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Histological profile of jejunum and liver of mallard duck fed with madre de agua (*Trichanthera gigantea*) leaf meal

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Abstract

This study aimed to evaluate the morphology of jejunum and liver of improve Philippine mallard duck fed with madre de agua (*Trichanthera gigantea*) leaf meal within 14 weeks of feeding trial. A total of 225-day-old ducklings were assigned at dietary levels of 0, 3, 6, 9, and 12%. Mallard ducks were fed following the recommended feeding guide for mallard ducks and provided water *ad libitum* throughout the experiment. Jejunal segment of the small intestine and liver were collected using a scalpel blade by making a 2-cm thickness transverse cut and near of its margin, respectively. Samples collected were fixed and stored in 10% formalin solution for over 24 hours and 5-micron thick hematoxylin-eosin-stained sections were prepared following paraffin embedding and histological processing. Data on growth performance and histological evaluation were recorded. Data gathered were subjected to analysis of variance of Completely Randomized Design. Based on the results of the study, the inclusion of madre de agua leaf meal on the intestinal villi of mallard ducks did not affect significantly in terms surface area of villi, however, significant differences in the crypt depth were observed. The results are in congruence to final body weight, gain in weight and FCR. On the other hand, the microscopic evaluation of the liver of improved Philippine mallard ducks had no observed pathological changes which shows that the madre de agua have no toxic component that can cause damage to the liver with prolonged feeding. The use of madre de agua leaf meal is safe as feed ingredient, hence recommended. The leaf meal can be included in the improved Philippine mallard ducks' diet without any detrimental effects on their health performance. However, further research is needed to include the use of commercial duck feeds to compare and to obtain a more conclusive result.

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Introduction

Poultry is a high-quality animal protein source, and various strategies have been used to boost poultry production profitability. Determination of nutrient requirements of different types of poultry is necessary to use efficiently the genetic potential of these birds for specific production goals (Pym, 1990). Dietary nutrient density is the most critical nutritional factor in commercial production, not only because it has a significant effect on growth performance, carcass quality, and health of poultry, but also because of economic inputs and outputs (Scott, 2002; Sterling *et al*., 2005; Brickett *et al*., 2007).

Duck rising is a lucrative livestock industry in the Philippines because of its eggs. Ducks are next to chickens in terms of their economic importance as a source of eggs as well as meat. Local farmers and entrepreneurs ventured into duck farming because of some economic value and a significant role in the Filipino culture. Duck farming does not require costly and elaborate housing facilities which needed slight space for rearing purposes. It can succeed in a wide range of climatic and nutritional conditions (Dagaas and Chang, 2004), is resilient to avian diseases, and can subsist on various feedstuffs.

The Philippine native mallard ducks (*Anas platyrynchos* Lin.) are raised by smallholders that provide low-cost animal protein and income for the poorer sector of the population in the rural areas through the selling of duck eggs, either fresh or boiled. Duck eggs have a demand for higher prices than table eggs of chicken. Ampode and Espina (2019) reported that duck egg sizes are large, thick shells and suitable for processing into value-added products such as salted, boiled, and *balut* (embryonated egg).

The perception of consumers on raw and cooked meat quality has created significant interest in increasing the understanding of digestive physiology and the dynamics of the gut microflora (Dibner and Richards, 2005). Physiological studies reveal that a functional gastrointestinal tract is vital for the digestion and absorption of nutrients required for the bird's maintenance and growth (Mateos *et al*., 2002; Baurhoo *et al*., 2009).

The digestive tract is the main site for digestion and absorption of nutrients in animals. It also acts as the largest immunological organ in the body, as it is the first point of protection against exogenous pathogens that enter the body, preventing the pathogens from colonization and entering the host cells and tissues (Choct, 2009). A balanced gut microorganism population, consisting of less pathogenic bacteria, and an increase in beneficial bacteria, may increase the availability of nutrients (Hashemi and Davoodi, 2010).

The liver is regarded as an essential and biggest gland of the body, and it plays a crucial role in numerous physiological processes such as synthesis of blood proteins, production and secretion of bile, detoxification, nutrients absorption, metabolism of several substances, and storing metabolites (Saez *et al*., 2012; Odokuma and Omokara, 2015).

Several additives from plants have been reported to contain nutrient properties that affect gut microflora, intestinal morphology, and meat quality of poultry. One of the possible sources of cheap protein is the leaf meal of some tropical legumes and browse plants to boost their income through the poultry business (WAC, 2006). Leaf meal supplements have been also included in the diets of poultry birds as means of increasing weight, improving gut health, and thereby reducing the cost of production (Nworgu and Fapohunda, 2002; Esonu *et al*., 2013; Nworgu *et al*., 2012).Traditionally, supplementation of protein to ducks during egg production is practiced. Unfortunately, sources of supplements such as snails and small shrimps have become scarce. In this regard, there is a next to explore potential locally available plants as a protein source to lessen feed costs and increase the profit of duck raisers (Lacayanga, 2015). Trichantera (*Trichantera gigantea*), also known as Nacedero, can be considered for this purpose. It is a fodder tree that adapts well to tropical conditions and grows easily between plantation crops, its protein content ranges from 17% to 22% on a DM basis and has high calcium content compared to other fodder trees (Rosales, 1997; Garcia *et al.*, 2008). Hence, this study was conducted to evaluate the morphology of jejunum and liver of mallard duck fed with amdre de agua leaf meal.

Materials and methods

Madre de agua leaves were collected and gathered using pruning shear from the Itik Farm Project at Isabela State University, Echague, Isabela. The age of madre de agua leaves used was at 90 days of regrowth.

After harvest, the leaves were immediately chopped and air-dried in a plastic net, turning the leaves occasionally for uniform dryness. The basis for monitoring dryness was the brittleness, texture, and color. The dried leaves were then hammer-milled through a 2-mm sieve. This was mixed with other feed ingredients to make a formulated ration appropriate for the improved Philippine mallard ducks.

Nutrient requirement of the mallard ducks was used to determine and as basis in the formulation of the diets.

Two hundred twenty-five 14-day old mallard ducks were randomly selected and distributed to treated and control groups with 15 animals per group with 3 replications. The treatments used in the study were:

T_1 – Control (Formulated feeds without leaf meal

T_2 – Formulated feeds with 3% Madre de agua leaf meal (MALM)

T_3 – Formulated feeds with 6% Madre de agua leaf meal (MALM)

T_4 – Formulated feeds with 9% Madre de agua leaf meal (MALM)

T_5– Formulated feeds with 12% Madre de agua leaf meal (MALM)

The composition of the experimental diet was least-cost formulated to contain the recommended nutrients for growing ducks for optimal performance. All nutrients met or exceeded the Philippine Recommendation for Poultry and Livestock Feed Formulation. The five dietary treatments were the basic diet supplemented with 0 (control), 3, 6, 9, and 12% madre de agua leaf meal. The experimental diet was given every 7:00 in the morning, 12:00 noon, and 4:00 in the afternoon, following the recommended feeding guide for mallard duck as *the* basis. The feed given was weighed and recorded, and a separate feed container was provided for every treatment. Feed refuse was collected and weighed. Further, clean drinking water was provided throughout the feeding experiment. The experimental ducks were treated equally as to other environmental requirements throughout the experimental period.

At 14 weeks of age, two ducks were randomly selected per treatment and fasted with feed for six hours, and water was offered *ad libitum*. Body weights were recorded before they were subjected to manual slaughter. Ducks were exsanguinated by a unilateral neck that severed the right common carotid artery and external jugular vein. After bleeding, scalding, plucking, and washing followed, the feet and head were removed. Carcasses were eviscerated manually, and the internal organs were removed.

The jejunal segment of the small intestine was collected using a scalpel blade. This was obtained by making a transverse cut in the jejunum with a 2-cm thickness. This segment of the intestine was selected since the jejunum serves as the site where a majority of nutrients are absorbed.

A 2-cm thickness of tissue sample was also collected from the lobe of the liver near its margin.The samples collected were fixed and stored in 10% formalin solution for over 24 hours and 5-micron thick hematoxylin-eosin-stained sections were prepared following paraffin embedding and histological processing.

The histological sections were evaluated using standard light microscopy. Measurements were made from photomicrographs taken at 100× magnification. Major changes can occur in the intestine due to postmortem interval and fixation time and considerable variation in the quality of sections can occur. It is important to minimize these effects and to use only those regions of the intestinal sections presenting good morphology. Therefore, the length of both the villi and crypt regions three adjacent to each other were measured for each sample. From the same photomicrograph regions, measurements of areas for a determined length of the intestinal segment were made for corresponding total mucosa and crypt regions. Villi measurements were taken, the villus height or length was obtained by measuring the distance from the apex to the base of the villi, and villus width was obtained by measuring the distance from the base of the villi to the junction to the basement membrane of the epithelial cell at the bottom of the crypt.

The liver was also examined using a light microscope and the portal triad was located and the neighboring hepatocytes were evaluated for any pathological changes.

Villi were selected from each section (3 villi per section) that demonstrated the longitudinal view from base to tip. The villus height was measured from the tip to the bottom excluding the crypt, while the villus width was measured at the basal and apical parts (Plate 4). Surface area was computed using the formula below:

Surface Area = (Villi Height) (Villi width × 2)

All data gathered were subjected to Analysis of Variance of Completely Randomized Design and comparison of treatment means was done using Least Significant Difference (LSD) Test. Analysis was carried out using the Statistical Tool for Agricultural Research (STAR).

Results and discussion

Histology of jejunum of the small intestine and the liver of mallard duck was conducted including the measurement of the jejunum villi height, jejunum villi width, and jejunum crypt depth and the histological evaluation of the liver portal area, hepatic sinusoids, and hepatocyte.

Intestinal morphology of the mallard ducks

The surface area of villi and crypt depth of the jejunum of mallard ducks fed with madre de agua leaf meal is presented in Table 1. No significant differences were observed in jejunal surface area of villi; however, a significant difference was observed in the crypt depth of the mallard duck. Nonetheless, the mallard ducks fed with 6% madre de agua have the greatest surface area of villi among treatments with a mean value of 1040374.73 µm (T3) while in Treatment 2, ducks fed with 3% madre de agua leaf meal obtained the lowest surface area of villi with a mean value of 329754.02 µm. An increase in villi length results in an increase in surface area, and therefore more area for absorption of nutrients to take place (Parsaie *et al.*, 2007; Saeid *et al.*, 2013).

In terms of jejunum crypt depth, significant differences among treatments were observed. Statistically, Treatments 2, 5, and 3 obtained deeper crypt depth with comparable means of 705.01 (T2), 596.84 (T5), and 583.57 (T3) respectively, the latter 2 Treatments (T5 and T3) were comparable crypt depth of Treatments 4 and 1 with a mean value of 478.55 (T4) and 433.59 (T1). as an increase in crypt depth and a decrease in villi height can lead to increase secretions into the gastrointestinal tract, resulting in diarrhea, a decrease in disease resistance, and a decreased animal performance (Parsaie *et al.*, 2007; Catalá-Gregori *et al.*, 2008). Deep crypts are a sign of a high turnover of cells along the villi, and high demand on the crypts to produce new cells for villi growth (Xu *et al.*, 2003). Enterocytes are damaged by pathogen bacteria in the digestive tract, which leads to an increase in crypt depth (Parsaie *et al.*, 2007).

High demand for tissue turnover results in an increase in the energy requirements for maintenance of the digestive tract (Choct, 2009). Shallower crypts are associated with a lower tissue turnover and therefore less demand for new tissue.

Table 1. Means of jejunal intestinal surface area of villi and crypt depth of the mallard duck fed with madre de agua leaf meal

Treatments	Jejunum	
	Surface area of villi (µm)	Crypt depth (µm)
T_1- Control	653469.73	433.59^{b}
T_2- Formulated Ration with 3% MALM	329754.02	705.01^{a}
T_3- Formulated Ration with 6% MALM	1040374.73	583.57^{ab}
T_4- Formulated Ration with 9% MALM	435287.80	478.55^{b}
T_5- Formulated Ration with 12% MALM	762527.89	596.84^{ab}
ANOVA	ns	*
C.V. (%)	53.70	16.57
LSD 0.05		168.67

Note: Means with the same letter are not significantly different.

Ns= not significant, *= significant at 5% level

This also results in fewer enterocytes in the secretory stage, therefore fewer secretions, and more villi enterocytes along the longer villi with absorptive functions, resulting in better nutrient absorption (Saeid *et al.*, 2013). Therefore, the villi to crypt ratio of the small intestine play an important role in the absorptive ability and digestive capacity of the small intestine (Saeid *et al.*, 2013).

Growth performance of the mallard duck

The production performance of mallard duck fed with madre de agua leaf meal in terms of the final body weight, cumulative feed consumption, gain in weight, feed conversion ratio, and feed conversion efficiency are shown in Table 2.

A significant result was obtained on the final body weight of mallard duck among treatments with a mean value ranging from 1287.39 to 1309.02 grams. Treatments 4 (9% MALM) and 3 (6% MALM) were comparable with each other with a mean value of 1302.70 and 1309.02 grams but not significantly different to Treatments 2 (3% MALM) and 1 (control) with a mean value of 1287.39 and 1288.89 grams. Treatment 5 (12% MALM) with a mean value of 1266.36 were not significantly different to Treatments 2 and 1. The significant result at the end of the study is in agreement with the findings of Sarria and Preston (1995).

In terms of the cumulative feed consumption of the mallard ducks, no significant differences were observed among treatment with a mean value ranging from 8363.66 grams to 8716.48 grams. The mallard duck with 3% MALM in the formulated duck ration got the highest feed consumption among all the treatments while the lowest was observed on Treatment 1 (control).

This result showed that madre de agua leaf meal is palatable and increasing levels did cause reduction in the feed intake of the improved Philippine mallard duck. This is supported by Rosales (1997) and Morbos *et al.* (2016), saying that madre de agua forage is highly palatable to animals. It could also be due to its bulky character with rather lower nutrient concentration per unit weight. Thus, birds eat more to satisfy their needs (Adeyemi *et al.*, 2012). Sarria and Preston (1995) also supports the comparable results of the study in terms of feed consumption.

The results revealed no significant differences among treatment means on the gain in weight of the mallard ducks with a mean value ranging from 1049.90 grams to 1091.83 grams. However, the pattern of differences showed a generally decreasing trend in the gain in weight with increasing level of madre de agua leaf meal supplementation. According to Samadi (2006) and Morbos *et al.* (2016), growing birds have a definite limit for protein accretion and this ability is mainly governed by genotype. The crude protein contents in leaf meals are much higher than farm by-products (Tesfaye *et al.*, 2013; Sugiharto *et al.*, 2018; Libatique, 2021). It has been acknowledged that specific foliage contains several bioactive compounds that are advantageous to the health of chickens. These compounds include vitamins, phenolic acids, flavonoids, isothiocyanates, tannins as well as saponins (Vergara-Jimenez *et al.*, 2017).

Table 2. Growth performance of the mallard duck fed with madre de agua leaf meal

Treatments	Production parameters				
	Final body weight (g)	Cumulative feed consumption	Gain in weight (g)	Feed conversion ratio	Feed conversion efficiency
T_1- Control	1288.89[ab]	8363.66	1072.54	7.81	12.84
T_2- Formulated Ration with 3% MALM	1287.56[ab]	8716.48	1070.10	8.15	12.29
T_3- Formulated Ration with 6% MALM	1309.02[a]	8522.75	1091.83	7.80	12.82
T_4- Formulated Ration with 9% MALM	1302.70[a]	8599.86	1085.09	7.93	12.63
T_5- Formulated Ration with 12% MALM	1266.36[b]	8603.81	1049.90	8.19	12.21
ANOVA	*	ns	ns	ns	ns
C.V. (%)	1.14	3.40	1.72	3.96	4.02
$LSD_{0.05}$	26.95				

Note: Means with the same letter are not significantly different from each other.

ns = not significant, *= significant at 5% level

Feed conversion ratio is the ratio between the total feed consumed over the total weight gain of mallard ducks. The lower the value, the more efficient are the mallard ducks in converting feed to live weight. Based on the result of the study, numerically, the data showed that all the different treatments have statistically the same amount of feeds consumed to produce a kilogram of weight with an average ratio of 7.80 to 8.19. Although not significant, data revealed that mallard ducks in Treatment 3 with 6% MALM had higher average feed conversion ratio and lowest FCR was observed in treatment with 12% MALM. The mallard ducks with the inclusion of 6% TLM in the ration had better feed conversion ratio compared to other treatments. This can be correlated with the feed consumption of the experimental mallard ducks which can be linked to nutritional factors affecting efficiency (Ampode *et al.* 2020). This study also corroborates the results obtained in the study of Bejar (2017).

The same trend was observed in terms of feed conversion efficiency of mallard duck supplemented with and without madre de agua leaf meal. Based on the result of the study, the mallard ducks have an average efficiency means of 12.21% to 12.84%. It should be noted that as birds grow older, they become more efficient in utilizing feed containing madre de agua leaf meal (Morbos *et al.*, 2016).

In terms of villus surface area Treatment 3 (6% MALM) have the greatest surface area and has a shallow crypt depth compared to other treatments which indicate efficiency in the absorption of nutrients. The results are in congruence with the growth performance of mallard ducks fed with and without madre de agua leaf meal. Treatment 3 obtained a final body weight (1309.02 g), gain in weight (1091.83 g), feed conversion ratio (7.80), feed conversion efficiency (12.82), and cumulative feed consumption (8522.75) which indicates that the 6% MALM resulted in better nutrient absorption because of tall villi height and shallow crypt depth. Tall villi height and shallow crypt depth indicate that intestinal segments in Treatment 3 have more absorptive cells and also a slow turnover of secretory cells from the intestinal crypt which shows an efficient absorption of nutrients in this segment when compared with other treatment groups.

The epithelial cells lining the villi facilitate the absorption of fluids and nutrients, secrete electrolytes and fluids, and regenerate and replace damaged cells or those lost to normal attrition (Hoerr, 2001).

In terms of nutritional attributes, phenol was present on madre de agua. Phenol content was extremely variable, from 0.045 to 5% of DM, which has been suggested to be the cause of the large variations of the nutritional value observed in feeding trials (Rosales, 1997). Active compounds, including phenols, polyphenols, terpenoid, polypeptides, lectin, alkalis, and essential oils stimulate digestion (Lee *et al.*, 2003; Cross *et al.*, 2007) and promote growth. The study of Omar *et al.* (2020) reported that phenolic-rich onion extract (PROE) increases the final body weight "FBW," bodyweight gain "BWG," and feed consumption was observed ($P < 0.05$) at different PROE levels. The positive effect of phenolic-rich onion extract on villus height and width, crypt depth, mucous thickness, and goblet cell count of duodenum, jejunum, and ileum may be attributed to the fact that including herbs in poultry diets promotes the development and enzymatic activity of the intestinal structure. The dietary addition of phenolic-rich onion extracts can improve the growth rate of broiler chicken by improving the AID% of amino acids and intestinal histology (Omar *et al.*, 2020). A large number of previous studies have suggested an increase in the production performance of chickens fed with propolis and/or bee pollen.

These effects could be related to the effect of propolis extract on gastrointestinal microbiota, which increases levels of beneficial bacteria and decreases pathogenic types (Kacaniova, 2012). This modulation of microbiota could promote intestinal health since the beneficial bacteria could provide improved feed digestibility and protection against pathogens via competitive exclusion through a variety of mechanisms (Ao and Choct, 2013; Duarte, 2014). Previous studies have already confirmed that longer intestinal villi indicate an improved ability to absorb nutrients in the intestine (Caspary, 1992; Awad *et al.*, 2006). In addition, it has been proven that longer villi are associated with active cell mitosis, which provides a greater absorptive potential of villi for various nutrients (Samanya *et al*, 2002; Onderci, 2006). Deeper intestinal villi crypts indicate a rapid metabolism of tissue in order to allow the renewal of the intestinal villi, if there is a need for its regeneration (Hamedi *et al.*, 2011). Lowering the height of the villi or reducing crypt depths of intestinal villi may lead to a reduction in the absorption of nutrients (Saeid *et al.*, 2013).

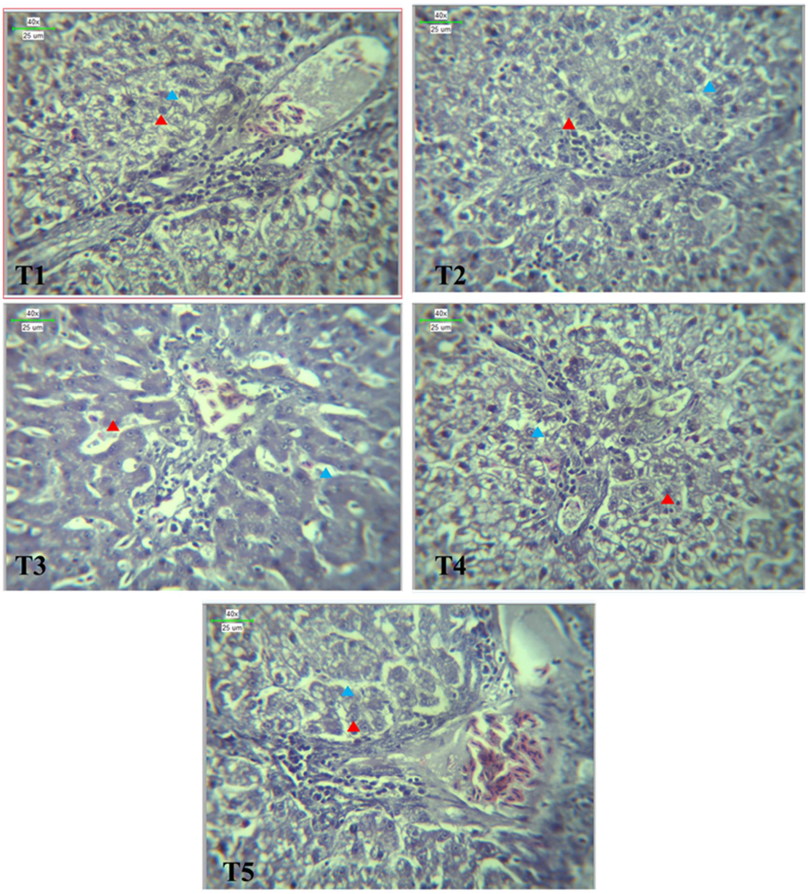

Fig. 1. Photomicrograph of liver from five treatment groups of ducks fed with different levels of made de agua leaf meal (*T. gigantea*). Hepatic sinusoids (blue arrowhead); hepatocytes (red arrowhead).

Histological evaluation of the liver of mallard ducks

Microscopic evaluation of the liver shows that the hepatic parenchyma of ducks is composed of hepatocytes radially organized (Fig. 1). These hepatocytes are intact and are arranged in cords and with evenly spaced hepatic sinusoids in all treatment groups which indicates that there are no observed pathological changes which shows that the madre de agua have no toxic component that can cause damage to the liver with prolonged feeding. Hence this indicates that the formulated ration in different levels is safe for ducks. This finding was compatible with the study of (Khaleel *et al.*, 2017) the hepatic parenchyma in both species of birds consisted of hepatocytes that were organized as irregular plates or cords of thick cells, which radiated around the central vein, forming small acini or lobules, and the blood sinusoids were distributed among those plates.

Conclusion

The dietary supplementation of madre de agua (*T. gigantea*) leaf meal on the intestinal villi of mallard ducks did not affect significantly in terms of jejunal surface area of villi, however, significant differences in the crypt depth were observed. The results are in congruence to final body weight, gain in weight, and FCR.

The inclusion of the madre de agua (*T. gigantea*) leaf meal in the production performance of mallard duck shows a significant effect on the final body weight of mallard duck. No significant effect on the cumulative feed consumption, gain in weight, FCR, and FCE.

On the other hand, the microscopic examination of the liver of improved Philippine mallard ducks had no observed pathological changes which shows that the madre de agua (*T. gigantea*) have no toxic component that can cause damage to the liver with prolonged feeding.

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