Effect of *Trichanthera gigantea* (Ketum Ayam) Leaf Supplementation on Growth Performance, Ileal Histomorphology and Caecal Microflora in Saga Chickens

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Abstract

The Saga chicken, developed to support sustainable poultry production in Malaysia, is valued for its meat quality but constrained by a relatively slow growth rate. It is essential to identify effective, locally available feed supplements to improve its productivity and sustainability. *Trichanthera gigantea* (Ketum Ayam) leaf meal has been traditionally used in poultry feeding, however, its effects on growth performance and intestinal integrity in Saga chickens is still lacking. This study evaluated the effects of dried *Trichanthera gigantea* leaf meal in varying inclusion levels (0%, 1%, 3% and 5%) on the body weight gain, feed intake, feed conversion ratio, ileal histomorphology and caecal coliform count in 120 four-week old male Saga chickens over an eight-week feeding trial. Body weight gain was optimal at the 1% inclusion level ($P<0.05$), whereas feed intake, feed conversion ratio and coliform counts were not significantly affected ($P>0.05$), suggesting preserved feed efficiency and microbial stability. Birds fed with 1% *Trichanthera gigantea* also exhibited significantly improved ileal morphology, characterised by reduced crypt depth and a higher villus height-to-crypt depth ratio ($P<0.05$), indicating enhanced intestinal absorptive capacity. These results confirm that 1% *Trichanthera gigantea* supplementation provides optimal intestinal integrity, supporting enhanced nutrient utilisation and offering a sustainable strategy to improve the productivity of the Saga chickens.

Keywords: *Trichanthera gigantea*, ileal histomorphology, villus height, crypt depth, caecal coliform, Saga chickens

Introduction

Poultry meat is one of the most widely consumed sources of protein globally, and Malaysia ranks among the highest consumers in Asia, with an annual per capita consumption of 49.3 kg of chicken meat (FAO, 2020 and DVS, 2024). Despite high domestic consumption, a significant portion of Malaysia's protein needs are met through imports (Mohd Rais, 2024), highlighting the urgent need to strengthen the domestic poultry farming sector.

One of the emerging segments in poultry farming is village chicken farming, which is gaining popularity due to birds' leaner meat and greater resistance to diseases. To meet growing consumer preferences for traditional village chicken meat, the Malaysian Agricultural Research and Development Institute (MARDI) has developed Saga chicken, a superior village chicken breed with desirable meat quality. However, Saga chicken has a slower growth rate, requiring 8 weeks to reach a slaughter weight of 1 kg (Nor Izwan Hakimi et al., 2024). Comparatively, commercial broiler breeds can achieve a body weight of 2 kg within 5 weeks (Shehata and Elsokary, 2024).

Growth-promoting agents in animal farming are acknowledged as an important tool to accelerate poultry growth (Nasarudin et al., 2024). Moreover, there is growing awareness among farmers regarding use of local and indigenous herbs as a means to support sustainable agricultural practices (Nasarudin et al., 2024). Therefore, to enhance the growth of Saga chicken it is essential to explore the use of locally available alternatives, including indigenous herbal feed supplements. *Trichanthera gigantea* (Ketum Ayam) has attracted attention due to its widespread availability in tropical agriculture (Rosales, 1997). This plant is widely cultivated and utilised by poultry farmers due to its high nutritional content and adaptability. It can serve as a component in formulated poultry diet, either fresh or processed into leaf meal. *Trichanthera gigantea* (Ketum Ayam) leaves have low levels of anti-nutritional factors and are highly palatable for animal consumption, making them a promising candidate as a natural feed supplement (Morbos et al. 2016). These attributes suggest that *Trichanthera gigantea* (Ketum Ayam) may enhance growth performance and support gut health by improving nutrient utilisation and intestinal function. However, there is limited scientifically published literature on the effects of *Trichanthera gigantea* leaves on poultry, particularly related to growth performance (body weight gain, feed intake and feed conversion ratio), intestinal histomorphology and caecal coliform counts. The ileum is important for nutrient absorption and changes in mucosal architecture, such as villus height, crypt depth and villus height-to-crypt depth ratio, and together with growth performance and caecal coliform counts, provide a holistic evaluation of the potential impact of feed supplements on gut health and intestinal integrity.

Therefore, this study aims to evaluate the optimal inclusion level of dried *Trichanthera gigantea* (Ketum

Ayam) leaves to enhance growth performance, elucidate intestinal integrity via ileal histomorphology, and assess caecal microbial impact in the Saga chicken as a holistic assessment of gut health and productivity.

Materials and Methods

Experimental Design and Animal Husbandry

This experiment was conducted under the approval of MARDI Animal Ethics Committee (AEC no. 20220418/R/MAEC00109). A total of 120 Saga male birds aged 4 weeks, with similar average weight distribution were used in this study. The birds were randomly assigned to four dietary treatment groups, each consisting of six replicates (cages), with five birds per replicate. Each cage served as the experimental unit for growth performance parameters.

Prior to the feeding trial, all birds were raised on a deep litter flooring system (during brooding period) and fed a commercial starter diet containing 21% crude protein and 12 MJ/kg metabolizable energy from hatch to four weeks of age. The experimental feeding trial was conducted for eight weeks, during which the birds were housed in 2-tier battery cages with each cage measuring 157cm (length) x 57cm (width) x 62cm (height) in an enclosed house system. The average temperature and humidity for the trial were 28.0 °C and 65.0%, respectively with 12-hour lighting schedule. Throughout the feeding trial, both feed and water were provided ad libitum.

Dietary Treatments and Feed Preparation

During the experimental period, the birds were fed an isocolaric and isonitrogenous grower diet containing 19% crude protein and 11 MJ/kg metabolizable energy. The basal diet served as the control (0% *Trichanthera gigantea*) and was formulated according to standard nutrient requirements for Saga chickens as shown in Table 1.

Dried *Trichanthera gigantea* (Ketum Ayam) leaf meal was incorporated into the basal diet at at inclusion levels of 1%, 3% and 5% *Trichanthera gigantea* leaves. The *Trichanthera gigantea* leaves were harvested oven-dried at 60^{o}C until it reached constant weight and subsequently ground using a standard laboratory grinder to get a fine meal with 2mm in diameter. All experimental diets were prepared in a mash form.

Proximate Analysis of Trichanthera gigantea Leaf Meal

The dried *Trichanthera gigantea* (Ketum Ayam) leaf meal was subjected to proximate analysis to determine crude protein, crude fibre, crude fat, ash and dry matter content following standard AOAC procedures and presented in Table 2.

Table 1: Ingredient composition of basal diet

Ingredients	Ingredient Composition % (Basal diet)
Maize	40.75
Wheat pollard middlings	20.00
Soya bean meal (44%)	18.00
Rice bran	9.36
Maize gluten meal (60% crude protein)	5.25
Palm kernel expeller	1.70
Limestone	1.53
Palm oil	1.10
Dicalcium phosphate, 20%P	1.06
Lysine HCl	0.51
Salt	0.40
Methionine	0.14
Mineral premix	0.10
Choline chloride (60%)	0.07
Vitamin premix	0.03

Table 2: Proximate analysis of *Trichanthera gigantea* (Ketum Ayam) leaf meals

Parameters	Proximate Analysis
Dry matter (DM)	18.40%
Gross energy	15.37 MJ/kg DM
Crude protein	15.40%
Crude fat	1.19%
Crude fibre	17.63%
Ash	13.95%

Note: 1. Values of the chemical composition are tabulated as mean

Data Collection and Laboratory Methods

Body weight data was recorded at the beginning and end of the experimental period to calculate the body weight gain. The formula used for body weight gain (kg) equates to final body weight of Saga chicken - initial body weight of Saga chicken. Feed intake (FI) was measured on a cage basis as the total amount of feed consumed during the trial. Feed conversion ratio (FCR) was calculated using the following formula:

FCR = Feed Intake / Body Weight Gain

At the end of the feeding trial, one bird from each replicate cage was sacrificed by exsanguination and slaughtered. About 2 cm of the ileum segment was collected approximately 5 cm proximal to the ileocecal junction, and placed in a 10% formalin solution. Upon fixation, the ileum segment was stained with hematoxylin and eosin. From each bird, three well-oriented sections were analysed. From each section, ten intact villus and corresponding crypts were microscopically measured. The villus height (μm), crypt depth (μm) and the villus height:crypt depth ratio was calculated to assess the ileal villus height and crypt depth.

For total coliform plate count determination, one gram of the caecal content was collected from one bird from each replicate cage. The caecal contents were then homogenised and subjected to ten-fold serial dilutions in peptone water which were plated on MacConkey agar for selective enumeration of coliform bacteria. Plates were incubated at 37^{o}C for 24 hours and coliform counts were expressed as colony forming units (cfu/g) of caecal content The caecal coliform counts (cfu/g) were transformed to logarithmic form ($\log_{10}$ cfu/g) prior to statistical analysis.

Statistical Analysis

The data obtained were analyzed statistically using one-way Analysis of Variance (ANOVA) with dietary treatment as the fixed factor, using SAS software version 9.3. Cage means were used as the experimental unit for growth performance parameters (body weight gain, FI and FCR). When significant differences were detected ($P<0.05$), treatment means were compared using Duncan's Multiple Range Test.

Results and Discussion

Growth Performance (Body Weight Gain, Feed Intake and Feed Conversion Ratio)

The effects of the supplementation of dried *Trichanthera gigantea* (Ketum Ayam) leaf meal on the growth performance of the Saga chickens are shown in Table 3. Birds fed the control diet and those supplemented with 1% *Trichanthera gigantea* (Ketum Ayam) leaves recorded significantly higher ($P<0.05$) body weight gain at 1.51kg compared to those with 3% and 5% inclusion levels. However, no significant differences ($P>0.05$) were observed among treatments for total feed intake or feed conversion ratio.

The absence of significant differences in feed intake indicates that inclusion of *Trichanthera gigantea* leaf meal up to 5% did not adversely affect feed palatability. Similarly, the lack of variation in feed conversion ratio suggests that overall feed efficiency in terms of feed consumed per unit of weight gain remain comparable across all dietary treatments. Therefore, the reduced body weight gain observed at higher inclusion levels (3% and 5%) cannot be attributed to differences in feed intake or feed conversion ratio. Instead, the reduction in body weight gain at higher treatment levels may be related to higher levels of leaf meal that likely increase the dietary fibre load introduced by the leaf meal, which may have reduced nutrient availability through reduced digestibility. Such effects may not always be reflected in the feed conversion ratio values, but could still negatively impact growth performance especially in monogastric animals such as poultry.

These findings are consistent with previous studies reporting that higher inclusion levels of *Trichanthera gigantea* leaf meal resulted in reduced body weight gain without necessarily affecting feed intake. For instance, Paguia et al. (2024) evaluated inclusion levels of 0%, 5% and 15% fermented *Trichanthera gigantea* leaf meal and reported that only the control group achieved the highest body weight gain, which was significantly higher than both the 5% and 15% treatment groups. Similarly, Morbos et al. (2016) found that increasing *Trichanthera gigantea* leaves concentration beyond the optimal level led to reduced body weight gain. This trend was not just limited to chickens; other poultry, such as mallard duck also showed a decreasing trend in weight gain with higher levels of *Trichanthera gigantea* supplementation (Marcos et al., 2024). One possible explanation is that, increasing the concentration of *Trichanthera gigantea* leaf concentrations beyond the optimal levels also increased the dietary fiber content. Overall, these results indicate that supplementation of *Trichanthera gigantea* (Ketum Ayam) leaf meal at 1% supports optimal growth performance in Saga chickens, whereas higher inclusion levels may exceed the physiological tolerance for young monogastric poultry.

Table 3. Body weight gain and ileal histomorphology of the birds

Parameters	Inclusion of dried *Trichanthera gigantea* leaves				P-Value
	Control (0%)	1%	3%	5%	
Body weight gain (kg)	1.51^{a}	1.51^{a}	1.42^{b}	1.39^{b}	0.0042
Total feed intake (kg)	5.62	5.74	5.60	5.71	0.9309
Feed conversion ratio	3.72	3.81	3.96	4.13	0.3628
Villus height (µm)	762.56^{b}	895.94^{a}	860.52^{ab}	885.94^{a}	0.0552
Crypt depth (µm)	157.44^{a}	132.63^{b}	143.46^{ab}	160.00^{a}	0.0081
Villus height: crypt depth ratio (v/c)	5.24^{c}	7.50^{a}	6.81^{ab}	6.00^{bc}	0.0007
Caecal coliform count (log_{10} cfu/g)	6.75^{a}	6.65^{a}	6.63^{a}	5.24^{b}	0.0656

Note: ^{abc} means with different superscripts within the same row differ significantly (P<0.05)

Ileal Histomorphology and Intestinal Integrity

Dietary supplementation with *Trichanthera gigantea* (Ketum Ayam) leaf meal significantly influenced ileal mucosal architecture (as shown in Table 3). The birds fed with 1% *Trichanthera gigantea* diet exhibited a significantly higher (P<0.05) villus height-to-crypt depth (V:C) ratio of 7.5 compared to the control and higher inclusion levels. This improvement was mainly driven by a reduction in crypt depth, indicating lower epithelial turnover and improved intestinal stability.

Although the birds fed with 3% and 5% *Trichanthera gigantea* exhibited relatively high villus height values, these treatments were associated with deeper crypts, resulting in lower overall V:C ratios A higher V.C. ratio is reflective of better ability of the intestine to absorb and utilize nutrients (Ogbuewu et al., 2024). Hence, the reduced V:C ratio reflects the increased metabolic demand for intestinal tissue renewal and it is also indicative of the birds' response to intestinal stress or inflammation. This may partially explain the reduced body weight gain observed at higher inclusion levels, despite similar feed intake and feed conversion ratio. Histological observations support these findings. Figure 1 shows the histology section of control group while Figures 2, 3 and 4 illustrate the results in treatment 1, 2 and 3, respectively. Figure 2 illustrates the elongated villi with shallow crypts and a well-organised epithelial structure in birds fed with 1% *Trichanthera gigantea* diet. In contrast, Figures 3 and 4 show comparatively deeper crypts and less uniform villus organisation at the 3% and 5% inclusion levels, which may reflect increased epithelial turnover and intestinal adaptation potentially associated with increased dietary fibre contribution at higher plant inclusion levels. Similar improvements in intestinal morphology have been reported in broilers fed with low inclusion levels of tropical leaf mixtures (Ogbuewe et al., 2024) and in mallard

ducks supplemented with *Trichanthera gigantea* leaf meals (Marcos et al., 2024).

The significantly higher V:C ratio observed at the 1% inclusion level indicates enhanced intestinal absorptive capacity and reduced epithelial turnover, reflecting a more functionally efficient ileum. Although the present study did not characterise the bioactive composition of *Trichanthera gigantea* leaf meal, previous studies have reported the presence of phenolic and saponin contents ranging from approximately 0.012-0.023% and 6.45-9.67%, respectively (Abdul Rashid et al., 2025). These compounds are widely associated with antioxidant and anti-inflammatory activities that may support epithelial stability and reduce excessive crypt proliferation. Therefore, the collective findings suggest that the intestinal benefits of *Trichanthera gigantea* are dose-dependent and optimally expressed at low inclusion levels, where the nutritional and functional components of the leaf meal support intestinal absorptive function without imposing excessive metabolic or structural stress on the gut.

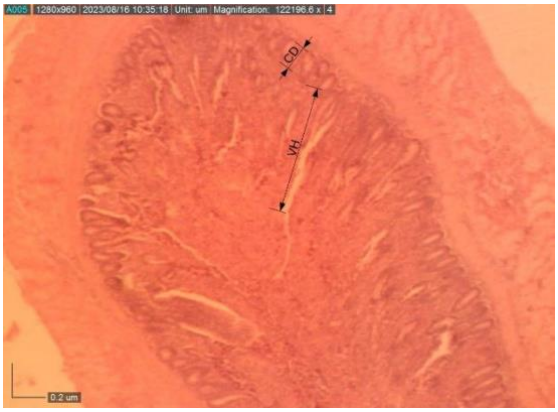

Figure 1: Histology section of the ileum from the Control group

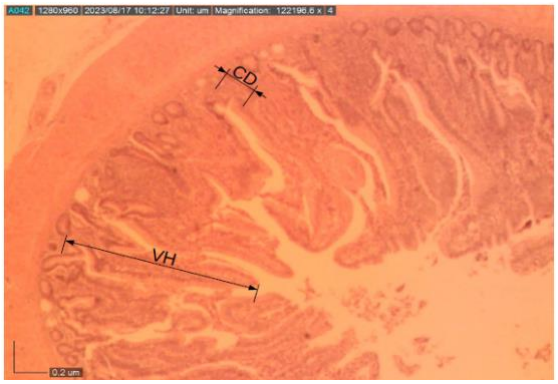

Figure 2: Histology section of the ileum from 1% *Trichanthera gigantea* supplementation

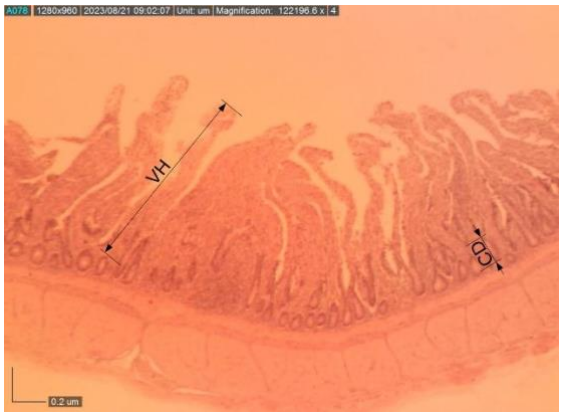

Figure 3: Histology section of the ileum from 3% *Trichanthera gigantea* supplementation

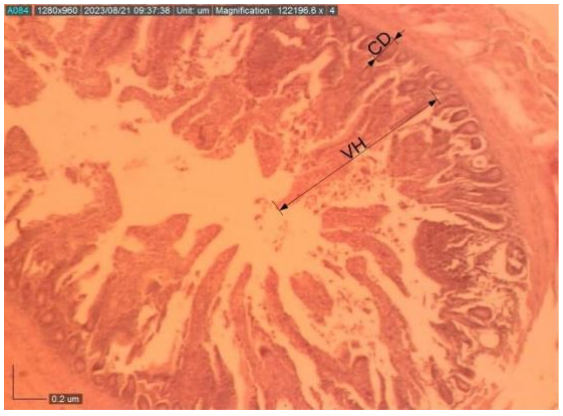

Figure 4: Histology section of the ileum from 5% *Trichanthera gigantea* supplementation

Caecal Coliform Count and Microbial Stability

Caecal coliform counts were not significantly affected (P>0.05) by the supplementation of *Trichanthera gigantea* (Ketum Ayam) leaves at any of the inclusion levels (Table 3). This result indicates that these inclusion levels neither promoted nor inhibited coliform proliferation in the caecum of Saga chickens.

The absence of significant changes in coliform counts suggests that supplementation with *Trichanthera gigantea* leaf meal at the levels studied, did not disrupt the existing microbial balance within the caecum. From a gut health perspective, maintaining microbial stability is desirable, as excessive suppression or proliferation of coliform bacteria may negatively affect digestive function and intestinal homeostasis. Therefore, the present findings indicate that *Trichanthera gigantea* supplementation was microbiologically neutral and compatible with the maintenance of a stable gut environment.

Comparable outcomes have been reported in broiler chickens fed with phytogenic feed additives such as *Moringa oleifera* leaf powder, where no significant differences in total viable bacterial or coliform counts were observed across various inclusion levels from 0%, 1%, 5% and 7.5% (Abd El-Ghany and Eraky,2020). These findings support the view that not all plant-based feed supplements exert pronounced antimicrobial effects and their main contribution may lie in supporting gut function without perturbing microbial equilibrium.

Overall, this study demonstrates that low-level inclusion of *Trichanthera gigantea* leaf meal can beneficially modulate growth performance and intestinal morphology in Saga chickens without adversely affecting feed intake, feed efficiency or caecal coliform populations. The lack of performance improvement at higher inclusion levels shows the importance of dosage optimisation when incorporating fibre-rich leaf meals into Saga chicken diets. These findings support the use of *Trichanthera gigantea* leaf meal as a locally available feed supplement that supports intestinal functionality and sustainable productivity for village chicken like Saga chickens.

Conclusion

In conclusion, dietary supplementation with 1% dried *Trichanthera gigantea* (Ketum Ayam) leaf meal represents the optimal inclusion level for Saga chickens, effectively enhancing growth performance with maximised body weight gain while enhancing intestinal integrity without disrupting feed intake, feed efficiency or caecal microbial stability. This efficacy is supported by superior ileal histomorphology, characterised by reduced crypt depth and an increased villus height-to-crypt depth ratio, indicating improved intestinal absorptive function and nutrient utilisation. These overall findings provide compelling evidence for the strategic use of a sustainable, indigenous botanical resource to overcome growth limitations in the Saga chicken, offering a path toward improved feed efficiency and strengthen

the resilience of village chickens in Malaysia.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Abd El-Ghany WA, Eraky RD. Influence of dietary Moringa oleifera on broilers performance, intestinal microbial population and humoral immune competence. J. Hellenic Vet. Med. Soc. 2020; 70(4): 1805-1810.

Abdul Rashid R, Idris LH, Abu Hassim H, Mohd Noor MH. Effect of *Trichanthera gigantea* (Humb & Bonpl.) Nees Leaf Harvested at Different Stages of Maturity on Its Nutrient and

Secondary Metabolites Compounds. Pertanika J. Trop. Agric. Sci. 2025; 48(6): 2119-2136.

Department of Veterinary Services, Malaysia. Livestock Statistics 2023/2024. Ministry of Agriculture and Food Security, Malaysia; 2024.

Erener G, Ocak N, Altop A, Cankaya S, Aksoy HM, Ozturk E. Growth performance, meat quality and caecal coliform bacteria count of broiler chicks fed diet with green tea extract. Asian Australas. J. Anim. Sci. 2011; 24(8): 1128-1135.

FAO et al. The state of food security and nutrition in the world: Repurposing food and agricultural policies to make healthy diets more affordable. Rome: Food and Agriculture Organisation; 2022 [Online]. Available: https://doi.org/10.4060/cc0639en. Accessed 1 June 2025.

Marcos MJL, Sumalbag JG, Cauilan JD. Histological profile of jejunum and liver of mallard duck fed with Madre de agua (*Trichanthera gigantea*) leaf meal. Int. J. Biosci. 2024; 25(2): 249-258.

Mohd Rais, MH. Revolutionising Malaysia's chicken farming industry. Business Today Malaysia [Online]. 2024 Jan 8. Available: https://www.businesstoday.com.my/2024/01/08/revolutionising-malaysias-chicken-farming-industry/. Accessed 1 June 2025.

Morbos CE, Dinah ME, Loliot CB. Growth performance of Philippine native chicken fed diet supplemented with varying levels of Madre de Agua (*Trichanthera gigantea* Nees) leaf meal. Ann Trop Res. 2016; 38(1): 174-182.

Nasarudin MAS, Abdul Razak AS, Mohamad Termizi AZA, Hairolnizam HFA, Amalina F, Ab Hamid MR, Samat, N. Alternative plant protein sources *Trichanthera gigantea* (Ketum Ayam) for poultry feed: A review. Constr. 2024; 4(2): 238-243.

Nor Izwan Hakimi NA, Mohd Noor Hisham MN, Roziatul Erin AR, Sarah R, Norham I, Muhammad Rusydi R, Harivanthan M, Noraini S. Effect of different levels of lysine and fibre-degrading enzyme on growth rate and gut development in the starter phase of slow-growing Ayam Saga. Mal. J. Anim. Sci. 2024; 27(2): 1-19.

Ogbuewu IP, Mabelebele M, Mbajiorgu CA. Determination of performance

response of broilers to fermented tropical leaf meal supplementation using meta-analytical method. Trop. Anim. Health Prod. 2024; 56(98): 1-16.

Paguia HM, Paguia RQ, Peralta RA, Esaga T, Balba CM, Corpuz MNC. Effect of fermented Madre de Agua leaf meal (*Trichanthera gigantea*) on growth performance of heritage free-range chicken (*Gallus domesticus* Linn.) GPH J. Agric. & Res. 2024; 7(2): 43-50.

Rosales M. *Trichanthera gigantea* (Humboldt & Bonpland) Nees: A review. Livest. Res. Rural. Dev. 1997; 9(4): 46-53.

Shehata SF, Elsokary MMM. Influence of various marketing ages on the growth, carcass characteristics, and economic indices of broiler chickens. Mansoura Vet. Med. J. 2024; 25(1): 61-68.

Wall DC, Malheiros RD, Anderson K, Anthony N. Comparative intestinal histological features observed in 1940 leghorn vs. 2016 leghorn-based commercial laying hens fed representative diets. Int. J. Pl. Anim. Environ. Sci. 2016; 13(4): 116-125.