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Laying Performance of Heritage Chicken (*Gallus domesticus*) Fed with Madre De Agua Leaf Meal (*Trichanthera gigantea*) as a Plant Protein Ingredient

Balba, C. M.[1]*, Paguia, H. M.[1], Esaga, A. R[1], Nonan Jr., L. G[1]
[1]College of Agriculture and Fisheries, Bataan Peninsula State University, Abucay, Bataan, Philippines
*Corresponding Author: cmbalba@bpsu.edu.ph
Email: hmpaguia@gmail.com
Email: adresaga@bpsu.edu.ph
Email: ljgnonan@bpsu.edu.ph
Tel: (+63) 09088759865

Abstract

This study examined the nutritional potential of Madre de Agua leaf meal (MDALM) as a plant-based protein source for heritage layer hens (*Gallus domesticus*). The experiment lasted 60 days and used a completely randomized design (CRD). Nine-month-old heritage hens were randomly assigned to three treatment groups, with four replicates in each group. The experimental diets were formulated as follows: T1 (control, 0% MDALM), T2 (10% MDALM), and T3 (15% MDALM). The parameters assessed included laying percentage, feed consumption, feed conversion ratio (FCR), feed cost per egg produced, income over feed cost (IOFC), egg weight, eggshell weight, eggshell thickness, and egg yolk color intensity. The results revealed that the inclusion of MDALM, up to 15%, did not significantly influence ($P>0.05$) laying percentage, feed consumption, feed conversion ratio, egg weight, eggshell weight, or eggshell thickness; all production metrics remained comparable to those of the control group. Furthermore, feed consumption was consistent across the treatments (112.71 to 114.33 g/bird/day), indicating the satisfactory palatability of the MDALM-supplemented diets. The egg yolk coloration intensity also exhibited a significant increase ($P<0.01$) which corresponded with the quantity of MDALM added. The control group had the least mean value of 5.7 on the DSM yolk color fan while T2 and T3 groups had significantly higher values of 6.95 and 7.85, which corresponded to improvements of 21.9% and 37.7%, respectively. Although economic data showed numerical trends toward higher feed costs per egg and a decrease in IOFC with greater MDALM inclusion, these differences were not statistically significant ($P>0.05$). Thus, the results recommend that Madre de Agua leaf meal (MDALM) can be included in layer diets, up to 15%, without negatively affecting production or the quality of the eggs. The observed enhancement in egg yolk color intensity indicates that MDALM functions effectively as both a viable alternative protein source and a beneficial natural pigment. Therefore, a 10% MDALM inclusion level is

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recommended for practical application, given its considerable impact on yolk color enhancement and its minimal negative influence on economic outcomes. This study's findings endorse the utilization of locally available MDALM as a sustainable feed ingredient for small-scale poultry operations, potentially reducing dependence on expensive conventional protein sources while simultaneously improving egg quality attributes.

Keywords:

Madre de Agua leaf meal, heritage chicken, laying performance, egg quality, yolk color, plant-based protein, alternative feed ingredient.

1) INTRODUCTION

Poultry production is an essential part of the Philippine economy, it is a business with a market demand for poultry meat and eggs. Most of the egg produced in the Philippines is for domestic consumption. As of September 30, 2023, the nation's total chicken laying flock was estimated to be 71.18 million birds. The inventory of layer chicken rose by 7.3 percent, while the inventory of native/improved chickens increased by 1.8 percent, of the total laying flock, layer chickens shared 66.9 percent, while the remaining 33.1 percent were native/ improved chickens (PSA, 2023). The income of the native chicken growers is relatively low since native chicken production is still a backyard family economic undertaking with limited marketable product volume. Native/ improved chickens are generally raised free range. The usual feedstuffs consisting of corn/cracked corn, rice bran, home mixed ration, filled/unfilled palay and rice milled were more or less the same pullets, cockerels, hens and roosters but amount varies depending on the stage of growth of chickens. These feeds are generally broadcast on the ground (Durasan, 2012).

Optimizing production efficiency in commercial laying operations is contingent upon a consistent supply of nutritionally complete feed. To maintain standardized marketable weights, producers have traditionally depended on expensive commercial rations to meet the high metabolic demands of the birds (Mananghaya, 2017). Similar, to any domesticated poultry species, the highest cost of production comes from feeds, which accounts for about 50 to 70%. The feed can go as high as 75% of the total cost of production in view of commercial feeds, being the major portion of the variable costs (Dozier et al., 2008). Considering the high cost of important feed ingredients (e.g., soybean meal) for poultry production, the utilization of locally produced plantbased protein sources as a practical feed ration is becoming more popular (Paguia et al., 2022; Tecson and Catubis, 2022). Madre de Agua is a tropical leguminous tree native to Central and Northern South America.

It has been introduced and cultivated in the Philippines as fodder and fed to animals (Rosales, 1997). Recent findings suggest that *Trichanthera gigantea* has considerable potential as a nutritious and sustainable source of animal fodder, the leaves and pods of this plant are notably rich in protein, essential amino acids, and minerals, thereby constituting a beneficial supplement to poultry diets. It was found that dry madre de agua leaves (MDALM) contain 88.44% dry matter. The leaves also have 18.21% crude protein, 12.5% crude fiber, 2.66% crude fat, 21.80%

ash, and 11.56% moisture. In addition, the leaves contain 5% calcium, 0.41% total phosphorus, and provide 2,983 cal/kg of gross energy (Jaya et al., 2008).

Using Madre de Agua (*Trichanthera gigantea*) in poultry feed has shown considerable promise as a sustainable alternative. (Morbos et al., 2016; Libatique, 2020) adding fermented leaf meal increases weight gain and also results in improved feed conversion ratios (FCR). It has been shown through previous research that overall egg production can be significantly improved through the addition of fermented leaf meals. Hien et al. (2017) showed that *Trichanthera gigantea* leaf meal induced higher values of yellow skin darkness in chickens, improved egg yolk color and increased palatability rate for meat and egg as the result of its degree of natural pigment. According to Bejar (2017), quail's egg yolk color was significantly improved, final weight gain and feed conversion ratio were better; return on investment was higher even though laying delay occurred when *Trichanthera gigantea* leaf meal at 15% was fed in their diets. Villar et al. (2022) also reported that replacing 10% of the Japanese quail diet with Madre de Agua leaf meal led to a very highly significant increase in egg yolk pigmentation and enhanced egg yolk weight and final weight without affecting production rate or feed efficiency. This evaluates the economic and productive viability of incorporating MDALM into laying production systems, aiming to provide smallholder farmers with a practical solution for increasing profitability through locally available feed resources, hence this study.

2) MATERIALS AND METHODS

A. Experimental Animal and Treatment Diets

The feeding trial involved nine-month-old heritage chickens, specifically from the Dominant CZ layer breeds. Prior to the start of the study, the experimental birds were examined to determine their initial physical characteristics. The examination confirmed their good health, showing no signs of illness or external parasites. The experimental diets were formulated using feed ingredients that were available locally. These included yellow corn, rice bran, vegetable oil, salt, fish meal, soybean meal, and madre de agua (*Trichanthera gigantea*). The leaf meal of madre de agua was prepared by air-drying and subsequently oven-drying until a final moisture content of 14% was attained. Thereafter, all experimental diets were analyzed in the laboratory to determine their nutrient composition, including crude protein, crude fat, crude fiber, ash, moisture, calcium, phosphorus, and nitrogen-free extract. The formulated diets were standardized across treatment groups and were provided in mash form. Proximate and nutrient composition of formulated feeds are shown in Table I.

TABLE 1. FEED INGREDIENTS, INCLUSION LEVELS, AND CALCULATED ANALYSIS FOR THE TREATED DIETS

Feed Ingredients	Control	10% MDALM	15% MDALM
Yellow Corn	54.00	46.60	41.60
Madre de agua leaf meal	-	10.00	15.00
Fish Meal	6.00	10.00	10.00
Rice Bran D1	8.30	10.00	10.00
Soybean meal	18.40	10.00	10.00
MDCP	2.30	2.50	2.50
Coconut oil	2.10	2.10	2.10
Limestone	8.60	8.50	8.50
Refined Iodized Salt	0.10	0.10	0.10
Vitamin Premix	0.10	0.10	0.10
Mineral Premix	0.10	0.10	0.10
Total	100	100	100
	Test Results		
Calculated Analysis	T1- Control	T2- 10% MDALM	T3- 15% MDALM
Crude Fat	5.07	5.84	6.30
Crude Fiber	2.71e	4.09	4.46
Moisture Content	10.98	10.98	10.99
Crude Ash	16.25	19.80	18.15
Crude Protein	15.16 e	12.33 e,o	13.50 e
Calcium	4.25	5.98	4.96
Phosphorous	0.88	0.89	0.81
Nitrogen Free Extracts	49.83	46.96	46.60

Chemical and nutritional composition of Madre de Agua leaf meal samples (table 1) was determined according to standard AOAC methods.

B. Experimental Design and Feeding Trial.

The study was conducted from September to November 2023 at the poultry research facility of Bataan Peninsula State University, Abucay Campus, Philippines. A 60-day feeding trial was conducted using a Completely Randomized Design (CRD) to evaluate the influence of Madre de Agua (Trichanthera gigantea) leaf meal (MDALM) on heritage layer chickens. Nine-month-old birds were randomly assigned to three different diets, with four independent groups for each diet to improve the statistical reliability of the study. The diets included basic control diets 0%(T1), 10% (T2) and 15% (T3) Madre de Agua leaf meal. The birds were raised in a controlled, fulllitterfloor experimental poultry house for the entire study. All birds were fed a standard feeding regime consisting of 120g/bird (2 × 60 g/bird) provided once in the morning and in the afternoon. To monitor the physiological and productive response to the treatments, data recording for performance and health parameters was conducted daily. This systematic arrangement allowed for a precise quantification of how varying MDALM inclusion levels modulate the performance and vitality of heritage breeds under local conditions.

C. Data collection

The production performance and egg quality characteristics of the heritage layers was evaluated using several key metrics such as Laying percentage, Percentage od second class eggs, Feed consumption per bird per day, Feed conversion ratio (FCR), Grams of feed per egg, Egg weight, Egg shell weight, Egg shell thickness, Egg yolk color intensity and Income over feed cost (IOFC) were determine and recorded weekly.

D. Statistical Analysis

Parameters were compared using analysis of variance ($P < 0.05$) for single factor experiment in Complete Randomized Design (CRD). Statistical analysis was performed using a statistical package software, Statistical Tool for Agriculture Research.

3) RESULTS AND DISCUSSIONS

Table 2 shows the production performance parameters of heritage layer chicken fed diets containing different levels of Madre de Agua leaf meal (MDALM) as a plant-based protein source. This study found no significant differences between performance, feed efficiency indicators and economic returns achieved with three dietary treatments. Likewise, the laying percentage exhibited a declining trend as MDALM inclusion increased, statistical analysis revealed no significant differences among treatment means ($F = 1.39$, $p > 0.05$), the inclusion of MDALM at levels up to 15% did not adversely affect egg production rate, despite T1 (64.14% ± 53.13) has a higher mean value compared to T2 (54.98% ± 50.19) and T3 (51.74% ± 42.05). All treatments had a stable proportion of second-class eggs, including those with soft shells, cracks and deformation (2.40% to 2.63%). Statistical analyses showed no significant treatment effect ($F=0.07$; $p > 0.05$), indicating that MDALM supplementation did not lead to eggshell quality damage or an increase in egg abnormalities.

Daily feed intake per bird and group was similar among hens in the treatment groups. There were no statistically significant differences (mean values between 112.71 g and 114.33

g, F = 0.27, p > 0.05). This shows that the addition of MDALM to the feed did not influence how much these birds decided to eat. This indicates that the experimental diets were acceptable with a MDALM level up to 15%, as compared to control diets. All the experimental units had similar feed conversion ratio (FCR) values ranging from 2.00 to 2.08. According to feed efficiency, MDALM has the potential to be a good alternative for conventional protein sources in the diet (F = 1.75, p > 0.05). The absence of a significant treatment effect on eggshell quality (F = 0.07, p>0.05) indicates that MDALM supplementation did not adversely affect shell quality or increase the egg abnormality incidence.

From the economic deal, there was a quantitative escalation of feed prices per egg along with various levels of inclusion of MDALM. The cost per egg increase from ₱5.24 in treatment group T1 to ₱8.27 in treatment group T3. Statistical analysis did not display any differences between the treatments (F = 3.71, p > 0.05). Conversely, the income over feed cost (IOFC) decreased as these values were respectively identified for T1 (1.62 ± 1.33) and T3 (1.15 ± 0.93), although these differences are not statistically significant (F = 2.34; p > 0.05). The decrease in income over feed cost (IOFC) resulting from higher levels of Madre de Agua leaf meal (MDALM) incorporation is in agreement with the increased feed cost per egg observed in the treatment units. Overall, the results suggest that accumulation of Madre de Agua leaf meal (up to 15%) in layer diets does not cause significant negative effects on performance during laying, egg quality, feed efficiency and economically.

TABLE 2. PRODUCTION PERFORMANCE (MEAN ± SD) OF *Gallus Domesticus* HERITAGE CHICKEN FED DIETS WITH VARYING INCLUSIONS OF MADRE DE AGUA LEAF MEAL AS PLANT BASED PROTEIN

Production Performance Parameters	T1 (Control)	T2 10% MDALM meal	T3 15% MDALM meal	*F* value
Laying Percentage (%)	64.14 ± 53.13	54.98 ± 50.19	51.74 ± 42.05	1.39^{ns}
Percentage of second class egg (%)	2.40 ± 02.03	2.63 ± 2.71	2.47 ± 2.33	0.07^{ns}
Feed consumption per bird per day (gram)	113.21 ± 97.08	114.33 ± 98.67	112.71 ± 96.71	0.27^{ns}
Feed Conversion Ratio	2.01 ± 1.73	2.08 ± 1.79	2.00 ±1.73	1.75^{ns}
Grams of Feed per Egg (gram/egg)	164.33 ± 141.51	168.29 ± 144.15	179.28±155.39	3.17^{ns}
Feed Cost per Egg Produced	5.24 ± 5.08	7.33 ± 6.23	8.27 ± 7.90	3.71 ns
Income Over Feed Cost	1.62 ± 1.33	1.22 ± 1.11	1.15 ± 0.93	2.34 ns

^{ns} = Not significant at a 5% level of confidence

The egg quality parameters of heritage chickens fed diets with varying inclusions of Madre de Agua leaf meal (MDALM) as a plant-based protein source are presented in Table 3. The parameters evaluated included egg weight, eggshell weight, eggshell thickness, and egg yolk color intensity, all of which are important indicators of egg quality and consumer acceptability. Egg weight showed minimal variation across treatments, with mean values ranging from 51.36 ± 45.09 g in T2 (10% MDALM) to 55.06 ± 46.98 g in T3 (15% MDALM), compared to 54.59 ± 46.70 g in the control group. Statistical analysis revealed no significant differences among treatments ($F = 1.15$, $p > 0.05$), indicating that MDALM inclusion did not adversely affect egg size.

Eggshell weight exhibited a numerical increasing trend with higher MDALM inclusion, from 6.70 ± 5.76 g in the control group to 7.35 ± 6.44 g in T3. Eggshell thickness measurements ranged from 0.373 ± 0.33 mm in T2 to 0.437 ± 0.36 mm in the control group.

Statistical analysis indicated no significant treatment effect ($F = 2.13$, $p > 0.05$), demonstrating that MDALM inclusion did not compromise shell integrity. This finding is particularly important because the thickness of an eggshell is a key factor in reducing breakage during handling and storage. Egg yolk color intensity improved significantly as the amount of MDALM increased ($F = 13.20$, $p < 0.01$). The control group (T1) showed the lowest average value of 5.7 ± 5.00 on the DSM yolk color fan. In contrast, T2 (10% MDALM) and T3 (15% MDALM) had significantly higher values of 6.95 ± 5.90 and 7.85 ± 6.92, respectively.

TABLE 3. EGG QUALITY PERFORMANCE (MEAN ± SD) OF *Gallus Domesticus* HERITAGE CHICKEN FED DIETS WITH VARYING INCLUSIONS OF MADRE DE AGUA LEAF MEAL AS PLANT BASED PROTEIN

Egg Quality Parameters	T1 (Control)	T2 10% MDALM meal	T3 15% MDALM meal	*F* value
Egg Weight (gram)	54.59 ± 46.70	51.36 ± 45.09	55.06 ±46.98	1.15^{ns}
Eggshell Weight	6.70 ± 5.76	6.88 ± 6.13	7.35 ± 6.44	3.19^{ns}
Eggshell Thickness	0.437 ± 0.36	0.373 ± 0.33	0.404 ± 0.36	2.13^{ns}
Egg Yolk Color Intensity	5.7 ± 5.00^{b}	6.95 ± 5.90^{a}	7.85 ± 6.92^{a}	13.20**

**= highly significant, ^{ns} = not significant at a 5% level of confidence

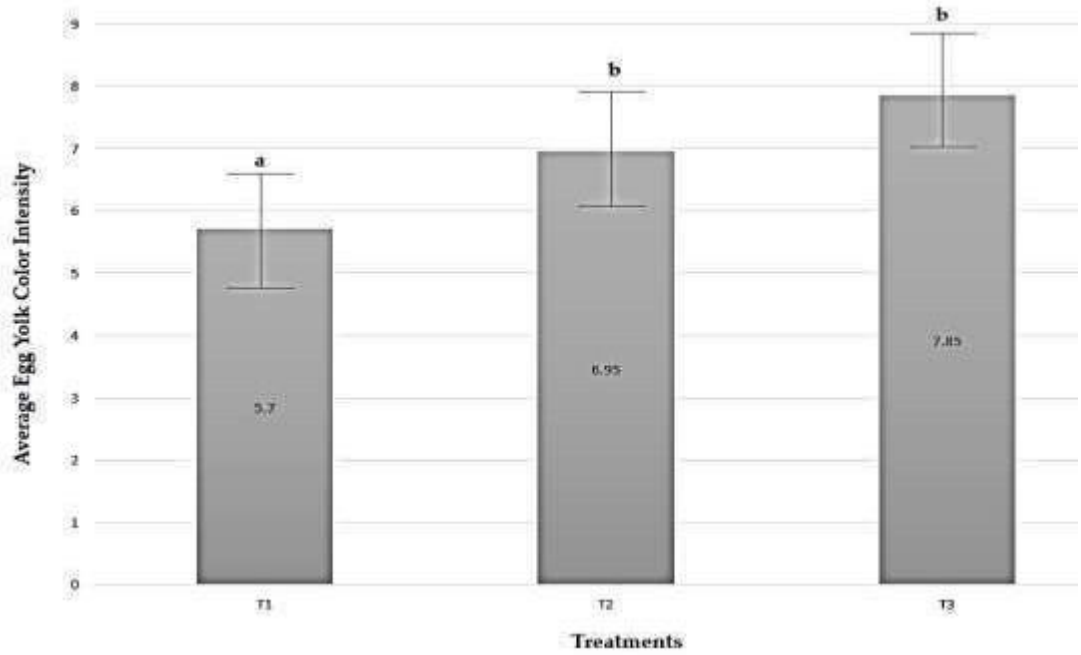


Figure 1. Comparison of average mean egg yolk color intensity of the experimental birds fed diets containing varying levels of Madre de Agua leaf meal as plant protein ingredients

Figure 1 presents the analysis of egg yolk color intensity of treatment groups, with mean values listed to allow easy comparison between dietary treatments. The data show average yolk color scores scored with the DSM yolk color fan, with higher numbers indicating more intense yellow pigmentation. The control (T1) had the lowest yolk color intensity mean value of 5.7 with the Madre de Agua leaf meal (MDALM) treatment groups displaying gradually increasing measures. By using the 10% MDALM inclusion level (T2), the treated sample intensity was determined as 6.95 showing the increment of +21.9% compared to control. The yolk color intensity was highest for T3 treatment group (15% MDALM) that had a mean value of 7.85 and entitled to a 37.7 % preferable comparing control.

In the current study, the increase in yolk color intensity from T1 to T3 exhibited a significant quantity dependence on MDALM supplementation. The value of incremental improvement at each inclusion level shows that increasing dietary levels of MDALM have subsequent, higher pigmented egg yolks. The present findings thus clearly demonstrate that Madre de Agua leaf meal is a potential alternative protein source as well as a valuable natural pigmenting agent for layer diet. Specifically, this study's outcome agrees with previous findings by Bejar (2017) who observed that incorporation of *Trichanthera gigantea* leaf meal improved significantly egg yolk color in quails since the carotenoid pigments from that plant species are bioavailable. Similarly, Villar et al. reported highly significant differences ($P<0.01$) in yolk pigmentation among Japanese quails fed diets containing Madre de Agua leaf meal, with the most pronounced effects occurring at a 10% inclusion level, which correlates with the dose-dependent response observed with Madre de Agua inclusion in the current investigation. Hien et al. *Trichanthera gigantea* leaf meal was shown to effectively enhance yolk pigmentation in addition to improving palatability of meat and egg (2017).

4) CONCLUSION

Results of this study confirm that up to 15% inclusion level of Madre de Agua (*Trichanthera gigantea*) leaf meal can be included in the diet of heritage layer chickens without affecting overall production performance. Adding MDALM did not lead to any statistically significant negative impact on production parameters such as laying percentage, feed consumption, feed conversion ratio and mortality rate that were similar between the experimental and control groups during the study period. Likewise, egg quality parameters including egg weight, eggshell weight, and eggshell thickness were not influenced by dietary treatments which suggests that the inclusion of MDALM ensures the physical integrity and marketability of eggs. Egg yolk color intensity was remarkably improved in a dose dependent manner, at highly significant ($P<0.01$) level with increased levels of MDALM supplementation. The gradual increase from 5.7 for the control group to 6.95 and 7.85 for the treatments with the inclusion of 10 and 15% MDALM in percentage was around (13%) between these two percentages, or (37.7) % in the highest level compared to control group. These results demonstrate that carotenoid pigments from Madre de Agua leaves are bioavailable and accumulate efficiently in egg yolks, providing a convenient approach to enhance this commercially desirable trait. Although numerical trends in metrics of economics over MDALM inclusion (feed cost/egg, income/feed cost) suggest decreased profitability with increasing MDALM levels, these differences were not statistically significant. Based on the general absence of significant differences between both groups it can be concluded that MDALM is a potential alternative protein source to replace parts of conventional ingredients in layer diets and improved yolk color significantly.

5) RECOMMENDATIONS

This study, therefore, recommends the inclusion of Madre de Agua leaf meal in layer diets as a practical plant-based protein supplement and suggests that inclusion levels up to 15% in layer diets are practical based on results showing no significant adverse effects on laying percentage, feed consumption, feed conversion ratio (FCR), egg weight, eggshell weight, or eggshell thickness among all treatment groups. The significantly higher egg yolk color intensity at 10% and 15% respectively provides strong evidence to suggest that MDALM is an effective natural pigmenting agent for enhancing this commercially relevant quality trait of eggs. However, based on the numerical trends for increasing feed cost per egg produced and decreasing income over feed cost at higher inclusion levels, 10% may be practical to utilize as it provides more yolk color enhancement while minimizing the numeric decreases in economic returns seen at the 15% level. Additionally, MDALM should be evaluated in a more diverse spectrum of poultry species and production types including broilers for meat production, breeders for reproductive performance, native chicken's genotypes under free range or semi intensive systems.

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