ISSN 1682-8356
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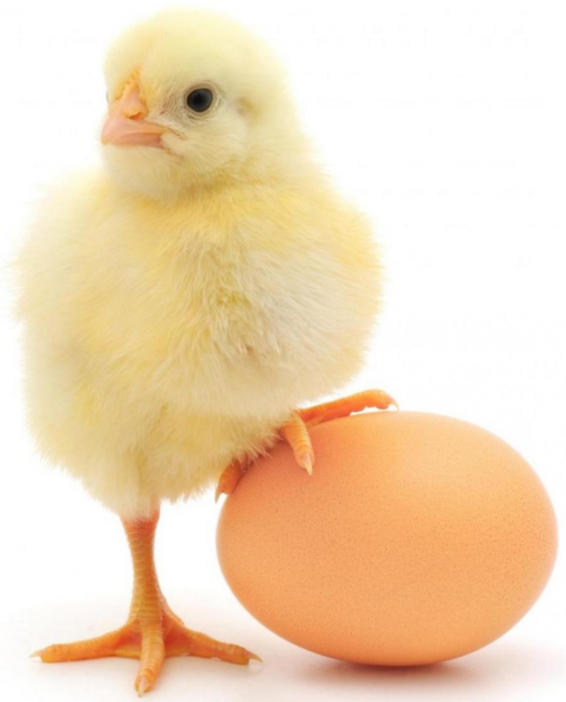

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.508.514

Research Article
The Effect of Diet Supplementation with Dried Mao (*Antidesma* sp.) Pomace on Egg-Laying Performance, Egg Qualityand Cholesterol Levels in the Egg Yolks of Laying Hens

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Abstract
Background and Objective: There is growing interest in the use of industry by-products in the animal and poultry husbandry. This experiment was conducted to determine the effects of mao (*Antidesma* sp.) pomace (MP) on egg-laying performance, egg quality and cholesterol levels in the egg yolks of laying hens. **Materials and Methods:** Ninety laying hens (Charoen Pokphand Brown)at 59 weeks of age were divided into 6 treatment groups; each group had 5 replicates, with 3 birds per replicate. The six groups of laying hens were fed a corn and soybean meal-based diet supplemented with 0, 1, 2, 3, 4 or 5 g kg^{-1} of diet of MP. Laying performance, egg quality and cholesterol levels in the egg yolks were monitored for 16 weeks. The diets contained 2,750 kcal kg^{-1} of metabolizable energy and 17.50% crude protein. **Results:** Diets supplemented with MP did not affect average daily feed intake (ADFI), laying rate, average egg weight, egg mass, ADFI:egg mass ratio or cholesterol levels in egg yolk ($p>0.05$). **Conclusion:** Neither the performances of laying hens nor the cholesterol levels in egg yolks were affected by the inclusion of MP in the diet. However, 5 g kg^{-1} MP diet supplementation may lead to the development of light egg-yolk color, as demanded by specialty markets.

Key words: *Antidesma* sp., egg quality, egg yolk color, laying hen diet, laying performance

Received: June 18, 2019 Accepted: July 07, 2019 Published: October 15, 2019

Citation: Kanda Lokaewmanee, 2019. The effect of diet supplementation with dried mao (*Antidesma* sp.) Pomace on egg-laying performance, egg qualityand cholesterol levels in the egg yolks of laying hens. Int. J. Poult. Sci., 18: 508-514.

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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

Poultry farming has shown the highest increase in production intensity compared to other livestock farming, with high growth rates and feed efficiency being the two main targets to ensure high production over time[1]. To further these production targets, antibiotics, for many years, been used by the poultry industry to advance profit by improving weight gain, feed conversion ratios and flock uniformity[2]. However, as the feed industry develops, the negative effects of antibiotics added to feed has become an increasingly prominent issue, as it has been reported that the widespread use of antimicrobial agents has led to the emergence of antimicrobial drug resistant organisms[3]. Because of the consumer concerns over safety, as well as a ban imposed by the European Union and other countries on antibiotic growth promoters used in feed, recent investigations have been conducted into the naturally occurring plant extracts as an alternatives[4].

Farmers of laying hens will eventually need to stop using antibiotics in feed. Therefore, a number of studies have been conducted that investigate the use of natural plants in order to enhance animal performance, egg yield and the egg quality of laying hens[5,6]. For example, hens fed mash diets containing mulberry leaves laid eggs with dark yellow yolks[7], while diets based on yellow maize supplemented with chili leaves had adverse effects on eggs feed intake and the feed conversion ratio[8]. Moreover, there has been a considerable increase in environmental pollution from the animal industry with wastes being incinerated and disposed of in landfills. This process results in the emission of global warming gases. In contrast, it has been reported that the by-products of food processing are one of the richest sources of natural antioxidants[9].

Antidesma is a diverse genus of tropical plants that occurs in Southeast Asia, Australia, tropical Asia and islands in the Pacific Oceans[10]. The fruits of *Antidesma* are acidic, like grapes, although they are less acidic and slightly sweet when fully ripe. The mao luang (*Antidesma thwaitesianum* Muell Arq.) fruit is the most popular variety used for beverages in Thailand. Mao juice and mao wine have become increasingly popular and its waste products, such as mao pomace (MP) from the processing, are plentiful. A large quantity of mao pomace is produced in Thailand during the rainy season. This fruit is often used for juice extraction, which creates problems when disposing of the pomace. Several researchers have discovered that mao pomace contains organic acids, amino acids and sugars[11]. Puangpronpitag *et al.*[12] also found that mao marcs were an abundant source of polyphenols and suggested that these polyphenolic compounds exhibited similar antioxidant potential similar to grape seed extract. Phenolic compounds have been successfully used to reduce lipid oxidation in meat products[13,14]. Moreover, phenolics exhibit a wide spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic as well as the ability to modify gene expression[15].

In our previous study, we reported an increase salable in net returns and net-profit returns per bird in broilers after being fed dried mao pomace[16]. Mao pomace, included total carbohydrate, total dietary fiber, insoluble dietary fiber, total calories, vitamin B1, vitamin E in alpha-tocopherol, cystine, lysine, tartaric acid, malic acid and citric acid[17]. Moreover, a previous study[18] reported that dried mao pomace from the juice industry contained anthocyanin at 11.36 mg mL^{-1}. Anthocyanins are pigments that give a purple color to grapes and their products[19]. Mao pomace is a natural pigment and may be used an alternative to pigment additives. To date, there are few publications regarding the use of mao pomace as a supplement in the diets of laying hens. No previous studies have been carried out to investigate the effects of mao pomace anthocyanins on egg-yolk color or egg yolk cholesterol levels. Although, a reduction in yolk cholesterol levels is expected.

Any effort towards improving the performance of laying hens without their health is of fundamental importance. The present study was conducted to evaluate the effect of mao (*Antidesma* sp.) pomace on laying performance, egg-quality and cholesterol levels in the egg yolks of laying hens.

MATERIALS AND METHODS

Preparation of the MP: The MP was collected from the Wanawong industry, Sakon Nakon, Thailand. It was dried for 1 day in an oven at 65°C and then ground and passed through a 2 mm screen. The MP was stored in plastic bags at ambient temperature (21-24°C) before being mixed into the feed.

Experimental design, birds and management: A 16 weeks (from 59-74 weeks of age) feeding experiment was conducted at the Animal Farm, Kasetsart University Chalermprakiat Sakon Nakhon Province Campus, Thailand, using hens at 59 weeks of age until 74 weeks of age. The animal care and protocol was approved by the Kasetsart University, Thailand. A completely randomized design was used with different levels of MP supplementation as treatments.

A total of 90 laying hens (Charoen Pokphan Brown)were randomly allocated into 30 feeding units (each with 5 cages in 6 levels and 3 birds/cage) that were then randomly assigned to 6 dietary treatments (5 units/treatment). Each metal wire cage (46×50×44 cm) was equipped with an independent feeder and 2 nipple drinkers. Feeding units were located randomly inside a ventilated house. The house was maintained at a temperature of 22±2°C with a daily photoperiod of 16L:8D during the entire experimental period.

A corn and soybean meal-based diet (Table 1) formulated to meet, or slightly exceed nutrient requirements[20] was left unmodified in the control group. The treatment groups were given this feed supplemented with 1, 2, 3, 4 or 5 g kg^{-1} of diet above the prepared MP (treatments are denoted as MP1, MP2, MP3, MP4 and MP5, respectively). The MP was first mixed with a premixture and then subsequently mixed with other dietary ingredients and then stored in plastic bags before feeding. The experimental diets were prepared every 2 weeks and commenced after an adaptation period of 1 week. All feeding conditions were the same between the adaptation and experimental periods. The diet was offered to the laying hens twice daily *ad libitum* and all hens had free access to water. Mortality incidence and the health status of the experimental laying hens were usually observed and recorded daily throughout the entire experimental period.

Laying performance and egg quality: The feed residue in each feeding unit was weighed at the end of each feeding week to obtain the average daily feed intake (ADFI). The number of eggs from each feeding unit was recorded and the weights of individual eggs were measured daily to determine the daily egg mass (g day^{-1} per hen) and laying rate. Eggs from each group were collected monthly to measure the following egg qualities: shell thickness, shell strength, Haugh unit, yolk color, lightness (L*) of the egg yolk, redness (a*) of the egg yolk, yellowness (b*) of the egg yolk, albumen ratio, yolk ratio and shell ratio. Egg weight was measured using an electronic digital balance. The breaking strength of the eggshell (N) was measured using a breaking strength measuring device[21]. Individual eggs were broken on a metal plate. The shell thickness measurement was based on the average thickness measured at the air cell, equator and sharp end using a pair of micrometer calipers. Egg 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[21] 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 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). For each egg, the shell membrane and shell were rinsed with warm water, dried at 60°C overnight and then weighed[22].The weight of the albumen was determined as the difference between the total egg 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[23].

Table 1: Ingredient and calculated composition of basal diets

Items	Percentage
Ingredient composition	
Corn	55.67
Defatted rice bran oil	6.00
Soybean meal (46% CP)	22.98
Fish meal	5.00
Rice bran oil	1.05
Oyster shell	7.85
Dicalcium phosphate	0.45
Salt	0.35
DL-methionine	0.15
Premix[1]	0.50
Total	100.00
Chemical composition	
Crude protein	17.50
Crude fiber	3.76
Crude fat	8.20
Calcium	3.51
Available phosphorus	0.35
Lysine	0.96
Methionine	0.75
ME (kcal kg^{-1})	2750.00

[1]Concentrate mixture including (per kg of diet); Vitamin A: 10000 IU, Cholecalciferol: 2000 IU, Vitamin E: 0.25 IU, Vitamin K_3: 2 mg, Vitamin B_{12}: 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

Determination of egg yolk cholesterol levels: Yolk cholesterol levels were determined using 60 eggs (10 eggs from each group) collected during the final 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. Twenty milliliters of freshly prepared methanolic potassium hydroxide solution (1.0 M) and 10 mL of isopropanol were then added, and the mixture was heated using 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 passed through a filter paper. The resulting eluent solution was used to determine the egg-yolk cholesterol level. Based on the recommendations of the commercial kit (Boehringer Mannheim GmbH Biochemical, Darmstadt, Germany), the cholesterol content of each egg was analyzed using a spectrophotometer (T 60U) according to Dresselhaus and Acker[24].

Data analysis: Data collected were subjected to one-way analysis of variance (ANOVA) following the General Linear

Model procedure[25]. Differences between treatments were tested using Duncan's new multiple range test at 5% significance level[26]. The results of the statistical analyses are shown in the tables as means with standard errors.

RESULTS AND DISCUSSION

The number of studies investigating the impact of mao (*Antidesma* sp.) pomace on poultry, especially laying hens, is limited. Research indicates that diets supplemented with 0.5%, mao pomace can reduce the feed cost per dozen eggs of layer chickens[27]. In this study, the inclusion of dried mao (*Antidesma* sp.) pomace (MP) supplementation to laying hen diets did not have any significant effect on ADFI, laying rate, average egg weight, egg mass, or ADFI:egg mass ($p>0.05$; Table 2). In addition, as the nutritional composition of the diets in all of the groups was the same, laying performance was not significantly different. No mortality occurred and all hens appeared healthy throughout the study period (data not shown). These results are in agreement with those of Mansoori *et al.*[28], who reported that adding increasing levels of dry tomato pomace (0-100 g kg^{-1}) in laying hen diets did not affect egg production, feed intake, or egg mass. A similar result was reported by Kara *et al.*[29] who found that supplementation with grape pomace at 6% did not show any significant effect on feed consumption, egg production or the feed efficiency of laying hens.

Feeding a diet supplemented with MP did not influence shell thickness, shell strength, Haugh unit, redness (a*) of the egg yolk, yellowness (b*) of the egg yolk, albumen ratio, yolk ratio or shell ratio ($p>0.05$; Table 3). Taking into consideration current egg quality parameters, these results suggest that supplementation up to 5 g kg^{-1} of dietary MP would have no detrimental effects on egg quality. Akbar *et al.*[30] has reported that egg quality changes in accordance with the level of production and age of the hen. With advancing age, the properties of the yolk increases, while the proportions of albumen and shell thickness decrease[31]. In this study, the laying rate and ages of the hens in all groups were the same and the egg quality was not found to be significantly different. Our findings that the inclusion 5 g kg^{-1} MP in the diets of laying hens did not have any significant effects on egg quality are in the line with those reported by Silici *et al.*[32], who demonstrated that the addition of grounded grape seed into breeder quail diets at 0.5, 1.0 and 1.5% did not affect the Haugh unit or eggshell thickness. These results are also supported by Kara[33] who found that supplementing the diets of laying hens with 2% grape pomace did not alter eggshell thickness, eggshell weight or the albumen index.

Table 2: Effect of dietary supplementation of dried mao (*Antidesma* sp.) pomace on laying hen performance during 16 week period of the feeding experiment (n = 5)

	Dietary treatments[1]							
	Control	MP1	MP2	MP3	MP4	MP5	SEM	p-value
ADFI (g day^{-1} per hen)	110.40	111.85	113.58	112.41	110.87	111.46	1.20	0.30
Laying rate (%)	84.52	83.71	83.57	82.38	82.50	84.76	0.65	0.50
Average egg weight (g)	62.38	62.10	62.15	62.84	62.49	62.92	0.68	0.10
Egg mass (g day^{-1} per hen)	52.72	51.98	51.94	51.77	51.55	53.33	0.38	0.22
ADFI: egg mass (g g^{-1})	2.09	2.15	2.19	2.17	2.15	2.09	0.02	0.19

[1]Control. MP1, MP2, MP3, MP4 and MP5 were laying hens fed the basal diet and the basal diet supplemented with MP at a concentration of 1, 2, 3, 4, or 5 g kg^{-1}, respectively

Table 3: Effect of dietary supplementation of dried mao (*Antidesma* sp.) pomace on egg quality of laying hens during 16 week period of the feeding experiment (n = 5)

	Dietary treatments[1]							
	Control	MP1	MP2	MP3	MP4	MP5	SEM	p-value
Shell thickness (mm)	0.36	0.37	0.36	0.36	0.38	0.37	0.01	0.38
Shell strength (N)	27.57	27.02	26.19	25.14	26.21	25.98	1.00	0.38
Haugh unit	82.17	79.15	81.16	80.08	79.14	76.67	1.20	0.50
Yolk color	13.25^{a}	13.25^{a}	13.00^{a}	13.0^{a}	12.99^{ab}	12.00^{b}	0.42	0.04
L* of egg yolk	45.58^{b}	45.35^{b}	46.10^{b}	47.04^{ab}	48.26^{ab}	49.07^{a}	0.76	0.03
a* of egg yolk	22.03	21.51	21.49	21.64	20.99	21.92	0.42	0.67
b* of egg yolk	45.48	43.25	44.25	46.91	44.54	44.46	1.11	0.49
Albumen ratio (%)	63.42	62.26	64.21	61.88	62.41	62.47	0.29	0.21
Yolk ratio (%)	26.28	27.22	25.21	27.25	26.28	26.20	0.33	0.22
Shell ratio (%)	10.29	10.44	10.57	10.86	11.05	10.57	0.11	0.27

[a-b]Means within a row with different letters differ ($p<0.05$), [1]Control. MP1, MP2, MP3, MP4 and MP5 were laying hens fed the basal diet and the basal diet supplemented with MP at a concentration of 1, 2, 3, 4, or 5 g kg^{-1}, respectively

Table 4: Effect of dietary supplementation of dried mao (*Antidesma* sp.) pomace on cholesterol in egg yolk of laying hens during 16 week period of the feeding experiment (n = 5)

	Dietary treatments[1]							
	Control	MP1	MP2	MP3	MP4	MP5	SEM	p-value
Cholesterol in egg yolk (mg/100 g)	7.56	7.80	8.78	8.32	8.16	7.17	0.27	0.18

Supplementing diets with MP at 5 g kg^{-1} decreased the egg-yolk color in this study, which can be attributed to the augmentative effect of MP on the lightness (L*) of egg yolks (p<0.05; Table 3). The results of the present study suggest that including MP in the diets of laying hens at 5 g kg^{-1} will decrease the egg-yolk color. Frões *et al.*[34] has reported that anthocyanins in grape pomace flour increased the density of yolk pigmentation when included in diets at the 6% level. Conversely, a study conducted by Kara *et al.*[29] indicated that the inclusion of grape pomace in the diet of laying hens did not alter the egg yolk color.

Consumers associate yolk color with egg quality and adequate nutrition and prefer yellow-orange yolks[35]. However, there is a worldwide consumer trend of preferring natural pigment sources and rejecting synthetic ones[34]. The required degree of pigmentation from light yellow to dark red varies among countries and even between regions of the same country[36]. Yolk color is known to be influenced mostly by the hen diet[37,38] and a main source of red and yellow pigments is xanthophylls, which are found in alfalfa meal, corn and gluten meal[39]. As the level of corn in the diet for all groups was the same in this study, the result of egg-yolk color was significantly different. It is not clear at present why the darkest egg-yolk color appeared in the 5 g kg^{-1} group but it is possibly related to the components of MP.

Feeding mao pomace-supplemented diets had no observable negative effects on the cholesterol levels of egg yolks in the experimental group compared with the control group (p>0.05; Table 4). Eggs are an excellent source of amino acids, vitamins and minerals and contain approximately 213 mg of cholesterol[40]. Aydin *et al.*[41] reported that dietary manipulations that decreased yolk cholesterol levels also decreased egg size and egg production. In a study in which the laying rate was relatively unaffected, oral administration of pravastatin to laying hens significantly lowered egg and yolk weight, as well as decreased egg cholesterol levels[42]. Mao pomace supplementation has, however, been shown to decrease serum cholesterol levels in laying hens[43]. Cholesterol is primarily bio synthesized in the liver of laying hens and incorporated into the vitellogenin, along with very low density lipoprotein particles, which are secreted into the bloodstream and subsequently taken up by growing oocytes via receptor-mediated endocytosis[44]. Another report demonstrated that the decreased egg-yolk cholesterol is dependent on decreases in the amount of cholesterol synthesized in the liver[41]. In the present experiment, cholesterol in the egg yolk of the MP groups did not differ significantly from the control group. However, the effect of the inclusion of mao pomace on performance, egg quality and cholesterol levels in the egg yolk of poultry can be influenced by several factors, including total anthocyanin content, which will depend on the mao variety and the soil where it is grown.

CONCLUSION

The results of the present study show that the dietary supplementation of mao pomace at a ratio of 5 g kg^{-1} in layer hen diets has no effect on their performance or egg-yolk cholesterol levels (p>0.05). However, mao pomace supplementation increases the lightness of egg yolks, which results in decreasing egg yolk color (p<0.05). Further studies are required to better understand the effects of MP on the levels of anthocyanin in eggs, oxidative stability and the storage properties of eggs.

SIGNIFICANCE STATEMENT

This study investigated the use of mao pomace as a feed additive to improve hen egg-laying performance, egg quality and egg yolk cholesterol levels. Mao pomace provides a source of crude protein, organic acids and anthocyanin. The feed of egg-laying hens that contain of anthocyanin has been shown to increase the lightness of egg yolks and decrease egg-yolk color.

ACKNOWLEDGMENTS

Special gratitude is extended to the Office of the Higher Education Commission and the Thailand Research Fund (TRF) who provided the Project Code: MRG6080021 for the financial support of this study. I am also thankful to the Faculty of Natural Resources and Agro-Industry, Kasetsart University Chalermprakiat Sakon Nakhon Province Campus, Thailand. I would like to thank Suwapa Detprom, Sutichok Mongkol and Kaewtip Jamonmam for their help with the experiment.

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