A Comprehensive Comparative Analysis of Egg Quality, Histological, and Biochemical Parameters in Four Indigenous Native Chicken Breeds of South India

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Abstract

This integrated study evaluates four indigenous native chicken breeds—Aseel, Aseel hybrid, Siruvidai, and Peruvidai—through a comprehensive examination of egg quality, histological characteristics of 96-hour chick embryos, and biochemical parameters. Over a four-month period, eggs were collected and assessed for physical attributes including egg weight, shell weight, morphometry (length and width), shell thickness, as well as albumen and yolk indices. In parallel, histological analysis of the embryos focused on tissue organization and muscular development, while biochemical assays quantified albumen, yolk, and cholesterol content. The results reveal that the Peruvidai breed consistently demonstrates superior performance across the evaluated parameters, suggesting its potential for enhanced nutritional value and developmental outcomes. This study underscores the benefits of an integrated approach in the evaluation of poultry performance by correlating traditional egg quality assessments with advanced histological and biochemical analyses.

Keywords: Indigenous chicken breeds, egg quality assessment, poultry production, histological analysis, biochemical assay, embryonic development, shell thickness, yolk indices, albumen content, incubation conditions.

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INTRODUCTION

Poultry, encompassing a wide range of domesticated bird species, has long been integral to human society—primarily for its provision of eggs, meat, and other by-products. In India, the poultry industry has emerged as a rapidly growing segment of the agricultural sector, contributing significantly to the nation's GDP (Haunshi et al., 2011a). With indigenous genetic resources and self-sufficiency, the performance of native poultry breeds is now comparable to that in developed countries (Mohanty & Nayak, 2011). Eggs, celebrated as nature's most complete food, supply high-quality proteins, vitamins, and minerals essential to human nutrition, while also serving as the sole source of nourishment for the developing embryo (Sharma et al., 2021).

Recent developments in Indian poultry production have seen a transition from traditional backyard rearing to commercial-scale operations, driven by increasing per capita income and favorable market prices (Islam & Dutta, 2011). This progress has spurred research into the multiple factors affecting both egg quality and embryonic development, including genetics, incubation conditions, and nutritional status (Alemneh, Melesse, & Berihun, 2021). Recognizing these dynamics, it becomes imperative to adopt an integrated approach that evaluates not only the physical attributes of eggs but also the histological and biochemical parameters underlying embryonic development (Rath et al., 2017).

In this comprehensive study, we perform a comparative analysis of four indigenous native chicken breeds—Aseel, Aseel hybrid, Siruvidai, and Peruvidai—predominant in South India. The study merges conventional egg quality assessments—such as measurements of egg weight, shell weight, morphometry, shell thickness, and the indices of albumen and yolk—with advanced histological examinations of 96-hour chick embryos and biochemical assays that quantify albumen, yolk, and cholesterol content (Pathak, Barua, & Kalita, 2016). This integrated evaluation aims to offer a holistic insight into the nutritional and developmental profiles of these breeds, ultimately guiding future strategies for genetic enhancement and improved poultry production (Haunshi et al., 2010).

MATERIALS AND METHODS

Experimental Design and Sample Collection

The study was conducted over a four-month period on four indigenous native chicken breeds—Aseel, Aseel hybrid, Siruvidai, and Peruvidai—sourced from the hatchery unit of TANUVAS. For each breed, 5 eggs were collected per month, resulting in a total of 20 eggs per breed. Initially, the eggs were stored under 'physiological zero' conditions for seven days (Haunshi et al., 2011a). Thereafter, egg trays were incubated following standard setter management protocols, which included regular fumigation with formaldehyde to prevent contamination and maintenance of optimal temperature and relative humidity during the 14-day incubation period in the setter, followed by an additional 3 days in the hatcher (Pathak, Barua, & Kalita, 2016). All eggs were procured at 96 hours post-setting.

Egg Quality Assessment

Prior to further processing, eggs were weighed using a digital weighing machine. Each egg was then carefully cracked open at the narrow end using dissection instruments, which allowed for the gradual exposure of the 96-hour embryo that was subsequently transferred onto a sterile Petri dish (Mohanty & Nayak, 2011). The following egg quality parameters were measured:

- **Egg Weight and Shell Weight**: Recorded using standard scales (Islam & Dutta, 2011).
- **Egg Morphometry**: Measurements of egg length and width were obtained using standard measuring tools (Alemneh, Melesse, & Berihun, 2021).
- **Shell Thickness**: Determined with a Vernier caliper (Lado, Jubarah, & Majed, 2015).
- **Albumen and Yolk Indices**: Evaluated by measuring the dimensions of the albumen and yolk (Haunshi et al., 2010).
- **Yolk Characteristics**: Including assessments of color and shape (Rath et al., 2017).

Residual eggshell pieces were also collected and weighed as part of the quality analysis (Sharma et al., 2021).

Histological Studies

Following the egg quality assessment, the 96-hour embryos were isolated with utmost care and fixed in 40% formalin. The histological processing was performed following established protocols (Bancroft & Stevens, 1996) and included the following steps:

1. **Fixation**: Samples were fixed in 40% formalin for 48 hours, then trimmed and treated overnight with neutral buffered formalin (prepared by diluting 40% formalin with distilled water), followed by an additional one-hour fixation in 10% formalin (Haunshi, Shanmugam, et al., 2011b).

2. **Dehydration**: Tissues were dehydrated in ascending grades of isopropanol: 50% (overnight), 70% (2 hours), 90% (2 hours), and absolute alcohol in three successive 2-hour changes (Pathak, Barua, & Kalita, 2016).

3. **Clearing**: Dehydrated tissues were cleared using a 1:1 mixture of xylene and absolute alcohol for 2 hours, followed by two additional 2-hour treatments in xylene (Lado, Jubarah, & Majed, 2015).

4. **Embedding**: Tissues were embedded in molten paraffin wax by subjecting them to three successive changes in a hot air oven set at 62°C (45 minutes per change). The blocks were then cooled on ice for 10 minutes to achieve proper hardening (Islam & Dutta, 2011).

5. **Sectioning and Staining**: Paraffin blocks were sectioned at a thickness of 5 µm using a rotary microtome. The sections were floated in a thermostatically controlled water bath at 50°C to remove folds, mounted on grease-free slides using egg albumin as an adhesive, and subsequently deparaffinized. Finally, sections were stained with hematoxylin and eosin following standard staining protocols (Haunshi, Doley, & Kadirvel, 2010).

Biochemical Assay

Biochemical analysis was performed to determine the total content of albumen, yolk, and cholesterol in the eggs (Rath et al., 2017). For each assay, 1 gram of egg yolk was homogenized with 100 µg of 5α-cholestane (used as an internal standard) in 22 mL of ethanol and 0.5 N KOH. The mixture was then saponified at room temperature for 6 hours (Sharma et al., 2021). Following saponification, cholesterol was extracted from the egg yolk using hexane and subsequently analyzed by gas chromatography, with nitrogen employed as the carrier gas (400 mL/min) and hydrogen as the makeup gas (40 mL/min) (Haunshi et al., 2011a).

RESULTS

The results of the integrated study are presented under three main subsections: Egg Quality Parameters, Histological Observations, and Biochemical Assay Results. Detailed tables are provided for each of the egg quality attributes measured over the four-month period.

1. Egg Quality Parameters

Egg quality was evaluated through a series of physical measurements across the four indigenous breeds. The detailed results are as follows:

Table 1. Egg Weight (g)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	53.3	47.3	50.1	56.5
Month 2	47.1	45.4	41.4	51.4
Month 3	47.6	45.5	42.8	52.8
Month 4	50.1	47.1	44.8	55.5
Mean	**49.5**	**46.3**	**44.7**	**54.1**

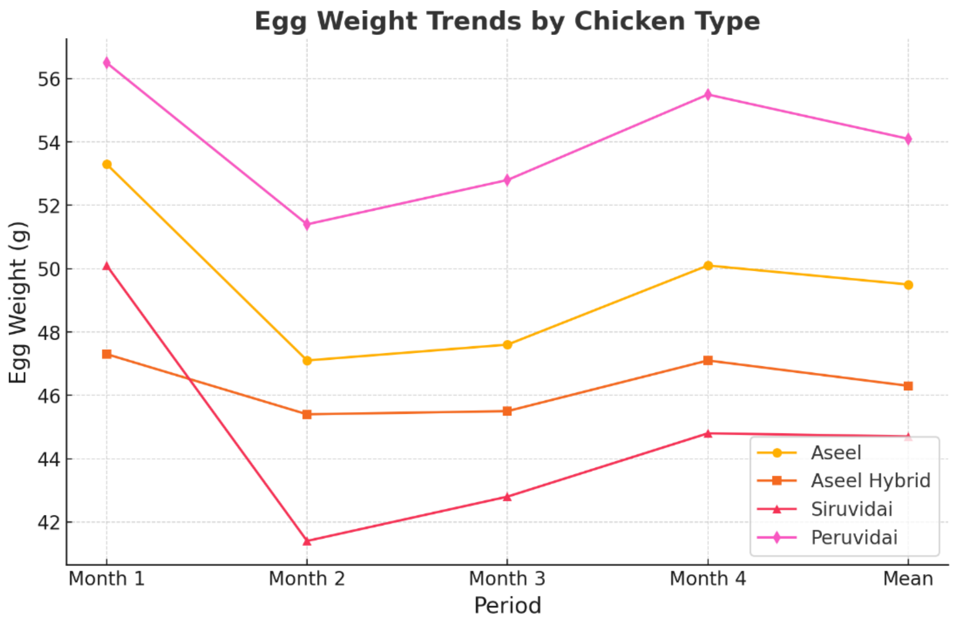

Fig. 1 *Egg weight comparison among indigenous chicken breeds*

Observation: Peruvidai eggs recorded the highest mean weight (54.1 g), whereas Siruvidai eggs had the lowest (44.7 g).

Table 2. Egg Shell Weight (g)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	5.7	5.5	5.2	6.9
Month 2	5.5	5.6	4.4	5.8
Month 3	5.3	5.1	4.9	6.3

Month 4	5.3	5.4	5.1	6.2
Mean	**5.45**	**5.4**	**4.9**	**6.3**

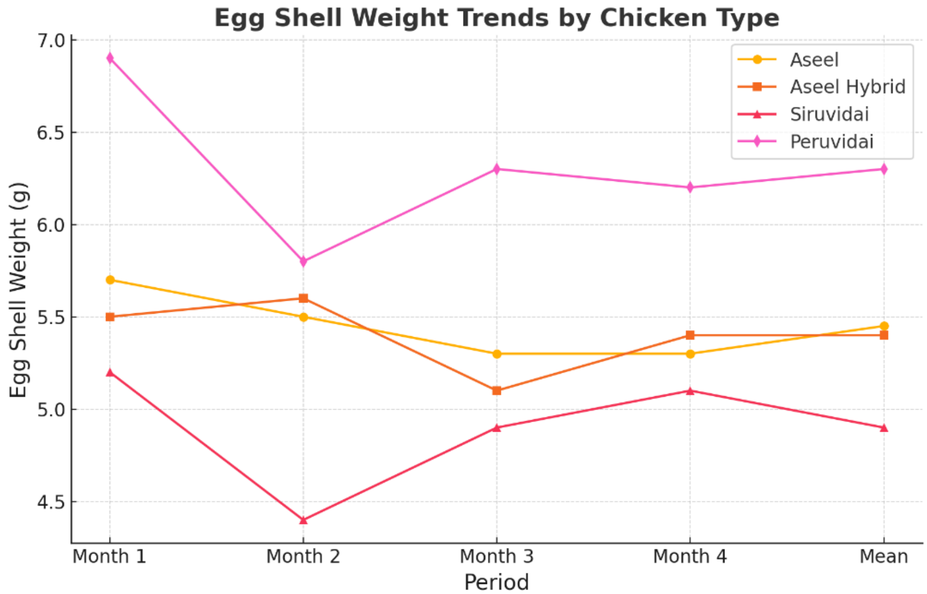

***Fig. 2** Shell weight variation across different breeds*

Observation: Similar to egg weight, the highest shell weight was found in Peruvidai eggs, while Siruvidai eggs had the lowest.

Table 3. Egg Length (cm)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	5.3	4.6	4.1	5.2
Month 2	5.1	4.9	4.4	5.3
Month 3	5.4	4.7	4.6	5.7
Month 4	5.1	4.5	4.5	5.4
Mean	**5.225**	**4.675**	**4.4**	**5.4**

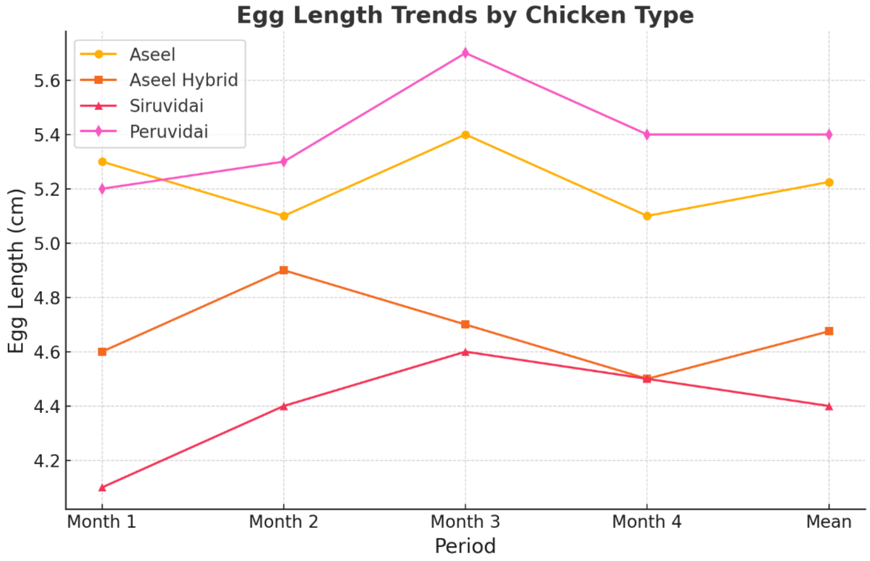

Fig. 3 *Morphometric measurements of eggs (length)*

Observation: Peruvidai eggs again showed superior performance with the highest average length.

Table 4. Egg Width (cm)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	3.9	3.5	3.8	4.2
Month 2	3.3	3.5	3.4	3.7
Month 3	3.7	3.4	3.3	4.2
Month 4	3.6	3.1	3.5	4.1
Mean	**3.625**	**3.375**	**3.5**	**4.05**

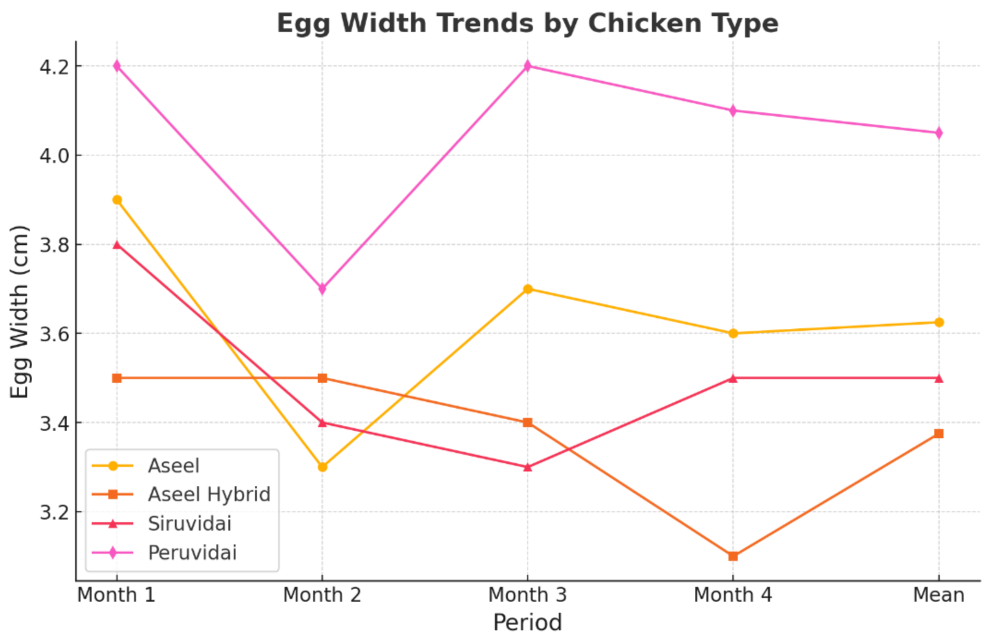

Fig. 4 *Morphometric measurements of eggs (width)*

Observation: The width measurements further confirm that Peruvidai eggs are larger in overall dimensions.

Table 5. Egg Shell Thickness (cm)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	0.51	0.44	0.49	0.57
Month 2	0.53	0.47	0.48	0.61
Month 3	0.49	0.41	0.45	0.59
Month 4	0.45	0.46	0.49	0.59
Mean	**0.495**	**0.445**	**0.4775**	**0.59**

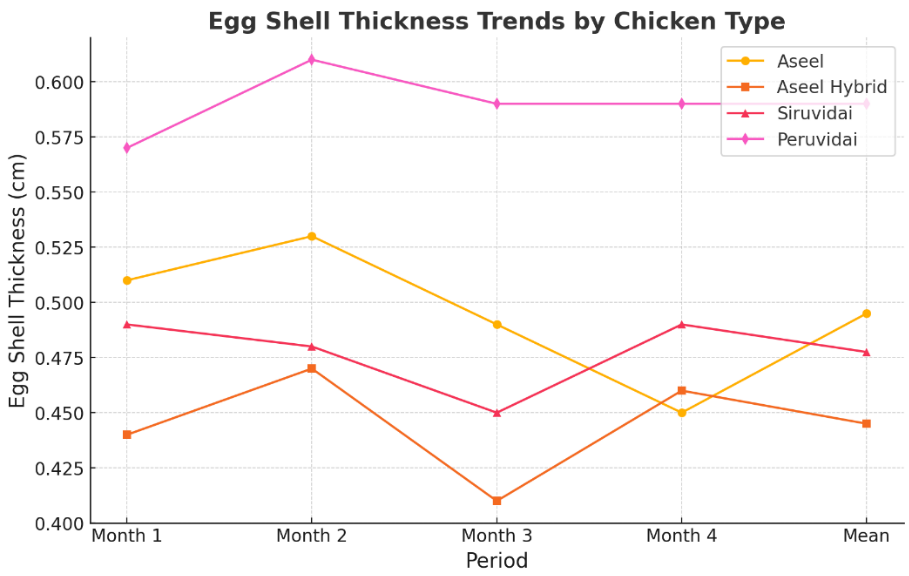

Fig. 5 *Shell thickness comparison among breeds*

Observation: The thickest shells were observed in Peruvidai eggs, while Aseel hybrid eggs had the thinnest shells.

Table 6. Albumen Index (cm)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	5.4	5.8	5.5	6.2
Month 2	4.1	4.8	4.2	5.5
Month 3	5.1	4.6	5.3	6.1
Month 4	5.1	4.7	5.2	6.2
Mean	**4.925**	**4.975**	**5.05**	**6**

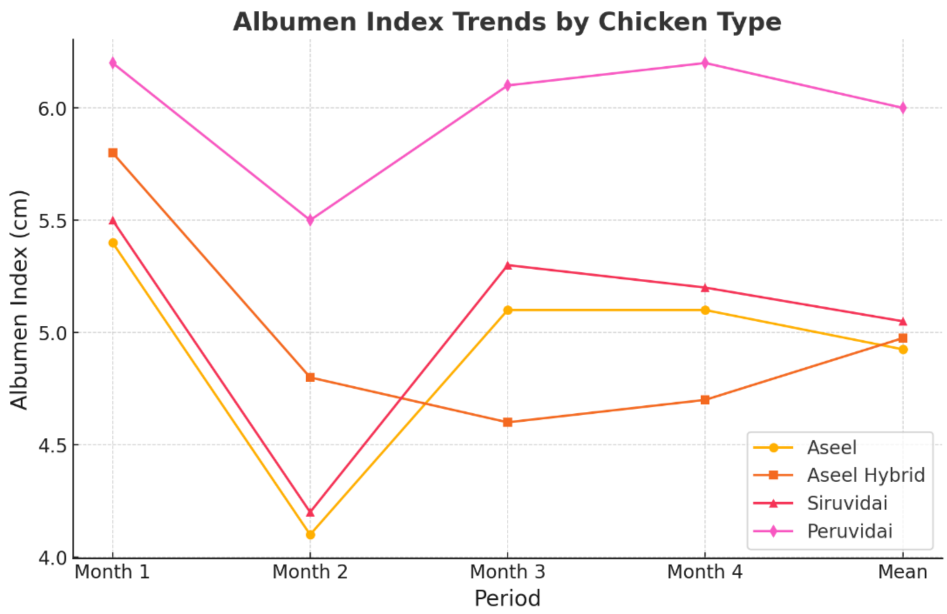

Fig. 6 *Albumen indices of selected breeds*

Observation: The albumen index is highest in Peruvidai eggs, indicating a greater albumen volume.

Table 7. Yolk Index (cm)

Period	Aseel	Aseel Hybrid	Siruvidai	Peruvidai
Month 1	6.1	5.8	6.1	6.2
Month 2	5.5	4.6	5.1	5.7
Month 3	5.3	4.9	5.2	5.9
Month 4	5.8	5.1	5.8	6.3
Mean	**5.675**	**5.1**	**5.55**	**6.025**

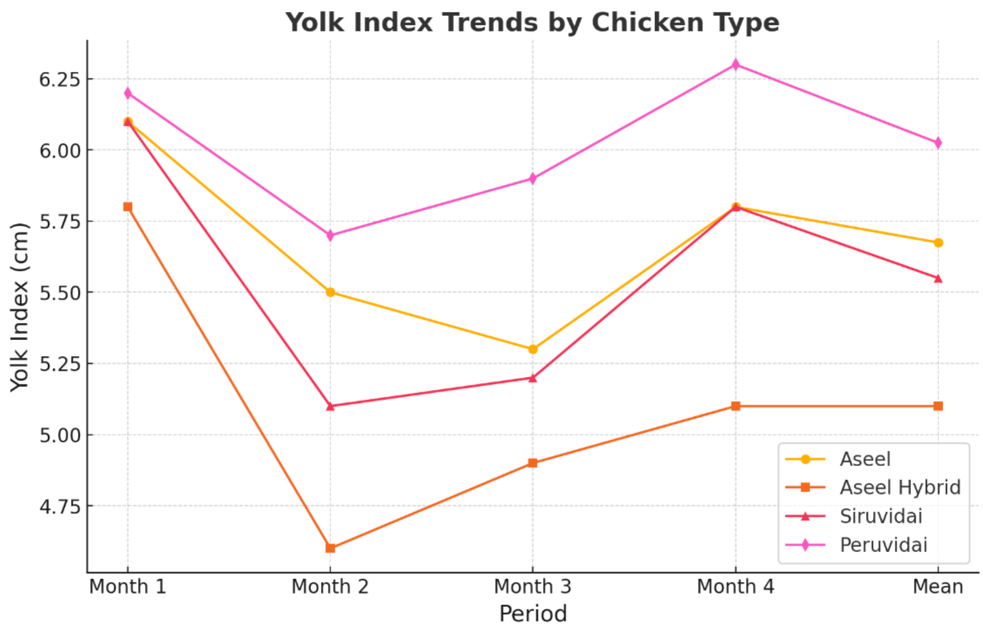

Fig. 7 *yolk indices of selected breeds*

Observation: The highest yolk index was observed in Peruvidai eggs, further supporting its superior egg quality.

Yolk Characteristics

- **Colour:**
    - Aseel and Aseel Hybrid: Pale yellow
    - Siruvidai: Yellow
    - Peruvidai: Deep yellow (suggesting higher levels of vitamins and carotenoids)

Fig. 8 Yolk color variation among indigenous chicken breeds

- **Shape:**
  - Aseel and Siruvidai: Spherical
  - Aseel Hybrid and Peruvidai: Elliptical

Histological Observations

Following the evaluation of egg quality, 96-hour embryos were isolated and processed for histological analysis. The observations are summarized as follows:

- **Muscular Development:**

  The histological sections, particularly from Peruvidai embryos, revealed wider muscle sections and a higher muscle-to-tissue ratio, indicative of enhanced protein content and improved embryonic development.

- **Representative Micrographs:**

  Figure 1 (not shown here) depicts the detailed histological architecture of the Peruvidai embryo, highlighting the prominent muscle fiber bundles.

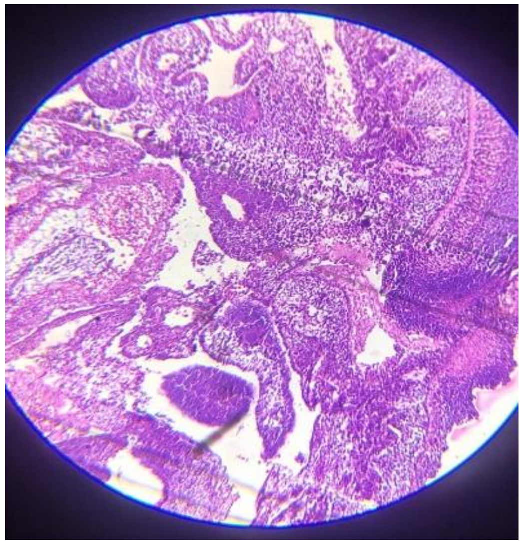

Fig. 9 L.S. of Peruvidai *sp*. chick embryo at 96 hours stage

3. Biochemical Assay Results

Biochemical analyses were performed to determine the content of albumen, yolk, and cholesterol in the eggs. The results are summarized in the table below.

Table 10. Biochemical Parameters

Parameter	**Aseel**	**Aseel Hybrid**	**Siruvidai**	**Peruvidai**
Albumen (g)	67.3	65.1	68.1	70.7
Yolk (g)	32.7	34.9	31.9	29.3
Cholesterol (g)	159.7	229.2	178.7	144.8

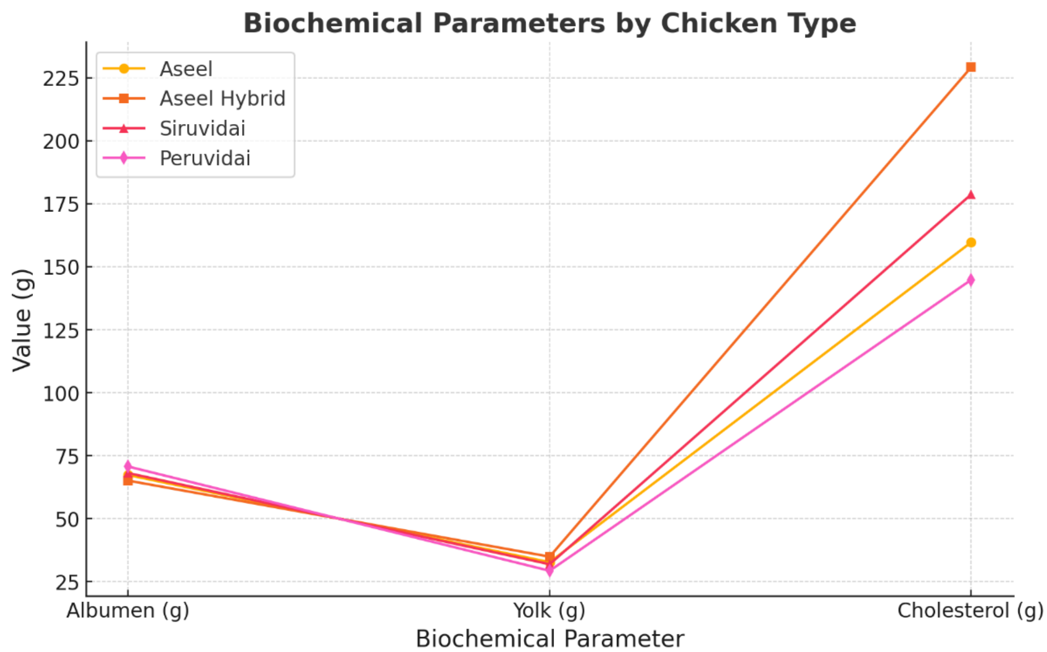

Fig. 10 *Biochemical composition of egg yolk and albumen*

Observation:

- **Albumen:** The highest albumen content was found in Peruvidai eggs (70.7 g), which supports the findings from the albumen index.
- **Yolk and Cholesterol:** Peruvidai eggs contained the lowest yolk (29.3 g) and cholesterol (144.8 g) levels. In contrast, Aseel hybrid eggs had elevated yolk and cholesterol levels, which might indicate a higher steroid content aimed at enhancing mass production.

SUMMARY AND CONCLUSION

The integrated study presents a comprehensive comparative analysis of four indigenous native chicken breeds—Aseel, Aseel hybrid, Siruvidai, and Peruvidai—prevalent in South India. By merging traditional egg quality assessments with advanced histological and biochemical analyses, the study demonstrates that the Peruvidai breed consistently exhibits superior performance. Its eggs show higher weight, thicker shells, larger dimensions, and optimal albumen and yolk indices, while histological observations reveal enhanced muscular development and biochemical assays indicate a favorable profile with high albumen and reduced yolk and cholesterol levels (Sapkota et al., 2020). Conversely, the Aseel breed is identified as the next most beneficial based on several measured parameters (Sohail et al., 2013).

These integrated findings underscore the potential of the Peruvidai breed for improved nutritional and developmental outcomes in poultry production. Similar studies on indigenous breeds, such as Kadaknath and Sakini chickens, have highlighted the importance of genetic selection in improving egg quality and productivity (Dinesh et al., 2024; Sapkota et al., 2020). Additionally, research on Naked-Neck and Aseel chickens has shown that breed-specific differences influence egg weight, shell strength, and overall production performance (Usman et al., 2014). The study highlights the value of a multifaceted approach in evaluating poultry performance and recommends future research into gene expression and hormonal profiling to further understand and enhance the genetic and physiological factors contributing to these observed differences (Mishra et al., 2022).

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