.67 ± 17.03c** |
| **16 weeks** | **655.67 ± 20.12c** |
| **20 weeks** | **870 ± 35.50b** |
| **24 weeks** | **1106.0 ± 11.37a** |
| **P- value** | **0.00\*\*** |

\*\* Parameters with different subscript in a single row are significantly different (p<0.01)

\* Parameters with different subscript in a single row are significantly different (p<0.05)

 NS- Non significant

**Table 3: Weight of the thymus in White Leghorn chickens from 0 day to 24 weeks of age (gm)**

|  |  |  |
| --- | --- | --- |
| **Age group** | **Weight of thymus (gm)** | **P value** |
| **Left thymus** | **Right thymus** |
| **0 day** | **0.1466 ± 0.03d** | **0.1533 ± 0.02d** | **0.59NS** |
| **4 weeks** | **0.9581 ± 0.12ab** | **0.9389 ± 0.08ab** | **0.66NS** |
| **8 weeks** | **1.1244 ± 0.00a** | **0.9985 ± 0.01ab** | **0.06NS** |
| **12 weeks** | **1.2129 ± 0.08a** | **1.0720 ± 0.12a** | **0.53NS** |
| **16 weeks** | **0.7401 ± 0.16bc** | **0.7376 ± 0.07bc** | **0.11NS** |
| **20 weeks** | **0.6112 ± 0.13c** | **0.5531 ± 0.13c** | **0.99NS** |
| **P value** | **0.00\*\*** | **0.00\*\*** |  |

\*\* Parameters with different subscript in a single row are significantly different (p<0.01)

\* Parameters with different subscript in a single row are significantly different (p<0.05)

 NS- Non significant

**Table 4: Length of the thymus (left and right) in White Leghorn chickens from 0 day to 24 weeks of age (mm)**

|  |  |  |
| --- | --- | --- |
| **Age group** | **Length of thymus (mm)** | **P value** |
| **Left thymus** | **Right thymus** |
| **0 day** | **38.33 ± 2.03e** | **34.00 ± 3.21d** | **0.45NS** |
| **4 weeks** | **52.21 ± 0.87d** | **51.81 ± 0.89c** | **0.79NS** |
| **8 weeks** | **63.52 ± 0.88c** | **59.63 ± 2.39b** | **0.15NS** |
| **12 weeks** | **64.57 ± 2.04c** | **67.95 ± 2.68a** | **0.48NS** |
| **16 weeks** | **70.39 ± 0.48b** | **62.81 ± 0.91ab** | **0.33NS** |
| **20 weeks** | **79.89 ± 0.85a** | **53.26 ± 0.39c** | **0.18NS** |
| **P value** | **0.00** | **0.00** |  |

\*\* Parameters with different subscript in a single row are significantly different (p<0.01)

\* Parameters with different subscript in a single row are significantly different (p<0.05)

 NS- Non significant

**Table 5: Width of the thymus (left and right) in White Leghorn chickens from 0 day to 24 weeks of age (mm)**

|  |  |  |
| --- | --- | --- |
| **Age group** | **Width of thymus (mm)** | **P value** |
| **Left thymus** | **Right thymus** |
| **0 day** | **4.33 ± 0.67b** | **3.67 ± 0.67b** | **1.00NS** |
| **4 weeks** | **7.74 ± 0.18a** | **6.82 ± 0.36a** | **0.16NS** |
| **8 weeks** | **8.09 ± 0.71a** | **7.37 ± 0.63a** | **0.95NS** |
| **12 weeks** | **8.33 ± 0.69a** | **8.06 ± 0.29a** | **0.23NS** |
| **16 weeks** | **7.98 ± 0.33a** | **6.83 ± 0.17a** | **0.18NS** |
| **20 weeks** | **7.79 ± 0.46a** | **6.79 ± 0.95a** | **0.16NS** |
| **P value** | **0.00\*\*** | **0.00\*\*** |  |

\*\* Parameters with different subscript in a single row are significantly different (p<0.01)

\* Parameters with different subscript in a single row are significantly different (p<0.05)

 NS- Non significant

**Table 6: Thickness of the thymus (left and right) in White Leghorn chickens from 0 day to 24 weeks of age (mm)**

|  |  |  |
| --- | --- | --- |
| **Age group** | **Thickness of thymus (mm)** | **P value** |
| **Left thymus** | **Right thymus** |
| **0 day** | **7.33 ± 0.33f** | **6 ± 0.58c** | **0.56NS** |
| **4 weeks** | **10.91 ± 0.48d** | **10.10 ± 1.77b** | **0.10NS** |
| **8 weeks** | **12.20 ± 0.20bc** | **11.11 ± 0.48ab** | **0.11NS** |
| **12 weeks** | **14.28 ± 0.18a** | **13.34 ± 0.03a** | **0.03\*** |
| **16 weeks** | **12.78 ± 0.41b** | **11.92 ± 0.47ab** | **0.77NS** |
| **20 weeks** | **11.43 ± 0.09cd** | **11.54 ± 0.59ab** | **0.11NS** |
| **P value** | **0.00\*\*** | **0.00\*\*** |  |

\*\* Parameters with different subscript in a single row are significantly different (p<0.01)

\* Parameters with different subscript in a single row are significantly different (p<0.05)

 NS- Non significant

**Table 7: Number of lobes of thymus (left and right) in White Leghorn chickens from 0 day to 24 weeks of age**

|  |  |  |
| --- | --- | --- |
| **Age group** | **No. of lobes of thymus** | **P value** |
| **Left thymus** | **Right thymus** |
| **0 day** | **5.33 ± 0.33a** | **5.33 ± 0.33bc** | **1.00 NS** |
| **4 weeks** | **4.67 ± 0.33a** | **4.67 ± 0.33c** | **1.00 NS** |
| **8 weeks** | **6 ± 0.58a** | **6.33 ± 0.33a** | **0.64 NS** |
| **12 weeks** | **5.67 ± 0.33a** | **4.33 ± 0.33c** | **0.04\*** |
| **16 weeks** | **6 ± 0.57a** | **6.67 ± 0.33a** | **0.37 NS** |
| **20 weeks** | **5.67 ± 0.33a** | **6.67 ± 0.33ab** | **0.10 NS** |
| **P value** | **0.306 NS** | **0.00\*\*** |  |

\*\* Parameters with different subscript in a single row are significantly different (p<0.01)

\* Parameters with different subscript in a single row are significantly different (p<0.05)

 NS- Non significant

4. Conclusion

The weight of the White Leghorn chicken increased steadily from hatching to 24 weeks of age. Thymus weight rose until 12 weeks, then gradually declined. The left thymus was deeply located near the esophagus, trachea, blood vessels, and nerves, while the right thymus lay closer to the skin and farther from neck structures, extending from the third cervical vertebra to the upper thoracic region. Both thymuses were pink to creamy white, bean or button-shaped, and soft in texture. Thymus weight, width, and thickness peaked at 12 weeks, with the right thymus reaching maximum length at 12 weeks and the left at 20 weeks. The highest lobe count occurred at 8 and 16 weeks. By 24 weeks, thymic involution was complete, with lobes replaced by adipose tissue.

**ETHICAL APPROVAL:**

Prior to starting the experiment, approval was secured from the Institutional Animal Ethical Committee (IAEC) at the College of Veterinary Sciences, Central Agricultural University, Selesih, Aizawl, Mizoram, under Approval Reference Number CVSC/CAU/IAEC/23-24/P-17dated 29.07.2024.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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