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Effects of Acidified Turmeric and its Role as Acidifier and Pigmenter in Early Laying Performance of Chicken

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ABSTRACT

A 10-week study evaluated the effect of acidified turmeric on layer hens' early laying performance and egg quality traits. A total of 50 heads of ready-to-lay chicken were laid out in RCBD and were assigned to the following drinking water: A—control-tap water, B—2% v/v water: turmeric rhizome, C—2% v/v water: turmeric rhizome with Lactic Acid Bacteria Serum acidifier, D—2% v/v water: turmeric rhizome with kamias acidifier, and E—2% v/v water: turmeric rhizome with calamansi acidifier. Data were analyzed using ANOVA for RCBD, and differences among treatments were determined using Tukey's HSD Test and declared significant at α=0.05. The results revealed that supplementation of the three acidifiers for turmeric showed improved hen-day egg production, feed conversion ratio (FCR) per dozen eggs, yolk color, the sale value of eggs, and income over feed and supplement cost. However, no significant differences ($p>0.05$) were observed in FCR per kg egg mass, egg weight, shell weight, shell thickness, albumen, and yolk weight. In conclusion, acidified turmeric can be used as an acidifier and pigmenter with remarkable improvement in the early laying performance of chicken.

Keywords: acidified turmeric, acidifier, egg quality, laying performance, pigmenter

INTRODUCTION

The market value of eggs greatly relies on the color of their yolks, an essential factor for consumers in evaluating their quality (Valentim et al. 2019). In the European Union, consumers prefer yolk colors within the range of 8 to 14 on the Roche scale (Grčević et al. 2019). Additionally, preferences for more intense yolk color vary depending on regions, countries' specific demands, and cultural importance (Filipiak-Florkiewicz et al. 2017; Martínez et al. 2021). However, it has been found that synthetic pigments commonly used in chicken's diet have a detrimental effect on human health. That is why some countries, like Sweden, have banned the use of synthetic pigments. Excessive use of synthetic pigments such as canthaxanthin in chicken feed could result in crystal formation in the retina,

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posing a possible risk to human eyesight (Grashorn & Steinberg, 2002). As a result, the search for alternatives to synthetic pigments has been increasing, and there is a rising interest in utilizing natural pigments. Among the potential natural pigment sources, turmeric (*Curcuma longa* Linn.) and its yellow pigment curcumin have emerged as possible alternatives.

Moreover, due to limitations imposed on antibiotic use in poultry production, numerous alternatives have emerged, one of which involves utilizing organic acids. However, the majority of the available organic acids are synthetic and expensive, making them unavailable to most farmers, particularly those in rural areas. The use of natural acidifiers such as lactic acid bacteria serum (LABS), kamias (*Averrhoa bilimbi* L.), and calamansi (*Citrus microcarpa* Bunge Wijnands) should be explored. The acidifier action reduces pH in the gastrointestinal tract and inhibits pathogenic microorganisms' growth (Tripathi, 2017). Furthermore, administering pigments (Pérez-Vendrell & Hernandez 2006) and acidifiers (Bouassi et al. 2016) via drinking water is becoming more relevant. So far, there is limited literature on the combined utilization of pigment and acidifiers in drinking water. Hence, this study was conducted to determine the effects of acidified turmeric using various extracts on early laying hen performance, egg quality, and income over feed and supplementation costs in the early laying phase of layer chicken.

MATERIALS AND METHODS

Experimental Animals and Layer Management

A total of 50 heads of ready-to-lay chicken (18 weeks old) were used in the experiment for ten weeks. These were placed in layer cages with dimensions of 18x14x18 inches. The hens were randomly distributed and given a one-month adaptation period before data collection began at 50% hen-day egg production. A polyvinyl chloride (PVC)-based linear feeder was used, providing 3-4 linear inches of feeding space per bird. Water was provided through practical plastic drinker cups attached to 1-liter bottles placed outside the cages. Lighting was controlled with a recommended 15-hour light period, consisting of 3 hours of artificial light from 6 p.m. to 9 p.m. and 12 hours of daylight. Four 40-watt incandescent lamps were placed 7-8 feet high to provide artificial light. The housing structure and surroundings were disinfected before the hens were placed inside the cages, and cleanliness was maintained throughout the study. Adequate ventilation and measures to prevent extreme weather conditions were implemented.

The study followed a recommended health and sanitation guidelines for poultry. The cages were disinfected seven days before the birds' arrival to provide a downtime period. Biosecurity measures were strictly enforced, and the control and prevention of external parasites were regularly carried out. The feed was offered based on the recommendations (110 g/hen/day) for a 2x/a day feeding (6:00 a.m. & 3:00 p.m.). The birds underwent a one-week acclimatization period before gradually shifting to the treatments used in the study. Water consumption was measured at 5:00 p.m., and consistent management practices were implemented throughout the experiment.

Chemical and Colorimetric Assay

The proximate analysis of turmeric was analyzed according to AOAC (2005). The turmeric rhizome has a dry matter of 85.05%, with 4.59% crude protein, 6.96% crude fat, and 2.16% crude ash. The samples were sent to a private laboratory for 2,2-diphenyl-1-picrylhydrazyl (DPPH)-Free Radical Scavenging Activity (FRSA) analysis, phenolic content, ascorbic content, pH, titratable acidity, total soluble solid, and colorimetric values of acidified turmeric.

Preparation of Acidified Turmeric

The acidified turmeric was prepared following the methods described by Sugiharto et al. (2020) shown in Figure 1.

Limitations of the Study:

This study was limited to adding 2 percent (Pratama et al. 2021) acidified turmeric per liter of drinking water. The early laying performance data gathering started at 50 percent of hen-day egg production and lasted ten weeks.

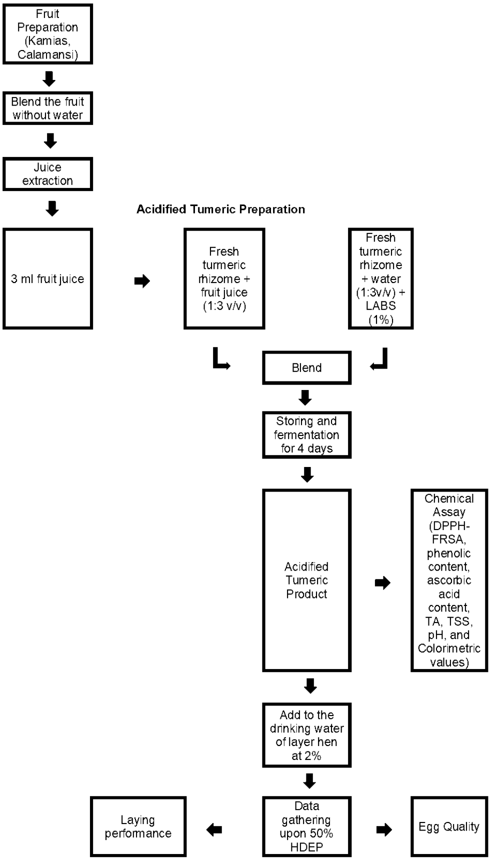

Figure 1. Process flow chart of the experimental procedure

Experimental Designs and Treatments

A randomized complete block design (RCBD) was employed in the experiment. The birds were grouped into five treatments having five blocks based on the location of the cages as the blocking factor. Each block has two birds per cage for each treatment. The following treatments were used in the study: A – control (tap water), B – 2% v/v water: turmeric rhizome, C – 2% v/v water: turmeric rhizome with LABS (lactic acid bacteria serum) acidifier, D – 2% v/v water: turmeric rhizome with kamias extract, and E – 2% v/v water: turmeric rhizome with calamansi extract.

Laying Performance and Egg Quality

Daily egg collection was performed, and weekly egg production was calculated by dividing the total number of eggs by the number of days. Feed conversion ratio (FCR) per dozens of eggs, FCR per kilogram egg mass (EM), and water intake (L) were also calculated weekly.

The following egg quality indicators were evaluated: egg weight, shell weight, shell thickness, yolk color, yolk, and albumen weight using a Roche yolk color fan, eggshell thickness gauge (ORKA, Food Technology, Israel), digital vernier caliper, and digital weighing scale.

Statistical Analysis

The data were analyzed in the analysis of variance (ANOVA) for RCBD using the general linear model of SAS (University Edition). Differences among treatments were determined using Tukey's Honestly Significant Difference (HSD) Test. The results were presented as mean values and standard error of the means.

RESULTS AND DISCUSSION

DPPH-FRSA, Total Phenolic, and Ascorbic Acid Content

The 2,2-diphenyl-1-picrylhydrazyl-Free Radical Scavenging Activity (DPPH-FRSA), total phenolic content (TPC), and ascorbic acid (AA) content are shown in Table 1. As shown, the respective values of DPPH-FRSA and TPC of acidified turmeric increased compared to turmeric: water treatment. Regarding AA content, the amount ranged from 0.04-0.35%, in which turmeric: calamansi was found to have a greater concentration. The obtained result indicated that fermentation improved the antioxidant activity of turmeric by adding organic acid and lactic acid bacteria. According to Bayliak et al. (2016), medicinal herbs possess greater antioxidant abilities when extracted using a solvent with an acidic pH. In addition, Sugiharto et al. (2020) mentioned that regardless of the acidification effect, herbs such as turmeric have strong antioxidants. It must be noted that antioxidants, such as phenolic compounds and ascorbic acid, play a crucial role in scavenging these free radicals and neutralizing their harmful effects. Bhimte et al. (2018) stated that antioxidant molecules prevent the detrimental effects caused by free radicals, cellular damage, toxic byproducts of metabolism, the aging process, and various diseases.

Table 1. Concentrations of antioxidant activity, phenolic and ascorbic acid content of acidified turmeric

CHEMICAL COMPOSITION	TURMERIC: WATER	TURMERIC: LABS	TURMERIC: KAMIAS EXTRACT	TURMERIC: CALAMANSI EXTRACT
DPPH -FRSA (µmol TE/100g)	224.30	248.60	258.80	261.00
TP content (%)	3.95	6.58	11.84	7.89
AA content (%)	0.04	0.06	0.04	0.35

Data presented as means and not statistically analyzed. Analysis was conducted on two samples;

Abbreviation: DPPH-FRSA (2,2-diphenyl-1-picrylhydrazyl-Free Radical Scavenging Activity); TP Total Phenolic; AA Ascorbic Acid

Total Acidity, Total Soluble Solids, pH, and Colorimetric Value

Table 2 presents the measurements of total acidity (TA), total soluble solids (TSS), pH, and colorimetric value for acidified turmeric. There was an increase in total acidity, which was aligned with the decrease in both pH and total soluble solids. Based on the findings of this recent study, treatment E had greater percent values in total acidity at 64.39 and total soluble solids at 3.73 after fermentation. Rodriguez et al. (2019) and Sugiharto et al. (2021) reported that acid fruits show characteristics that make them suitable for cultivating LAB with probiotic potential. The result reveals that acidified turmeric can also be a probiotic source because, according to Jayabalan et al. (2014) and Zubaidah et al. (2019), the increase in total acidity that occurs during fermentation results from modifications in glucose by bacteria and yeast. These changes led to the production of organic acids, which reduced the pH value. As expected, TSS in the current study decreased when subjected to fermentation. These findings affirm a previous study by Managa et al. (2021), who found that certain strains of lactic acid bacteria (LAB) are responsible for decreased TSS observed during smoothie fermentation. Similarly, Thorat et al. (2017) found that microbes utilize the sugars in vegetables during fermentation, reducing TSS.

After the acidification of turmeric, L* (lightness) and b* (yellowness) values of acidified turmeric were higher than turmeric: water treatment. On the other hand, the a* (redness) value of the acidified turmeric was lower than turmeric with water, except when turmeric was acidified with calamansi. Similarly, Madhusankha et al. (2018) discovered a significant and direct relationship between the amount of curcumin present and the L*a*b values. Their findings revealed that higher curcumin content in a sample resulted in higher L* and b* values. In addition, Chew et al. (2018) also revealed that the a* value increased significantly when calamansi juice was added to unpasteurized and pasteurized sugarcane juice at a concentration of 3.0% (v/v).

Table 2. Percent total acidity, total soluble solids, pH, and colorimetric values of acidified turmeric

ITEMS	TURMERIC: WATER	TURMERIC: LABS	TURMERIC: KAMIAS	TURMERIC: CALAMANSI
Total Acidity (TA), %				
Non-fermented	0.48	0.64	12.66	48.15
Fermented	-	0.76	13.36	64.49
Total Soluble Solids (TSS), %				
Non-fermented	0.83	0.87	3.63	5.37
Fermented	-	0.70	3.38	3.73
pH				
Non-fermented	6.20	5.50	3.20	2.62
Fermented	-	5.47	2.27	2.24
L*a*b values				
Non-fermented	38.85	-	-	-
	10.94	-	-	-
	17.24	-	-	-
Fermented				
L*	-	39.48	55.68	56.73
a*	-	8.25	9.01	17.97
b*	-	18.66	41.3	44.74

Data presented as means and not statistically analyzed. Analysis was conducted on two samples;

Abbreviation: A - control (tap water), B - turmeric: water (with no fermentation), C - turmeric: Lactic acid bacteria serum (LABS), D -(turmeric: kamias); DPPH -FRSA (2,2-diphenyl-1-picrylhydrazyl -Free Radical Scavenging Activity); AA (Ascorbic Acid); L* -lightness, a* -redness, and b* -yellowness

Hen-day Egg Laying Performance, feed conversion ratio, and water intake of hens given acidified turmeric in drinking water

Data for laying performance is presented in Table 3. Based on the results, there were significant differences in Hen-day Egg Production (HDEP) ($p<0.0001$), FCR/dozens of eggs ($p<0.0001$), and water intake ($p=0.0032$). However, FCR/kg egg mass was not significant. A higher HDEP was observed when laying hens received water with acidified turmeric compared with the negative control. The combined effects of curcumin and organic acids enhance overall health in layer chickens. Buniowska-Olejnik et al (2023) reported that curcumin promotes the growth of various lactic acid bacteria and inhibits the colonization of harmful bacteria in the intestines, as demonstrated by Nascimento et al (2019).

Furthermore, organic acids such as propionic, formic, acetic, and lactic acid create an unfavorable environment for the growth of spoilage and pathogenic microorganisms. These findings suggest that the synergistic effect of curcumin and organic acids may improve gut health and overall well-being in layer chickens resulting from increasing HDEP. The better feed conversion ratio (dozen eggs) from acidified turmeric supplementation in the present study agrees with Rahman et al. (2008), who found a significant effect on FCR per dozen eggs with a diet supplemented with various levels of organic acid. Additionally, the FCR per kg egg mass (EM) of this study ranged from 1.99 to 2.04, which coincides with the 2.2 or below FCR per kg egg mass (EM) according to the Poultry Expert System (n.d.). Hence, supplementation of acidified turmeric via drinking water had no adverse effect on FCR per kg EM. The effect of the acidifier on water intake was most pronounced when using calamansi extract throughout all weeks. However, water intake was comparable with other acidifiers such as LABS and kamias extract. According to Zhang et al. (2022), the broiler chicken that consumed acidified water had a higher water intake than the control (p<0.05).

Additionally, in a study conducted by Gentle in 1972, it was found that chickens (day old to maturity) show a reaction to chemical stimulants like hydrochloric acid and acetic acid, even in low concentrations. Also, the drinking water's color may influence the current study's increased water intake. Ham and Osorio (2007) reported that color contrast is crucial for attracting chickens. Moreover, Gulizia and Downs (2021) mentioned that chickens preferred blue, followed by green, yellow, and red.

Table 3. The effects of acidified turmeric via drinking water on early laying performance

PARAMETERS	TREATMENT					SEM	P-value
	A	B	C	D	E		
HDEP (%)	89.00^{c}	92.29^{bc}	96.43^{a}	94.86^{ab}	96.57^{a}	0.88	<0.0001*
FCR/dozen of egg	1.49^{c}	1.44^{bc}	1.37^{a}	1.40^{ab}	1.37^{a}	0.01	<0.0001*
FCR/kg EM	2.04	2.02	1.99	2.01	1.99	0.04	0.8732^{ns}
WI (L)	16.69^{b}	17.42^{b}	17.85^{ab}	17.77^{ab}	18.99^{a}	333.21	0.0032*

^{ab} values with similar superscripts are significant at (P<.05);
Abbreviation: A- control (tap water), B- turmeric: water (with no fermentation), C- turmeric: Lactic acid bacteria serum (LABS), D- (turmeric: kamias); HDEP Hen-day egg production; FCR Feed conversion ratio; EM Egg Mass; WI Water intake; SEM Standard error means

Egg Quality

Table 4 presents the data on egg weight, shell weight, shell thickness, albumen, and yolk weight, respectively, of laying hens given acidified turmeric in terms of egg quality characteristics. The result indicated no significant differences in all of these egg traits for ten weeks during early laying performance. These findings suggest that factors such as the age and size of the layer hen may have a greater influence on egg quality. According to Hafez and Kamar (1995), the gradual physiological changes in the ovary as the hen ages could partially explain the similarity in egg weight during the early laying performance. Moreover, Roberts et al. (2013) mentioned that shell weight and thickness increase during the early-mid laying stages before declining in late laying. Silversides and Scott (2001) also observed that albumen weight decreases as hens age.

Furthermore, the increase in yolk weight with hen age can be attributed to the higher rate of synthesis and deposition of lipoprotein (Nasri et al. 2019). A significant result (<0.0001) was observed in the yolk color score (YCS) of layer hen. Treatment E had the highest YC at 9.01, followed by Treatment C, Treatment D, Treatment B, and Treatment A at 8.84, 8.36, 8.22, and 7.07, respectively. The YCS values obtained in this study were higher than those reported by Kinati et al. (2022), where layer hens were given turmeric powder supplementation and recorded a YCS value of 6.97. According to a study conducted by Nuningtyas et al. (2020), the yellow pigment called curcumin found in turmeric rhizomes has the potential to enhance the color stability of egg yolk. Additionally, turmeric that has undergone fermentation exhibits improved bioavailability (Pianpumepong et al. 2012). This increased bioavailability may contribute to the enhancement of yolk color in eggs.

Table 4. The effects of acidified turmeric given via drinking water on egg quality

PARAMETERS	A	B	C	D	E	SEM	P-VALUE
Egg weight (g)	58.42	58.57	59.56	59.11	59.24	0.94	0.9034^{ns}
Shell weight (g)	5.67	5.78	5.71	5.77	1.37	0.07	0.6158^{ns}
Shell thickness (mm)	0.447	0.453	0.461	0.459	0.457	0.003	0.0585^{ns}
Albumen weight (g)	33.73	34.21	35.46	34.46	34.70	0.93	0.7538^{ns}
Yolk weight (g)	14.54	14.94	15.25	14.74	15.30	0.25	0.1924^{ns}
YCS	7.07^{c}	8.22^{b}	8.84^{ab}	8.36^{ab}	9.01^{a}	0.16	<0.0001*

^{abc} values with similar superscripts are significant at P<.05);
Abbreviation: A- control (tap water), B- turmeric: water (with no fermentation), C- turmeric: Lactic acid bacteria serum (LABS), D- (turmeric: kamias); HDEP Hen-day egg production; FCR Feed conversion ratio; WI Water intake; SEM Standard error means; YCS Yolk color score

Income over Feed and Supplementation Cost of Acidified Turmeric

The sale value of eggs and income over feed and supplementation cost (IOFSC) of layer hens given acidified turmeric in their early laying performance are shown in Table 5. Results revealed a significant difference (p<0.05) in the sale value of eggs and IOFSC. Treatment E (2% v/v water: turmeric rhizome with calamansi acidifier) had the highest sale value of eggs with Php. 544.00. It was followed by treatment C (2% v/v water: turmeric rhizome with LAB acidifier), treatment D (2% v/v water: turmeric rhizome with kamias acidifier), and treatment B (2% v/v water: turmeric rhizome) with Php. 536.00, Php. 528.00, and Php. 512.00, respectively. In terms of IOFC, it can be observed that treatment C (2% v/v water:

turmeric rhizome with LAB acidifier) had the highest return at Php. 267.34, followed by treatment D (2% v/v water: turmeric rhizome with kamias acidifier) (Php. 250.85), treatment E (2% v/v water: turmeric rhizome with calamansi acidifier) (Php. 252.33), 2% v/v water: turmeric rhizome (Php. 245.60) and pure water (234.20). Higher IOFST from layer hens supplemented with turmeric-treated drinking water can be attributed to the higher number of eggs produced, resulting in a high sale value.

Table 5. Sale value of eggs and income over feed and supplementation cost of layer hen given acidified turmeric

ITEM	TREATMENT				
	A	B	C	D	E
Total eggs, pcs	62	64	67	66	68
Price/egg	8.00	8.00	8.00	8.00	8.00
Sale value of eggs, Php	496.00 ^{c}	512.00 ^{bc}	536.00 ^{ab}	528.00 ^{ab}	544.00 ^{a}
Feed consumed, kg	7.70	7.70	7.70	7.70	7.70
Total cost of feed consumed, Php	261.80	261.80	261.80	261.80	261.80
DWS, Php	0.00	4.60	6.86	15.35	29.87
Total Cost	261.80	266.40	268.66	277.15	291.67
IOFSC	234.20 ^{b}	245.60 ^{ab}	267.34 ^{a}	250.85 ^{ab}	252.33 ^{ab}

^{ab}figures with similar superscripts are significantly different (P<.05)

egg production per bird for 70 days

control (tap water), B- turmeric: water (with no fermentation), C- turmeric: Lactic acid bacteria serum (LABS), D- (turmeric: kamias); DWS- Drinking water supplement; IOFSC – Income over feed and supplementation cost (sale value of eggs – TFSC)

The suggested retail price per kilogram of feed is based on the market price as of 2023. Price per kg is Php. 34.00

CONCLUSION

Supplementation of acidified turmeric given via drinking water had no adverse effect on laying performance and egg quality. In conclusion, any of the three acidifiers of turmeric significantly improved hen-day egg production, water intake, FCR per dozens of eggs, egg yolk color, the sale value of eggs, and income over feed and supplementation costs. On the other hand, FCR per kg egg mass, egg weight, shell thickness, albumen weight, and yolk weight were not affected. Future studies are required to investigate the effect of these drinking media on the mid-to-late-laying performance of layers and further elucidate its effects as acidifiers and pigmenters. Additionally, it is suggested to conduct curcumin analysis, pH levels in the digestive tract, intestinal morphology, and immune response studies on layer hens.

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