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Dietary Inclusion of Kulitis (*Amaranthus viridis*) to Enhance Laying Hens' Egg Quality

Kian Matthew S. Del Mundo[1], Mike Luigi G. Mario[1*], and Kirsten Khyla C. Zarate[1]
[1] *Lagro High School*
**corresponding author: mario.136534100295@depedqc.ph*

Ma. Kristine E. Tamayo[1], *Research Adviser*
Master Teacher II, Lagro High School

Abstract: Philippine poultry manufacturers choose selectively bred hens over native hens in commercial egg-laying operations due to the native hens' lower egg production and lower egg weights. As such, it was decided to ascertain whether including 15% Kulitis (*Amaranthus viridis*) leaves in the hen diet can enhance egg quality, specifically overall egg weight, yolk color, and eggshell thickness. Two 26-week-old Darag hens weighing 1 kg were selected and fed standard feed (untreated hen) and Kulitis-infused feed pellets (treated hen) for 21 days. Water was given ad libitum. The first five eggs laid by each hen were collected and evaluated for overall egg weight and eggshell thickness, and the colors of the yolks were labeled utilizing the DSM Yolk Color Fan's hex color codes and their corresponding DSM values (1-16). Independent Samples T-test showed that the Kulitis-infused feed pellets increased egg weight and darkened the yolk color significantly ($P<0.05$), while eggshell thickness was not significantly affected ($P>0.05$). Overall, Kulitis-infused feed pellets enhanced overall egg weight and yolk color without affecting eggshell thickness.

Keywords: Kulitis leaf; feed additive; overall egg weight; eggshell thickness; yolk color

1. INTRODUCTION

1.1. Background of the Study

Native chicken farming in the Philippines has become a crucial industry, comprising 45% of the total chicken stock in the nation (PCAARRD, 2020). However, there is still room for improvement in egg output and quality as poultry product manufacturers in the Philippines still prefer genetically modified breeds for commercial egg-laying operations due to native hens' lower egg production and smaller egg weights. Research by Berkhoff et al. (2017) shows that weight and price determine consumer choice when buying farm eggs, with the color of the yolk being the most important physical characteristic. Moreover, Yalcinalp (2013) emphasizes the significance of eggshell thickness in egg quality, as poor eggshell quality can result in decreased chick quality, poor hatchability, and economic losses.

Globally, antibiotics have been regularly added to layer diets to improve performance and prevent diseases over the past few decades, resulting in the manufacture of high-quality eggs (Steiner, 2006), but were fully restricted in several countries due to the upsurge of antibiotic-resistant microorganisms in humans and the growing consequences for product safety (Salim et al., 2018). To mitigate this, the poultry industry analyzed the drivers of egg quality, such as genetics, body weight, nutrition, and lighting programs (The Poultry Site, 2018). Various research studies also verified the protein's impact on feeds and egg quality (Shim et al., 2013); however, using protein layers in excess to produce larger eggs puts hens at risk for kidney failure (Algawany, et al., 2016). Due to these limitations, the quest for an alternative to antibiotics that contained such bioactive compounds resulted in the addition of plants that contained the necessary chemicals to the feed of hens (Dilawar et al., 2021).

Kulitis (*Amaranthus viridis*) belongs to the Chaulai (Amaranthaceae) family and is a widespread wild vegetable weed mainly cultivated in continents like Asia and Africa, as well as in cultural regions, mainly in Latin America (Iqbal et al., 2012). It contains medicinal constituents such as linoleic acid, tannins, alkaloids, and saponins, with Kulitis leaves specifically containing 45.2% linoleic acid (Abdel-Alim,

2023). Linoleic acid plays a significant role in influencing laying hens' performance and egg quality, and its synthetic form is commonly used in the egg industry (Wang et al., 2017). However, the global market priced linoleic acid extracts at around $50 or roughly 3000 Philippine pesos per kilogram (Wuhan Dujiang Industrial Co., Ltd., 2021). In addition, linoleic acid in the Philippines is usually sold only as supplements for human consumption. For that reason, local poultry farmers suffer from the inaccessibility of linoleic acid extract, which is mainly imported from other countries.

1.2. Objectives

The researchers have undertaken a study aimed at investigating the potential of Kulitis leaves as an economical and readily accessible additive for chicken feed. This research focuses on assessing the impact of incorporating Kulitis leaves into the diet of Darag hens to enhance the quality of eggs by examining factors such as overall egg weight, egg yolk color, and eggshell thickness.

1.3. Scope and Limitations

The research involved only two Darag hens, one treated and one untreated, with the treated hen being fed Kulitis-infused feed for 21 days. The study specifically examined the effects of chicken feeds containing 15% Kulitis leaves on egg quality without evaluating other concentrations of Kulitis in the feed. The experiment took place on a backyard farm in Bagumbong, Caloocan City, as laboratory facilities for hens were unavailable. The chickens were exclusively handled and fed by the farm owner, and the feeding period occurred during the cooler months of December and January to attain the standard environmental temperature from Holik (2009). The egg weight analysis in this study solely focused on overall egg weight, following the Philippine National Standard for Table Eggs of the Bureau of Agriculture and Fisheries Standards. Furthermore, due to the unavailability of a physical DSM yolk color fan, a digital alternative was used to assess the yolk color. It is also crucial to note that this study solely focuses on specific egg traits like overall weight, shell thickness, and yolk color. This research did not consider other potential effects of Kulitis leaves on the health of laying hens and the chemical composition of the eggs.

1.4. Definition of Terms

The chicken egg, a vital food group rich in protein and fat, is the dependent variable and will be assessed in terms of overall weight, yolk color, and shell thickness. Its color value affects consumer preference. Darag refers to a native Philippine chicken breed characterized by specific physical attributes. The DSM yolk color fan, a 16-scale color index, is used to assess egg yolk color, which impacts consumer preference. Kulitis leaves were used as a feed additive and are incorporated into the chicken feed to enhance egg qualities such as overall weight, yolk color, and eggshell thickness. Linoleic acid, present in Kulitis leaves, is known to influence egg quality. Overall egg weight represents the combined weight of the eggshell, egg white, and egg yolk, measured in grams. Lastly, a stress-free environment was maintained, ensuring optimal temperature, housing, and handling for the Darag hens during the study.

2. METHODOLOGY

2.1. Theoretical Framework

This study is anchored on Anatoliyivna, Borisovich, Changzhong, Georgievna, and Viktorivna's (2021) theory about the production of poultry products with phytogenic drugs, wherein it can help boost antioxidant function, immunity, growth, egg quality, and industry sustainability.

In light of this, Quisenberry (1965) hypothesized that linoleic acid is one of the variables affecting the weight and production of eggs in laying chickens. His experiment showed that the use of grain sorghum as the only grain component in laying diets results in lower egg output and egg size due to differences in linoleic acid content.

Furthermore, Shi et al. (2009) found that egg weight can be used to assess egg quality traits, with yolk weight percentage declining as egg weight increased. This study establishes the groundwork for researchers to determine the significance of egg weight in relation to egg shape and shell measurement, Haugh unit, albumen height, and albumen and yolk weight percent.

2.2. Permit & Plant Identification

A permit to use Darag hens from Del Mundo's Backyard Farm for this study was signed by the backyard farm's owner, Mr. Michael Z. Del Mundo. The Jose Vera Santos Herbarium (PUH) Institute of Biology at UP Diliman assisted in the identification of the Kulitis plant.

2.3. Material Gathering

The researchers purchased 1.5 kg of Kulitis bundles from Lagro Central Talipapa, Quezon City. The CJ Legion 3 Maintenance feed was bought from a poultry supply store in Caloocan City.

2.4. Environmental Conditions of the Darag Hens

Mr. Del Mundo's farm in Bagumbong, Caloocan had Darag hens in scratch pens that were 70x97x70 cm in size. Each hen had a space of 91.44 cm^2, meeting the minimum requirement of 45.72 cm^2 to 91.44 cm^2 per hen (Penn State Extension, 2020). The hens had their own food and water bowls, and the area temperature was maintained at 24°C–24.5°C, which complies with the standard environmental temperature for laying hens, according to Holik (2009). The Darag hens used in the study were only handled and fed by the farm owner to ensure proper handling and care.

2.5. Production of Kulitis-infused Feed Pellets

The Kulitis-infused feed pellets were made per batch at Del Mundo's backyard farm in Caloocan. The Kulitis leaves were first washed thoroughly with water. For each batch lasting a week, 126 g of Kulitis leaves were blended with 300 ml of water. The ratio of Kulitis leaves to feed mixture was 3:20, with 150 g of Kulitis leaves for every 850 g of powdered feed, which was anchored on Manyelo et al. (2022). Using a mortar and pestle, 714 grams of CJ Legion 3 Maintenance feed were ground, and then the blended Kulitis was added and mixed through stirring and kneading until soft and crumbly. Afterward, the feed mixture was pelleted through the mechanical meat grinder and underwent sun-drying for 3 hours until hardened. The overall yield of the Kulitis-infused feed pellets weighed 840 g, enough to feed the treated Darag hen for seven days. This procedure was repeated three times for three weeks with a 6-day interval for each batch to ensure the freshness of the feed pellets used throughout the study.

2.6. Chicken Feeding

A feeding adjustment had been made for the treated Darag hen, in which the Kulitis-infused feed pellets were gradually introduced to the treated hen's diet by providing it with 50% regular feed and 50% Kulitis-infused feed pellets for the first four days (Biggs, 2022). Subsequently, the hens were fed 120 g of feed (normal feed for the untreated hen; Kulitis-infused feed pellets for the treated hen) daily, divided into two portions (morning and late afternoon), along with unlimited access to water for three weeks. The experimentation timeframe was based on a study by Klein (1977), wherein egg quality traits were assessed after three weeks of plant dietary inclusion.

2.7. Egg Collection

The study used non-bred Darag hens that began laying eggs 24 days after the feeding experiment. The first five eggs per hen were taken for testing. In total, ten unfertilized eggs were collected for the study, with both the treated and untreated hens providing five samples each.

2.8. Measuring Overall Egg Weight

The effects of the Kulitis on the weight of the eggs were determined when the eggs were measured in grams using a digital gram scale. The researchers recorded the weights of the egg samples, with the requirement of having the same number of egg samples per hen.

2.9. Measuring Eggshell Thickness

A vernier caliper was utilized to measure the eggshell thickness following the procedure outlined in Advanced Instructional Systems, Inc. and North Carolina State University (2010). The eggshell was placed between the caliper's fixed jaw and the lower jaw. Measurements from the first mark on the Vernier scale were recorded in the data table to determine the shell thickness.

2.10. Determining Yolk Color

A yolk separator was used to separate the yolks from the egg whites. Each yolk was then photographed using a Redmi Note 10 Pro camera under consistent lighting. The

colors of the yolks were assessed by comparing them to the hex color codes of the DSM yolk color fan, following Grashorn (2016). Since the DSM yolk color fan was not available in the Philippines, a digital alternative was used, as explained further in the data analysis.

2.11. Descriptive Analysis for Overall Egg Weight

To assess if the weights of eggs from hens fed with Kulitis-infused feed were higher than those from untreated hens, the recorded egg weights of both groups were compared. Egg sizes were subsequently determined using the Philippine National Standard for Table Eggs (Bureau of Agriculture and Fisheries Standards, 2017).

2.12. Descriptive Analysis for Yolk Color

The researchers used Canva's color picker to detect the hex color codes of each blade of the DSM yolk color fan and then tabulated them for assessment. Thereupon, the pictures of the yolks were analyzed using Canva's color dropper tool to match and label the color of the yolk based on its corresponding yolk color number (DSM value) in the DSM yolk color fan. After labeling the yolk color, the increasing or decreasing DSM value was used to interpret the lightness or darkness of the yolk color. Additionally, the most preferred DSM value (DSM=12) for egg yolks globally, as per DSM's article, "YolkFan™: Measuring Egg Yolk Color," was used to rate if the average DSM value of the yolks from each hen fit the standard.

2.13. Statistical Analysis for Egg Quality

The researchers used an Independent Sample T-test in JASP software to compare egg sample quality between two setups. The study involved two groups: eggs from hens given Kulitis-infused feed and eggs from hens fed normal feed. Data on egg weights, yolk DSM values, and eggshell thickness were collected for 10 eggs (5 per hen) and organized in an Excel spreadsheet. This method compared treatment and control groups in terms of overall egg weight, yolk color, and eggshell thickness.

3. RESULTS AND DISCUSSION

A. *Effects of Kulitis Leaves on the Enhancement of the Overall Egg Weight*

Table 1.

Overall Egg Weights Laid by Darag Hens in the Untreated and Treated Group

Group	E#1 WT (g)	E#2 WT (g)	E#3 WT (g)	E#4 WT (g)	E#5 WT (g)	Ave. (g)
U	42	39	41	43	42	41.4
T	50	54	51	51	50	51.2

The measurements of egg weights (in terms of grams) from the untreated and treated groups are shown in Table 3, with the treated group's eggs (E) weighing 51.20 grams on average, while the untreated group's eggs (U) weighed an average of 41.40 grams.

Table 2.

Grouped Data of Overall Egg Weight Values from the Untreated and Treated Hen

Overall	Group	N	Mean	SD	SE	CV
Egg Weight	U	5	41.400	1.517	0.678	0.037
	T	5	51.200	1.643	0.735	0.032

Table 3.

Statistical Analysis of Overall Egg Weight Values from the Untreated and Treated Hen w

Overall Egg	t	df	p	Mean Diff.	SE Diff.
Weight	-9.800	7.949	< .001	-9.800	1.000

The statistical results for the significant increase in

overall egg weight among untreated and treated hens are presented in Tables 2 and 3. There was a significant increase in egg weight between untreated and treated hens, as the computed value of p = 0.00000493403 is less than the significance level (α= 0.05).

B. *Effects of Kulitis Leaves on the Enhancement of the Yolk Color*

Table 4.

The DSM Values of the Egg Yolks from the Untreated Hen

Picture of the Yolk	Hex Color Code	DSM
	#FEBD02	10
	#FFA502	13
	#FFA502	13
	#FFAA01	12
	#FFB400	11
	Ave. DSM value	**11.8**

Table 5.

The DSM Values of the Egg Yolks from the Treated Hen

Picture of the Yolk	Hex Color Code	DSM
	#FFA502	13
	#FFA502	13
	#FFA502	13
	#FF980C	14
	#FFAA01	12
	Ave. DSM value	**13**

The five egg yolks from the untreated hen and their corresponding DSM values are represented in Table 4. The second and third yolks had similar DSM values of 13, while the average DSM value was 11.8, 0.02 points below the most preferred DSM value (DSM=12). The five egg yolks from the treated hen had similar DSM values of 13, and the average DSM value was 13, 1 point above the most preferred DSM value (DSM=12).

Table 6.

Grouped Data of Yolk DSM Values from the Untreated Hen and Treated Hen

DSM	Group	N	Mean	SD	SE	CV
Value	U	5	11.800	1.304	0.583	0.110
Weight	T	5	51.200	1.643	0.735	0.032

Table 7.

Statistical Analysis of Yolk DSM Values from the Untreated Hen and Treated Hen

DSM	t	df	P	Mean Diff.	SE Diff.
Value	-2.021	6.817	0.042	-1.400	0.693

Tables 6 and 7 display the statistical results regarding the significant change in the yolk color. Based on the JASP, statistical analysis showed that the eggs of the treated hen had significantly darker yolk colors than those of the untreated hen. The computed value of p = 0.042 is less than the significance level, suggesting that the yolk colors of the treated hen are significantly darker than those of the untreated hen since P<0.05.

C. *Effects of Kulitis Leaves on the Enhancement of the Eggshell Thickness*

Table 8.

Eggshell Thickness of the Eggs Laid by Darag Hens in Both Untreated and Treated Group

Group	E#1 THK (mm)	E#2 THK (mm)	E#3 THK (mm)	E#4 THK (mm)	E#5 THK (mm)	Ave. THK (mm)
U	0.55	0.9	1	0.5	0.5	**0.680**
T	1	1	1	1	1	**1.000**

Table 8 displays the measured eggshell thickness (THK) in the untreated and treated groups. The measurements acquired from the untreated group were comparably lower than those from the treated group. The eggshell thickness of the untreated hen was 0.680 millimeters on average, while the treated hen's measured 1.000 millimeters.

Table 9.

Grouped Data of Eggshell Thickness Values from the Untreated and Treated Hen

Shell	Group	N	Mean	SD	SE	CV
THK	U	5	0.680	0.249	0.111	0.366
	T	5	1.000	0.000	0.000	0.000

Table 10.

Statistical Analysis of Eggshell Thickness Values from the Untreated and Treated Hen

Shell	t	df	p	Mean Diff.	SE Diff.
THK	-2.874	4.000	0.052	-0.320	0.111

Tables 9 and 10 present the results of the JASP analysis regarding eggshell thickness between treated and untreated hens. The computed value of $p = 0.052$ is greater than the significance level ($\alpha = 0.05$), suggesting that the data support the null hypothesis (H0: 1>2). As a result, $P>0.05$ indicates that there is insufficient evidence to prove that eggs laid by hens fed chicken feed infused with Kulitis leaves have a noticeably thicker shell than eggs laid by hens fed normal feed.

4. CONCLUSIONS

In conclusion, feeding Darag hens with 15% Kulitis (*Amaranthus viridis*) leaves improved their egg traits. The treated group had heavier eggs and darker yolks compared to the untreated group. However, there was no significant difference in eggshell thickness between the two groups.

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