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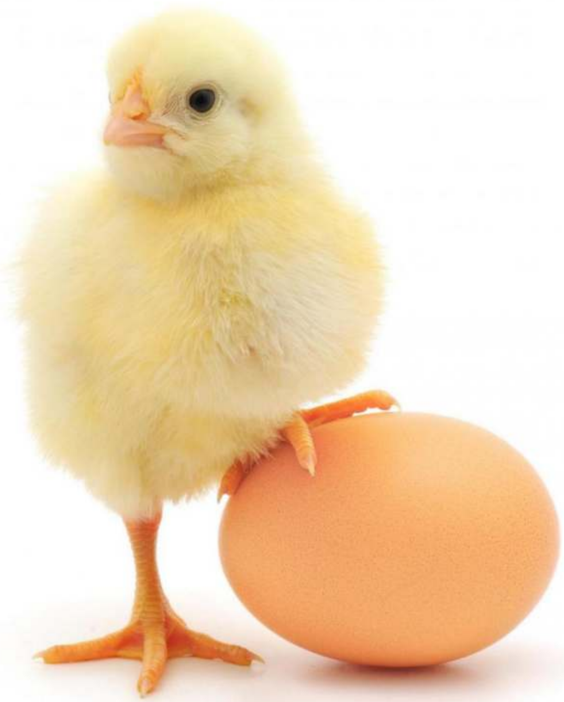

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Research Article
Effect of Chili Leaf Powder on Laying Hen Performance, Egg Quality and Egg Yolk Cholesterol Levels

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Abstract

Objective: This study was conducted to determine the effect of chili leaf on laying hen performance, egg quality and egg yolk cholesterol levels. **Materials and Methods:** The completely randomized design involved evaluation of a control (no chili leaf powder) diet and experimental diets supplemented with chili leaf powder (CLP) at 1, 2 or 3%. One hundred and twenty laying hens (Charoen Pokphand Brown) at 61 weeks of age were divided into 4 treatments, each with 10 replicates (3 birds per replicate). Each group was randomly allocated to one of the treatments for 5 weeks. All diets were isonitrogenous and isocaloric. **Results:** The results of the effect of supplementation with CLP at 0, 1, 2 and 3% showed that the body weight gain, egg yield, egg weight and cholesterol were not significantly different among the treatments ($p>0.05$). However, supplementation with CLP at 0 and 1% resulted in a higher feed intake and feed conversion ratio than those obtain with CLP supplementation at 3% ($p<0.05$). There were no significant differences among treatments in egg quality, breaking strength, shell thickness, yolk color, yolk percentage, shell percentage, albumen percentage, Haugh unit, lightness (L*) of yolk color, redness of yolk color (a*) and yellowness (b*) of yolk color ($p>0.05$). **Conclusion:** Dietary supplementation with 3% CLP can be used as alternative feed additive for laying hens despite an adverse effect on feed intake and feed conversion ratio, as feeding 3% CLP had no adverse effects on egg quality.

Key words: Chili leaf powder, egg quality, egg yolk cholesterol, feed additive, feed conversion ratio, feed intake

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There is increasing demand for functional foods for human consumption that provide various benefits in addition to nutrients. Eggs can be enriched with certain nutrients through dietary manipulation of the laying hen to create specialty products that could possibly provide health benefits to humans[1]. Among the possible alternatives to antibiotic growth promoters (AGPs), phytogenic and herbal products are of interest because they are now considered more reliable and acceptable among consumers as safe and natural additives[2]. Many natural additives have been investigated and tested, such as mulberry leaves[3], skullcap extract[4], garlic and thyme[5], mango skin and paprika extract[6], broccoli stems and leaves[7], strawberry leaves[8], black cumin[9], olive leaves[10] and mao pomace[11].

In Thailand, particularly in the Northeastern region, chili is traditionally used in many kinds of food because of its nutritional value and medicinal effects that have been studied with regard to human health. Lokaewmanee *et al.*[3] reported that *Capsicum* fruits are important sources of keto-carotenoids (capsanthin) and pungent capsaicinoids (capsaicin). Capsaicin prompts the release of substance P, neurokinin A, vasoactive intestinal polypeptide and calcitonin gene-related peptide from the peripheral endings of afferent nerves[12]. Moreover, chili is a good source of vitamin A, vitamin C[13] and carotenoid compounds, with well-known antioxidant properties[14]. Commercial processing of chili produces a large amount of diverse agricultural wastes representing one of the most important and promising energy-rich and protein-rich sources of animal feed. The chili leaves are left after harvesting the chili. There have been a limited number of studies on the effects of diets supplemented with chili leaf powder (CLP) associated with the effect of diets supplemented with it on the production performance, feed conversion ratio and egg cholesterol in poultry. Thus, the objective of this study was to assess the effects of dietary CLP on egg performance, egg quality and cholesterol in egg yolk.

MATERIALS AND METHODS

Study site: The present study was performed in November 2017 at the Animal farm, Kasetsary University Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon Province, Thailand.

Preparation of the CLP: Chili leaves were obtained as a waste product from a fresh chili farm in Nhong Hoi village, Sakon Nakon Province, Thailand. The chili leaf samples were dried in a hot-air oven at 50°C for 2 days. Finally, the leaves were ground using an electronic grinder. The materials were kept at room temperature until they were mixed with the basal diet. Dry matter, crude protein, crude fiber, crude fat and crude ash were determined using methods according to the Association of Official Analytical Chemists[15] and are shown in Table 1.

Animals, diets and feed treatments: The experiment was managed in accordance with the guidelines and rules for animal experiments, Kasetsart University, Thailand. One-hundred and forty-four CP Brown laying hens were used in this study. At the start of the experiment, the birds were 61 weeks old and were 66 weeks old at the end of the experiment. Hens were allocated randomly to 4 treatment groups of 30 hens each. Each group was distributed into 10 replicates with 3 hens per replicate. Hens were fed a basal diet based of corn and soybean meal (Table 1), which was balanced to meet the nutrient requirements for laying hens[16]. The four treatment groups received the following diets: group 1 basal diet (control); group 2 basal diet+1% CLP; group 3 basal diet+2% CLP and group 4 basal diet+3% CLP. The chemical composition of the CLP (Table 2) was measured. Feed and water were provided *ad libitum*.

Recording performance and egg quality: The body weight of individual birds was recorded at the beginning and end of the

Table 1: Ingredients and nutrients composition of the basal diet (g kg^{-1})

Ingredients	
Corn	552.20
Defatted rice bran oil	60.00
Soybean meal	229.80
Fish meal	50.00
Rice bran oil	15.00
Oyster shell	78.50
Dicalcium phosphate	4.50
Salt	3.50
DL-methionine	1.50
Premix[a]	5.00
Nutrient composition	
Crude protein (g kg^{-1})	175.00
Crude fiber (g kg^{-1})	37.60
Crude fat (g kg^{-1})	82.00
Calcium (g kg^{-1})	35.10
Available phosphorus (g kg^{-1})	3.50
Lysine (g kg^{-1})	9.60
Methionine (g kg^{-1})	7.50
Metabolizable energy (Mj kg^{-1})[b]	11.51

[a]Concentrate mixture including (per kg of diet); Vitamin A: 10000 IU, Cholecalciferol: 2000 IU, Vitamin E: 0.25 IU, Vitamin K3: 2 mg, Vitamin B12: 10 µg, Choline: 250 mg, Folacin: 1 mg, Niacin: 30 mg, Pantothenic acid: 10 mg, Pyridoxine: 3 mg, Riboflavin: 6 mg, Thiamin: 2 mg, Ethoxyquin: 125 mg, Choline: 1500 mg, Copper: 10 mg, Iron: 60 mg, Iodine: 0.5 mg, Iodine: 0.5 mg, Manganese: 40 mg, Zinc: 50 mg, Selenium: 0.2 mg, Preservative: 6.54 mg and Feed supplement: 26 mg. [b]Calculated values

Table 2: Chemical composition of chili leaf powder (CLP)

		Dietary of CLP (%)			
	CLP	0	1	2	3
Crude fiber (g kg^{-1})	146.10	41.30	41.80	42.20	43.20
Crude protein (g kg^{-1})	47.00	186.70	182.10	181.20	180.90
Crude fat (g kg^{-1})	79.10	47.80	41.80	41.80	42.30
Ash (g kg^{-1})	51.60	121.20	101.70	102.70	104.10
Gross energy (Mj kg^{-1})	16.79	16.60	16.60	16.60	16.60

experiments (at 61 and 66 weeks of age, respectively). All eggs were collected and recorded on a daily basis. Egg laying rates were calculated. In addition, eggs were individually weighed. Feed intake (in grams per hen per day), feed efficiency and body weight gain (in grams) were calculated.

By the end of the experimental period, 30 eggs per treatment had been evaluated to measure egg quality. The breaking strength of the eggshell (N) was measured using a breaking strength measuring device[17]. Eggs were broken and their contents were separated and weighed individually to determine eggshell membrane thickness and eggshell thickness. The eggshell thickness measurement was based on the average thickness measured at the air cell, equator and sharp end using a pair of micrometer calipers. For each egg, the shell membrane and shell were rinsed with warm water, dried at 60°C overnight and then weighed. Haugh units and yolk color were determined using an egg multitester instrument (EMT 7300, Tohoku Rhythm Co., Ltd., Japan). The yolk was separated from the albumen and weighed. Yolk color was determined using an egg multitester instrument (EMT 7300, Tohoku Rhythm Co., Ltd, Japan). Yolk color was also determined according to the CIE[18] system using the L* a* b* scale, where L* is color brightness or lightness, a* is redness and b* is yellowness. Color measurements were performed at 24 h postmortem, using a CR-310 Chroma Meter (Minolta CR-310, Osaka, Japan). The instrument was calibrated on the CIE LAB color space system using a white calibration plate (Calibration Plate CR-A43, Minolta Cameras). Then, the weight of the albumen was determined as the difference between the weight and the yolk and shell weights. The values of the yolk ratio, albumen ratio and eggshell ratio were calculated for each individual egg according to Tilki and Saatci[19].

Determination of egg yolk cholesterol level: Yolk cholesterol was determined using 40 eggs (10 eggs from each group) collected in the last week of the study. After transferring 0.5 g of egg yolk into a 50 mL cup, 1.0 g of salt was added to the yolk. Next, 20 mL of freshly prepared methanolic potassium hydroxide solution (1.0 M) and 10 mL of isopropanol were also added and the egg yolk mixture was heated inside a magnetic stirrer for 30 min. The addition of isopropanol was performed after the mixture's temperature had cooled to 20-25°C. The mixture was then filtered through a filter paper and the resulting eluent solution was used to determine the egg yolk cholesterol level. Based on the recommendations of the commercial kit (Boehringer Mannheim GmbH Biochemica, Darmstadt, Germany), the cholesterol content of the eggs was analyzed using a spectrophotometer (T 60U) according to Dresselhaus and Acker[20].

Data analyses: All parameters considered were subjected to an analysis of variances using the proc GLM software program (SAS Institute[21]). Differences between treatments were tested using Duncan's new multiple range test (significance, $p<0.05$; Steel and Torrie[22]). Each result of the statistical analysis is shown as a mean value with standard error in the tables.

RESULTS AND DISCUSSION

The results of the present study are given in Table 3 and 4. There were no statistically significant differences in body weight gain, egg yield or egg weight after feeding dietary CLP. This corresponded with another study that reported no adverse effects observed in body weight gain, egg yield and egg weight in layers fed dietary olive leaf powder[10]. El Bagir *et al.*[23] showed that dietary black cumin at 1 or 3% significantly ($p<0.01$) increased the final body weight of laying hens. On the other hand, another study showed that the addition of black cumin seeds into the diet significantly decreased the body weight of the chickens[24]. Based on the present findings, the dietary chili leaf powder added at 3% decreased the feed intake and feed conversion ratio of laying hens compared to the control and dietary chili leaf powder added at 1% ($p<0.05$). The decreases in feed intake and feed conversion ratio were dose dependent. Because the decrease in the feed intake of the laying hens was positively correlated with the feed conversion ratio, a reduction in the feed intake was accompanied by a decrease in the feed conversion ratio. Voemesse *et al.*[25] reported that *Moringa oleifera* leaves did not limit feed consumption. The results of an experiment with layers demonstrated that olive leaf powder had no effect on

Table 3: Effect of dietary chili leaf powder (CLP) on body weight gain, daily feed intake, feed conversion ratio, egg yield, egg weight and egg yolk cholesterol of laying hens

		Dietary CLP (%)				
	Control	1	2	3	SEM	p-value
Body weight gain (g)	224.40	205.80	202.80	194.50	14.24	0.46
Daily feed intake (g)	141.10[a]	139.32[a]	131.38[ab]	127.14[b]	1.90	0.02
Feed conversion ratio	2.69[a]	2.69[a]	2.51[ab]	2.41[b]	0.29	0.02
Egg yield (%)	81.62	80.00	81.62	87.21	1.04	0.07
Egg weight (g)	51.77	52.61	52.41	53.00	0.34	0.32
Cholesterol (g kg^{-1})	9.00	9.00	8.00	8.00	0.20	0.80

SEM: Standard error of means, [a,b]Means in the same row with different superscript letters are significantly different ($p<0.05$)

Table 4: Effects of dietary chili leaf powder (CLP) on quality of egg of laying hens

		Dietary CLP (%)				
	Control	1	2	3	SEM	p-value
Breaking strength (N)	21.85	27.08	27.28	30.03	4.20	0.21
Shell thickness (mm)	0.04	0.04	0.05	0.06	0.01	0.37
Yolk color	13.10	12.89	12.65	12.62	2.30	0.05
Yolk ratio (%)	26.33	26.65	26.82	27.24	4.23	0.76
Shell ratio (%)	9.47	9.55	9.66	10.31	10.04	0.16
Albumen ratio (%)	63.30	63.36	63.63	63.69	1.54	0.97
Haugh unit	85.32	83.31	85.39	88.63	13.14	0.35
Lightness of yolk color (L*)	49.42	46.91	52.37	50.84	7.88	0.14
Redness of yolk color (a*)	22.31	22.30	23.81	21.30	3.54	0.32
Yellowness of yolk color (b*)	44.95	46.43	45.43	46.55	7.26	0.53

SEM: Standard error of means

the feed intake and feed conversion ratio[10]. In the present study, there were no incidences regarding animal disease in any experimental group since the experimental conditions were well controlled and healthy birds were used. The improvement in the feed conversion ratio during the present study might be attributed to the antioxidant properties of chili leaf.

There was no difference between eggs collected from treated hens with respect to their cholesterol ($p>0.05$; Table 3). Aydin *et al*.[26] reported that diets supplemented with 2 or 3% black cumin significantly decreased egg cholesterol per gram of yolk. Similarly, another study showed that different dietary levels of olive leaf powder decreased the egg yolk cholesterol content in laying hens[10]. Eggs are an excellent source of amino acids, fatty acids, vitamins and minerals and contain approximately 213 mg of cholesterol[27]. Understanding the metabolic process of cholesterol formation in the egg yolk of laying hens may provide further enlightenment. Hargis[28]; Bok *et al*.[29]; Kim *et al*.[30]. cited many reports on the metabolic process of egg yolk cholesterol formation in domestic fowl and modifying factors. The primary controlling factor in cholesterol synthesis is the formation of mevalonic acid via HMG-CoA reductase. Cholesterol is primarily biosynthesized in the liver of laying hens and incorporated into vitellogenin and very low density lipoprotein particles, which are secreted into the bloodstream and subsequently taken up by growing oocytes via receptor-mediated endocytosis[31]. Therefore, it was suggested that the decrease in the egg yolk cholesterol was dependent on the decrease in cholesterol synthesized in the liver. The present study showed that the control group had the highest cholesterol content in (9.00 mg g^{-1}) but this was not statistically greater than the cholesterol content for the group fed a diet supplemented with 3% CLP. The layer diet supplemented with 3% CLP decreased the egg cholesterol level by approximately 10% compared to the control eggs, although the mechanism involved is not yet known. Further research is needed to determine the actual mode of action in decreasing egg yolk cholesterol.

The egg quality was appraised using the breaking strength, shell thickness, yolk color, yolk ratio, shell ratio, albumen ratio, Haugh unit, lightness (L*) of yolk color, redness (a*) of yolk color and yellowness (b*) of yolk color and there were no significant differences among treatments ($p>0.05$; Table 4). The present results agreed with the findings of Cayan and Erener[10], who reported that olive leaf powder produced a significant difference in egg quality. The present results were consistent with Lu *et al*.[32], who reported no adverse effects on egg quality in layers fed dietary *Moringa oleifera* leaf. Further investigation should be carried out using nontraditional feed resources that can be used in poultry feed to replace high cost, traditional feed resources that are believed to help in producing functional eggs for developing populations. After

66 weeks of age, the feed intake and feed conversion ratio decreased with increasing dietary CLP levels. These results indicate that a 3% CLP supplementation is best able to improve hen performance in tropical regions.

CONCLUSION

Dietary supplementation of chili leaf powder (CLP) at a ratio of 3% in the layer diet decreased the feed conversion ratio due to the reduced feed intake and due to the numerical but not significant, reduction in egg yolk cholesterol. However, studies evaluating the supplementation of chili leaf powder in laying hen diets are still limited. More detailed studies are needed to elucidate the effects of chili leaf powder on plasma biochemistry and organ histopathological indices.

SIGNIFICANCE STATEMENTS

The present findings can be considered the first report presenting the effect of chili leaf powder on laying hen performance, egg quality and egg yolk cholesterol levels. Furthermore, chili leaf powder may be an alternative feed additive for laying hens. This study found that adding chili leaf powder at a high dose reduced the feed conversion ratio by lowering the feed intake.

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