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Research article

Effect of marigold-derived products as pigment source on growth performance, antioxidant activity and liver enzymes of broiler chickens

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Abstract

The effect was evaluated of marigold-derived products on the growth performance, pigment deposition, antioxidant activity and liver enzymes of broilers. In total, 1,400 one-day-old Arbor Acres male broiler chicks were randomly allocated into 7 treatments (10 replicates with 20 birds each) using a 2×3 factorial in a completely randomized design with two marigold-derived products (marigold meal, MGM; marigold extract, MGE) and three levels of xanthophylls (50 parts per million (ppm), 100 ppm, 150 ppm) with one control group. There were no significant interactions of the source and level of marigold-derived products on the growth performance, meat and skin pigmentation, and liver enzymes of broilers. Feeding marigold-derived products improved the body weight gain (BWG; $p < 0.05$) and tended to improve the feed conversion ratio ($p = 0.072$) of broilers. An increase in the level of xanthophylls tended to increase BWG ($p = 0.0510$). The highest BWG (2,326.1±57.1 g) and the lowest FCR (1.52±0.03) were in the MGE 100 group. The supplementation of marigold-derived products increased the redness (a*; $p < 0.01$) and yellowness (b*; $p < 0.01$) values of breast muscle and increased the a* ($p < 0.01$) and b* ($p < 0.01$) values of breast skin. An increase in the level of xanthophylls from the marigold-derived products decreased serum malondialdehyde ($p < 0.01$). The supplementation of marigold-derived products decreased the aspartate transaminase level ($p < 0.01$). The source and level of marigold affected xanthophylls deposition. The supplementation of marigold-derived products improved the meat and skin color and the antioxidant status of broilers.

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Introduction

The color of the skin and meat of broiler chickens is an important factor in determining consumer's preferences in many countries (Wang et al., 2017; Lizana et al., 2020). Pigmentation of the meat, skin and egg yolk is influenced by the quantity and source of carotenoid pigment in the diets. (Al-Harthi et al., 2018, Perez-Vendrell et al., 2001). Corn can be used as a pigment for poultry products, but its production is limited in many countries. Therefore, broken rice, rice bran, cassava and wheat are commonly used as energy-feed ingredients to substitute corn (Attia et al., 2003; El-Ghamry et al., 2005; Khempaka et al., 2016). However, these feed ingredients lack pigment and usually do not produce the desirable yellow color in eggs or poultry meat (Saha et al., 1999; Ghazalah et al., 2018). Natural sources of carotenoid pigment are tomatoes, peppers, olive, carrots and marigold (Al-Harthi et al., 2019; Lizana et al., 2020). Marigold is the most widely accepted natural pigment in commercial poultry feeds (Nuraini et al., 2017). It contains high amounts of xanthophylls with potent antioxidant properties to stimulate immune responses and reduce inflammation in poultry (Delgado-Vargas et al., 1998; Ak et al., 2020). Marigold products for inclusion in a poultry diet are available in the form of marigold meal (MGM) and marigold extract (MGE). *In vitro* studies have shown the effective radical scavenging capacity of several differently prepared extracts of marigold flowers (Cetkovic et al., 2003; Miliauskas et al., 2004). Supplementation of marigold extract improves growth performance (Rajput et al., 2012), and increases the yellowness value (b*) of the carcass and meat quality in broiler chickens (Wang et al., 2017). Marigold extract supplementation also elevates the total antioxidant capacity and superoxide dismutase activity in the liver (Wang et al., 2017). However, a recent study showed that marigold petal extract did not increase antioxidant enzymes (Pirman et al., 2021). It is possible that the diet compositions level and form of marigold, of or unfavorable environmental conditions may contribute to inconsistent results. Beheshti-Moghaddam et al. (2016) indicated that increases in blood alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in broilers induced liver damage by carbon tetrachloride, and feeding marigold oil extract was able to reduce this adverse effect. However, studies of marigold meal and marigold extract supplementation on liver function by measuring blood ALT and AST are scarce. Investigating the effect of marigold meal and marigold extract on liver enzymes in conjunction with the antioxidant activity in blood may benefit the health status of broilers. Although commercial marigold extract products are widely used and accepted in the poultry industry, commercial products have the disadvantage of being expensive. In contrast, the preparation of marigold flowers in a meal form, using the local breed of marigold, is easy and inexpensive. Therefore, the objective of this study was to evaluate the effect of marigold-derived products from two sources—marigold flower meal developed by Kasetsart University (Kamphaeng Saen-80-DO breed) and marigold flower extract produced commercially—as pigment sources on the growth performance, antioxidant activity and their effect on liver enzymes in broiler chickens.

Materials and Methods

This study was approved by the Ethics Committee of Animal Care and Use for Scientific Research at Kasetsart University (Approval No. ACKU 61-AGK-012).

Animal and dietary treatments

In total, 1,400 one-day-old Arbor Acres male broiler chicks were used. The chicks were randomly allocated into 7 dietary treatments with 10 replications each (20 birds per replication) using a 2×3 factorial with one control in a completely randomized design. There were two marigold-derived products (marigold meal and marigold extract). The marigold meal was produced from whole fresh marigold flowers (Kamphaeng Saen-80-DO breed). The whole fresh marigold flowers were sun-dried for 48 hr and then oven-dried at 50°C for 3 hr. The dried marigold flowers were ground through a 0.75 mm sieve, stored in ultraviolet bags, and kept at 4°C in a refrigerator (Pornpotsupakit, 1979). The marigold meal contained 10 g of total xanthophylls per kilogram. The marigold extract used was the commercial xanthophylls product (Oro GLO® 20 Dry). This product is a dry stabilized, natural source of yellow xanthophylls from saponified marigold extract. The marigold extract product contained 20 g of total xanthophylls per kilogram. Three levels of xanthophylls (50 parts per million (ppm), 100 ppm and 150 ppm) were used. One control group without marigold-derived products was included. The chicks were housed under an evaporative cooling system and lighting and vaccination were managed according to standard commercial practices. Feed in a mash form and water were provided *ad libitum* throughout the experiment. Birds were fed a standard commercial corn-rice based diet (Table 1) to meet nutrient recommendations (National Research Council, 1994). The starter, grower and finisher diets were fed from age 1–21 d, 22–31 d and 32–37 d, respectively.

Table 1 Ingredients and nutrient composition of basal diets

Item	Starter (0–21 d)	Grower (22–31 d)	Finisher (32–37 d)
Ingredient (%)			
Corn	30.0	30.0	30.0
Soybean meal (48% crude protein)	29.5	21.9	18.2
Broken rice	28.6	35.0	38.9
Full fat soybean	6.00	8.00	8.00
Rice bran oil	1.68	0.844	1.10
Mono-dicalcium phosphate (21% phosphorus)	1.35	1.23	0.997
Limestone	0.772	0.896	0.786
Broiler premix[1]	0.600	0.600	0.600
Salt	0.420	0.393	0.391
L-lysine	0.400	0.410	0.401
DL-methionine	0.300	0.256	0.247
L-threonine	0.189	0.181	0.140
Choline chloride (60%)	0.140	0.140	0.140
Toxin binder	-	0.100	0.100
Salinomycin	0.0500	0.0500	-
Calculated nutrient composition (%)			
Metabolizable energy for poultry (kcal/kg)	3,100	3,100	3,150
Crude protein	21.5	19.0	17.5
Digestible lysine	1.28	1.15	1.06
Digestible methionine	0.520	0.470	0.450
Digestible methionine + cystine	0.913	0.834	0.794
Calcium	0.900	0.900	0.800
Total phosphorus	0.717	0.666	0.599
Available phosphorus	0.479	0.450	0.400

[1] Premix contained (per kilogram of diet): 70 mg Fe, 16 mg Cu, 89 mg Zn, 120 mg Mn, 1.29 mg I, 0.30 mg Se, 0.30 mg Biotin, 27,500 IU vitamin A, 5,500 IU vitamin D_3, 3.50 mg vitamin K_3, 3.04 mg vitamin B_1, 8.00 mg vitamin B_2, 60.36 mg vitamin B_3, 15.03 mg vitamin B_5, 5.45 mg vitamin B_6 and 0.03 mg vitamin B_{12}

Growth performance

Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and the mortality rate were determined for the total period (1–37 d). The birds were weighed at the end of the period and FI was measured daily. The FCR was calculated as the FI per day per bird divided by the BWG, with all measurements in grams. All birds were checked daily; any mortality was recorded. The day of death and the weight of each dead bird were considered to calculate the corrected FI and FCR.

Meat and skin color evaluation

At age 37 d, two broilers per replicate were randomly selected for breast meat and skin color evaluation. The intensity of breast meat and skin color were evaluated for luminosity values (L*), redness values (a*) and yellowness values (b*). The measurement was conducted 45 min after air exposure to allow blooming using a Miniscan EZ 4500L spectrophotometer (Hunter Associates Laboratory, Virginia, USA) as described by Castaneda et al. (2005).

Blood sampling

At age 35 d, two birds per replicate were randomly selected for blood sampling. Blood samples (5 mL each) were collected from the wing veins. Two mL of blood samples were transferred to sterile, heparinized, vacutainer tubes, then centrifuged at 1,500×*g* for 10 min to collect the plasma that was immediately stored at 20°C until liver enzyme analysis. The remaining 3 mL of each blood sample were transferred to a dry, clean, centrifuge tube without any coagulant and allowed to clot for 15 min at room temperature. Then the tube was centrifuged at 1,500×*g* for 5 min at 25°C to obtain clean supernatant serum that was used to determine malondialdehyde (MDA).

Antioxidant activity

The MDA content was measured using the thiobarbituric acid (TBA) method at absorbance of 532 nm. The results were expressed as nanomoles per milliliter of protein, as described by Esterbauer et al. (1991).

Liver enzymes

Liver function enzymes (both ALT and AST) were measured using standard enzymatic methods based on the ILabs Chemistry System (IL Test™ ALT/GPT Liquid and IL Test™ AST/GOT Liquid) at the laboratory of the Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Thailand.

Statistical analysis

All data were subjected to analysis in a 2×3 factorial with one control in a completely randomized design using the PROC GLM procedure of the SAS Studio 3.8 Basic Edition software (SAS University Edition, 2018). Each replicate was considered the experimental unit, with 10 replicates per treatment. The model included the main effects of marigold-derived products source, xanthophyll level and their interaction. The analysis was in accordance with the following model:

$$Yijk = \mu + A_i + B_j + (AB)_{ij} + E_{ijk};$$

where μ is the population mean, A_i is the effect of xanthophyll source (i = 1 to 2), B_j is the effect of xanthophyll level (j = 1 to 3), $(AB)_{ij}$ is the interaction of the source and level of xanthophylls and E_{ijk} is the residual error.

The results were presented as mean ± SD and pooled standard error of the mean (SEM) values. Statistical differences among treatments were determined based on Tukey's test. Comparisons between the control group and the marigold-derived products sources were detected using specific contrasts details (Tables 3, 5 and 7). Statistical significance was tested at $p < 0.05$.

Results and Discussion

Growth performance

There was no significant interaction effect between the source and level of marigold-derived product supplementation on the growth performance from age 1–37 d (Table 2.). The MGE supplementation had no significant effect on FI nor on the mortality rate but significantly increased BWG and tended to improve the FCR of broilers ($p = 0.072$) compared to that of the MGM group. An increase in xanthophylls level of the MGE group tended to increase BWG ($p = 0.0510$) but not the FI ($p > 0.05$) nor the FCR ($p > 0.05$). Neither MGM nor MGE in the diets affected broiler performance compared to the control group (Table 3.) The highest BWG (2,326 g) and the lowest FCR (1.52) were in the MGE 100 group. Dried powder of marigold flower supplementation at 5 g/kg or 10 g/kg of the broiler diet was reported to not increase the FI during age 1–42 d (Foroutankhah et al., 2019). Hasin et al. (2006) showed that the FI of pullets was not affected by 4% marigold in the feed, possibly due to being no effect of the marigold product on the palatability of the diet. Rajput et al. (2012) reported that MGE at 200 mg/kg supplementation improved the live body weight ($p < 0.05$), but not the FI nor the FCR ($p > 0.05$) of broilers compared to the other lower levels of MGE (0 mg/kg, 100 mg/kg or 150 mg/kg). In quails, marigold extract supplementation in the diet at 15 ppm improved production performance (Nuraini et al., 2017). Tunio et al. (2013) reported a significant improvement in the live body weight of broilers fed a diet containing lutein at 25 mg/kg feed ($p < 0.05$). Similarly, Shanmugasundaram and Selvaraj (2011) reported that lutein supplementation increased turkey production by decreasing inflammatory parameters and improving the antioxidant efficiency of the broilers. In the current study, MGE improved the BWG of the broilers, perhaps because MGE contains many pharmaceutical active ingredients such as carotenoids, flavonoids, glycosides, steroids and sterols quinines, volatile oil and amino acids (Vahed et al., 2016; Ashwlayan et al. 2018; Ak et al., 2020). Many active compounds may increase the antioxidant status and decrease the oxidative stress of broilers, resulting in a subsequent improvement in broiler performance.

Meat and skin color evaluation

There was no significant interaction between the source and level of marigold-derived products supplementation on broilers, breast meat and skin pigmentation at age 37 d (Table 4). The MGE supplementation increased the redness value (a*) and yellowness value (b*) but decreased the luminosity value (L*) of breast muscle ($p < 0.01$) compared to the MGM group. An increase in xanthophylls levels increased

the redness value (a*; $p < 0.01$) and yellowness value (b*; $p < 0.01$) of breast muscle. The highest a* value was observed at 100 ppm for the MGE group. The highest b* value was observed at 150 ppm for the MGE group. These results were consistent with the work of Wang et al. (2017), who reported that the supplementation of marigold extract linearly increased the redness values (a*) of the thigh muscle, especially in the 120 mg/kg group ($p < 0.01$). The current results showed that the MGE supplementation increased the redness value (a*; $p < 0.01$) and yellowness value (b*; $p < 0.01$) but decreased the luminosity value (L*; $p < 0.05$) of breast skin compared to the MGM group. The xanthophylls level did not affect the color of the breast skin ($p > 0.05$). Birds fed with marigold-derived products had a decreased luminosity value (L*; $p < 0.01$) but an increased redness value (a*; $p < 0.01$) and yellowness value (b*; $p < 0.01$) of the breast compared to the control group (Table 5). Birds fed marigold-derived products also increased the a* ($p < 0.05$) and b* ($p < 0.01$) values of the skin. Similarly, Rajput et al. (2012) found that broilers fed diets supplemented with marigold extract products had a higher yellow color in shank skin than birds fed diets without marigold extract products. These authors also demonstrated that among 100 ppm, 150 ppm and 200 ppm of marigold extract products, 200 ppm provided the highest shank skin color ($p < 0.01$). In addition to the pigment concentrations, other factors can play an important role in determining the final skin color of the birds (Sirri et al., 2010). In the current study, comparing at the same level, birds fed MGE had redness (a*) and yellowness (b*) values of breast meat and skin higher than for the MGM group ($p < 0.01$). It was possible that the variation in the xanthophylls content in the MGM group was caused by variations in the processing conditions from each batch. The level of oxidation in the MGM group may have reduce the xanthophylls content during processing and storage, leading to an inconstant absolute xanthophylls intake of the birds that consequently affected the deposition rate of xanthophylls in the meat and skin. Xanthophylls are sensitive to light and high temperatures (Kadian et al., 2013). An antioxidant agent is normally added to commercially produced MGE to prevent oxidation activity and prolong shelf life. However, in the current study, there was no antioxidant supplementation to the MGM product; therefore, the xanthophylls were subjected to damage by oxidation compared to those in the MGE group. Jensen and Cardon (2005) found that differences in the color of marigold products normally resulted from the different forms of the product and production methods.

Table 2 Growth performance at age 37 d of age of broiler chickens fed different sources and levels of xanthophylls from age 1–37 d

Treatment	Level of xanthophylls (ppm)	Initial BW (g)	BWG (g)	FI (g/bird)	Mortality (%)	FCR
MGM	50	44.02±0.49	2,259.2±65.3	3,524.8±145.2	2.50±2.64	1.56±0.07
	100	44.40±0.26	2,253.6±79.2	3,495.6±110.8	2.50±3.54	1.55±0.06
	150	44.32±0.27	2,195.6±80.9	3,526.2±162.5	2.00±4.83	1.61±0.08
MGE	50	44.51±0.61	2,274.2±59.0	3,537.7±152.1	2.00±2.58	1.54±0.07
	100	44.13±0.56	2,326.1±57.1	3,537.2±67.4	2.00±2.58	1.52±0.03
	150	44.20±0.50	2,263.9±108.0	3,508.1±166.0	1.50±2.41	1.54±0.06
Pooled SEM		0.0029	9.17	16.1	0.37	0.0078
Means for main effect of marigold-derived products						
	MGM	44.25±0.38	2,236.1±78.5[b]	3,515.5±137.0	2.33±3.65	1.58±0.07
	MGE	44.28±0.56	2,288.1±80.5[a]	3,527.7±132.0	1.83±2.45	1.53±0.06
Means for main effect of level of xanthophylls						
	50	44.27±0.59	2,266.7±61.0	3,531.2±145.0	2.25±2.55	1.55±0.07
	100	44.27±0.45	2,289.8±76.8	3,516.4±91.8	2.25±3.02	1.54±0.05
	150	44.26±0.39	2,299.7±99.3	3,517.2±160.0	1.75±3.73	1.57±0.07
p-values for main effects and interactions						
Source of marigold-derived products (A)			*	NS	NS	0.072
Level of xanthophylls (B)			0.051	NS	NS	NS
Interaction (A×B)			NS	NS	NS	NS

MGM = marigold flower meal; MGE = marigold flower extract; Initial BW = initial body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio; ppm = parts per million; SEM = standard error of the mean

Mean ± SD within a column superscripted with different lowercase letters differ significantly ($p < 0.05$).

*significant at $p < 0.05$; NS = not significant

Table 3 Growth performance at age 37 d of broiler chickens fed different sources and levels of xanthophylls from age 1–37 d, compared with specific contrast

Treatment	Level of xanthophylls (ppm)	Initial BW (g)	BWG (g)	FI (g/bird)	Mortality (%)	FCR
Control	0	44.32±0.29	2,243.2±75.8^{ab}	3,539.1±106.8	2.00±2.58	1.57±0.07
	50	44.02±0.49	2,259.2±65.3 ^{ab}	3,524.8±145.2	2.50±2.64	1.56±0.07
MGM	100	44.40±0.26	2,253.6±79.2 ^{ab}	3,495.6±110.8	2.50±3.54	1.55±0.06
	150	44.32±0.27	2,195.6±80.9 ^{b}	3,526.2±162.5	2.00±4.83	1.61±0.08
	50	44.51±0.61	2,274.2±59.0^{ab}	3,537.7±152.1	2.00±2.58	1.54±0.07
MGE	100	44.13±0.56	2,326.1±57.1^{a}	3,537.2±67.4	2.00±2.58	1.52±0.03
	150	44.20±0.50	2,263.9±108.0^{ab}	3,508.1±166.0	1.50±2.41	1.54±0.06
Pooled SEM		0.0029	9.17	16.1	0.374	0.0078
p-value		NS	*	NS	NS	NS
			p-values for contrasts			
Control versus MGM			NS	NS	NS	NS
Control versus MGE			NS	NS	NS	NS
Control versus Marigold-derived products			NS	NS	NS	NS

MGM = marigold flower meal; MGE = marigold flower extract; Initial BW = initial body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio; ppm = parts per million; SEM = standard error of the mean.

Mean ± SD within a column superscripted with different lowercase letters differ significantly ($p < 0.05$).

*significant at $p < 0.05$; NS = not significant

Table 4 Color of breast and skin at age 37 d of broiler chickens fed different sources and levels of xanthophylls from age 1–37 d

Treatment	Level of xanthophylls (ppm)	Breast			Skin		
		L*	a*	b*	L*	a*	b*
MGM	50	57.06±1.39	3.89±0.26	17.02±1.06	73.27±0.73	3.55±0.67	14.24±1.31
	100	57.48±1.82	3.83±0.85	17.83±1.46	73.23±0.86	3.38±0.70	14.52±1.42
	150	57.38±1.90	4.33±0.53	19.40±1.09	73.32±0.78	3.88±0.73	15.47±1.11
MGE	50	56.25±2.48	4.66±0.60	20.16±1.66	72.80±0.82	3.92±1.08	16.41±1.58
	100	56.05±1.50	5.40±0.70	22.21±1.15	72.59±0.78	4.33±0.77	17.58±1.57
	150	55.64±1.73	5.32±0.44	22.98±1.34	72.79±1.71	4.09±1.19	17.28±2.37
Pooled SEM		0.220	0.0717	0.148	0.120	0.108	0.198
Means for main effect of marigold-derived products							
	MGM	57.31±1.67^{a}	4.02±0.63^{b}	18.08±1.54^{b}	73.27±0.77^{a}	3.60±0.71^{b}	14.74±1.35^{b}
	MGE	55.98±1.90^{b}	5.13±0.63^{a}	21.78±1.81^{a}	72.73±1.15^{b}	4.11±1.01^{a}	17.09±1.88^{a}
Means for main effect of level of xanthophylls							
	50	56.66±2.00	4.28±0.66^{b}	18.59±2.10^{b}	73.04±0.80	3.74±0.90	15.33±1.80
	100	56.77±1.79	4.62±1.10^{ab}	20.02±2.58^{ab}	72.91±0.87	3.86±0.86	16.05±2.14
	150	56.51±1.98	4.83±0.67^{a}	21.19±2.19^{a}	73.05±1.32	3.99±0.97	16.38±2.02
p-values for main effects and interactions							
Source of marigold-derived products (A)		**	**	**	*	**	**
Level of xanthophylls (B)		NS	*	**	NS	NS	NS
Interaction (A×B)		NS	NS	NS	NS	NS	NS

MGM = marigold flower meal, and MGE = marigold flower extract; L* = luminosity; a* = redness; b* = yellowness; ppm = parts per million; SEM = standard error of the mean.

Mean ± SD within a column superscripted with different lowercase letters differ significantly ($p < 0.05$).

* and ** = significant at $p < 0.05$ and $p < 0.01$, respectively; NS = not significant

Table 5 Color of breast and skin at age 37 d of broiler chickens fed different sources and levels of xanthophylls from age 1–37 d compared with specific contrast

Treatment	Level of xanthophylls (ppm)	Breast			Skin		
		L*	a*	b*	L*	a*	b*
Control	0	59.54±1.88^{a}	2.57±0.64^{d}	14.46±0.70^{e}	73.45±1.00	3.11±1.02	12.24±1.90^{c}
MGM	50	57.06±1.39^{ab}	3.89±0.26^{c}	17.02±1.06^{d}	73.27±0.73	3.55±0.67	14.24±1.31^{bc}
	100	57.48±1.82^{ab}	3.83±0.85^{c}	17.83±1.46^{cd}	73.23±0.86	3.38±0.70	14.52±1.42^{b}
	150	57.38±1.90^{ab}	4.33±0.53^{bc}	19.40±1.09^{bc}	73.31±0.78	3.88±0.73	15.47±1.11^{ab}
MGE	50	56.25±2.48^{b}	4.66±0.60^{ab}	20.16±1.66^{b}	72.80±0.82	3.92±1.08	16.41±1.58^{ab}
	100	56.05±1.50^{b}	5.40±0.70^{a}	22.19±1.15^{a}	72.59±0.78	4.33±0.77	17.58±1.57^{a}
	150	55.64±1.73^{b}	5.32±0.44^{a}	22.98±1.34^{a}	72.79±1.71	4.09±1.19	17.28±2.37^{a}
Pooled SEM		0.220	0.0717	0.148	0.120	0.108	0.198
p-value		**	**	**	NS	NS	**
		p-values for contrasts					
Control versus MGM		**	**	**	NS	NS	**
Control versus MGE		**	**	**	0.0532	**	**
Control versus Marigold-derived products		**	**	**	NS	*	**

MGM = marigold flower meal; MGE = marigold flower extract; L* = luminosity; a* = redness; b* = yellowness; ppm = parts per million; SEM = standard error of the mean

Mean ± SD within a column superscripted with different lowercase letters differ significantly ($p < 0.05$).

* and ** = significant at $p < 0.05$ and $p < 0.01$, respectively; NS = not significant

Table 6 Effect of marigold-derived products on serum malondialdehyde (MDA) and liver enzymes in broiler chickens at 35 days of age

Treatment	Level of xanthophylls (ppm)	MDA (nmol/ml of serum)	ALT (U/L)	AST (U/L)
MGM	50	0.58±0.10^{a}	4.75±2.03	379.9±96.7
	100	0.47±0.12^{ab}	4.30±1.55	360.4±60.7
	150	0.40±0.07^{b}	4.65±3.28	314.8±78.1
MGE	50	0.48±0.11^{ab}	4.85±2.10	350.7±48.0
	100	0.45±0.05^{ab}	3.50±2.16	316.3±78.8
	150	0.46±0.09^{ab}	3.75±1.44	349.7±42.1
Pooled SEM		0.0107	0.275	10.1
Means for main effect of marigold-derived products				
	MGM	0.48±0.12	4.57±2.33	351.7±81.9
	MGE	0.47±0.08	4.03±1.95	338.9±58.8
Means for main effect of level of xanthophylls				
	50	0.53±0.11^{a}	4.80±2.01	365.3±75.8
	100	0.46±0.09^{ab}	3.90±1.88	338.3±72.1
	150	0.43±0.08^{b}	4.20±2.51	332.2±63.6
	p-values for main effects and interactions			
Source of marigold-derived products (A)		NS	NS	NS
Level of xanthophylls (B)		**	NS	NS
Interaction (A×B)		*	NS	NS

AST = aspartate transaminase, ALT = alanine transaminase, and MDA = malondialdehyde.

MGM = marigold flower meal, and MGE = marigold flower extract

Mean ± SD within a column superscripted with different lowercase letters differ significantly ($p < 0.05$).

* and ** = significant at $p < 0.05$ and $p < 0.01$, respectively; NS = not significant

Table 7 Effect of marigold derived products on serum malondialdehyde (MDA) and liver enzymes in broiler chickens at 35 days of age comparing with specific contrast

Treatment	Level of xanthophylls (ppm)	MDA (nmol/ml of serum)	ALT (U/L)	AST (U/L)
Control	0	0.54±0.08[a]	4.60±2.93	442.2±142.2[a]
MGM	50	0.58±0.10[a]	4.75±2.03	379.9±96.7[ab]
	100	0.47±0.12[ab]	4.30±1.55	360.4±60.7[ab]
	150	0.40±0.07[b]	4.65±3.28	314.8±78.1[b]
MGE	50	0.48±0.11[ab]	4.85±2.10	350.7±48.0[ab]
	100	0.45±0.05[ab]	3.50±2.16	316.3±78.8[b]
	150	0.46±0.09[ab]	3.75±1.44	349.7±42.1[ab]
Pooled SEM		0.0107	0.275	10.1
p-value		**	NS	*
		p-values for contrasts		
Control versus MGM		NS	NS	**
Control versus MGE		*	NS	**
Control versus marigold-derived products		*	NS	**

AST = Aspartate transaminase; ALT = Alanine transaminase; MDA = malondialdehyde; MGM = marigold flower meal; MGE = marigold flower extract; ppm = parts per million; SEM = standard error of the mean

Mean ± SD within a column superscripted with different lowercase letters differ significantly ($p < 0.05$).

* and ** = significant at $p < 0.05$ and $p < 0.01$, respectively; NS = not significant

Antioxidant capacity

There was a significant interaction effect between the source and level of marigold-derived products on the serum MDA of broilers. An increase in the xanthophylls level from the marigold-derived products decreased the MDA in the serum ($p < 0.01$), as shown in Table 6. Birds fed diets supplemented with marigold-derived products decreased their MDA levels compared to the control group ($p < 0.05$; Table 7). The serum MDA level of the birds fed the MGM at 50 ppm was not different from the control birds. However, increasing the MGM to 150 ppm resulted in a lower level of serum MDA than for the control group. Among the MGE groups, increasing the levels of MGE to 100 ppm or 150 ppm did not affect the serum MDA level compared to MGE at 50 ppm. Wang et al. (2017) reported that the MDA level did not decrease when the supplemented marigold extract contained lutein at 15 mg/kg, 30 mg/kg, 60 mg/kg or 120 mg/kg in the diets. The mechanism is unclear, but it could be because MDA may respond to MGE at a very high concentration (perhaps more than 150 mg/kg), as seen in the growth performance response. In the work of Wang et al. (2017), although MGE did not affect the MDA level, the highest dose of MGE (containing lutein at 120 mg/kg) significantly increased the activity of superoxide dismutase in the liver and thigh muscle of broilers (linear, $p < 0.01$). Unfortunately, the authors did not determine other antioxidant parameters; therefore, the effect of marigold-derived products on the antioxidant capacity was not pronounced in that study. Determination of catalase, superoxide dismutase, MDA, total antioxidant capacity and the ratio of MDA:total antioxidant capacity could be useful in drawing an overall picture of the antioxidant status (Attia et al., 2020). Chkhikvishvili et al. (2016) demonstrated for the first time that both the flavonoid and carotenoid constituents of French marigold (*Tagetes patula* L.) extract could protect Jurkat T-cells from hydrogen peroxide-caused oxidative stress. The current findings aligned with the antioxidant and anti-inflammatory properties of marigold preparations used in folk medicine and confirmed in animal and human studies.

Liver enzymes

There was no significant interaction effect between the source and level of marigold-derived products supplementation on the levels of AST and ALT in broilers at age 35 d ($p > 0.05$). Furthermore, neither the source nor the level of marigold-derived products affected the levels of AST and ALT ($p > 0.05$; Table 6). Marigold has potential hepatoprotective effects on liver damage due to its antioxidant properties (Hamzawy et al., 2013). Toliba et al. (2018) indicated that the marigold flower has a high antioxidant capacity because it contains high levels of total phenol, total flavonoids and

oleoresins—these substances have properties that help reduce inflammation in poultry. It was possible that the marigold-derived products helped to protect the liver cells. Supplementation of marigold-derived products reduced the AST level compared to the control group ($p < 0.01$; Table 7), indicating that liver damage was reduced when broilers were fed diets with the marigold-derived product.

Marigold-derived products in the MGE supplementation (100 ppm of xanthophylls) improved the growth performance, enhanced yellowness (b*) and redness (a*) values of the breast meat and skin and also increased the antioxidant capacity. Supplementation of marigold-derived products reduced the AST level in broiler chickens compared to the control group. The current study also suggested that the commercial marigold extract form was absorbed better than the marigold meal, suggesting that marigold extract at the optimum level of 100 ppm of xanthophylls in the diet could improve performance, enhance pigmentation of the meat and skin and increase the antioxidant capacity of broiler chickens.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Ak, G., Zengin, G., Sinan, K.I., et al. 2020. A comparative bio-evaluation and chemical profiles of *Calendula officinalis* L. extracts prepared via different extraction techniques. Appl. Sci. 10: 5920. doi.org/10.3390/app10175920

Al-Harthi, M.A., Attia, Y.A., Al-Sagan, A., Elgandy, M.F. 2018. The effects of autoclaving or/and multi-enzymes complex supplementation on performance, egg quality and profitability of laying hens fed whole *Prosopis juliflora* pods meal in the diet. Europ. Poult. Sci. 82: 248. doi: 10.1399/eps.2018.248.

Al-Harthi M.A., Attia, Y.A., Elgandy, M.F. 2019. The effect of pelleting and enzyme supplementation on performance, carcass and blood parameters of broilers fed on different concentrations of olive cake. Europ. Poult. Sci. 83: 280. doi: 10.1399/eps.2019.280.

Ashwlayan, V.D., Kumar, A., Verma, M., Kumar, V., Gupta, S.K. 2018. Therapeutic potential of *Calendula officinalis*. Pharm. Pharmacol. Int. J. 6: 149–155. doi: 10.15406/ppij.2018.06.00171

Attia, Y.A., Al-Harthi, M.A., El-Maaty, H.M.A. 2020. The effects of different oil sources on performance, digestive enzymes, carcass traits, biochemical, immunological, antioxidant, and morphometric responses of broiler chicks. Front. Vet. Sci. 7: 181. doi.org/10.3389/fvets.2020.00181

Attia, Y.A., Qota, E.M.A., Aggoor, F.A.M., Kies, A.K. 2003. Value of rice bran, its maximal utilization and upgrading by phytase and other enzymes and diet- formulation based on available amino acid for broiler chicks. Arch. Geflügelk 67: 157–166.

Beheshti-Moghaddam, S., Kermanshahi, H., Vahed, R., Nasiri-Moghaddam, H. 2016. The protective effects of marigold (*Calendula officinalis*) extract in liver damage by CCl4 in broiler chicken. Vet. Res. Biol. Prod. 28: 60–69.

Castaneda, M.P., Hirschler, E.M., Sams, A.R. 2005. Skin pigmentation evaluation in broilers fed natural and synthetic pigments. Poult. Sci. 84: 143–147. doi.org/10.1093/ps/84.1.143

Cetkovic, S.G., Dilas, S.M., Canadanovic-Brunet, J.M., Tumbas, V.T. 2003. Thin-layer chromatography analysis and scavenging activity of marigold (*Calendula officinalis* L.) extracts. Acta Period. Technol. 34: 93–102. doi.org/10.2298/APT0334093C

Chkhikvishvili, I., Sanikidze, T., Gogia, N., Enukidze, M., Machavariani, M., Kipiani M., Vinokur, Y., Rodov, V. 2016. Constituents of French marigold (*Tagetes patula* L.) flowers protect Jurkat T-cells against oxidative stress. Oxid. Med. Cell. Longev. 2016: 4216285. doi.org/10.1155/2016/4216285

Delgado-Vargas, F., Paredes-Lepez, O., Avila-Gonzalez, E. 1998. Effects of sunlight illumination of marigold flower meals on egg yolk pigmentation. J. Agric. Food Chem. 46: 698–706. doi.org/10.1021/jf9702454

El-Ghamry, A.A., Al-Harthi, M.A., Attia, Y.A. 2005. Possibility to improve rice polishing utilisation in broiler diets by enzymes or dietary formulation based on digestible amino acids. Arch. Geflügelk. 69: 49–56.

Esterbauer, H., Schaur, R.J., Zollner, H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic. Biol. Med. 11: 81–128. doi.org/10.1016/0891-5849(91)90192-6

Foroutankhah, M., Toghyani, M., Landy, N. 2019. Evaluation of *Calendula officinalis* L. (marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers. Anim. Nutr. 5: 314–318. doi.org/10.1016/j.aninu.2019.04.002

Ghazalah, A.A., Abd-Elsamee, M.O., AbdEl-Halim, H.A.H. 2018. Impact of using cassava root meal and different coloring agents on laying hen performance and egg yolk color. Egypt. Poult. Sci. 38: 959–968. doi: 10.21608/EPSJ.2018.22388

Hamzawy, M.A., El-Denshary, E.S.M., Hassan, N.S., Mannaa, F.A., Abdel-Wahhab, M.A. 2013. Dietary supplementation of *Calendula officinalis* counteracts the oxidative stress and liver damage resulted from aflatoxin. ISRN Nutr. 2013: 538427. doi.org/10.5402/2013/538427

Hasin, B.M., Ferdaus, A.J.M., Islam, M.A., Uddin, M.J., Islam, M.S. 2006. Marigold and orange skin as egg yolk color promoting agents. Int. J. Poult. Sci. 5: 979–987. doi: 10.3923/ijps.2006.979.987

Jensen, P.C.M., Cardon, D. 2005. Plant Resources of Tropical Africa 3. Dyes and Tannins. PROTA Foundation. Wageningen, the Netherlands.

Kadian, S.S., Sharma, A., Sood, D.R. 2013. Effect of light and heat on stability of crude carotenoid extract from natural sources. Int. J. Pharm. Sci. Res. 4: 2415–2418.

Khempaka, S., Hokking, L., Molee, W. 2016. Potential of dried cassava pulp as an alternative energy source for laying hens. J. Appl. Poult. Res. 25: 359–369. doi.org/10.3382/japr/pfw020

Lizana, R.R., Mendoza, O.Z., Perales, C.V. 2020. Evaluation of tarsus pigmentation in chickens fed with different levels of xanthophyll pigment: A practical application of the CIELab system. Int. J. Poult. Sci. 19: 265–269. doi: 10.3923/ijps.2020.265.269

Miliauskas, G., Venskutonis, P.R., van Beek, T.A. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 85: 231–237. doi.org/10.1016/j.foodchem.2003.05.007

National Research Council. 1994. Nutrition Requirements of Poultry, 9th ed. National Academy Press. Washington DC, USA.

Nuraini, N., Mirzah, M., Djulardi, A. 2017. Marigold flower extract as a feed additive in the poultry diet: Effects on laying quail performance and egg quality. Int. J. Poult. Sci. 16: 11–15. doi: 10.3923/ijps.2017.11.15

Perez-Vendrell, A.M., Hernandez, J.M., Llaurado, L., Schierle, J., Brufau, J. 2001. Influence of source and ratio of xanthophyll pigments on broiler chicken pigmentation and performance. Poult. Sci. 80: 320–326. doi.org/10.1093/ps/80.3.320

Pirman, T., Rezar, V., Vrecl, M., Salobir, J., Levart, A. 2021. Effect of olive leaves or marigold petal extract on oxidative stress, gut fermentative activity, and mucosa morphology in broiler chickens fed a diet rich in n-3 polyunsaturated fats. J. Poult. Sci. 58: 119–130. doi.org/10.2141/jpsa.0200026

Pornpotsupakit, S. 1979. Use of marigold meal (*Tagetes erecta* and *Tagetes patula*) as a source of xanthophyll in poultry ration. M.Sc. thesis, Faculty of Agriculture, Kasetsart University. Bangkok, Thailand.

Rajput, N., Naeem, M., Ali, S., Rui, Y., Tian, W. 2012. Effect of dietary supplementation of marigold pigment on immunity, skin and meat color, and growth performance of broiler chickens. Braz. J. Poult. Sci. 14: 233–304. doi.org/10.1590/S1516-635X2012000400009

Saha, P.K., Chowdhury, S.D., Das, S.C., Saha, S.K. 1999. Replacement value of two Bangladeshi varieties of yellow corn for wheat in the diet of laying chicken. Asian-Australas. J. Anim. Sci. 12: 776–782. doi.org/10.5713/ajas.1999.776

SAS University Edition. 2018. SAS Studio Release: 3.8 (Basic Edition). SAS Institute Inc. Cary, NC, USA.

Shanmugasundaram, R. Selvaraj, R.K. 2011. Lutein supplementation alters inflammatory cytokine production and antioxidant status in F-line turkeys. Poult. Sci. 90: 971–976. doi.org/10.3382/ps.2010-01150

Sirri, F., Petracci, M., Bianchi, M., Meluzzi, A. 2010. Survey of skin pigmentation of yellow-skinned broiler chickens. Poult. Sci. 89: 1556–1561. doi.org/10.3382/ps.2009-00623

Toliba, A.O., Egorov, M.A., Sukhenko, L.T., Akmaev, E.P. 2018. Physicochemical properties and food application of marigold flower extracts prepared by conventional and supercritical CO_2 methods. Int. J. Adv. Res. 6: 876–885. doi.org/10.21474/IJAR01/7883

Tunio, M.T., ShuMing, Y., Zhijun, C., Zubair, M., Qiu, J., Zhao, Y., Chen, G., Chow, Y., Chen, A. 2013. Effect of pigments with different origins on pigmentation and performance of broilers. Pakistan J. Zool. 45: 1715–1725.

Vahed, R., Kermanshahi, H., Nasiri H.M., Hassanabadi, A., Beheshti Moghaddam, S. 2016. Effect of different levels of marigold (*Calendula officinails*) oil extract on performance, blood parameters and immune response of broiler chickens challenged with CCl_4. Iran. J. Anim. Sci. Res. 7: 447–455.

Wang, S., Zhang, L., Li, J., Cong, J., Goa, F., Zhou, G. 2017. Effects of dietary marigold extract supplementation on growth performance, pigmentation, antioxidant capacity and meat quality in broiler chickens. Asian-Australas. J. Anim. Sci. 30: 71–77. doi.org/10.5713/ajas.16.0075