Mesopotamia J. of Agric. ISSN: 2224 - 9796 (Online) مـجلــة زراعــة الـرافديـن
Vol. (47) No. (1) 2019 ISSN: 1815 - 316 X (Print) المجلد (47) العدد (1) 2019

Effect of garlimmune addition in drinking water and the laying time on egg external, internal traits and hatchability of broiler breeder

Nidhal AG. Mustafa
Animal resources Dept./ Agriculture College/ Salahaddin University -Erbil/Iraq
Email: nidhal.mustafa@su.edu.krd

ABSTRACT

The adding garlimmune in broiler breeder drinking water were tested experimentally for evaluation of its effect on egg production, external and internal traits of egg and hatchability at the different time of laying(T1: 8:30 AM, T2: 10:30 AM, T3: 1:30 PM and T4: 8:30 AM- 1:30 PM). The results indicated that the use of garlimmune had a good effect on egg production (HD %), egg weight, feed conversion ratio (FCR), eggshell thickness (mm), eggshell weight (g) and eggshell strength (kg/cm^2), total protein, PUFA, HDL in egg, hatchability and set eggs (%), hatched chicks weight (g) and post hatch antibody titer against ND, IBD and IB by ELISA. However, MUFA, LDL, atherogenic index, hatch window (h), embryonic mortality (1-21day) and culled chicks were significantly ($p \leq 0.05$) decreased in garlimmune treatment compared with the control at different laying time, but there is on-significant difference in total lipid among the treatments. Noticed the time laying 10:30 AM in the both groups was more effectiveness in all parameters of the study.

Key Words: garlimmune, egg weight, shell traits, hatchability, chicks immunity.

Received: 3/12/ 2018, Accepted: 19/3/2019

INTRODUCTION

The use of natural feed additives as substitutes for antibiotic in poultry production has become an area of great interest. Medicinal plants or herbs consists of many pharmacologically active chemical compounds which have antimicrobial, antioxidant, antifungal, antiviral, anti-inflammatory effects as well as immune-modulatory properties (Toghyani *et al.,* 2015). Also herbals have received increased attention as possible antibiotic growth promoter replacements (Faghani *et al.,* 2014). The compounds which comprise the essential oil of thyme have been identified as phenolic compounds suchas thymol, carvacrol and γ-terpipene. Thyme may stimulates digestive enzymes such as amylase, protease and lipase and consequently improve digestibility of food elements (Bolukbaşi and Erhan, 2007). Cinnamaldehyde is the important component of cinnamon, creating about 65 percent of the extracted essential oil. Immune system stimulating effects has been reported for cinnamaldehyde (Faghani *et al.*, 2014), in addition different flavonoids have free-radical-scavenging activities and antioxidant properties. The herb cinnamon, and thyme infusions used as drinking water did not improve the performance of broilers. However, cinnamon and herbal mix infusions significantly improved the immune response of birds to the NDV vaccine (Sadeghi *et al*., 2012). The antibody production were promoted to defend against invading pathogens, essentially the less energy expended by chickens on the non-specific immune system, the more energy that is available for growth and pro-duction (Huangand Lee, 2018). This study aimed to know the effect of garlimmune from Miavit- Germany (an extract which is composed of garlic 40000, thyme 40000, thymol 14000, cinnamaldehyde 14400 mg/l, it's an immune stimulator, antibacterial, antiviral, antioxidant and appetizer) at different laying time on reproductive

characteristics of broiler breeder females by measuring egg quality and hatching traits. Furthermore the effect of garlimmune on the immunological response of post hatching chick.

MATERIALS AND METHODS

This study was carried out in Taq Taq poultry breeder farm and Hatchery project / Kosar company/Koya-Erbil**.** A completely randomized design with 600 females and 72 males of broiler breeder (Ross-308) at age 30 wk. were divided into 2 groups as follows: group 1 control (basal diet) and group 2 garlimmune each group contain 4 treatments of different laying time (T1: 8:30 AM, T2: 10:30 AM, T3: 1:30 PM and T4: 8:30 AM - 1:30 PM) was used to evaluate the effects of adding 0.5 % garlimmune extract 2 wk at different laying time and this study continued for 2 month. The farm temperature was controlled under 26-27°C, the broiler breeder males and females were exposed to a 16-h photoperiod a long an experimental period. water was available *ad libitum* and feed consumption presented according to Ross 308 broiler breeder guide. The production ration of broiler breeder was formulated according to the NRC (NRC, 1994), the basal composition of the diet detailed in Table (1).Hen day production (H.D)%, Feed intake (FI.) and feed conversion ratio (FCR) were calculated throughout the experiment. At the end of the experiment about 12 eggs were taken from each treatment groups evaluate egg quality measurements.

One gram of each egg yolk was homogenized with 15 ml of chloroform-methanol 2:1 (by volume), sonicated, and filtered as previously described by Elkin and Rogler (1990). Total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) concentrations were determined using the same reagent kits as those used for serum analysis and the atherogenic index was calculated as the ratio of LDL/HDL. Whole egg protein (g/l) concentration using commercial kits measured by Spectrophotometer. Monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) measured by Gas Chromatograph (GC).

At age 30.5 weeks 600 eggs were taken from each treatment in both garlimmune and control at different laying time were set for 18 d in an incubator at (37.75 °C) and 55% relative humidity, then transferred to the hatchery for 3d at (37.25 °C) and 65% relative humidity until the end of 21st day of incubation. At the end of hatching all live and dead chicks were counted, the hatchability of set and fertile eggs, total embryonic mortality, culled chicks and chicks weight were determined. Serum was harvested after blood centrifuged from 1d old chicks to measure the antibody titer of Newcastle Disease (ND), Infectious Bursa Disease (IBD) and Viral Infectious Bronchitis (IB) were measured by direct ELISA Synbiotics (Biocheck – ELX 800).

Table (1): Broiler Breeder diets ingredients (%)

Ingredients[1]	Males	Females	Approximately analysis		
Wheat	64.00	55.36	Crude protein %	13.84	18.00
Wheat bran	15.00	6.50	Fat %	3.250	4.45
Wheat flour	9.00	7.00	ME.Kcal/kg	2765	2802
Soya bean meal	6.70	18.50	C/P ratio	199.78	155.82
Bredmix	2.50	2.50	Crude fiber	4.23	3.91
Soybean oil	1.00	2.50	Available Ca %	1.15	3.05
Lime stone	1.50	7.30	Available P %	0.39	0.38
Salt	0.05	0.05	Na %	0.20	0.21
Methionine	0.01	0.03	Lysine %	0.55	0.81
Lysine	0.01	0.03	Methionine %	0.38	0.42
Anti toxcin	0.03	0.03			
Multi-Minerals	0.05	0.05			
Multi-Vitamins	0.10	0.10			
Enzymes	0.05	0.05			

[1]All feed ingredients taken from Kosar Company in Erbil 2 Bredmix -1kg contain: Vitamins: 334000IU A,، 67000 mg E,D3 500mg ,B1، 167 mg,B21000mg, B6، 0.66 mg, B12 67mg, Niacin 1000mg. Minerals: Fe 1.667mg, Mn 3.334mg, Colin 17000mg, folic acid 17mg, Butin 1.33mg, Zn 2.667mg, Cu 334mg, I 17mg. Methionin 27.000mg, Zn-Bastracin 667mg, Anti-oxidant 3.333 ppm, P 10.6% and Na 4-4.5%. [3] NRC: Chemical analysis of ingredients depending on NRC (1994). [4] A sample of feed analyzed in laboratory of Science College of Baghdad University.

All data were analyzed by using CRD (Complete Randomize Design) by SAS (SAS, 2005), significant differences among treatment means were determined by Duncan's multiple range tests at level 0.05 (Duncan, 1955).

RESULTS AND DISCUSSION

Table 2. evaluated the broiler breeder dietary addition with garlimmune extract in drinking water on body performance at different laying time. The results were significantly ($p \leq 0.05$) increase hen day production (H.D%) , egg weight (g), and the feed conversion ratio (FCR) improved in the all treatments which added garlimmune at different laying time compared with the control at the same laying time. The results in Table 3. Indicates significantly ($p \leq 0.05$) increased in eggshell thickness (mm), eggshell weight (g) and eggshell strength (kg/cm^2) in the treatments which added at laying time T2 (10:30 AM), T1 (8:30 AM) and T4 (8:30 AM - 1:30 PM) compared with the T3 (1:30 PM) of garlimmune addition and with all control treatments at different laying time.

The improvement in egg production, feed conversion ratio (FCR) and egg weight specially in garlimmune group related to contain allicin, free fatty acid (oleic acid: 8.40%), Iodine, saponification, Acid, Ca, P, K, Mg, Mn, Cu (Bagudo and Acheme, 2014), all these nutrients enhanced egg production, egg weight, FCR and eggshell quality especially eggshell strength. Also garlic allicin is the most predominant

thiosulphate in garlic that is responsible for the characteristic odor and has an antibacterial effect (Cunha *et. al*., 2012).

Table (2): Effect of garlimmune addition in drinking water and laying time on broiler breeder production trait.

Traits	Treatments	Time of egg laying (h)				MSE
		T1 8:30 AM	T2 10:30 AM	T3 1:30 PM	T4: 8:30 AM - 1:30 PM	
Hen day production (H.D %)	C	79.30 ^{c}	81.14 ^{bc}	77.96 ^{c}	79.48 ^{bc}	2.11
	G	87.15 ^{ab}	88.68 ^{a}	86.08 ^{b}	87.55 ^{ab}	1.03
Egg weight(g)	C	63.21 ^{b}	63.18 ^{b}	63.12 ^{b}	63.38 ^{b}	1.06
	G	67.15 ^{a}	66.71 ^{a}	66.82 ^{a}	67.03 ^{a}	0.93
Feed conversion ratio (FCR) (g)/egg	C	2.473 ^{a}	2.348 ^{a}	2.408 ^{a}	2.403 ^{a}	0.235
	G	2.168 ^{bc}	2.036 ^{c}	2.279 ^{b}	2.185 ^{bc}	0.183

C: control (standard diet), G: garlimmune extract, ^{a,b,c} different superscripts within rows and columns for the same trait indicate significant differences at ($P \leq 0.05$), MSE: mean of standard error.

Table (3): Effect of garlimmune addition in drinking water and laying time on broiler breeder eggshell characteristics.

Traits	Treatments	Time of egg laying (h)				MSE
		T1 8:30 AM	T2 10:30 AM	T3 1:30 PM	T4: 8:30 AM - 1:30 PM	
Eggshell thickness (mm)	C	0.37 ^{ab}	0.38 ^{ab}	0.30 ^{b}	0.35 ^{b}	0.07
	G	0.44 ^{a}	0.48 ^{a}	0.39 ^{ab}	0.44 ^{a}	0.05
Eggshell weight (g)	C	11.45 ^{ab}	11.71 ^{ab}	10.82 ^{b}	11.33 ^{b}	0.43
	G	12.15 ^{s}	12.46 ^{a}	11.56 ^{ab}	12.06 ^{a}	0.25
Eggshell strength (kg/cm^2)	C	3.52 ^{b}	3.82 ^{ab}	3.29^{b}	3.55^{b}	0.15
	G	4.17 ^{a}	4.49 ^{a}	3.73 ^{b}	4.13 ^{a}	0.12

C: control (standard diet), G: garlimmune extract, ^{a,b,c} different superscripts within rows and columns for the same trait indicate significant differences at ($P \leq 0.05$), MSE: mean of standard error.

The results in Table 4. shows the broiler breeder dietary addition with garlimmune extract in drinking water on body performance at different laying time. The results were significantly ($p \leq 0.05$)increased total protein in whole egg, PUFA and HDL in egg yolk in the all treatments of garlimmune addition compared with control treatments at different laying time. While, the results of MUFA, LDL and atherogenic index in egg yolk were significantly ($p \leq 0.05$) decreased in garlimmune treatment compared with control treatments at different laying time. However there is no-significant differences among the treatments of control and garlimmune addition in total lipid.

Garlimmune extract in drinking water was effect on the normal physiological function, especially blood serum constituents Low Density Lipids (LDL) which might be due to the garlic (Reuter *et al*., 1996). Spices and their extracts have lipotropic effects, some of the active components in spices affect lipid metabolism through fatty acid transportation and therefore it can be increased lipid utilization (Cross *et al*, 2007).

Table (4): Effect of garlimmune addition in drinking water and laying time on broiler breederegg protein , lipid and cholesterol profiles.

Traits		Treatments	Time of egg laying (h)				MSE
			T1 8:30 AM	T2 10:30 AM	T3 1:30 PM	T4: 8:30 AM - 1:30 PM	
Total Proteinin whole egg (g/100 g egg)		C	6.48^{bc}	7.19^{b}	6.04^{c}	6.57^{bc}	0.413
		G	7.63^{ab}	8.45^{a}	7.38 ^{b}	7.82^{ab}	0.366
Egg yolk (g/100 g egg)	Total lipid	C	19.48 ^{a}	19.76 ^{a}	19.10 ^{a}	19.45 ^{a}	0.762
		G	19.83 ^{a}	20.03 ^{a}	19.41 ^{a}	19.76 ^{a}	0.589
	MUFA	C	43.52^{b}	36.94 ^{c}	49.05 ^{a}	43.18^{b}	1.67
		G	28.15^{ed}	24.35^{e}	32.61^{d}	28.39^{ed}	1.28
	PUFA	C	20.15^{d}	24.16^{c}	19.43^{d}	21.25^{cd}	1.17
		G	34.46^{ab}	36.07^{a}	30.95 ^{b}	33.81^{ab}	0.83
Cholesterol (mg/100 g egg)	Total CH	C	176.32^{ab}	158.00 ^{b}	180.94^{a}	172.80^{ab}	7.02
		G	143.57^{c}	119.77 ^{e}	145.11^{c}	136.16^{d}	4.91
	LDL	C	109.53 ^{ab}	98.65 ^{ab}	116.93 ^{a}	108.37 ^{ab}	3.47
		G	85.22 ^{c}	77.85 ^{d}	91.18 ^{b}	84.75 ^{c}	2.08
	HDL	C	41.67 ^{cd}	43.38 ^{c}	39.75 ^{d}	41.60 ^{cd}	2.17
		G	47.05 ^{b}	54.17 ^{a}	44.08 ^{bc}	48.43 ^{b}	1.05
	Atherogenic index	C					
		G	2.63 ^{ab}	2.27 ^{b}	3.02 ^{a}	2.64 ^{b}	0.327
			1.81 ^{c}	1.44 ^{d}	2.07 ^{bc}	1.77 ^{c}	0.136

C: control (standard diet), G: garlimmune extract, ^{a,b,c} different superscripts within rows and columns for the same trait indicate significant differences at ($P \leq 0.05$). MSE: mean of standard error. MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, CH: cholesterol, LDL: low density lipoprotein, HDL: high density lipoprotein, Atherogenic index: the ratio of LDL cholesterol to HDL cholesterol (LDL/HDL).

The results in Table 5. shows significantly ($p \leq 0.05$) increased in the hatchability of fertile eggs (%),hatchability of set eggs (%) and hatched chicks weight (g) in the all treatments of garlimmune treatment compared with control group at different laying time. However, hatch-window (h), Total embryonic mortality (1-21) days (%) and

culled chicks (%) significantly ($p \leq 0.05$) decreased in the treatments of garlimmune addition compared with control group at different laying time.

Michalska and Stępińska (1996) showed a positive correlation between the level of HDL cholesterol and fertility. These authors also found a significant ($P \leq 0.05$) correlation between yolk HDL concentration and yolk cholesterol concentration, may be important during the production of hatching eggs. Also Rahimi *et al.*, (2015) were referred the addition of herbs extract contain thyme and garlic in quails drinking water seems to improve the quality of produced eggs, especially as it reduces the yolk cholesterol and intestinal population of bacteria (the bacteria population competes with birds for feed nutrients), which improve the performance of egg-laying birds in terms of quantitative traits, egg weight, shell strength and egg quality. It regard to their biological activities, in order to improve fertility and hatching percentage of incubated eggs.

Table (5): Effect of garlimmune addition in drinking water and laying time on hatching traits.

Traits	Treat-ments	Time of egg laying (h)				MSE
		T1 8:30 AM	T2 10:30 AM	T3 1:30 PM	T4: 8:30 AM - 1:30 PM	
-Hatching of fertile eggs %	C	82.89 ^{c}	88.21 ^{b}	81.33 ^{c}	84.48 ^{bc}	2.48
	G	93.20^{ab}	94.30 ^{a}	91.13 ^{ab}	92.88 ^{ab}	1.95
-Hatching of set eggs %	C	85.33 ^{c}	86.79 ^{bc}	83.57 ^{c}	85.23 ^{c}	2.29
	G	90.12 ^{ab}	92.50 ^{a}	88.85 ^{b}	90.49 ^{ab}	2.06
-Hatching window [1](h)	C	11.15 ^{ab}	10.06 ^{b}	11.45 ^{a}	10.89 ^{ab}	1.03
	G	6.22 ^{d}	4.37 ^{e}	7.51 ^{c}	6.03 ^{d}	0.57
Total embryonic mortality (1-21) days %	C	8.42 ^{ab}	7.80 ^{b}	9.38 ^{a}	8.53 ^{ab}	0.67
	G	5.03 ^{c}	3.33 ^{e}	6.10 ^{bc}	4.82 ^{d}	0.42
-Culled chicks%	C	6.25 ^{ab}	5.41 ^{b}	7.05 ^{a}	6.23 ^{ab}	0.58
	G	4.85 ^{c}	4.17 ^{d}	5.05 ^{bc}	4.62 ^{c}	0.41
-Hatched chicks weight (g)	C	40.19 ^{c}	42.07 ^{b}	39.79 ^{c}	40.68 ^{bc}	1.29
	G	42.73 ^{ab}	44.20 ^{a}	41.80 ^{b}	42.91 ^{ab}	0.87

C: control (standard diet), G: garlimmune extract, ^{a,b,c} different superscripts within rows and columns for the same trait indicate significant differences at ($P \leq 0.05$).
MSE: mean of standard error.
[1] Hatch window: period (hour) between the first and the last chick that hatched in the basket.

The results of antibody titer against ND, IBD and IB by ELISA of post hatch illustrated in the Table (6). The titer of the pos thatch ND and IB was significantly enhanced ($p \leq 0.05$) in the garlimmune treatment in all laying time compared with the control groups at the same time. Also noticed significant increases in pos thatch ND at laying time (10:30)AM, (8:30AM- 1:30PM), (8:30AM) respectively compared with the laying time (10:30AM) in both garlimmune and control groups. The results of IB

titer of the post hatch was significantly higher ($p \leq 0.05$) in the garlimmune treatments in all laying time compared with the control groups at the same time. Post hatch IB was significantly increased at laying time (10:30AM), (8:30AM-1:30PM), (8:30AM) and (10:30AM) respectively in garlimmune groups addition, while, the results in control groups recorded insignificant differences among the laying times.
The antibody titers of ND, IBD and IB in the all garlimmune and control group the laying time (10:30AM) recorded more improvement compared with other, but the time (1:30AM) recorded the lowest titers.

Garlic is uncommonly used as plant supplement in poultry feeds. It was found that it has some positive effects on animal health and immune response which reflected in positive effects on the animal performance (Fanelli *et al*., 1998). In the laying hens garlic inclusion resulted an increased TWBC which reflecting good immune response(Birrenkott *et al*., 2000). Herbs that are rich in such flavonoids as thyme act as antioxidants and may therefore enhance the immune function (Rahimi *et al*., 2011; Jameel *et al*., 2014).Khaligh *et al*., (2011) and Narimani *et al*., (2011) concluded the addition of herbs as garlic, thyme and menthol in drinking water caused to increasing antibody titer against ND compare to control group.

Table (6): Effect of garlimmune addition in drinking water and laying time on post hatch maternal immunity(ng/ml) by ELISA.

Antibody titer	Treatments	Time of egg laying (h) T1 8:30 AM	T2 10:30 AM	T3 1:30 PM	T4: 8:30 AM - 1:30 PM	MSE
ND	C	4613 ^{cd}	5129 ^{c}	4317 ^{d}	4686 ^{cd}	315
	G	7015 ^{ab}	8207 ^{a}	6885 ^{b}	7369 ^{ab}	233
IBD	C	3895^{c}	4083 ^{c}	3590^{c}	3690 ^{c}	401
	G	5035 ^{b}	5572 ^{a}	5011^{b}	5206 ^{ab}	298
IB	C	2691^{cd}	3026 ^{c}	2350^{d}	3002^{c}	187
	G	3745 ^{ab}	3933 ^{a}	3379^{b}	3686 ^{ab}	203

C: Control, G: Garlimmune, ND: Newcastle Disease, IBD: Infectious Bursal Disease, IB: Viral Infectious Bronchitis disease. ^{a,b,c} different superscripts within rows and columns for the same trait. indicate significant differences at ($P \leq 0.05$)

CONCLUSION

The beneficial addition of garlimmune in broiler breeder drinking water at different laying time observed improvements in egg production percentage, egg weight, feed conversion ratio, eggshell quality, egg total protein, HDL, PUFA, hatchability and reduced embryonic mortality% also improved maternal immunity of pos thatch by ELISA then produce good quality chicks. In the present study the laying time 10:30AM of garlimmune and control groups achieved more improvement in the productive, egg quality and immune response for hatched chicks.

AKNOWLEGEMENT

I express my appreciation and great thankful for M. Muhsin Mohammed Ameen the owner of TaqTaq Poultry breeder and hatchery project/ Kosar company and Dr. Mahbuba AG. Mustafa for their assistance and facilities to carry this work.

تأثير اضافة garlimmune في ماء الشرب لأمهات فروج اللحم ووقت وضع البيض في الصفات الخارجية والداخلية للبيض ونسبة الفقس

نضال عبدالغني مصطفى
قسم الثروة الحيوانية/ كلية الزراعة/ جامعة صلاح الدين-أربيل/ العراق
Email: nidhal.mustafa@su.edu.krd

الخلاصة

أختبر تجريبيا تأثير إضافة garlimmune في ماء الشرب لأمهات فروج اللحم على انتاج البيض، صفات البيض الخارجية والداخلية ونسبة الفقس في أوقات مختلفة من وضع البيض (8:30 ص، 10:30 ص، 1:30 ب.ظ ، 8:30 ص - 1:30ب.ظ). أشارت النتائج الى ان استخدام garlimmune له تاثير جيد في نسبة انتاج البيض ووزنه و كفاءة التحويل الغذائي وسمك القشرة ووزن القشرة، قوة مقاومة القشرة للكسر والبروتين الكلي والأحماض الدهنية غير المشبعة المتعدد و البروتينات الدهنية عالية الكثافة في البيض ونسبة الفقس من البيض المخصب والحاضن ووزن الفرخ الفاقس ورفع من مستوى الاجسام المضادة لمرض النيوكاسل والكمبورو والتهاب القصبات الفيروسي ، في حين انخفضت والأحماض الدهنية غير المشبعة الأحادية (MUFA) والبروتينات الدهنية واطئة الكثافة في البيضLDL ونسبة LDL/HDL ونسبة الأجنة الهالكة الكلية (1-21) يوما ونسبة الأفراخ المستبعدة في معاملات إضافة garlimmune مقارنة مع معاملة السيطرة وفي الأوقات المختلفة من وضع البيض. بينما لم توجد أية فروقات معنوية في مستوى الدهون الكلية بين معاملات التجربة ولكلا المجموعتين. لوحظ ان وقت وضع البيض في الساعة 10:30 كان أكثر فاعلية في جميع الصفات وفي كلا المجموعتين.

الكلمات الدالة: garlimmune ، وزن البيض، صفات القشرة ، نسبة الفقس، مناعة الأفراخ.

تأريخ استلام البحث: 2018/12/3، تأريخ قبول البحث 2019/3/19

REFERENCES

Bagudo, B. U. and Acheme O. D. (2014).Chemical analysis of locally cultivated garlic and it's oil. Der Chemical Sinica, 5(1):128-134.

Birrenkott, G.P., G.E. Brockenfelt, J.A. Greer and M.D. Owens, (2000). Topical application of garlic reduces northern fowl mite infestation in laying hens. *Poult. Sci*., 79: 1575-1577.

Bolukbaşi, SC and Erhan MK. (2007). The effect of feeding thymus, sage and rosemary on laying hen performance, cholesterol and some proteins ratio of egg yolk and Escherichia coli count in feces. Archiv fur Geflugelkunde, 72 231-237.

Cross, D.E., Mcdevitt, R.M., Hillman, K., Acamovic. T. (2007). The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Br. Poult. Sci., 48: 496-506.

Cunha, Camila p., Eulália s. S. Hoogerheide, Maria i. Zucchi, Mariza monteiro and José b. Pinheiro. (2012). New microsatellite markers for garlic, Allium Sativum (Alliaceae). American Journal of Botany: 99 (1): e17–e19, doi: 10.3732/ajb.1100278.

Duncan, D.B. (1955). Multiple ranges and multiple f. test. Biometric., 11:42.

Elkin, R. G., and J. C. Rogler. (1990). Reduction of the cholesterol content of eggs by the oral administration of lovastatin to laying hens. J. Agric. Food. Chem. 38:1635–1641.

Faghani, M, Rahimian Y, Rafiee A and Namjoo AR. (2014). Effect of garlic and cinnamon in comparison to virginiamycine on performance and some hematological parameters in broiler chicks. Research Opinions in Animal & Veterinary Sci., 4(9) 504-507.

Fanelli, S.L., G.D. Castro, E.G. De Toranzo and J.A. Castro, (1998). Mechanisms of the preventive properties of some garlic components in the carbon tetrachloride-promoted oxidative stress. Diallyl sulfide; diallyl disulfide; allyl mercaptan and allyl methyl sulfide. Res. Commun. Mol. Pathol. Pharmacol., 102: 163-174.

Huang, C. M. and T. T. Lee. (2018). Immunomodulatory effects of phytogenics in chickens and pigs - A review. Asian-Australas J Anim Sci., 31:617-627.

Jameel, Yasser J.; Abed, Ali R. and Al-Shimmary, Fateh O. (2014). Influence of adding garlic and thyme and their combination on immune response and some blood parameters in broiler. International Journal ofAdvanced Biological Research.4 (2): 211-215.

Khaligh, F., G.H. Sadeghi, A. Karimi and A. Vaziry, (2011). Evaluation of different medicinal plants blends in diets for broiler chickens. J. Medicinal Plants Research, 5(10): 1971-1977.

Michalska, E. and Stępińska M. (1996) Egg yolk HDL-cholesterol content and its relation to total plasma and yolk cholesterol and some production traits in three lines of Japanese quail. Animal Science Papers and Reports 14 (2): 141-146.

Narimani-Rad, M., A. Nobakht, H.A. Shahryar and A.R. Lotfi.(2011). Influence of dietary supplementation of medicinal plants mixture ziziphora, mentha pulagum and peppermint on some serum biochemical and immunological measures of broiler chickens. J. Middle-East. Sci. Research, 8: 457-459.

NRC (National Research Council). (1994). Nutrient Requirements of poultry, 9th rev. Ed. National academy press, Washington , D.C.

Rahimi, S., Teymouri Zadeh1, Z., Karimi Torshizi, M. A., Omidbaigi R. and Rokni H. (2011). Effect of the Three Herbal Extracts on Growth Performance, Immune System, Blood Factors and Intestinal Selected Bacterial Population in Broiler Chickens. J. Agr. Sci. Tech. 13: 527-539

Rahimi, Behnamifar, A.S.; Karimi T. M.A.; Hasanpour, S. and Mohammadzade, Z. (2015).Effect of thyme, garlic and caraway herbal extracts on blood parameters, productivity, egg quality, hatchability and intestinal bacterial population of laying Japanese quail.Iranian Journal of Veterinary Medicine. 9 (3): 179-187.

Reuter, H.D., H.P. Koch and L.D. Lawson. (1996). Therapeutic Effects and Applications of Garlic and its Preparations. In: Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species, Koch, H.P. and L.D. Lawson (Eds.). Williams and Wilkins, Baltimore, MD., pp: 135-213.

Sadeghi, GH, A. Karimi, SH. Padidar Jahromi, T. Azizi, A. Daneshmand. (2012). Effects of Cinnamon, Thyme and Turmeric Infusions on the Performance and

Immune Response in of 1- to 21-Day-Old Male Broilers. razilian Journal of Poultry Science, 14 (1): 15-20.

SAS, Statistical analysis system (2005). User's guide for personal computer. Release 8.2 SAS Institute .Inc. Cary, NC, USA.

Toghyani, M., A. Ghasemi and S.A. Tabeidian. (2015). The Effect of Different Levels of Seed and Extract of Harmal (Peganum harmala L.) on Immune Responses of Broiler Chicks. Int. J. Biol. Food, Vet. Agri. Engi.,Vol:9, No:1.