

THE IMPACT OF POWDERS AND OIL ADDITIVES OF CINNAMON AND CLOVE IN QUAILS DIET AS ANTISTRESSOR AND ANTIOXIDANT DURING HOT MONTHS

M. A. Mustafa¹
Prof.

P. H. Wasman²
Assist. Lecturer

^{1,2}Animal Resources Dept., Coll. of Agriculture Engineering Sci., Salahaddin University-Erbil/Iraq
mahbuba.mustafa@su.edu.krd

ABSTRACT

The present investigation was carried out to compare the impact of cinnamon and clove powders and their oil additives as anti-stressors on quails under heat stress which is one of the riskiest environmental conditions that influence poultry all over the world in general and Iraq in particular. For this purpose, 420 quails (5 weeks old) were chosen and randomly grouped into seven treatments with different treatments (T0: control (standard diet), T1: 2 g clove powder/kg diet, T2: 1 ml clove oil/kg diet, T3: 2 g cinnamon powder, T4: 1 ml cinnamon oil/kg diet, T5: 1 g clove powder+1 g cinnamon powder/kg diet, T6: 0.5 ml clove oil+0.5 ml cinnamon oil/kg diet) added to standard diet. The quails were fed with these diets for 17 weeks. The results indicated that adding clove, cinnamon, and their oils to quail diet under heat stress led to significantly ($p<0.05$) lower relative density of heat shock protein HSP40, HSP70, HSP90, the concentrations of (corticosterone hormone, MDA and CK oxidative stimulator enzymes in blood plasma) and heterophil-to-lymphocyte (H/L) ratio, however, the concentrations of antioxidant enzymes AOA, GPx, SOD and CAT in blood plasma were significantly ($p<0.05$) higher in all additive treatments compared with the control group. It was also seen that treatment with the oil additives resulted in higher improvement than the powder additives.

Keywords: cinnamon, clove, heat shock proteins, antioxidant enzymes, corticosterone, H/L ratio, quail

مصطفى وسمان

مجلة العلوم الزراعية العراقية -2020: 51(3): 760-766

تأثير إضافة مسحوق و زيت القرفة والقرنفل في علائق طائر السمان كمضادات الإجهاد والأكسدة خلال الأشهر الحارة

باكية هميرة وسمان

محبوبة عبدالغني مصطفى

مدرس مساعد

أستاذ

قسم الثروة الحيوانية/ كلية الزراعة/ جامعة صلاح الدين - أربيل/ العراق

المستخلص

أجريت البحث الحالي لمقارنة تأثير إضافة مسحوق و زيت القرفة والقرنفل في السمان على أنها مضادات الإجهاد و الأكسدة في الأجواء الحارة والتي تعد واحدة من أخطر الظروف البيئية التي تؤثر على الدواجن في جميع أنحاء العالم بشكل عام والعراق بشكل خاص. لهذا الغرض ، تم اختيار 420 سمان (5 أسابيع) وتم توزيعها بشكل عشوائي في سبعة معاملات: T0 (عليقة قياسية)، T1 (2 غم مسحوق القرنفل/ كغم علف)، T2 (1 مل زيت القرنفل/ كغم علف)، T3 (2 غم من مسحوق القرفة)، T4 (1 مل زيت قرفة/ كغم علف)، T5 (1 غرام من مسحوق القرنفل + 1 غرام من مسحوق القرفة / كجم من الحمية ، T6 (0.5 مل زيت القرنفل + 0.5 مل زيت القرفة/ كغم علف) أضيفت الى العليقة القياسية. تم تغذية السمان بهذه العلائق لمدة 17 أسبوعاً. أشارت النتائج إلى أن إضافة مسحوق القرنفل والقرفة وزيتونها تحت ظروف الإجهاد الحراري أدت إلى انخفاض معنوي ($p<0.05$) في الكثافة النسبية لبروتينات الصدمة الحرارية HSP40 و HSP70 و HSP90 ، تراكيز (هرمون الكورتيكوستيرون والأنزيمات المحفزة للأكسدة MDA و CK) في بلازما الدم و نسبة الخلايا المتغايرة إلى اللمفاوية (H/L). بينما ارتفعت نسبة الأنزيمات المضادة للأكسدة (AOA و GPx و SOD و CAT) في بلازما الدم. ولوحظ تفوقت المعاملات المضافة بالزيوت على التي أضيفت إليها مسحوقي القرفة والقرنفل.

كلمات مفتاحية: قرفة، قرنفل، بروتينات الصدمة الحرارية، انزيمات مضادة للأكسدة، كورتيكوستيرون، هتروفييل/ ليمفوسايت، السمان

*Received:1/8/2019, Accepted:22/11/2019

INTRODUCTION

Heat stress has been proved to cause higher concentrations of HSP70 in broilers and laying hens (13 & 25). In hot regions, heat stress is one of the most significant stressors in the broiler industry (2, 3 & 15). It has been indicated that because broiler chickens do not have sweat glands, heat stress leads to an increase in their breathing frequency and core temperature, which in turn results in increased alveolar ventilation and cools the bird down by evaporation (17). Moreover, bird performance is negatively affected as a result of increased feed conversion ratio and decreased feed intake which are provoked by exposure to high temperatures (6). When there is environmental stress, the bird's body tries to keep its thermal homeostasis, leading to elevation in the levels of reactive oxygen species (ROS) as a result of which, the body undergoes oxidative stress, resulting in production and release of heat shock proteins (HSP) which are aimed at protecting the bird from the destructive cellular effects of ROS (19). Also, because of constant reactions of mitochondrial oxidative respiration and other cellular and non-cellular processes such as ionizing radiation, inflammatory reactions, and phagocytosis (20), cells continuously produce reactive nitrogen species (RNS) and reactive oxygen species (ROS), leading to disturbance of normal antioxidant and oxidant cellular homeostasis, resulting in oxidative stress (1 & 26). Since the biological activity of herbal substances is much higher than that of the plants from which they have been retrieved, food industry has recently become remarkably interested in using plant extracts like flavonoids, oleoresins, and essential oils as poultry nutrition (31). In this regard, research has indicated promising results, such that there are numerous reports on the fact that supplementation of some herbal substances has a positive effect on the growth performance of broiler chickens, their antioxidant status, and the oxidative stability of meat. Also functional feed additives contains cinnamon and its oil positively improved the oxidative statuses of quails under heat stress (23). In this regard, using natural herbal antioxidants in the process of fattening broilers has been referred to as an appropriate method to improve antioxidant status of

poultry and enhance oxidation stability of poultry meat during storage (21). Study has proved that cinnamon dietary supplementation improves growth of broilers because this herb possesses antioxidant activity (11). The clove oil reported to possess superoxide anion radicals scavenging activity (14). Also, clove (*Syzygium aromaticum*) and its essential oil have been proved to contain antioxidant and antimicrobial properties; therefore, it can be used to control heat stress (8). According to what was mentioned the above, the present study was conducted in order to measure and compare the effect of the dietary addition with cinnamon and clove powders and their oils as antistressor and antioxidant on quail under heat stress.

MATERIALS AND METHODS

Experimental design

This study was carried out on a total of 420 quails aged 5 weeks which were randomly assigned into seven treatments. Each treatment had three replicates that consisted of 20 birds. The quails were reared for a period of 12 weeks. The quails were obtained from Animal Resources Dept., College of Agriculture, Salahaddin University-Erbil, Iraq. The quails were reared in specially-designed cages which were 65 cm long, 60 cm wide, and 50 cm high. The cages were kept in a temperature-controlled room of 35-39 °C in the afternoon and 28-30 °C at night and in the morning. The feed was standard diet in control (T0), 2 g clove powder/ kg diet in T1, 1 ml clove oil / kg diet in T2, 2 g cinnamon powder in T3, 1 ml cinnamon oil / kg diet in T4, 1 g clove powder + 1 g cinnamon powder/ kg diet in T5, and 0.5 ml clove oil + 0.5 ml cinnamon oil/ kg diet in T6 added to standard diet.

Chemical analysis of cinnamon bark, clove bud powders and their oils Nutrient analysis of 100 grams clove powder that was used in this study indicated that it contained 308 kcal energy, 44.01% carbohydrate, 9.53% water, 6.28% protein, 6.93% total fat (lipid), 33.25% total fiber, 0.0% cholesterol, 0.748 gr calcium, 0.107 gr phosphor, and 451 µg total carotenes. The utilized cinnamon powder included 256 kcal energy, 39.51% carbohydrate, 9.97% water, 7.43% protein, 5.07% total fat (lipid), 38.02% total fiber, 0.0% cholesterol, 0.983 gr calcium, 0.071 gr phosphor, and 483 µg total

carotenes. Laying quails aged 6-17 weeks were fed a diet that included corn (48.43%), soybean meal (27.6%), wheat (15%), sunflower oil (1.0%), limestone (6.0%), dicalcium phosphate (DCP) (1.15%), vitamin premix (0.10%), mineral premix (0.10%), methionine (0.17%), and lysine (0.45%). The

calculated chemicals of diet (per kg) were metabolized energy (2800 kcal), crude protein (18.52%), crude fiber (4.3%), methionine (0.45%), lysine (1.02%), Ca (2.61%), available P (0.354%), and Na (0.176%). The two samples of clove and cinnamon oils were analyzed by GC (See Table 1).

Table 1. Chemical analysis of fatty acids and volatile oils in clove and cinnamon oils

	Compound	Clove bud oil	Compound	Cinnamon bark oil	
Fatty acids	SF	6.67	SF	0.27	
	MUFA	2.38	MUFA	0.14	
	PUFA	11.90	PUFA	1.55	
	Total UFA	14.28	Total UFA	1.69	
	Total Omega-3	259 mg	Total Omega-3	1.3 mg	
	Total Omega-6	148 mg	Total Omega-6	3.17 mg	
	Eugenol	80.71	E-cinnamaldehyde	78.10	
	Eugenyl acetate	13.60	Copaene	8.12	
	β -caryophyllene	4.45	Eugenol	7.26	
	Humulene	0.41	Caryophyllene	1.85	
volatile oils	Caryophyllene oxide	0.23	α -pinene	1.35	
	α -copaene	0.21	(Z)- β -ocimene	1.19	
	methyl eugenol	0.06	Borneol	0.795	
	iso eugenol	0.08	1,8-cineole	0.347	
	chavicol	0.15	E-Isoeugenol	0.278	
	methyl salicylate	0.10	α -Guaiene	0.71	
	SF: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, UFA: Unsaturated fatty acid.				

Heat shock proteins (HSP), antioxidant and corticosterone determinations

At the end of week 17, blood samples were obtained from the quails by sacrificing them. Blood sample were collected from 12 quails at the age 17 weeks for each treatment and placed in EDTA tubes to determine HSP concentration using the kit produced by Cusabio Biotech Co. For this purpose, the determination steps explained in the instruction of the kit of chicken HSP40, HSP70 and HSP90 were employed in various matrices and diluted with the sample diluent to produce samples with values within the dynamic range of the assay. The OD concentration was determined using ELISA. Moreover, twelve blood samples were collected from the birds of each treatment. They were placed in normal tubes and centrifuged to obtain the serum of the antioxidant enzymes determination: total antioxidant activity (AOA), glutathione peroxidase (GPx), catalase (CAT), super oxide dismutase (SOD), malondialdehyde (MDA) and creatine kinase (CK). Also corticosterone hormone concentration in plasma was determined using ELISA according to the

instructions of the kit included in the Buyer's Guide for Life Science Bio-compare.

Leukocytes determination

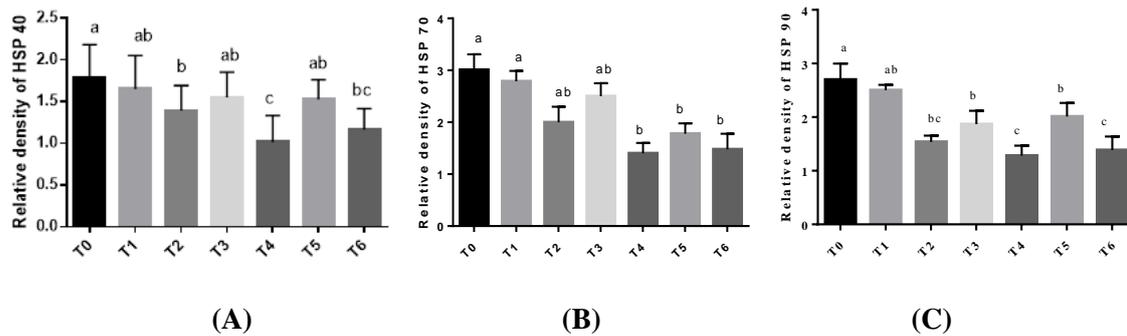
In order to determine H/L ratio, differential leukocyte count (heterophil and lymphocyte) was made on slides stained with Wright-Giemsa and observed in an optical microscope (100x) (7).

Statistical analysis

The collected data were analyzed through CRD (Completely Randomized Design) using the program SAS (28), and to compare differences among the treatments, Duncan's multiple range tests were employed (9).

RESULTS AND DISCUSSION

The results demonstrated that clove and cinnamon powders and their oil additives led to improvement in blood plasma heat shock protein (HSP) relative density in the quails that were under heat stress, such that HSP 40 was significantly lower in the treatments of oil additive T2, T4, and T6 than other treatments at a p-value of <0.05 (See Figure 1A). Moreover, T2, T4, T5, and T6 led to lower concentrations of HSP 70 (See Figure 1B). Also, HSP 90 declined as a result of T2, T3, T4, T5, and T6 (See Figure 1C).



Figures 1. A-C. The effect of clove and cinnamon powders and their oil additives on blood plasma and heat shock proteins HSP 40, HSP 70 and HSP 90 in quails at the age 12 week

Table 2. shows adding clove and cinnamon powders and their oil additives had a significant impact on corticosterone hormone concentration in blood plasma and heterophil to lymphocyte (H/L) ratio. A significant

decline was observed in the corticosterone plasma of quails in T2, T4, T5, and T6 (p<0.05) and H/L ratio in all addition groups compared with the control group.

Table 2. The influence of clove and cinnamon oil additives on serum corticosterone concentration (ng/100 ml) and H/L ratio in quails under heat stress

Traits	T0	T1	T2	T3	T4	T5	T6	MSE
Corticosterone	22.6 ^a	18.11 ^{ab}	16.09 ^b	17.54 ^{ab}	15.88 ^b	15.20 ^b	14.69 ^b	1.45
H/L ratio	0.812 ^a	0.638 ^b	0.416 ^b	0.439 ^b	0.405 ^b	0.468 ^b	0.412 ^b	0.058

T0: control (standard diet), T1: 2 g clove powder/kg diet, T2: 1 ml clove oil/kg diet, T3: 2 g cinnamon powder, T4: 1 ml cinnamon oil/kg diet, T5: 1 g clove powder+1 g cinnamon powder/kg diet, T6: 0.5 ml clove oil+0.5 ml cinnamon oil/kg diet. ^{a, b, c}, Mean values within the same row were significantly different (P<0.05).

Table 3. observe the adding clove and cinnamon powders and their oil additives had effect on the oxidative enzymes concentration in blood plasma. They were significantly (p<0.05) higher AOA, GPx and SOD in all treatment of clove, cinnamon and their mixture oils, also CAT in T4 and T6. So, AOA

and CAT were significantly (p<0.05) higher in (T2, T3, T4, T6), GPx in all additive treatments. While, MDA records significantly (p<0.05) lower in T2, T3, T4, T5, T6 and CK in all addition groups compared with the control group.

Table 3. The influence of clove or cinnamon powders and their oil additive on serum antioxidant enzymes profile (g/100 ml) of quail under heat stress

Traits	T0	T1	T2	T3	T4	T5	T6	MSE
AOA (mmol/L)	1.293 ^c	1.448 ^b	1.525 ^b	1.593 ^b	1.989 ^{ab}	1.702 ^{ab}	2.18 ^a	0.117
GPx (U/mL)	32.85 ^c	37.32 ^b	38.11 ^b	37.00 ^b	40.33 ^{ab}	39.14 ^b	44.35 ^a	3.05
CAT (U/mL)	3.46 ^b	4.08 ^b	5.91 ^{ab}	4.17 ^b	6.82 ^a	3.77 ^b	6.20 ^a	0.475
SOD (U/mL)	1.66 ^c	2.15 ^b	1.80 ^b	2.61 ^{ab}	3.69 ^a	2.53 ^b	3.19 ^a	0.339
MDA(μmol/ml)	19.71 ^a	17.35 ^{ab}	14.22 ^{bc}	16.08 ^{ab}	13.85 ^c	15.77 ^b	13.68 ^c	1.093
CK (U/ml)	97.3 ^a	73.00 ^b	62.35 ^c	70.84 ^b	53.09 ^{cd}	68.11 ^b	49.65 ^d	3.57

T0: control (standard diet), T1: 2 g clove powder/kg diet, T2: 1 ml clove oil/kg diet, T3: 2 g cinnamon powder, T4: 1 ml cinnamon oil/kg diet, T5: 1 g clove powder+1 g cinnamon powder/kg diet, T6: 0.5 ml clove oil+0.5 ml cinnamon oil/kg diet. AOA: Total antioxidant capacity, GPx: Glutathione peroxidase, CAT: Catalase, SOD: Superoxide dismutase, MDA: Malondi-aldehyde, CK: creatine kinase. ^{a, b, c}, Mean values within the same row were significantly different (P<0.05).

This study was an investigation into analyzing and comparing the effect of cinnamon and clove powders and oil additives on heat stress among quails. The results demonstrated that the quails with treatments containing cinnamon and clove powders and oil additives had a significantly lower heat stress compared to the control quails which received standard diet, because these dietary supplementations of cinnamon and clove led to a decrease in the

plasma of the birds, which in turn reduced their oxidative stress. As the results presented in Table 1 indicate, clove bud oil contains some important fatty acids including SF (6.67%), MUFA (2.38%), PUFA (11.90%), Omega-3 (259 mg), and Omega-6 (148 mg), and cinnamon bark oil contains SF (0.27%), MUFA (0.14%), PUFA (1.55%), Omega-3 (1.3 mg), and Omega-6 (3.17 mg). Volatile oils like eugenol (80.71%), eugenyl acetate

(13.60%), and β -caryophyllene (4.45%) were observed in the clove bud oil, and E-cinnamaldehyde (78.10%), copaene (8.12%), eugenol (7.26%) in the cinnamon bark oil. Similar to this result, existence of these fatty acids and volatile oils in clove and cinnamon has been reported by other studies (9). Based on the results of the present study presented in Figures 1A-C above, the control quails that received a standard diet had the highest density of heat shock proteins 40, 70, and 90. However, those groups of quails that were fed with powders and oil additives of cinnamon and clove had remarkably lower densities of HSP 40, 70, and 90. As seen in the above figures, quails in treatments 2, 4, and 6 that respectively received standard diet + 1 ml clove oil/kg, standard diet +1 ml cinnamon oil/kg diet in T4, and standard diet + 0.5 ml clove oil + 0.5 ml cinnamon oil/kg as their diets had significantly lower densities of HSP 40, 70, and 90. Therefore, as the results revealed, it can be stated that supplementing the standard diet with cinnamon oil (T4) led to the lowest density of HSP 40, 70, and 90, followed by supplementations of clove and cinnamon oils (T6), and clove oil (T2). Similar to this finding, (16) who reported supplementing the diet of quails with clove oil resulted in a significant drop in protein carbonyl, 8-hydroxy-2'-deoxyguanosine, malondi-aldehyde and creatinine in blood. This finding is also in agreement with those of the study carried out by (27) who concluded that adding clove powder and oil to the quails' diet led to reduction in proteins and subsequent heat stress. Similar to the effect of cinnamon oil on reduction of heat shock proteins and consequently heat stress, (5) concluded that utilizing cinnamon as a dietary supplementation for broiler chicken resulted in a significant drop in heat shock proteins and heat stress. (11) also indicated that adding cinnamon essential oil had a positive effect on oxidative status in broiler chickens. Dietary supplementation of cinnamon and turmeric either alone or together can effectively attenuate the negative effects of heat stress on the performance of broiler chickens by decreasing lipid peroxidation (4). The results of the present study showed that adding the mixture of cinnamon and clove oils to the

standard diet of quails led to a remarkable decrease in the density of heat shock proteins; however, this effect was not as strong the sole effect of each herb (See Figures 1A-C above). The effect of combination of clove and cinnamon oils has not been studied before; however, it is predictable give the sole effect of each plant. Also, as revealed by the results, oil additives of cinnamon and clove were more effective than their powders in terms of reducing heat shock proteins in the studied quails. This finding is in good agreement with those of the study carried out by (27). The above mentioned results are also in line with those of studies conducted by (12 & 13). The results of the current experiment also demonstrated that adding cinnamon and clove oils and their combination to the diet of the quails resulted in a significant decrease serum corticosterone, the expression of HSP90 and HSP70 in the major of cells blood. This finding is in line with those of the study carried out by (30) who observed that clove powder supplementation reduced serum enzymes in Japanese quails. Furthermore, it was shown that HSP90 was positively correlated with corticosterone. This finding was in good agreement with the results of the study conducted by (16). HSP90 transcripts declined in the chicken hypothalamus, while thermal stress at 35-39°C led to decrease in HSP40. Changes in gene expression as a result of heat stress are greatly significant in cell adaptation and resistance to stress (Figures 1A-C). Genes that were differently expressed were mainly related to response to metabolism, signal transduction, transport, and stress. HSP genes (i.e. HSP90 AA1, HSP70, and HSP25) and related chaperones were actually the major regulated groups in chicken testes after severe heat stress. Different functional clusters were related to heat stress effects, including those of energy metabolism, ion binding, extracellular space, and cytoskeleton. As a result, it can be stated that HSP expression in response to increased heat is a universal cellular mechanism that protects proteins against unfavorable changes, including misfiling and molecular mechanisms of HSR (29). Cells frequently generate reactive oxygen species (ROS) and reactive nitrogen species (RNS), which disturb normal

oxidant and antioxidant cellular homeostasis, leading to oxidative stress (1). These oxygen-containing compounds can be broadly categorized according to their oxygen-containing capacity: superoxide anion (O_2^-), hydroxyl radical (OH), alkoxyl radical (RO), peroxy radical (HOO), nitric oxide radical (NO), nitrogen oxide (NO_2), as well as potent non-radicals, such as hydrogen peroxide (H_2O_2) and oxygen singlet ($1O_2$) (22 & 24). Both ROS and RNS in accumulated levels are very reactive and more potent than normal oxygen and nitrogen, thus causing deleterious effects to the living system. In spite of all negativities associated with accumulated cellular ROS, several studies have shown that, at low or moderate levels of unknown concentration, ROS perform important cellular beneficial roles, including acting as secondary messengers in signal transduction, in immune defense, in antibacterial infections in the phagosome and vascular tone, as well as in ROS-induced programmed cell death in cancer cells (1 & 26). Based on the results of the present study, it was concluded that powders, oil additives, and mixtures of cinnamon or clove that include fatty acids and volatile oils helped decrease heat shock proteins HSP40, HSP70, and HSP90. As a result, it is recommended that supplementing the standard diet of quails with cinnamon or clove powders, oil additives, and mixtures can be quite useful in treating and managing various HSPs, which in turn leads to a reduction in corticosterone MDA and CK enzymes in blood plasma concentrations and H/L ratio during hot months. However, increased the GPx, concentrations of antioxidant enzymes AOA, SOD and CAT concentrations in blood plasma in all additive treatments compared with the control group, remarkably oil additives resulted in higher improvement than the powder additives.

REFERENCES

1. Alfadda, A.A. and R.M. Sallam. 2012. Reactive oxygen species in health and disease. *Journal of Biomedicine and Biotechnology*. doi:10.1155/2012/936486
2. Al-Hassani, D.H. and A. Al-Shukri. 2016. Comparative efficacy of different supplement with drinking water used to alleviate body temperature of heat-stressed broiler chickens. *Iraqi Journal of Agri-cultural Sciences*, 47 (special issue): 12-18
3. Altan, O., A. Pabuçcuoglu, A. Altan, S. Konyalioglu and H. Bayraktar. 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Sci.* 44: 545–550
4. Baghban, P., M. Daneshyar and R. Najafi. 2016. Effects of Cinnamon (*cinnamomum zeylanicum*) and Turmeric (*curcuma longa*) powders on performance, enzyme activity, and blood parameters of broiler chickens under heat stress. *Poultry Science Journal*, 4 (1): 47-53
5. Baghban, P., Kanani, M. Daneshyar, J. Aliakbarlu and F. Hamian. 2017. Effect of dietary turmeric and cinnamon powders on meat quality and lipid peroxidation of broiler chicken under heat stress condition. *Veterinary Research Forum*, 8 (2), 163-169.
6. Borges, SA., AV. Fischer da silva, A. Maiorka, DM. Hooge and KR. Cummings. 2004. Effects of diet and cyclic daily heat stress on electrolyte, nitrogen and water intake, excretion and retention by colostomized male broiler chickens. *International J. of Poultry Sci.* 3: 313-321
7. Cook, F.W. 1959. Staining fixed preparation of chicken blood cells with combination May-Grünwald-Right-Phloxine B stain. *Avian Dis.* 3:272–290
8. Cortés-Rojas, D.F., C.R. de Souza and W.P. Oliveira. 2014. Clove (*syzygium aromaticum*) a precious spice. *Asian Pacific Journal of Tropical Biomedicine*, 4 (2), 90–96. doi:10.1016/S2221-1691(14)60215-X
9. Dalkilic, B., M. Ciftci, T. Guler, I.H. Cerci, O.N. Ertas and M. Guvenc. 2009. Influence of Dietary Cinnamon Oil Supplementation on Fatty Acid Composition of Liver and Abdominal Fat in Broiler Chicken, *Journal of Applied Animal Research*, 35:2, 173-176
10. Duncan, D. B. 1955. Multiple range and multiple F test. *Biometrics*. 11:1-42
11. Faix, Š., Z. Faixová, I. Plachá and J. Koppel. 2009. Effect of cinnamomum zeylanicum Essential oil on antioxidative status in broiler chickens. *Acta Vet. Brno.* 78: 411-417
12. Garrido, C., S. Gurbuxani, L.R. and G. Kroemer. 2001. Heat shock proteins: endogenous modulators of apoptotic cell

- death. *Biochem Biophys Res Commun*, 286 (3): 433-442
13. Gu, X.H., Y. Hao and X.I. Wang. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 2. Intestinal oxidative stress. *Poultry Sci.* 91: 790–799
14. Gulcin, M. Elmastas and HY. Aboul-Enein, 2012. Antioxidant activity of clove oil - A powerful antioxidant source. *Arabian Journal of Chemistry*, 5: 489-499
15. Habeeb, G.R., D.H. Al-Hassani and I.H. Ismail. 2004. Effect of enzymes supplementation on some productive traits of broiler reared under hot climate. *Iraqi Journal of Agricultural Sciences*, 35 (1): 141-144
16. Hao Y. and X. Gu H., 2014. Effects of heat shock protein 90 expression on pectoralis major oxidation in broilers exposed to acute heat stress, *Poultry Science*. 93 (11): 2709–2717
17. Hartlova, H., J. Balba, M. Koubkova, J. Draslarovaz and A. Fucftoval. 2002. Influence of heat stress on the metabolic response in broiler chickens. *Scientia Agriculture Bohemoslovaca*, 33: 145- 149
18. Hussein, Mohamed, E.M. Abd El-Hack, M. Samir, S. Islam and A.S. Ayman. 2018. Effects of clove (*Syzygium aromaticum*) oil on quail growth, carcass traits, blood components, meat quality, and intestinal microbiota. *Poultry Sci.* 98 (10): 338-348
19. Lara, L.J. and M.H. Rostagno. 2013. Impact of heat stress on poultry production. *animals: journal from MDPI*, 3 (2): 356–369. doi:10.3390/ani3020356
20. Lobo, V., A. Patil, A. Phatak and N. Chandra. 2010. Free radicals antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.* 4: 118
21. Lu, J., K. Zhang, S. Nam, R.A. Anderson, R. Jove and W. Wen. 2010. Novel angiogenesis inhibitory activity in cinnamon extract blocks VEGFR2 kinase and downstream signaling. *Carcinogenesis*. 31: 481e-488
22. Lushchak, VI. 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chem.-Biol. Int.* 224: 164-175. (CrossRef) (PubMed)
23. Mustafa, Mahbuba AG., M. AG. Nidhal and S.S. Payam. 2017. Effect of functional feed additives on egg production, hatchability and hematological traits of Japanese quails during summer condition. *Iraqi Journal of Agricultural Sciences*, 48 (special issue): 80-85
24. Oyinloye, BE., AF. Adenowo and AP. Kappo. 2015. Reactive oxygen species, apoptosis, antimicrobial peptides and human inflammatory diseases. *Pharmaceuticals*. 8: 151-175. (Cross Ref) (PubMed).
25. Rajput, N., M. Naeem, R. Yan R, X. Zhong and T. Wang. 2013. Effect of dietary supplementation of curcumin on growth performance intestinal morphology and nutrients utilization of broiler chicks. *Journal of Poultry Sci.* 50:44- 52
26. Reisinger, N., T. Steiner, N. Nitsch, G. Schatzmayr and TJ. Applegate. 2011. Effects of a blend of essential oils on broiler performance and intestinal morphology during coccidial vaccine exposure. *J. Appl. Poultry. Res.* 20: 272-283
27. Salman, Khawla and Ibrahim, Dhia. 2012. Test the activity of supplementation clove (*eugenia caryophyllus*) powder, oil and aqueous extract to diet and drinking water on performance of broiler chickens exposed to heat stress. *International J. of Poultry Sci.* 11: 635-640. 10.3923/ijps.2012.635.640
28. SAS, Institute. 2005. SAS User's Guide: Version 9.2 review ed. SAS Institute Inc, Cary, NC
29. Surai, P.F. 2015. Antioxidant action of carnitine: molecular mechanisms and practice applications. *EC Vet. Sci.* 2: 66-84
30. Tariq, H., P.V. Rao, R.S. Raghuvanshi, B.C. Mondal and S.K. Singh. 2015. Effect of Aloe vera and clove powder supplementation on carcass characteristics, composition and serum enzymes of Japanese quails. *Veterinary world*, 8 (5): 664–668. doi:10.14202/vetworld.2015.664-668
31. Windisch, W., K. Schedle, C. Plitzner and A. Kroismayr. 2008. Use of phyto-genic products as feed additives for swine and poultry. *J. of Animal Science*, 86 (14): 140-148.