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Table of Contents

Original Research

Comparative Histomorphometric Analysis of the Proventriculus and Ventriculus of the Darag Philippine Native Chicken and Hubbard Redbro™

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Abstract

Background: Due to the increasing consumer's awareness of the impact of bird's welfare on meat quality and overall nutritional value, the global demand for chicken meat production has been gradually shifting to slow-growing breeds. However, comparative data on slow-growing commercial and native chicken breeds are scarcely available. **Methods:** Tissue samples of the proventriculus and ventriculus from *Darag* and Hubbard Redbro that were raised at different time periods were processed using standard staining technique for histomorphometric analysis. The measured histological parameters were then correlated with growth and selected production-related variables. **Results:** The proventricular deep gastric gland lobule area (DGGA) and depth (DGGD) as well as the ventricular tubular glands and lamina propria thickness (TGLPT) were comparable between breeds except during the 8th week period wherein *Darag* showed significantly higher TGLPT than Redbro. Interestingly, *Darag* exhibited a statistically significant strong positive correlation between DGGA and body weight, organ weight, and total feed consumption. Conversely, no significant correlation was found in Redbro strain. **Conclusion:** The findings of this study offer novel information regarding the histomorphological characteristics of the *Darag* chicken's stomach, which can potentially provide insights in further optimizing the current aspects of its management and farm practices.

Keywords: *Darag*, Histomorphometry, Proventriculus, Ventriculus, Philippine native chicken

1. Introduction

The Philippine native chicken is an important livestock resource in the country as it contributes to the supply of meat and eggs and considered as an income, savings, and insurance for rural Filipino farmers [1]. According to the chicken situation report released by the Philippine Statistics Authority (PSA) as of 30 September 2023, the native chicken population comprises the bulk of the 202.82 million total chicken population in the country accounting to 43.0% in comparison to the 34.7% and 22.3% share of the broiler and layer chickens, respectively [2]. This shows that the country's native chicken production is thriving and highly acceptable. Presently, there are many strains of native chicken in the country which include the *Banaba*, *Bolinao*, *Camarines*, *Paraokan*, *Boholano*, *ZamPen*, and *Darag* [3].

The *Darag* Philippine native chicken is an indigenous strain that is commonly raised in the Western Visayas area, specifically in the Island of Panay where it originated [2]. They are defined by their wheaten plumage color with hens having a penciled pattern and roosters displaying a plain pattern. They have medium to large single comb, white earlobes and skin color, orange irises, and mostly black shanks [4]. Adult males and females weigh around 1.30 and 1.00

kilograms, respectively, and are usually grown for 75-120 days to reach ready-to-slaughter age [3]. *Darag* chickens are usually preferred by rural Filipino farmers because of their lower rearing requirements compared to its commercial counterpart. They do not require specialized housing as they are normally grown free-range and are able to withstand the harsh natural environment without compromising their reproductive capability [5-6]. They are also relatively inexpensive as they do not need strict biosecurity measures and feeding requirements. Moreover, they are gaining popularity among local consumers as a healthier alternative due to their low cholesterol content not to mention their distinctive savory, gamey flavor [3].

At present, the demand for slow-growing chicken breeds like *Darag* and other native or indigenous chicken strains and some commercially available strains such as the Hubbard JA57, JA87, and Redbro has been consistently growing as influenced by the increasing knowledge of consumers on the impact of improved bird welfare on the nutritional and sensory characteristics of the meat [7-8]. Indeed, they are no longer just bred to cater a niche specialty market or serve as a meat source under a low-input system. The Redbro, which closely resembles *Darag*, is a hybrid broiler-type chicken that was developed by the Hubbard company to comply with the standards set forth by the Better Chicken Commitment (BCC) and Global Animal Partnership (GAP) to promote better welfare for chickens. It is reported to be more economical, yield better production performance, and produce a lower carbon footprint as compared to its older commercial counterpart [9-10].

However, despite their increasing popularity and utility, there is a marked scarcity of information to date on the comparative data of *Darag* and those slow-growing commercial strains especially in terms of the morphometry of their organs and its correlation with production and growth performance parameters. Recently, our group established a baseline information on the gross morphometry of the gastro- intestinal tract as well as the proportions of the lean, fat, and bone between *Darag* and Redbro thereby providing invaluable insights into the digestive capacity and nutrient utilization capability of these breeds [11]. In the present study, the comparative histomorphometric features of the stomach, particularly the proventriculus and ventriculus, of *Darag* and Redbro were investigated and then determined how these measured data relate to its growth and production performance. The findings of this work would provide better understanding of the histomorphological traits of *Darag* that will further aid in optimizing the current aspects of its management and farm practices.

2. Materials and Methods

2.1 Birds and Management

The birds used in the present study were provided by the completed "Genome-wide association study (GWAS) for growth and egg production traits of *Darag* native chicken" project of the Institute of Animal Science, College of Agriculture and Food Science (IAS-CAFS) which utilized 200 male day-old chicks consisting of 100 *Darag* and 100 Hubbard Redbro chicks [11]. The birds were raised for a total growing period of 15 weeks at the University Animal Farm of the IAS-CAFS (14.145485903561903, 121.25209186479026) and were housed under complete confinement system using elevated cages with the following pen dimensions: 4.0 ft (L) x 3.0 ft (W) x 2.5ft (H). They were maintained under a light exposure of 24 h for the first 7 days, 18 h from day 8 until day 21 followed by 12 hours for the remainder of the growing period until harvest. Environmental temperature, on the other hand, was kept at 31℃ from days 0 to 21, and then followed by 28℃ thereafter. The birds had free access to water and were fed with commercial booster feeds from 0 to 4 weeks of age, commercial starter feeds from 5 to 8 weeks of age, and commercial grower feeds from 9 weeks of age until harvest. All husbandry protocol and experimental procedures were carefully reviewed and approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee (UPLB-IACUC) with approval reference number, UPLB-2023-036 [11].

2.2 Sample Collection

Three (3) birds per group at different time periods (1, 2, 4, 6, and 8 weeks) were sacrificed via neck slaughtering wherein the trachea, esophagus, jugular vein, and carotid arteries were severed using a sharp knife [11]. A ventral coelomic incision was done to access the abdominal cavity, then the crop down to the rectum were collected. The proventriculus and ventriculus were carefully separated from any structures adhering to it, and the organ segments were then cut, weighed, and measured for total length. These segments were subsequently cut into smaller pieces and placed in a container with 10% neutral buffered formalin for at least 72 h.

2.3 Tissue Processing and Histomorphometric Examination

Tissue samples were subjected to routine paraffin technique and stained with hematoxylin and eosin (H&E). In detail, the formalin-fixed tissue samples were washed in running tap water and dehydrated in ascending concentrations of alcohol (70%, 80%, 90%, and 100%). These were then dipped in 100% xylene, embedded in paraffin, and cut into serial sections with 5 μm thickness using a microtome. For every four slides, one was stained with H&E in accordance with standard procedure. The sections were briefly deparaffinized in four changes of xylene for 5 min each, then were repeatedly immersed in a solution of 100% xylene and ethanol.

The slides were rehydrated by dipping it 10 times in decreasing concentrations of alcohol (100%, 90%, 80%, and 70%), followed by a 5 min rinsing over running water, and a 2 min staining with hematoxylin solution. Afterwards, the slides were dipped 5 times in 80% alcohol, stained for 5 to 10 min with eosin solution, and washed in ascending concentrations of alcohol. The slides were dipped in 100% alcohol, dipped 5 times in 100% xylene, and then soaked in two changes of xylene for 5 min each. Lastly, a drop of mounting medium was placed in the center of the slide and then covered with a clean coverslip.

The prepared H&E tissue sections were carefully examined using a compound light microscope (Amscope, China) to evaluate the histological and histomorphometric features of the proventriculus and ventriculus of both chicken strains that were collected at different time periods. Histomorphometric examination was carried out on the following parameters: the deep gastric gland lobule area (DGGA) and deep gastric gland lobule depth (DGGD) of the proventriculus and the combined tubular glands and lamina propria thickness (TGLPT) of the ventriculus. Using x10 magnification, the entire tissue section along with the micrometer was captured and was stitched together using the Photomerge feature of the Adobe Photoshop. DGGA, defined as the amount of surface that was covered by a lobule within the proventricular wall, was quantified by measuring at least 5 gland lobules. DGGD, defined as the distance from the wall of the gland to the tip of the epithelium of the gland, was quantified by measuring at least 5 depths from multiple lobules. Meanwhile, TGLPT, defined as the distance from the tip of the tubular gland to the base of the lamina propria, was similarly quantified by measuring the thickness of at least 5 different areas. Measurement of all parameters at each pre-determined time point was accomplished using Image J (Fiji software, National Institute of Health, Maryland, USA).

2.4 Correlation Analysis and Statistical Data

All data collected were analyzed using GraphPad Prism (San Diego, CA, USA). The mixed effects model was used to analyze the effect of age, strain, and their interaction on the different proventricular and ventricular parameters measured. The two-stage linear step-up method of Benjamini, Krieger, and Yekutieli was selected as the post hoc test to control the false positives that may be brought about by the mixed effects model. Furthermore, a two-tailed Pearson's correlation coefficient with a confidence interval of 95% was utilized to determine the relationship between the measured parameters and the animal's body weight, organ weight, and total feed consumption. All values with $P < 0.05$ were considered as significant, $P < 0.01$ were considered as highly significant, and $P < 0.001$ were considered as very significant.

3. Results

3.1 Proventricular DGGA and DGGD

The proventriculus, irrespective of the chicken strain, showed intact mucosal layers and prominent epithelial folds surrounding the centrally located lumen (Fig. 1A & 1B). The deep gastric glands, which are conspicuously occupying the bulk of the proventricular wall, presented some ovoid or pear-shaped lobules having multiple secretory tubules that are wrapped around with connective tissues and some muscle fibers (Fig. 1C). As shown in Fig. 1D, histomorphometric analysis of the proventricular DGGA did not significantly vary between *Darag* and Redbro at each of the examined time point. It was noted, however, that DGGA in *Darag* depicted a consistent upward trend starting at week 2 until week 8 whereas a steady increase was only observed in Redbro from week 1 to week 4. As for the DGGD, no statistically significant difference was likewise accounted between the two chicken strains throughout the experimental period (Fig. 1E).

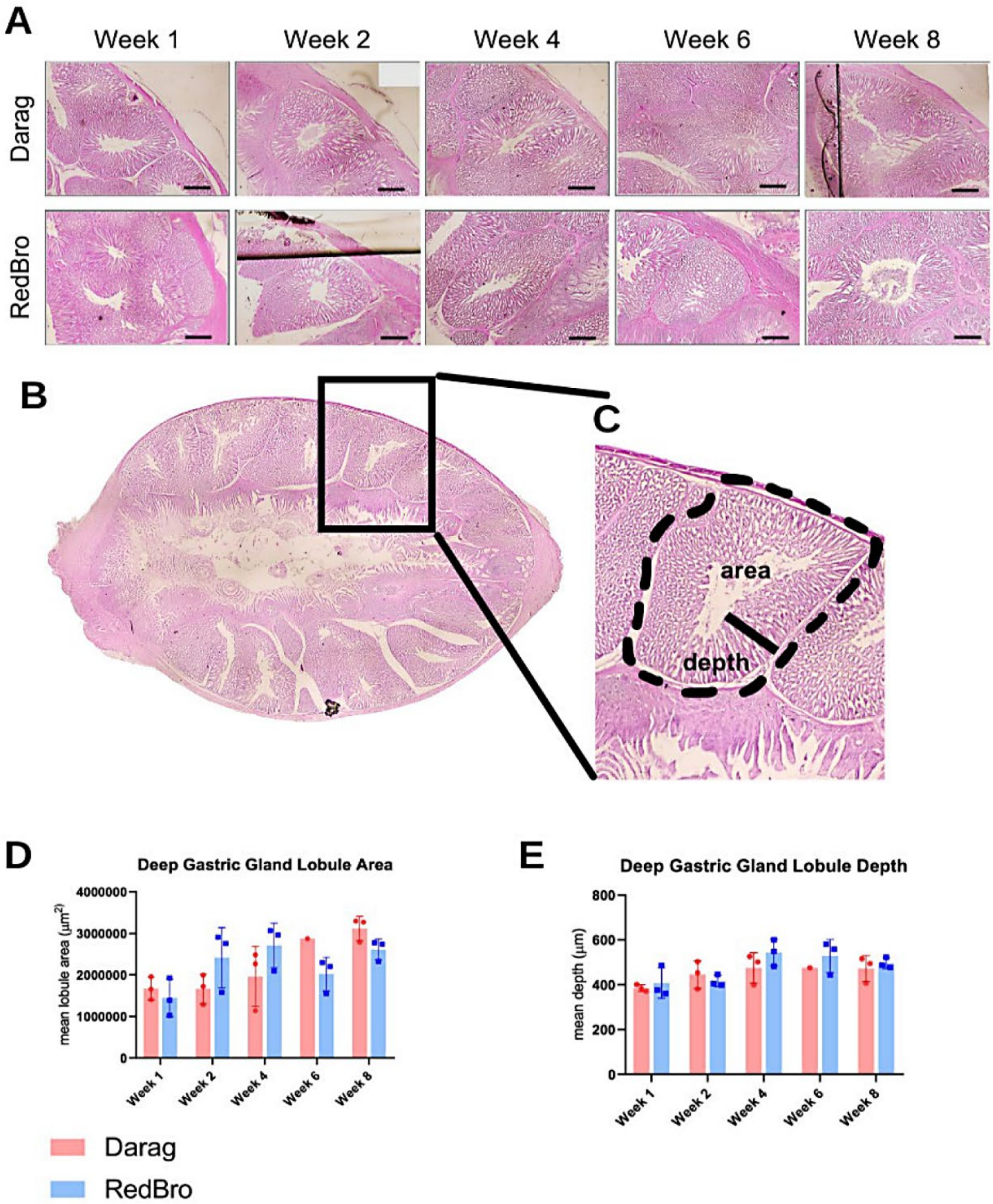

Fig. 1. Histomorphological findings of the proventriculus between the two chicken strains, *Darag* vs. Redbro show comparable parameters in all age points. (A) Representative images of the proventriculus sections from *Darag* and Redbro strains at weeks 1, 2, 4, 6, & 8. Scale bar = 500 µm. (B) Demonstrated is the whole proventriculus tissue section, and (C) the deep gastric gland lobule area (broken line) and depth (solid line). The bar graphs displaying (D) the mean deep gastric gland lobule area and (E) the mean deep gastric gland lobule depth of the proventriculus between the two groups at each examined time point. Data are shown as means ± standard deviation (SD) of 5 tissue sections per animal (n = 3 animals/group).

3.2 Ventricular TGLPT

Examination of the H&E sections of the ventriculus of both *Darag* and Redbro revealed a thick layer of keratinoid (cuticle) lining the mucosal tunic which presents columnar epithelium that are arranged in folds of varying height and glandular tubules that are divided by lamina propria. Also noticeable are the prominent muscular tunic consisting of the internal oblique, thick middle circular, and thin longitudinal layers (Fig. 2A-2C). Histomorphometric analysis of the ventricular TGLPT, on the other hand, unveiled statistically comparable results between the two chicken strains across all examined time points with the exemption of week 8 wherein a significantly increased TGLPT was exemplified by *Darag* as opposed to Redbro.

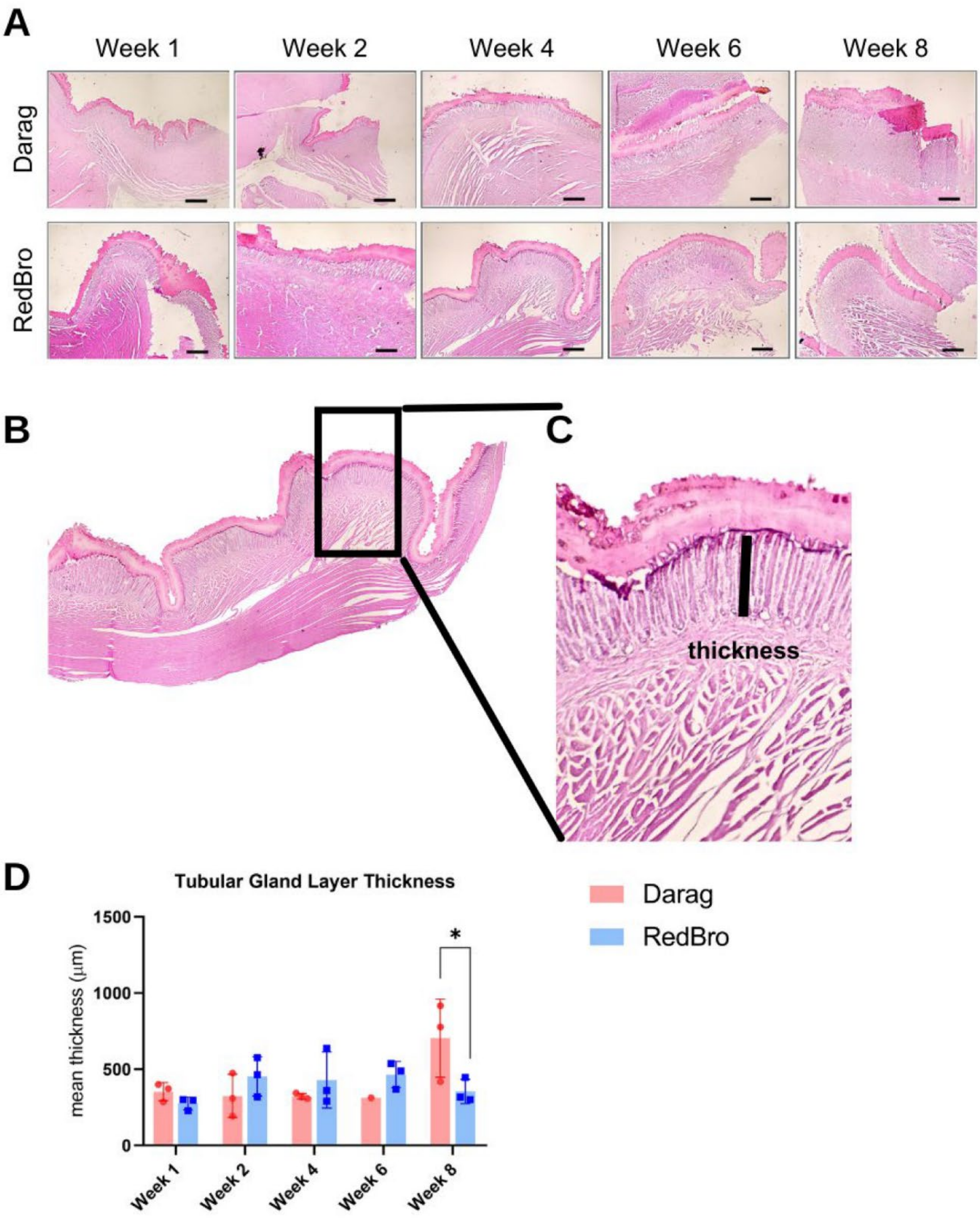

Fig. 2. Histomorphological findings of the ventriculus between the two chicken strains, *Darag* vs. Redbro show thicker tubular gland layers in the Darag strain. (A) Representative images of ventriculus sections from *Darag* and Redbro strains at weeks 1, 2, 4, 6, & 8. Scale bar = 500 μm. (B) Shown is the merged photo of the ventriculus. (C) The thickness of the tubular gland layer is demonstrated by the solid line. (D) The bar graph displaying the mean tubular gland layer and lamina propria thickness of the ventriculus between the two groups at each examined time point. Data are expressed as means ± standard deviation (SD) of 5 tissue sections per animal (n = 3 animals/group). * $P < 0.05$

3.3 Effects of Age and Strain on the Different Parameters, and their Interaction

Age had a highly significant effect on the proventricular DGGA and a significant effect on both proventricular DGGD and ventricular TGLPT. Interestingly, the interaction between age and strain only produced a highly significant effect on the variability of the mean ventricular TGLPT. Meanwhile, the strain did not appear to influence all examined parameters (Table 1).

3.4 Correlation between the different parameters and body weight

In sharp contrast to Redbro, the *Darag* strain exhibited a strong positive correlation between DGGA and body weight which achieved a high degree of statistical significance. The rest of the proventricular and ventricular parameters, regardless of chicken strain, did not yield any significant correlation with body weight (Table 2; Supplementary Table 1).

Table 1. Mixed-effects model test results for the effects of age and strain on the differences seen in each dependent variable.

Parameters	Age	Strain	Interaction
Proventriculus parameters			
DGGA	F (4, 18) = 6.0640 P = 0.0028**	F (1, 18) = 0.0080 P = 0.9294	F (4, 18) = 2.9030 P = 0.0513
DGGD	F (4, 18) = 4.4330 P =0.0114*	F (1, 18) = 1.5990 P = 0.2222	F (4, 18) = 0.6521 P = 0.6328
Ventriculus parameter			
TGLPT	F (4, 14) = 3.5010 P = 0.0351*	F (1, 4) = 0.0014 P = 0.971	F (4, 14) = 5.9600 P = 0.0051**

* Effect is significant at $P < 0.05$; ** Effect is significant at $P < 0.01$; DGGA, Deep gastric gland area; DGGD, Deep gastric gland diameter; TGLPT, Tubular gland layer and lamina propria thickness

Table 2. Two-tailed Pearson correlation coefficients between the histomorphometric parameters of the proventriculus and ventriculus of *Darag* and Redbro and body weight.

Parameters		*Darag*	Redbro
Proventriculus			
DGGA	Pearson's Correlation	0.966**	0.502
	Sig. (2-tailed)	0.008	0.389
	N	5	5
DGGD	Pearson's Correlation	0.702	0.663
	Sig. (2-tailed)	0.187	0.223
	N	5	5
Ventriculus			
TGLPT	Pearson's Correlation	0.764	0.173
	Sig. (2-tailed)	0.133	0.781
	N	5	5

**Correlation is significant at $P < 0.01$; DGGA, Deep gastric gland area; DGGD, Deep gastric gland diameter; TGLPT, Tubular gland layer and lamina propria thickness

3.5 Correlation between the different parameters and organ weight

In parallel with earlier findings on body weight, correlation analysis unveiled a highly significant strong positive relationship between proventricular DGGA and organ weight in *Darag* strain. Conversely, no significant correlation was determined between these factors in Redbro strain. Other measured parameters for both chicken strains did not appear to establish a significant correlation with organ weight (Table 3; Supplementary Table 2).

3.6 Correlation between the different parameters and total feed consumption

Among the different parameters measured, only the proventricular DGGA showed a statistically significant positive correlation with total feed consumption. This relationship, however, was notably observed in *Darag* alone but not in the Redbro strain (Table 4; Supplementary Table 3).

Table 3. Two-tailed Pearson correlation coefficients between the histomorphometric parameters of the proventriculus and ventriculus of *Darag* and Redbro and organ weight.

Parameters		*Darag*	Redbro
Proventriculus			
DGGA	Pearson's Correlation	0.968**	0.510
	Sig. (2-tailed)	0.007	0.380
	N	5	5
DGGD	Pearson's Correlation	0.725	0.819
	Sig. (2-tailed)	0.166	0.090
	N	5	5
Ventriculus			
TGLPT	Pearson's Correlation	0.663	0.354
	Sig. (2-tailed)	0.222	0.559
	N	5	5

**Correlation is significant at $P < 0.01$; DGGA, Deep gastric gland area; DGGD, Deep gastric gland diameter; TGLPT, Tubular gland layer and lamina propria thickness

Table 4. Two-tailed Pearson correlation coefficients between the histomorphometric parameters of the proventriculus and ventriculus of *Darag* and Redbro and total feed consumption.

Parameters		*Darag*	Redbro
Proventriculus			
DGGA	Pearson's Correlation	0.965**	0.685
	Sig. (2-tailed)	0.008	0.202
	N	5	5
DGGD	Pearson's Correlation	0.779	0.783
	Sig. (2-tailed)	0.121	0.117
	N	5	5
Ventriculus			
TGLT	Pearson's Correlation	0.674	0.337
	Sig. (2-tailed)	0.212	0.579
	N	5	5

**Correlation is significant at $P < 0.01$; DGGA, Deep gastric gland area; DGGD, Deep gastric gland diameter; TGLPT, Tubular gland layer and lamina propria thickness

4. Discussion

The gastro-intestinal tract morphometrics have been well established in literature to be intricately connected with the animal's digestive ability and nutrient absorptive capacity [12]. In relation to the bird's stomach, gross weight and dimensions (i.e. length, width, thickness) as well as the histomorphological features of the proventriculus and ventriculus have been extensively studied, as reported in diverse avian species including the ostrich [13], starling bird [14], red jungle fowl [15], turkey [16], Japanese quail [17], and even in commercial [18-20] and indigenous broiler chickens [21]. Recently, we have documented the comparative gross morphometry of the gastro-intestinal tract including the stomach tissues of the two slow-growing broiler strains, the *Darag* Philippine native chicken and a commercial strain, the Hubbard Redbro [11]. In the current study, the histomorphometric features of the proventriculus and ventriculus of these two chicken strains were further investigated to better understand the impact of genetic background on the morpho functional traits of the digestive organ. To our knowledge, our study belongs to the very few works that have attempted to investigate the histomorphometry of the gastric glands as majority have focused on determining the thickness of the layers or tunics of these gastric tissues.

Examination of the proventricular DGGA and DGGD revealed no significant difference between *Darag* and Redbro across all determined time periods. This coincided well with the gross morphometric findings which showed statistically comparable results on the normalized weight of the proventriculus between the two strains throughout the 8-week period [11]. On the other hand, the mixed effect model depicted a commensurate increase in both proventricular parameters with advancing age. These results essentially agree with the findings of Akter *et al.* (2018) [22] and Mehra and Kumar (2023) [23] using Cobb-500 broiler chickens. This is further supported by another study which accounted for the corresponding increase in the deep gastric gland diameter of the proventriculus as seen in Kadaknath fowl with increasing age [24]. The age-dependent increase in proventricular DGGA and DGGD may be attributed to increase in the number and size of the oxyntico-peptic cells lining the gland therefore resulting in higher digestive enzyme secretion and better digestion of ingesta [19]. This observation may also be explained in part by the fusion of two or more adjacently located glands as the bird ages thereby allowing formation of a large proventricular gland [24].

The ventricular TGLPT measured in both slow-growing chicken strains did not significantly deviate from one another during the first 6 weeks. However, a considerable increase in this parameter was notably observed on week 8 in *Darag* but not in the Redbro strain. This finding somewhat corroborated the result of the gross data except that significant difference in the normalized weight of the gizzard between the two strains was perceived as early as 3 weeks of age [11]. This apparent discrepancy implies that the expansion of other histological structures like the muscularis mucosa and the muscular layer might possibly occur in the ventriculus of *Darag* during these earlier growing periods wherein development of muscle fibers would be crucial in enhancing the muscle rhythm and digestive ability [12].

The mixed effect model provided confirmation that age, in combination with strain, truly influenced the variability of the mean ventricular TGLPT. Additionally, it showed that a linear relationship exists between TGLPT and age which is in consonance with previous studies [12,25]. The proportional increase in the thickness or height of the ventricular glands as a function of advancing age is critical for broilers and other avian species as this would consequently lead to an increase in the production of cuticle that is necessary for mechanical digestion. It has been previously shown that birds which are considered as insectivores, granivores, and herbivores possess a well-developed ventriculus with a prominently thick and abrasive cuticle layer [26-27]. Since the *Darag* native chicken breed has been particularly developed to be grown free-range and consume a variety of forages, insects, and grains instead of relying to commercial feeds like the Redbro strain, this would lend justification to the significantly greater ventricular TGLPT achieved during the 8th week of age. Moreover, it has been argued that availability of moderate amount of fiber in the diet of birds may enhance the size and development of the gizzard, which in turn could increase the flow of feed through this organ and allow higher contact between nutrients and digestive enzymes owing to the increase in the gastroduodenal refluxes [28].

Furthermore, it has been reported that fibrous material supplied by the range could enhance the production of insoluble non-starch polysaccharides (NSP) that may alter the development of gastro-intestinal tissues. In the case of the gizzard, this is typically reflected by a substantial increment in the size and weight of the tissue [20,29]. Future studies should incorporate analysis of the digestive secretions using histochemical staining methods [15,30-31] to gain better insights as to whether the significant increase in the size of the ventriculus along with its tubular glands in *Darag* would translate to more superior digestive function than in the Redbro strain.

Although gastric glands are integral component of the stomach as it facilitates efficient digestive process by elaborating essential enzymes and secretions that chemically act to reduce the complexity of ingested feeds and enhance the absorption of nutrients [32], the relationship between these structures and selected growth- and production-related variables are largely unknown. Therefore, in this present study, correlation analysis was finally performed between the measured proventricular and ventricular parameters and body weight, organ weight, and total feed intake in both slow-growing chicken strains. Results showed that *Darag*, in sharp contrast with the Redbro strain, demonstrated a statistically significant strong positive correlation between the proventricular DGGA and body weight, organ weight, and total feed intake. In support of our findings, Selim *et al.* [33] also found that birds fed post hatch had greater deep gastric gland lumen diameter along with greater mucosal fold and mucosal layer thickness of the proventriculus, versus the fasted group, therefore signifying that feed consumption exert an effect on the histomorphological structures of the proventriculus including its proventricular deep gastric gland [33].

On the other hand, the proventricular DGGD and ventricular TGLPT both failed to establish a substantial correlation with all the variables regardless of the chicken strain. These observations may be partly explained by the effect of intense selection pressure for desirable traits which tremendously disrupt the balance between growth performance and maturity of the organs [34]. As substantiated by previous works, the rapid increase in the muscle mass as especially observed in modern broiler chickens was associated with retarded growth of the digestive organs and presumably their associated gastric glands, when compared to unselected heritage lines of the same age [35-36]. Interestingly, our recent data unveiled a negative allometry for gastrointestinal growth in both chicken strains indicating that the growth of the gastro-intestinal tract proceeds at a relatively slower pace as compared to the increase in their body weight or muscle mass [11]. However, it should be noted that the ventricular TGLPT exemplified by *Darag* yielded a moderate to strong positive correlation with all the examined variables especially with respect to the body weight in contrast to the Redbro strain. In concordance with this, the *Darag* strain garnered a significantly higher normalized weight of the gizzard starting at week 3 until the end of the experimental period despite attaining a body weight that was more than 3-fold lower than those of the RedBro strain at any given point in time [11].

5. Conclusion

The findings of the present study essentially mirrored the gross morphometric findings showing comparable results among the measured proventricular and ventricular parameters between the two slow-growing broiler strains except for a significantly higher ventricular TGLPT exemplified by *Darag* during the 8th week period in contrast to the Redbro strain. Correlation analysis revealed that only the proventricular DGGA appears to correlate well with body weight, organ weight, and total feed intake as demonstrated by the statistically significant strong positive relationship in *Darag* but not in the Redbro strain. The novel information proffered by the present study may provide invaluable insights into further optimizing the current aspects of management and farm practices (i.e. feeding and nutrition) in *Darag* native chicken.

Availability of Data and Materials

All data are accessible to readers upon written request to the corresponding author.

Author Contributions

Conceptualization, M.J.M.D., V.A.M. and M.J.C.A.; Methodology, M.I.A.M.M., M.J.M.D., H.N.F.B. and M.J.C.A.; Investigation, M.I.A.M.M. and M.J.C.A.;

Writing – Original Draft, M.I.A.M.M., M.J.M.D. and M.J.C.A.; Writing – Review & Editing, M.I.A.M.M., M.J.M.D., V.A.M., H.N.F.B. and M.J.C.A.; Funding Acquisition, V.A.M.; Supervision, M.J.M.D., V.A.M. and M.J.C.A.; Resources, H.N.F.B. and V.A.M.

Ethics Approval and Consent to Participate

All experimental procedures adhered to pertinent international and local guidelines for the care and use of animals and were approved by the University of the Philippines Los Baños Institutional Animal Care and Use Committee with approval number UPLB-2023-036.

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