Inclusion of Garlic (Allium Sativum.) and Turmeric (Curcuma longa L.) powder to laying hens' diets on egg quality traits, bacterial population and intestinal histomorphology

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Inclusion of Garlic (*Allium Sativum.)* and Turmeric *(Curcuma longa L.)* powder to laying hens' diets on egg quality traits, bacterial population and intestinal histomorphology

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Abstract

This study was carried out in poultry field belong to Animal production, College of Agriculture, University of Anbar, for six periods (28 day/period) from July–January. The study aimed to determine the effect of adding natural additives garlic (*Allium Sativum)* and turmeric *(Curcuma longa L.)*on egg quality, and small intestine histomorphology. A total number of 112, 42 weeks old, laying hens (Shaver strain) were divided to seven treatments with four replicates per treatment (four hens in replicate). Birds in Treatment 1 were fed a basal diet (Control) without any addition. Birds in treatment 2, Treatment 3 and Treatment 4 were fed diets supplemented with 0.25, 0.5 and 1% of garlic powder sequentially, Laying hens in Treatment 5,Treatment 6 and Treatment 7 were fed diets supplemented with 0.25, 0.5 and 1% of turmeric powder sequentially. All the treatments had the same feed. During the experimental periods Shell weight, Shell thickness, Egg Shape index, Albumen weight, Yolk weight, Yolk index and Haugh units were recorded. Laying hens supplemented with garlic and turmeric treatments showed no overall effect on quality characteristics except Albumen weight, Yolk weight and Yolk index. Hens fed diets with garlic (0.25%) recorded high values in albumin weights as compared with laying hens fed diets with garlic (0.5 and 1%). Moreover, hens fed diets with 1% garlic powder recorded highest weights in yolk weight. Laying hens in control group recorded lowest values in yolk index as compared with other treatments. No significant differences recorded in live body weight, dressing percentage and relative weights of liver, heart, spleen and gizzard. Also, results obtained garlic have most effect on decrease of *total aerobic bacteria* ($P<0.05$) on comparison to turmeric treatments, but garlic treatment (1%) shows the least rate of *colibacilli* grown as comparison with other treatments ($P<0.05$). However, turmeric (1%) supplement could not decrease population of bacteria. No significant differences were recorded between treatments in intestine morphology which included villus height, crypt depth and villus width in duodenum and jejunum.

Keywords: Garlic, Turmeric, egg quality

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Introduction

Although antibiotics achieved good performance, their potential side effects became a real public health concern globally (Donoghue, 2003). Nowadays, antibiotics is using as a common methods to prevent diseases and increasing production of eggs and meat, which is consider as a main way to get higher productive. Also, the continuation of using antibiotics in feeds causes many problems one of that is increase of drug resistance, nevertheless, remaining drug in tissues which cause imbalance of microflora in digestive tract (Awad *et al*., 2009). Although, using antibiotics are less frequently used in laying hens' chicken feeds as compared to broiler, the increase of harmful bacterial resistance to antibiotics in poultry products and its transfer to humans is a major importance. The above reasons, researchers turned to use feed additives which preserve the production performance. One of the additives, herbs and their products which have great importance which considered harmless to humans and animals and do not have negative effects on environment and common health. Furthermore, their consumption is not complicate with drugs resistance. Previous studies has showed that herbs and their extracts have different biological functions in poultry, such as antibacterial properties, antiviral, antiparasitic and antioxidant (Botsoglou *et al*., 2002; Papageorgiou *et al*., 2003; Lee *et al*., 2004).Some researcher reported that herbs have the ability to stimulate endocrine glands and the immune system (Lee *et al*., 2004). Garlic (*Allium sativum*) has been a subject of great interest as a medicinal plant and therapeutic worldwide since thousands years (Shetty et *al*. 2013). The pharmacological effects of garlic (*Allium sativum*) due with its sulfur compounds (Tapiero*et al*., 2004). *In vitro* studies have showed that garlic (*Allium sativum*) have antibacterial, antiparasitic, antifungal, antiviral and antioxidant properties (Ankri and Mirelman, 1999). It has the ability to lowering triglycerides, plasma cholesterol; reduce blood pressure and cardiovascular diseases (Sterling and Eagling, 2001). Allicin, the major bioactive compound in garlic, has most pharmacological effects (Amagase*et al*., 2001). Previous studies showed that garlic had the ability to lowering cholesterol 7 α-hydroxylase activity 3-hydroxy-3-methylglutaryl reductase activity and 3-hydroxy-3-methylglutaryl reductase activity, triglycerides, blood glucose, plasma and egg cholesterol in animals and human (Chi *et al*., 1982; Konjufca *et al*., 1997; Lim *et al*., 2006; Azeke and Ekpo, 2008; Canogullari *et al*., 2009; Adebiyi*et al*., 2017). Turmeric (*Curcuma longaL.*) had subject interesting in various purpose in poultry nutrition due to its nutritional and medical effects such as anti-inflammatory, anti-microbial, anti-protozoal , anti-oxidant and anti-aging (Amalraj*et al*., 2017).The use of turmeric(*Curcuma longaL.*) as feed additive in poultry rations was reviewed by Khan *et al*. (2012). Curcumin is a major active material in turmeric which responsible for giving flavor and color to foods, has a potent antioxidant properties (Sreejayan, 1994; Ruby *et al*., 1995). It has also used for treatment inflammation (Durrani*et al*., 2006; Kermanshahiand Riasi, 2006; Malekizadeh*et al*., 2012). Moreover, turmeric (*Curcuma longaL.*) had active ingredients such as tetrahydrocurcuminoids (Osawa*et al*., 1995) bisdemethoxycutcumin, and demethoxycurcumin (Wuthi-udomler*et al*., 2000).The aim of this study is to investigate the effect of different levels of garlic and turmeric powder on egg quality characteristics, bacterial population and intestinal histomorphology of laying hens(shaver) .

Materials and Methods:

This study was carried out at the poultry farm belong to animal resources, College of Agriculture, University of Anbar, from July – January. A total number of 112, 42 week old shaver laying hens were randomly divided into seven treatments (each treatment contains four replicates with 4 hens). Feed and water were provided. The diets were formulated to meet the requirements of birds established by the (NRC, 1994) for laying hen (Table 1). Laying hens in treatment 1 were fed a basal diet without any addition (control) . Whereas treatments 2 , 3 and 4 were fed garlic powder at 0.25 , 0.5 and 1% respectively in addition basal diet, treatments 5 , 6 and 7 were fed turmeric powder at 0.25 , 0.5 and 1% respectively in addition basal diet. The garlic and turmeric powder were purchased commercially as dried herbs supplements. The chickens were reared in twenty-eight cages (40×50×45 cm) each replicate placed in pen. The study continued for four periods (28day/ period). House system was whole controlled,16 hours light was provided per day. Feed and water were available throughout the experiment. At weeks 46, 50, 54 and 58 of age, Eggs collected from each bird in each treatment. The internal egg characteristics were measured through breaking four eggs per replicate were taken randomly from each treatment and their quality traits were measured once weekly for 16 times. By using a sensitive electronic scale the weight of eggs laid by birds in each replicate were recorded. The egg quality parameters including egg shell quality (shell weight and membranes),shell thickness, albumen weight, albumin height and Haugh unit. Yolk quality parameters include yolk height which determined by using a vernier caliper. Shape index and yolk index determined according to (Romanoff and Romanoff, 1949):-

Yolk index (%)= (yolk height/yolk diameter)× 100.

Albumen index (%) = (Albumen height/average of albumen length and width) ×100.

Shape index (%) = (width / length)× 100.

The huagh unit was determined according to (Haugh ,1937):-

Huagh unit =100 log[H-($\sqrt{G}$(30W$^{0.37}$-100)/100 +1.9], where H= albumen height (mm); G= 32.2, and W= weight of egg (g).

At the end of the experimental period, three birds were chosen randomly from each treatment for slaughter test and carcass weights were determined and presented as a percentage of live body weight. At the end of study periods, one bird from each replicate was selected and slaughtered to determined the microbial tests. The contents of cecum were collected in petri dishes. The samples were put on ice until transferred to the laboratory and prepared for microbial culture to measure the microbial content, one gm. of cecal contents were serially diluted and 10 ml. of each dilution was spot on each plates count agar and MacConkey agar media to count total aerobic and *E. coli*, respectively. After incubation, the bacteria were counted in petri dishes and the number of bacteria in the initial volume was calculated using:

Number of bacteria = Number of colonies ×(1/Dilution) × Cultured volume.

For the histomorphological examination after slaughtering the bird, small intestine was removed immediately and from the middle part of two sections (duodenum, jejunum,) the fragments were separated by 3 to 5 cm. according to (McManus ,1948) and (Bradley *et al*.,1994). Data obtained from the study were analyzed using computer software by statistical analysis system (SAS, 2001) and significant differences among means were determined by using Duncan´s multiple range test (Duncan, 1955).

Table 1. Composition of the experimental laying hen basal diet and calculated chemical analysis.

Ingredients	(%)
Yellow corn	36
Wheat	30
Protein*	4
Soy bean meal (48%)	20
Oil	1
Di calcium phosphate	1
Limestone	7.7
Salt	0.3
Total	100
Calculated analysis**	
Metabolizable Energy(kcal/kg)	2804
Crude Protein %	17.7
ME: C.P	158
Calcium%	3.41
Available Phosphor%	0.48
Lysine	0.93
Methionine	0.36
Methionine+ Cystein	0.69

*Jordan origin : (40% crude protein, 2107 kcal metabolizable energy, 6% fat, 2.5% ash, 6-7% Ca, 3.3% ,P ,2.0% Methionine+ Cystin, 2.5% Lysine.
** Chemical analysis according to (NRC , 1994)

Results and Discussion

Data in Table (2) showed there were no significant differences among the treatments of adding garlic and turmeric powder to laying hens diets on Egg weight, Shell weight(%), Shell thickness, Egg Shape index and Haugh unit all over study periods except for Albumen weight ,Yolk weight, Yolk index and Albumen Index. These results disagree with Osawa *et al.,*(1995) who reported that curcuma stimulate protein synthesis by bird enzymatic system. Also, yolk index percentages were significantly higher for all treatments as compared with control groups. Nutrients in layer rations did not have any

beneficial effect on Haugh unit (Naber, 1979). Rabinokov *et al.*, (2000) mentioned that allicin in garlic converted into many disulfide derivative exerting that have anti-oxidative activity by reaction with sulfur compound. Antioxidant effects for allicin derivatives from garlic could maintain egg. Previous studies suggested that natural herbals like garlic and turmeric and their active compounds such allicin and curcumin have beneficial effects like antioxidant activity (Ramirez-Tortosa*et al.*, 1999).The inclusion of 0, 5, 10 and 15 g/kg garlic powder decreased yolk weight(Mottaghitalab and Taraz , 2002). Results of Lawson and Hughes (1992) reported that allicin an active material in garlic is unstable and poorly absorbed from digestive tract. Garlic supplementation enhanced the activities of the pancreatic enzymes and provided microenvironment for better nutrient absorption and utilization (Ramakrishna *et al.*, 2003).Pappas *et al.*, (2006) reported that the decline in albumen deterioration rate characterizes as a less of the antioxidant status of egg contents. In agreement with our results, Yalcin *et al.*, (2006) mentioned that no differences between treatments in yolk weight egg albumen index, egg shell index and haugh unit values by adding garlic powder at 5 and 10g/kg to laying hens' diets for 22 weeks. However, Lim *et al.*, (2006) reported that garlic powder with 30g/kg had no effects on egg quality except the haugh unit which linearly increased after 2 weeks of storage by increasing of dietary garlic powder**.** Safaa (2007) indicated that 2% addition of dietary garlic increased yolk weight and Haugh unit**.** These results agree with the finding of Radwan *et al*, (2008) who observed adding turmeric to the control diet at levels 0.5 and1.0% have no effect on external characteristics of eggs which included Shell weight , Shell thickness and Egg Shape index. Also agree with the our results the results of Behnamifar *et al.*, (2015) who mentioned that egg quality of quails which included Shell weight , Shell thickness , Haugh unit and Yolk weight were not significantly affected by adding of Herbal extracts of garlic , thyme and caraway to drinking water.

Table 2.Effect of adding garlic and turmeric powder on egg quality characteristics.

Items	Control	Garlic 0.25%	Garlic 0.5%	Garlic 1%	Turmeric 0.25%	Turmeric 0.5%	Turmeric 1%	SEM	*P*-Value
EW (g)	49.79	49.89	49.76	49.96	49.93	49.77	49.91	0.048	NS
SW (%)	10.23	10.48	10.70	10.61	10.72	10.70	10.67	0.085	NS
ST (mm)	0.347	0.352	0.352	0.354	0.354	0.356	0.358	0.001	NS
ESI (%)	74.28	74.26	74.91	74.72	75.16	74.77	74.34	0.202	NS
AW (%)	51.50 a	51.24 a	49.68 b	49.06 b	50.04 ab	50.58 ab	50.27 ab	0.255	0.0263
YW (%)	38.26 b	38.27 b	39.61 ab	40.32 a	39.24 ab	38.72 b	39.06 ab	0.235	0.011
YI (%)	46.05 c	50.36 ab	49.82 b	51.84 ab	52.19 a	51.19 ab	51.98 ab	0.361	0.034
HU	74.07	74.27	73.53	75.27	74.07	74.67	74.66	0.349	NS

a,b,c...= Means in the same row with different superscripts, differ significantly (P< 0.05).
SEM = Standard Error of Means.
NS = Non Significant (P>0.05).
EW: Egg weight, SW: Shell weight (g), ST: Shell thickness, ESI: Egg Shape index, AW: Albumen weight (g), YW: Yolk weight (g), YI: Yolk index , HU: Haugh unit.

As shown in table (3)Live weight, Dressing percentage and relative weights of Liver, Heart, Spleen and Gizzard were not effect by adding garlic and turmeric powder to laying hens diets. However, spleen weight increased. Hens fed 0.5 or 1.0% turmeric increased in spleen weight as compared to hens in control treatment. The increase of

relative weight of spleen may be due to activity of curcumin to stimulate immune which the active material in turmeric (Antony *et al.,* 1999). Our results were similar supported by Al-Sultan (2003) who mentioned that, higher relative weight of spleen was observed in chickens received feed contained 1.0% turmeric. In agreement with the current study, Radwan*et al*.,(2008) reported that hens fed turmeric 0.5 and 1% had no significant effect on carcass parameters and internal organs. Also, results were in agreement with findings of Vasko *et al*., (2015) who found supplemented garlic powder 0.05% to laying hens' rations had no effect on carcass weight and relative weights of internal organs.

Table 3.Effect of adding garlic and turmeric powder on carcass characteristics.

Items	Control	Garlic 0.25%	Garlic 0.5%	Garlic 1%	Turmeric 0.25%	Turmeric 0.5%	Turmeric 1%	SEM	*P*-Value
Live weight (g)	1826	1973	1906	1960	2023	1940	1883	25.86	NS
Dressing (%)	69.71	69.59	68.10	69.63	68.17	69.37	69.94	0.381	NS
Liver (%)	2.23	2.29	2.11	2.09	2.22	2.44	2.27	0.047	NS
Heart (%)	0.575	0.509	0.566	0.557	0.557	0.554	0.553	0.011	NS
Spleen (%)	0.167	0.166	0.169	0.161	0.165	0.170	0.187	0.006	NS
Gizzard (%)	1.995	1.771	1.864	1.631	1.852	1.529	1.785	0.059	NS

SEM=Standard Error of Means.
NS = Non Significant (P> 0.05).

In continue of Table 4, the effect of garlic and turmeric on populations of *Total aerobic bacteria* and *Colibacilli* is reported. The colony forming units of total aerobic bacteria and *Colibacilli* in digest of small intestine in garlic and turmeric treatments showed a significantly decrease compared with control treatment. Garlic have most effect on decrease of *total aerobic bacteria* (P<0.05) on comparison to turmeric, but garlic treatment (1%) shows the least rate of *Colibacilli* grown in all of treatments (P<0.05). However, turmeric (1%) supplement could not decrease population of bacteria. Multiple *in vitro* studies illustrated that garlic and turmeric represented antimicrobial activity against intestinal microorganisms such *Salmonella typhimurium*, *Clostridium perfringens* and *E. coli* (Bara andVanetti ,1992 ; Mekala*et al*., 2013).The mechanism of microbe's action is interceded by lipophilic property to break through the bacterial membranes, which releases membrane components from cells to external environment (Kim*et al*., 2008). Recently, studies have been examining adding herbs and their extracts as growth promoters and feed additives substitute of antibiotics. The mechanism of action based re-balance of useful microorganisms in digestive tract, stimulate of increasing of enzyme secretion, improvement in the immunity response, the morphohistological maintenance of intestinal tract and the activity of antioxidant (Brugalli, 2003). Previous studies have demonstrated their *in vitro* effects against many pathogens microbes, with antifungal, antimicrobial activity, in addition to antioxidant effects (Kamel, 2000).In study, Losa*et al*., (2001) demonstrate adding extracts of a herbal mixture decrease 70% of broilers infected with *Clostridium perfringens*. A significant decrease of *Clostridium perfringens* colonization in the intestine of birds fed diets contained mixture of curcumin and allicin (Mitsch*et al*.,2004) and it has been documented that garlic differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Rees*et al*.,1993).These findings were in thorough consistency with our results of decrease intestinal microflora of laying hens(shaver). It is important to consider that environment conditions and basal diet can

be affect with*in vivo* antimicrobial activity of garlic(*Allium Sativum)* and turmeric*(Curcuma longa L.) i*n laying hens.

Table 4.Effect of adding garlic and turmeric powder on the total aerobic bacteria and *Colibacilli* populations in laying hens.

Items,*log*10 *cfu*/g	Control	Garlic 0.25%	Garlic 0.5%	Garlic 1%	Turmeric 0.25%	Turmeric 0.5%	Turmeric 1%	SEM	*P*-value
Total aerobic bacteria	7.06 a	6.12 bc	5.46 c	5.45 c	6.53 ab	6.44 ab	6.32 ab	0.22	0.0065
Colibacilli	6.72 a	5.69 bc	5.72 bc	5.09 c	6.13 ab	5.92 b	5.68 bc	0.39	0.0684

a,b,c Means within a column with no common superscript differ significantly (P > 0.05).
SEM= Standard Error of the Means.
NS= Non Significant

In table 5, the effect of garlic and turmeric on intestine morphology in laying hens has been presented. Result of experiments on villus height, villus width and crypt depth in the duodenum and jejunum did not show significant differences between treatments (P>0.05), also crypt depth and villus width in the ileum have not significant differences (P>0.05).Villus height is an indicator of increasing enzyme digestion and absorption of nutrients (Miles *et al*., 2006). Sieo *et al*., (2005) reported that intestinal villus height and the ratio of villus height to crypt depth is an indication of the vast area for nutrient absorption and higher absorption function.

Table 5. Effect of adding garlic and turmeric powder on intestine morphology of laying hens.

Items	Control	Garlic 0.25%	Garlic 0.5%	Garlic 1%	Turmeric 0.25%	Turmeric 0.5%	Turmeric 1%	SEM	*P*-value
Duodenum									
Villus height (mm)	0.976	1.023	1.079	0.993	1.142	0.986	1.195	29.20	NS
Crypt depth (mm)	0.0508	0.0501	0.0459	0.0484	0.0494	0.478	0.483	21.80	NS
Villus Width (mm)	0.281	0.261	0.249	0.248	0.212	0.246	0.266	0.011	NS
Jejunum									
Villus height (mm)	0.922	0.929	0.901	0.943	0.936	0.988	0.979	30.54	NS
Crypt depth (mm)	0.0546	0.0518	0.0496	0.0520	0.0524	0.0522	0.0518	16.88	NS
Villus Width (mm)	0.209	0.232	0.229	0.216	0.176	0.222	0.194	0.008	NS

SEM=Standard Error of Means.
NS= Non Significant.

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