Plant Archives Vol. 19, Supplement 1, 2019 pp. 695-705 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF PHYSIOLOGICAL AND CHEMICAL NANO GARLIC SUPPLEMENTATION ON BROILER CHICKENS

Mohamed R. El-Gogary[1], Ayman Y. El-Khateeb[2*] and Asmaa M. Megahed[1]

[1]Department of Poultry Production, Faculty of Agriculture, Mansoura University, 35516, Egypt

[2] Department of Agricultural Chemistry, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt

Email: aymanco@mans.edu.eg

Abstract

The aim of this study was to investigate the effects of dietary supplementation with garlic extract 1g/kg and synthesized calcium nanoparticles with garlic extract (0.5 and 1 g/kg) on growth performance, blood profiles and histology in broilers. A total of one hundred twelve1-d-old unsexed broiler chicks were randomly allotted to 4 treatments with 4 replications per treatment and 28 chicks per pen floor. Feed and water were offered *ad libitum* till the termination of the trial after 42 days. Growth performance parameters and blood parameters were measured. The LBW, BWG, FI and FCR of broilers fed the control group, garlic extract 1 g/kg and Nano garlic 0.5g/kg were no significant but feeding the Nano garlic 1 g/kg produced significantly lowest means of LBW, BWG and FI as compared to other groups in the starter period. Dietary supplementation with garlic extract 1 g/kg, Nano garlic (0.5 and 1 g/kg) these no significant the plasma levels of glucose, total protein, albumin, total lipids, triglyceride and HDL. But, the experimental groups for Nano garlic 0.5 was lower concentration for LDL compared with other groups. The results related for humeral immune response of broiler chickens showed significant increases in immunoglobulin in trait groups (garlic extract, Nano garlic 0.5g/kg and Nano garlic 1 g/kg) compared with the control group. The garlic extract and Nano garlic (0.5 and 1 g/kg) administration to broiler diet significantly increased TAC compared to the control group but, the best response was for broilers fed 0.5 g/kg Nano garlic diet. Similarly, MDA was lower significantly by adding 0.5g/kg Nano garlic. The present outcomes show that supplemental Nano garlic at 0.5 g/kg of diet has beneficial effects on lipid profile, immunity, antioxidant status and histological observations of broiler chicken.

***Key words*:** Broilers, Nano garlic, garlic extract, lipids profile, immune response, antioxidant status, histology

Introduction

Garlic contains at least 33 substances containing sulphur, enzymes and amino acids, minerals including selenium. The main active components in garlic are allicin, ajoene, dialkyl polysulfides, s-allylcysteine (SAC), diallylsulfide, S–methyl–cystein sulfoxide and s–allylcysteine sulfoxide which may be responsible for healing effect of garlic (Togashi *et al*., 2008). The use of garlic as a feed additive in broiler diets has been shown to improve feed conversion ratios and to reduce mortality (Tollba and Hassan, 2003) in contrast to other studies showing that garlic paste had no effect on feed intake, body weight gain, or feed efficiency in broiler chickens (Choi *et al.,* 2010). Previous studies indicated that the effects of garlic addition on growth performance in broilers were not consistent (Aporn and Adcharatt, 2008). In broilers, it was reported that garlic, as a natural feed additive, improved broiler growth and feed conversion ratio, and decreased mortality rate (Puvača *et al.*, 2014). Improvement of broilers performance, blood lipid profile and tissues canbe achieved by supplementation of diets with garlic powder (Stanaćev *et al.,* 2011). Additionally, several components of garlic and garlic extracts have been shown to have antioxidant properties in both meat-type and egg-type chicken (Sallam *et al.,* 2004). Garlic has been confirmed to have antioxidant effects and immunomodulation and antimicrobial activities in poultry (Chowdhury *et al.,* 2002). Nanotechnology is one of the most important tools in modern agriculture, and agri-food nanotechnology is anticipated to become a driving economic force in the near future. The recent advances brought into methodology for biological and ecofriendly synthesis and characterization of herbal and medicinal plant-mediated nanoparticles were reported by Chauhan *et al.* (2012). Majeed *et al.* (2015) described that encapsulation of extracts increased their stability, durability and bioavailability. Hafeez *et al.* (2015) reported an improved performance for encapsulated essential oils than its powder form in broilers. Along these lines, this exploration was intended to assess the beneficial effect(s) of dietary Nano garlic on performance, blood biochemical, immune responses and histology of broiler chickens.

Materials and Methods

1. Preparation of investigated plant extracts

Extraction of the selected plants was prepared according to the method described by Dent *et al.,* 2013. Accurately 5g of plants powder were extracted separately using 100mL of Ethanol (30%) performed at 60°C for 30 minutes on a horizontal water bath shaker

(Memmert WB14, Germany). The extracts were then filtered through Whatman no. 1 filter paper (Whatman International Ltd., Kent, UK) using a Büchner funnel and the filtrates were adjusted to 100mL in volumetric flasks with appropriate deionized water. The extracts were stored at -18°C till analyses.

2. Synthesis of Metal Nanoparticles

Metal of Calcium nanoparticles were ecofriendly synthesized using the method reported by Yugandhar and Savithramma **(**2013**)** with a slight modification. Prepared by adding to previous plant extracts. Aqueous solution of calcium chloride dihydrate, S.D fine chemical limited, India (0.05 M) was prepared using deionized water and added slowly to the same volume of prepared extracts. The reaction mixture was stirred at 5000 rpm for 1 hour at room temperature 25±1ºC and incubated at a room temperature for 2-3 days. Then, lyophilized to a fine powder.

3. Nanoparticles Characteristic *via* UV-Vis spectroscopy

The reduction of pure Ca^{++} ions and capping of the resulting calcium nanoparticles were monitored using ATI Unicom UV-Vis Spectrophotometer vision software V 3.20, by detecting the UV-Vis spectra of the reaction mixture at different wavelengths. The UV- Visible spectra of the synthesized metal nanoparticles were recorded around 240-440 nm. The analysis was accomplished at 25ºC using quartz cuvettes (1 cm optical path).

4. Nanoparticles Characteristic *via* Transmission Electron Microscope (TEM)

The size, shape, surface area, crystal structure and morphological data of the obtained nanoparticles were characterized using transmission electron microscopy, TEM (JEOL TEM-2100) attached to a CCD camera at an accelerating voltage of 200 kV. Each sample of the synthesized metal nanoparticles was prepared by involving a suspension of the sample on grids of carbon coated with copper and the solvent was allowed to be evaporated slowly before recording the TEM images. TEM measurements were recorded at the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

5. Nanoparticles Characteristic *via* Zeta potential

Zeta potential analysis is a technique for determining the surface charge of nanoparticles in suspensions using Malvern Instruments Ltd Zeta Potential Ver. 2.3 at the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.

6. Birds, Management and Experimental Design

The experimental work of the present study was carried out in Private farms in Dakranis Dakahlia Governorate, Egypt from March to April 2018. The objective of the present study was to evaluate the effect Nano garlic on growth performance, carcass yield, some blood metabolites, immunity, lipid peroxidation and histology in broiler chickens.

Cobb 500 broiler chickens (n=112), one-day-old, were divided into four treatment groups, each of which include four replicates (pens floor). This experiment was divided into four groups. the groups were assigned to four diet treatments (0.0 control group, 1g/kg aqueous extract garlic, 0.5g/kgNano garlic group and 1g/kg Nano garlic group) with four replications of 28 birds for 42 d. Birds were reared in pens floor and the length, width of each pen were 70 and70 cm, respectively. Thus the pen floor area was 0.49 m^2 (70 × 70 cm). Chickens were reared to 42 days of age and fed a starter ration from one to 21 days of age (3199 kcal of ME/kg of diet and 23% CP) and grower ration from 22 to 42 days of age (3200 Kcal of ME/kg of diet 21%CP). Diets were formulated to cover or exceed the recommended requirements of broiler chicks according to **NRC, (1994).** Feed in mash form and water were provided freely. The composition and chemical analysis of the experimental diets are shown in Table 1.

7. Performance of broiler chickens

Live body weight (BW); feed intake (FI) and body weight gain (BWG) were measured weekly throughout the experimental period, then feed conversion ratio (FCR) was calculated (feed: gain g). Birds were individually weighted to the nearest gram in the early morning before receiving any food and water at weekly intervals during the experimental period. Live body weights of broilers were recorded at the beginning ofthe experiment and at weekly basis thereafter. Weekly records on FI and BWG of broilers were also recorded on a replicate group basis. Accordingly, FCR was calculated as the amount of feed consumed per unit of BWG.

8. Carcass Characteristics

At the termination of study (42 day of age), three chickens per group, whose LBW were around the average weight of their respective group, were chosen for slaughter test. The chicken was individually weighed, immediately sacrificed and reweighed after complete bleeding. Their carcasses were skinned and then eviscerated. Records on weights of carcasses and giblets (including liver, kidney, gizzard and heart) were maintained.

9. Blood sampling and biochemical analysis

Three birds from each treatment were chosen, slaughtered and blood samples were collected in heparinized tubes then centrifuged at 4000 rpm for 15 min. and the plasma obtained was stored at -20o C until analysis. Plasma biochemical constituents namely: glucose, (total cholesterol (Chol), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), total antioxidant capacity (TAC) and malondialdehyde (MDA) were also measured by commercial kits. Immunoglobulins (IgG, IgA and IgM) were determined by ELISA technique.

10. Tissues specimens and histological procedures

Representative tissue samples were taken from duodenum, bursa of fabricius and liver during slaughtering, immediately fixed in 10% formalin-saline solution, and then dehydrated in ascending concentrations of alcohol solutions ranged from 70% to absolute ethanol alcohol. Samples were cleared in xylene, and then embedded in melted paraffin wax, to obtain tissue blocks. They were then sectioned and stained with haematoxylin and eosin stain (Junquerira *et al.,* 1971). Sections were examined under light microscope and photographed by using a digital Camera.

11. Statistical Analysis

Measurable examination for the acquired information was performed by two-way investigation of variance using the method of least square analysis of Co-variance (SAS, 2006). Duncan's multiple range test was utilized to separate significant differences among means (Duncan, 1955).

Table1: Composition and Chemical Analysis of the Basal Diets.

Ingredients (%)	Starter	Grower	Chemical Analysis	Starter	Grower
Yellow corn %	628.3	691.8	ME, kcal/Kg	3199	3200
Soybean meal 44%	130	95.8	CP, %	23	21
Corn Gluten Meal 60.2%	185.4	167.5	Crude Fiber, %	2.53	2.41
Di calcium Phosphate %	18.2	13.5	Ether extract, %	2.95	3.12
Limestone %	14.6	15	Calcium, %	1	0.91
DL-methionine %	0.5	1.2	Av-Phosphorus, %	0.45	0.357
L-Lysine %	4	4	Methionine, %	0.52	0.553
Sodium chloride %	3	3	Meth, +Cys, (TSAA, %)	0.92	0.925
Vit+Min Premix[1] %	3	3	Lysine, %	1.1	1.1
Soya bean oil	13	5.2			

"[1]Premix provided the following per kilogram of diet: VA (retinyl acetate), 2654 µg; VD3 (cholecalciferol), 125 µ g; VE (dl-α-tocopheryl acetate), 9.9 mg; VK3 (menadione dimethylpyrimidinol), 1.7 mg; VB1 (thiamin mononitrate), 1.6 mg; VB12 (cyanocobalamin), 16.7 µ g; riboflavin, 5.3 mg; niacin (niacinamide), 36 mg; calcium pantothenate, 13 mg; folic acid, 0.8 mg; d-biotin, 0.1 mg; choline chloride, 270; BHT, 5.8; Fe (iron sulphate monohydrate), 50 mg; Cu (copper sulphate pentahydrate), 12 mg; I (calcium iodate), 0.9 mg; Zn (zinc oxide), 50 mg; Mn (manganous oxide), 60 mg; Se (sodium selenite), 0.2 mg; Co (cobalt sulphate), 0.2 mg. 2Calculated from data provided by **NRC (1994).**3 The respective diet formulated to contain 18.84, 218.84, 318.84, and 418.84 m g/kg VE and the dose titrations were achieved by addition of VE at the expense of soybean meal."

Results and Discussion

1. Nanoparticles Characteristic *via* UV-Vis spectroscopy

The synthesis of the calcium nanoparticles has been elucidated by scanning the UV–Vis spectra. As shown in Fig. (1), the maximum absorption peak that recorded at 280 nm is due to the characteristic surface plasmon resonance of the produced metal nanoparticles. The prepared calcium nanoparticles were found to be very stable due to possible presence of polyphenolic compounds present in the garlic extract that prevent accumulation. Polyphenols are an antioxidant agent with specific chemical structure have an essential role in the reduction process for synthesis of metal nanoparticles. The properties of synthesized nanoparticles were examined as a function of UV irradiation. The use of UV–Vis spectroscopic analysis is an effective method for demonstrating the presence of metal nano-structures [Sun *et al*. (2002) and Darroudi *et al*. (2011)]. The UV irradiation role was confirmed to describe the progress of calcium salt reduction in the presence of garlic extract at ambient temperature.

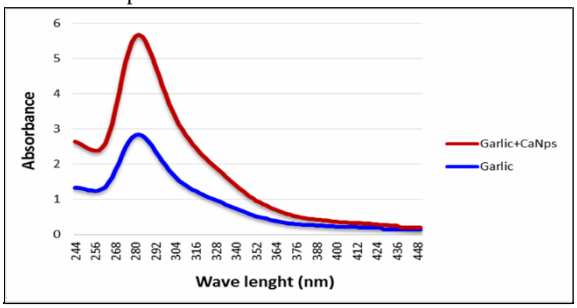

Fig. 1: UV-Vis spectroscopic measurements of garlic and its calcium nanoparticles.

2. Nanoparticles Characteristic *via* Transmission Electron Microscope (TEM)

Calcium nanoparticles were prepared using garlic extract were characterized by TEM measurements to confirm the presence of CaNP to estimate the shape, aggregation and particles size of synthesized nanoparticles according to Yugandhar and Savithramma (2013). As shown in Fig. (2), TEM was performed for the synthesized nanoparticles at 100 nm magnification value. The size of the particles between 23.70 to 38.45 nm. The shape of particles was spherical with square aggregation and less numbers were tetragonal. Smaller particles causing more surface area which a reason to more effective responses.

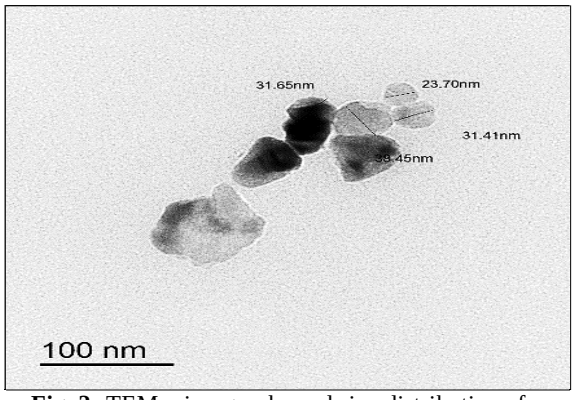

Fig. 2: TEM micrographs and size distributions for calcium nanoparticles synthesized by garlic extract at 100 nm magnification value.

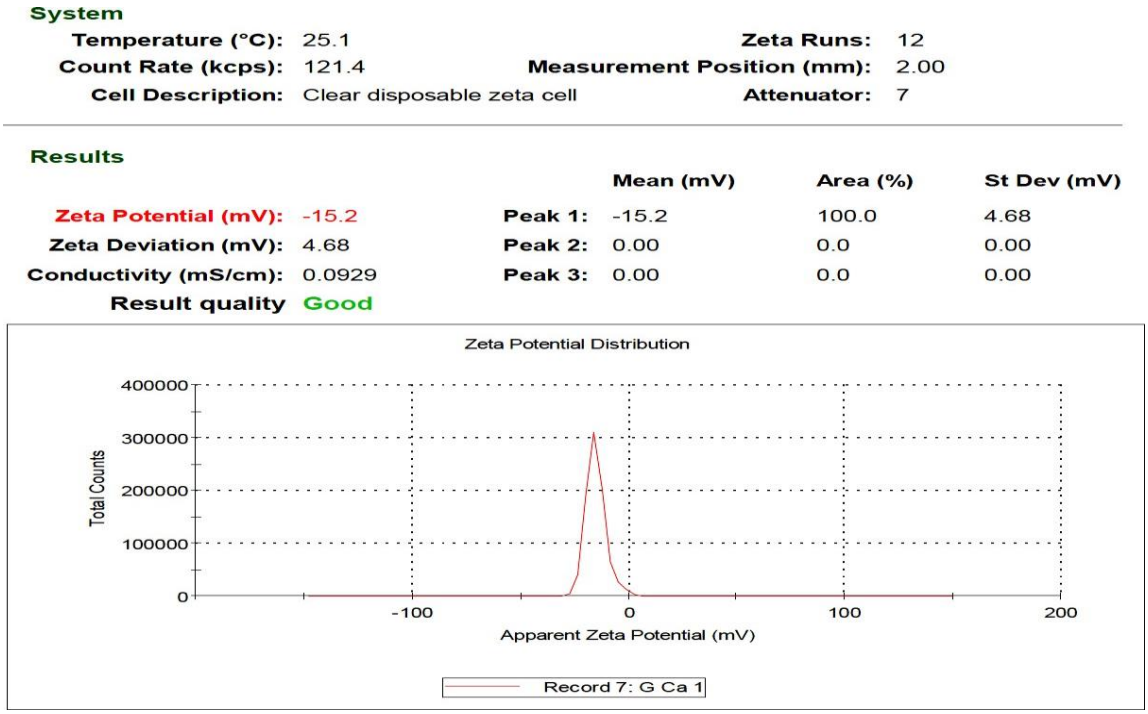

Fig. 3: Zeta potentialdistribution for calcium nanoparticles synthesized by garlic extract.

3. Nanoparticles Characteristic *via* Zeta potential

Zeta Potential is an important tool for understanding the state of the nanoparticle surface and predicting the long term stability of the nanoparticle. Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticle surface, Zeta Potential technique was used to determine the nanoparticles surface charge. Nanoparticles have double layer of ions travels as it diffuses throughout the solution, the electric potential at the boundary of the double layer is known as the Zeta potential of the particles and has values that typically ranged from +100 mV to -100 mV. Fig 3 showed that synthesized calcium nanoparticles using garlic extract has Zeta Potential value of -15.2 mVwhich were high stability because nanoparticles with Zeta potential values greater than +25 mV or lesser than -25 mV typically have high degrees of stability (Soheyla and Foruhe, 2013).

4. Broiler Investigation

The objective of the present study is to examine the effect of inclusion of Nano garlic in broiler chicken, 0-6 weeks on growth performance, carcass yield, relative organ weight, blood profile (glucose, total protein, albumin, total lipids, triglycerides, total cholesterol, HDL, LDL, immunoglobin G, TAC and MDH) and histology.

5. Growth Performance

The effects of dietary garlic extract and Nano garlic supplementation on broiler chicken's performance at 21 and 42 day of age are presented in Table (2). The LBW, BWG, FI and FCR of broilers fed the control group, garlic Extract 1 g/kg and Nano garlic 0.5g/kg were no significant but feeding the Nano garlic 1 g/kg produced significantly lowest means of LBW, BWG and FI as compared to other groups in the starter period. On the other hand, no significant effect of control group, garlic extract and Nano garlic (0.5 and 1 g/kg) on growth performance were observed during the whole experimental period. These results suggested that there were no beneficial effects of the dietary garlic powder on BW, feed consumption and feed efficiency Yalcin *et al.* (2007) and Chol *et al*. (2010). They explained that the strong odor of garlic does not act as a deterrent of feeding. El-Katcha *et al.,* 2016 reported that addition of dietary garlic extract supplementation by (0.0, 25, 50, 75 and 100mg allicin/kg) diet on body weight development of broiler chicken no significant difference between experimental groups at the starter period. But, dietary garlic extract supplementation at 0.1, 0.2 and 0.3 mg/kg diet significantly improved final body weight. In agreement with the current Navidshad *et al.,* 2018 reported that the addition of garlic extract had no effect on the BWG during the starter and growing periods. On the other hand, the addition of fermented garlic (0.1, 0.3 and 0.5%) to the broiler diets linearly increased average weight gain between 1-21 days, which partially explains the fermented garlic induced decrease in feed: gain ratio. Neither fermented garlic nor non-fermented garlic adversely affected the feed intake in broiler chickens. Thus, the underlying mechanism of the fermented garlic induced increase in daily weight gain is likely the consequence of improvements in nutrient digestibility, especially at early ages Lee *et al*., 2016. While, revealed that increasing both non-encapsulated and Nano encapsulated herbal extracts to 0.05% in finisher diets improved body weight gain in the period of 28–42 days and consequently the whole time from 1 to 42 days Meimandipour *et al.,* 2017.

5. Carcass Yield

Table 3 summarizes the effect of garlic extract and Nano garlic supplementation on carcass characteristics at 42 day of age. Compared with the control diet, carcass weight and yield as well as heart, gizzard, liver and spleen and giblets were not significantly influenced by the garlic extract and Nano garlic supplementation at the end of the experiment; however, the liver and bursa were significantly higher in chicken of control groups than other groups. These results are in agreement with Onibi *et al.* (2009) supplement of garlic powder in dose of 5000 mg/kg of feed portion improved live weight, but had no influence on yield of carcasses or quality of inner organs. Also, Raeesi *et al.* (2010) found that garlic supplementation at 1 or 3 % level had no significant effects on carcass or digestive organs among trails in poultry.

7. Blood Profile

The effect of dietary supplementation with garlic extract 1 g/kg diet and Nano garlic on glucose, total protein, albumin, total lipids, triglyceride, cholesterol, HDL and LDL of broiler chickens are shown in Table 4. Dietary supplementation with garlic extract 1 g/kg, Nano garlic (0.5 and 1 g/kg) these no significant the plasma levels of glucose, total protein, albumin, total lipids, triglyceride and HDL. But, the experimental groups for garlic extract and Nano garlic (0.5 and 1 g/kg) were higher concentration for HDL compared with control group. This result has agreed with that illustrated by Hoten *et al*., 1991 who found that total serum cholesterol and triglyceride concentration were not significantly affected by the supplementation of dietary garlic powder at 1 g/kg diet over a 35-day growth period for broiler chicken. Similar, study for broiler chicken was reported by Amouzmehr *et al*., 2012 who found that supplementation garlic at 0.3% or 0.6% to broiler diets containing approximately 2% of soybean oil did not affect serum cholesterol levels. On the other hand, the supplementation with 5% or 3% garlic powder plus α-tocopherol resulted in significantly lower total and low-density lipoprotein cholesterol levels and greater high-density lipoprotein cholesterol levels compared with the control in broiler chicken Chol *et al*., 2010. On the other hand, Jawad (2007) found that the supplementation of raw garlic 5% or 10% has not significantly for the total serum protein and glucose levels for broiler chicken. Similarly, El-Katcha *et al*., 2016 who found that garlic extract (allicin) supplementation at 25, 70, or 100 mg/kg had no significant effect on serum total protein and albumin concentrations in broiler chickens. Our results for dietary supplementation with garlic extract and Nano garlic 0.5 g/kg lower significant the plasma LDL concentration compared with the control group but, no found significant between control group and Nano garlic 1g/kg. This result has agreement with that described by Puvača *et al*., 2014 who found that the addition of garlic powder (0.5 and 1%) decreased LDL

levels compared to the level in broiler chickens of the control treatment. Similarly, this effect can be explained by the possible mechanism of antioxidant and anti-peroxide lowering action on LDL or the decrease in hepatic production of very low-density lipoprotein (VLDL) which serves as the precursor of LDL in the blood circulation (Kim *et al.,* 2009). Moreover, Leonarduzzi *et al.* (2002) reported that cholesterol oxidation products may occur in significant amounts in LDL particles. Therefore, the decrease in LDL cholesterol could also mirror the antioxidant effects of fermented garlic powder.

8. Immune response and antioxidant status

The effects of dietary supplementation with garlic extract 1 g/kg, Nano garlic 0.5g/kg and Nano garlic 1g/kg on immunoglobin G (IgG, IgA and IgM), total antioxidant capacity (TAC) and malondialdehyde (MDA)of broiler chickens are shown in table 5.The results related for humoral immune response of broiler chickens showed significant increases in immunoglobulin in trait groups (garlic extract, Nano garlic 0.5g/kg and Nano garlic 1 g/kg) compared with the control group. this result was agreement with Hanieh *et al.,* 2010 who found that supplementing chickens with garlic exerted enhancing effect on the humoral immune. Jameel *et al.,* 2014 suggest that supplementing broilers diet with mixture of 1% thyme plus 1% garlic could enhance the immune response and blood profile of broilers. The positive response of humoral non- specific defense mechanisms like lysozyme and ceruloplasm in activity after aged garlic extract and allicin treatment was reported in early-weaned piglets in a study performed by Tatara *et al.* (2008). But the literature is very limited regarding the effect of garlic on immune system in broilers.

It was reported by (Birrenkott *et al.,* 2000 and Fadlalla *et al.,* 2010) that including garlic in the laying hens garlic inclusion resulted in increased total white blood cells which reflecting good immune response. On the other hand, Jafari *et al.* (2008) who reported that inclusion of 1 and 3% of garlic powder did not enhance the serological response of broilers to Newcastle vaccine. It is of great interest to notice that garlic extract and Nano garlic (0.5 and 1 g/kg) administration to broiler diet significantly increased TAC compared to the control group but, the best response was for broilers fed 0.5 g/kg Nano garlic diet. Similarly, MDA was lower significantly by adding 0.5g/kg Nano garlic. These results are in agreement with other studies (Durak *et al.,* 2002 and El-Gogary *et al.,* 2018) which declared that garlic extract or oil decreased the level of MDA in the blood samples which demonstrates reduced oxidation reactions in the body. Garlic has many kinds of antioxidant compounds, mainly such as flavonoid and sulfur-containing compounds (Gorinstein *et al.,* 2005). Furthermore, Leonarduzzi *et al.* (2002) reported that cholesterol oxidation products may occur in significant amounts in LDL particles. Therefore, the decrease in LDL cholesterol could also mirror the antioxidant effects of fermented garlic powder.

Table 2: Effect of garlic extract and Nano garlic supplementation on performance of broiler chickens at different ages.

Main effects	Control	Garlic extract (1g/kg)	Nano garlic (0.5g/kg)	Nano garlic (1g/kg)	Pooled SEM	Sig.
LBW 0 –old(g)	40.62	40.57	40.50	40.50	0.078	NS
LBW 21-old(g)	652.8^{ab}	654.1^{ab}	690.1^{a}	631.8^{b}	14.47	*
LBW 42-old (g)	1970	1990	2004	1964	18.40	NS
BWg 21 –old(g)	612.2^{ab}	613.5^{ab}	649.6^{a}	591.3^{b}	14.46	*
BWg 42 –old (g)	1929	1950	1963	1924	18.41	NS
FI (0-21 old) per brid (g)	875.0^{a}	891.7^{a}	908.0^{a}	791.2^{b}	17.42	*
FI (0-42 old)per brid (g)	3774	3736	3816	3729	41.43	NS
FCR(0-21 old)	1.43	1.46	1.40	1.33	0.038	NS
FCR (0-42 old)	1.95	1.91	1.94	1.93	0.016	NS

Note: a-b: In each of the main effects, means in the same row with different superscripts differ significantly ($P \leq 0.05$).

Table 3: Impact of garlic extract and Nano garlic supplementation on carcass traits of broiler chickens at marketing age.

Main effects	Control	Garlic extract (1g/kg)	Nano garlic (0.5g/kg)	Nano garlic (1g/kg)	Pooled SEM	Sig.
LBW (Kg)	2.131[a]	1.996[b]	2.045[b]	1.975[b]	0.026	*
Carcass (Kg)	1.513	1.470	1.493	1.466	0.042	NS
Heart (g)	11.40	11.00	10.36	11.86	0.788	NS
Gizzard (g)	35.06	31.66	30.66	31.66	2.185	NS
Liver (g)	64.93[a]	49.16[ab]	50.93[ab]	42.20[b]	5.701	*
Spleen (g)	3.10	3.013	2.33	3.23	0.396	NS
Abdominal fat(g)	50.86	39.06	45.33	41.46	8.762	NS
Gabliets (g)	114.50	93.86	94.30	88.96	8.52	NS
Bursa (g)	1.733[a]	1.333[ab]	0.933[b]	1.166[b]	0.153	*

Note: a-b: In each of the main effects, means in the same row with different superscripts differ significantly (P≤ 0.05).

Table 4: Influence of garlic extract and Nano garlic supplementation on plasma glucose, total protein, albumin, total lipids, triglycerides, cholesterol, HDL and LDL in 6-week-old broiler chickens.

Main effects	Control	Garlic extract (1g/kg)	Nano garlic (0.5g/kg)	Nano garlic (1g/kg)	Pooled SEM	Sig.
Glucose(mg/dl)	123.0	115.6	115.6	116.6	7.04	NS
Tp(g/dl)	3.96	4.15	4.26	4.28	0.117	NS
Alb(g/dl)	2.34	2.25	2.19	2.30	0.113	NS
TL (mg/dl)	626.9	629.5	551.4	552.9	32.98	NS
Tri (mg/dl)	123.1	132.9	129.3	117.5	6.73	NS
Cho(mg/dl)	175.9	171.4	160.1	175.6	4.25	NS
HDL(mg/dl)	56.83	64.36	61.26	62.63	3.41	NS
LDL (mg/dl)	94.43[a]	80.50[b]	72.96[c]	89.46[a]	2.16	*

Note: a-c: In each of the main effects, means in the same row with different superscripts differ significantly (P≤ 0.05).

Table 5: Influence of garlic extract and Nano garlic supplementation on immune response and antioxidant status in 6-week-old broiler chickens.

Main effects	Control	Garlic extract (1g/kg)	Nano garlic (0.5g/kg)	Nano garlic (1g/kg)	Pooled SEM	Sig
IgG (ug/ml)	475.03[b]	604.06[a]	648.03[a]	647.43[a]	24.37	*
IgM(ug/ml)	114.16[b]	132.13[a]	135.10[a]	144.43[a]	5.29	*
IgA (ug/ml)	125.86[b]	141.50[ab]	147.80[a]	153.76[a]	4.95	*
MDA(Nmol/dl)	21.23[a]	18.50[ab]	16.50[b]	17.40[ab]	1.13	*
TAC (nmol/dl)	1.22[b]	1.43[ab]	1.46[a]	1.41[ab]	0.062	*

Note: a-b: In each of the main effects, means in the same row with different superscripts differ significantly (P≤ 0.05).

9. Histological Observations

9.1. Duodenum histology

Histological examination of the duodenal sections from birds fed garlic-supplemented diets is illustrated in 1-4. It is clear from these sections that the villi size and shape are greatly influenced by different treatments. The number of villi/microscopic filed was more abundant in the control section (Fig.4) than the other treatments. Moreover, the villi width and height were greater in chicks fed on garlic Nano (0.5 g/kg) and garlic Nano (1 g/kg) as shown in fig.3 and 4. However, the villi width was sharply increased in duodenal sections from chick fed garlic extract (fig.5) accompanied by many small developed villie originated from the crypts of lieberkühn. Both small and wide villi have many goblets cells and well-arranged columnar epithelium lining. This effect was also observed in the number and size of crypts which were greatly increased in sections from garlic extract (Fig.5); Nano-garlic (0.5g/kg) and then the control treatment (Fig.6 and Fig.4). But the size and number of crypts in Fig.7 (birds fed 1g/kg Nano garlic) were smaller than the other treatments. The muscular is mucosa layer was nearly similar in all sections.

It is assumed from the previous histological observation that the increased villus height is paralleled by an increase in the digestive enzymes activity and the absorptive function of the small intestine segments due to increase the absorption surface area. In this respect, Hodges (1974) claimed that the crypts of lieberkühn are the main source of epithelial cells lining of villi which contain numerous goblet cells, endocrine cells, lymphocytes and undifferentiated cells. It is well known that the crypts had the ability to secrete fluids containing different vital substances essential for enhancing the internal micro-environment of the GIT segments. These fluids are almost pure extracellular fluid with a neutral pH in the range of 6.5 to 7.5 which results in a watery vehicle supply for improving nutrients absorption, elaboration and production of antibodies and lymphocytes along with an increase in goblet cells that function as mucous secreting glands and (or) intestinal hormones, especially secretion (Denbow, 2015).

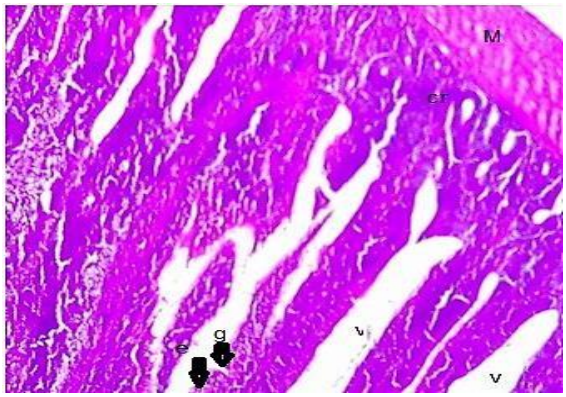

Fig. 4. T. S. of duodenum control group of broilers (H & Ex 100). Key: M= muscularis mucosa, cr= crypts of lieberkühn, V= villi, g = goblet cells, e= epithelial lining.

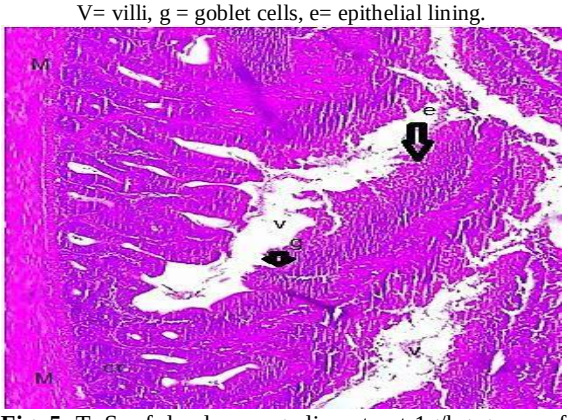

Fig. 5. T. S. of duodenum garlic extract 1g/kg group of broilers (H & Ex 100). Key: M= muscularis mucosa, cr= crypts of lieberkühn, V= villi, g = goblet cells, e= epithelial lining.

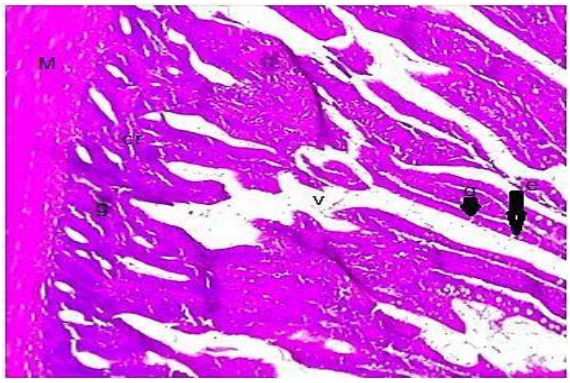

Fig. 6. T. S. of duodenum Nano garlic 0.5g/kg group of broilers (H & Ex 100). Key: M= muscularis mucosa, cr= crypts of lieberkühn, V=villi, g = goblet cells, e= epithelial lining.

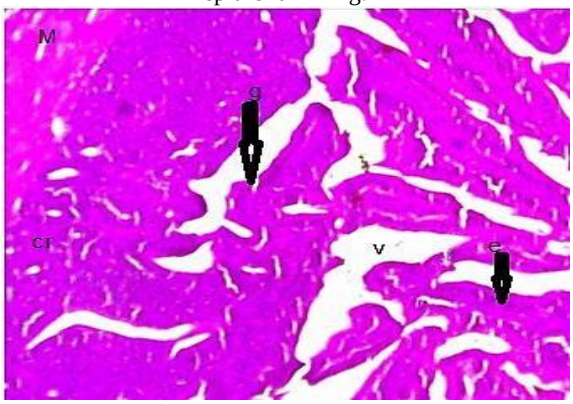

Fig. 7. T. S. of duodenum Nano garlic 1g/kg group of broilers (H & Ex 100). Key: M= muscularis mucosa, cr= crypts of lieberkühn, V= villi, g = goblet cells, e= epithelial.

9.2. Bursa of fabricius histology

The bursa of fabricius is a primary lymphoid organ in birds. In general, it is composed of about 15-20 plicae (fold) each of them contain several follicles (B). These follicles had two distinct areas, cortex and medulla (C, M) enclosed in a pseudostratified columnar epithelial layer (e) as clearly in Fig. 8 (1 g/kg garlic extract). The cortex is more deeply-stained than the medulla, due to the fact that it is composed of many small lymphocytes. The medulla is composed of undifferentiated epithelial cells and lymphoblasts which appeased as a pale-stained area. This structure was clearly observed in the control section, however, the bursa follicles were enlarged with relatively fine connective tissue septa between follicles and many small lymphoblasts in the medullary area (Fig. 9). Moreover, it appears from sections that garlic extract (1g/kg) and Nano garlic (0.5 g/kg) exert beneficial effects on the bursa structure (Fig.10 and 11),

in terms of well-defined bursal follicles with many large lymphocytes in the cortex area and very well epithelial layer converting. The lumenae between adjacent plicae are well opened, being filled with the secretary materials of bursal follicles. These observations did not observe in the bursa section from birds fed Nano-garlic at 1g/kg level (Fig.11) where the bursal follicles diameter was small with large medullary layer than the cortex one.

It is likely that forms and levels of garlic supplementation to broiler diets could stimulate bursal follicles to produce many lymphocytes that help improving the immune response of birds. This hyperactivity is associated with the presence of many lymphocytes in the medullary area with many phagocyte cells and macrophages in the luminal areas in between bursal plicae. There is also many plasma cells and dendritic secretary cells within the lumens of bursal follicles. These cells are responsible for phagocytosis and for maintaining B-cells production as reported by Glick (1983). The birds fed different Nano garlic - supplemented diets had better immunity in terms of higher plasma immunoglobulins level.

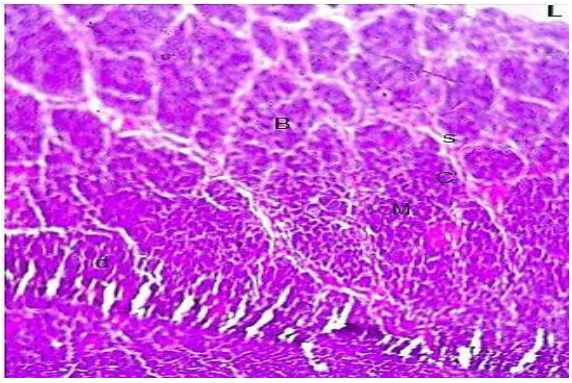

Fig. 8. T. S. of bursa of fabricius control group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen

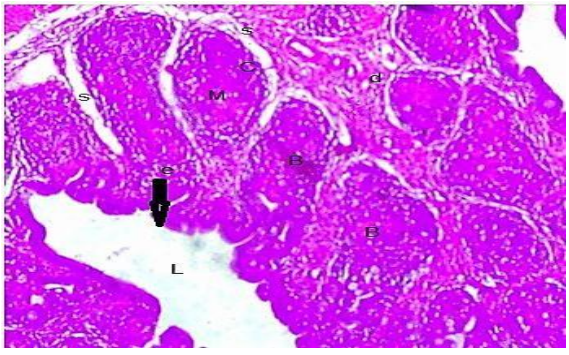

Fig. 9. T.S. of bursa of fabricius garlic extract 1 g/kg group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen,e= outer epith. Layer.

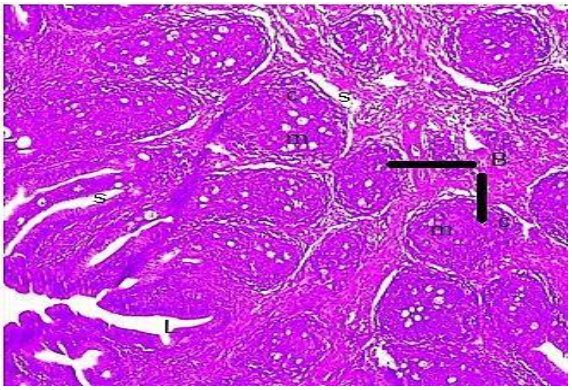

Fig.10. T. S. of bursa of fabricius Nano garlic 0.5 g/kg group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen.

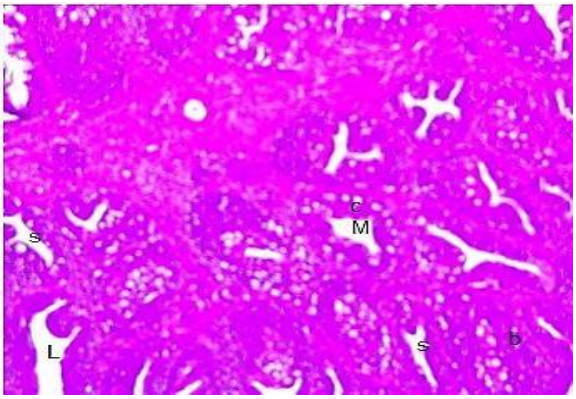

Fig. 11. T. S. of bursa of fabricius Nano garlic 1 g/kg group of broilers (H&Ex100). Key: B= bursal follicles, s= septa, c, cortex, m= medulla, d= undifferentiated epith. Cells, L= lumen.

Conclusion

Based on the present results, it can be concluded that dietary supplementation with garlic extract and CaNPs garlic can enhance the efficiency of feed utilization, and immune status of broiler chicken. In addition, supplemental Nano garlic and garlic extract can induce a beneficial effect on the lipid profile and oxidative status of broiler chicken. The CaNPs garlic can safely be used in diets of growing broiler chicken, since it has no adverse effects on their growth performance or carcass characteristics.

References

Amouzmehr, A.; Dastar, B.; Nejad, J.G.; Sung, K.I.; Lohakare, J. and Forghani, F. (2012). Effects of garlic and thyme extracts on growth performance and carcass characteristics of broiler chicks. Journal of Animal Science and Technology, 54: 185-190.

Aporn, S. and Adcharatt, S. (2008). Effect of garlic (*Allium sativum*) supplementation in diets of broilers on productive performance, meat cholesterol and sensory quality. Conference on International Research on Food Security, Natural Resource Management and Rural Development. University of Hohenheim, October 7–9.

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. Poultry Science, 79(11), 1575-1577.

Chauhan, R.P.S.; Gupta, C. and Prakash, D. (2012). Methodological advancements in green nanotechnology and their applications inbiological synthesis of herbal nanoparticles. International Journal of Bioassays.1(7): 6–10.

Choi, I.H.; Park, W.Y. and Kim, Y.J. (2010). Effects of dietary garlic powder and α-tocopherol supplementation on performance, serum cholesterol levels, and meat quality of chicken. Poultry Science 89 :1724–1731.

Chowdhury, S.R.; Chowdhury, S.D. and Smith, T.K. (2002). Effect of dietary garlic on cholesterol metabolism in laying hen. J Poult Sci., 7: 122–128.

Darroudi, M.; Ahmad, M.B.; Zak, A.K.; Zamiri, R. and Hakimi, M. (2011). Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-light. Int. J. Mol. Sci. 12(9): 6346–6356.

Denbow, M. (2015). Gastrointestinal anatomy and physiology. In: Sturkie's Avian Physiology. Sixth Edition, Scanes, C.G. ed. pp 337-367. Academic Press, UK, USA.

Dent, M.; Dragović-Uzelac, V.; Penić, M.; Brnčić, M.; Bosiljkov, T. and Levaj, B. (2013). The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (*Salvia officinalis* L.) extracts. Polyphenols from dalmatian wild sage. Food Technol. Biotechnol. 51(1): 84-91.

Duncan, D.B. (1955). Multiple range and multiple F tests. Biometrics, 11: 1-42.

Durak, Ä., H. S. Öztürk, E. Olcay, and C. Guven, 2002. Effects of garlic extract supplementation on blood lipid and antioxidant parameters and atherosclerotic plaque formation process in cholesterol-fed rabbits. Journal of herbal pharmacotherapy, 2(2), 19-32.

El-Gogary, M.R.; Mansour, A.M. and El-Said E.A. (2018). Blood biochemical and immunological responses to garlic oil administration in growing rabbits diet. J. of Agric. Sci., 10(1): 217-224.

El-katcha, M.I.; Soltan, M.A. Sharaf, M.M. and Hasen, A. (2016). Growth Performance, Immune Response, Blood serum parameters, Nutrient Digestibility and Carcass Traits of Broiler Chicken as Affected by Dietary Supplementation of Garlic Extract (Allicin).5Alexandria Journal of Veterinary Sciences 2016, Apr. 49 (2): 50- 64 ISSN 1110-2047, www.alexjvs.com DOI: 10.5455/ajvs.219261.

Fadlalla, I.M.T.; Mohammed, B.H. and Bakhiet, A.O. (2010). Effect of Feeding Garlic on the Performance and Immunity of Broilers. Asian Journal of Poultry Science, 4: 182-189.

Glick, B. (1983). Avain Biology. Academic Press Inc. New York.

Gorinstein, S., Drzewieki, J.; Leontowicz, H.; Leontowicz, M.; Najman, K. and Jastrzebski, Z. (2005). Comparison of the bioactive compounds and antioxidant potentials of fresh and cooked Polish Ukrainian, and Israeli garlic. J. Agric. Food Chem. 53: 2726–2731.

Hafeez, A.; Manner, K., Schieder, C. and Zentek, J. (2015). Effect of supplementation of phytogenic feed additives (powdered vs. encapsulated) on performance and nutrient digestibility in broiler chickens. Poult. Sci . 95(3): 622-629.

Hanieh, H.; Kiyoaki, N.; Mingzi, P.; Chaogetu, G.; Asaki, A. and Yasuhiro, K. (2010). Modulatory effects of two levels of dietary alliums on immune response and certain immunological variables, following immunization, in White Leghorn chickens. Anim Sci. J. vol. 81:673-680.

Hodges, R.D. (1974). The digestive system. In: The histology of the fowl. pp. 35-108. Academic press, Inc., NY.

Hoten, G.M.J.; Fennell, M.J. and Prasad, B.M. (1991). Effect of dietary garlic (*Allium sativum*) on performance, carcase composition and blood chemistry changes in broiler chickens. Canadian Journal of Animal Science 71: 939–942.

Jafari, R.A.; Ghorbanpoor, M. and Diarjan, S.H. (2008). Effect of dietary garlic on serum antibody titer against Newcastle disease vaccine in broiler chicks. J. Biol. Sci., 8 (7): 1258-1260.

Jameel, Y.J.; Abed, A.R. and Al-Shimmary, F.O. (2014). Influence of adding garlic and thyme and their combination on immune response and some blood parameters in broiler. I.J.A.B.R, 4(2): 211- 215.

Jawad, A.H. (2007). Some haematological and biochemical effects of garlic on broiler chicken. Bas.J.Vet.Res., 6(2) : 2007.

Junquerira, I.C.; Carnerior, J. and Long, J.A. (1971). Basic histology. Chapter 1, 5thed. Editorial guanabara koogan S. A., Riode Janerio, Brazial.

Kim, Y.J.; Jin, S.K. and YANG, H.S. (2009). Effect of dietary garlic bulb and husk on the physicochemical properties of chicken meat. Poultry Science, 88: 398-405.

Lee, K.W.; Kim, G.H.; Kim, J.H.; Yeon, J.S.; Cho, S.B.; Chang, B.S. and Kim, S.K. (2016). Effects of dietary fermented garlic on the growth

performance, relative organ weights, intestinal morphology, cecal microflora and serum characteristics of broiler chickens. Brazilian Journal of Poultry Science. 18(3): 511-518.

Leonarduzzi, G.; Sottero, B. and Poli, G. (2002). Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects: a review. J. Nutr. Biochem. 13: 700–710.

Majeed, H.; Bian, Y.; Ali, B.; Jamil, A.; Majeed, U.; Farid Khan, Q.; Lqbalk, H.J.; Shoemaker, C.F. and Zhong, F. (2015). Essential oil encapsulations: uses, procedures, and trends. J. Chem. Soci. 5: 58449-58463.

Meimandipour, A.; Emamzadeh, A.N. and Soleimani, A. (2017). Effects of nanoencapsulated aloe vera, dill and nettle root extract as feed antibiotic substitutes in broiler chickens. Arch. Anim. Breed., 60: 1–7.

Navidshad, B.; Maghsoodi, Z.; Nikbin, S.; Vahedi, V.; Adibmoradi, M.; Aghjehgheshlagh, F.M. and Moradi, H. (2018). The compensation effect of dietary garlic on chicken consuming a minimal level of choline, Italian Journal of Animal Science, 17(1): 175-179.

NRC, (1994). Metabolic Modifiers: Effects on the Nutrient Requirements of Food-Producing Animals. National Academy Press, Washington, DC.

Onibi, G.E.; Adebisi, O.E.; Fajemisin, A.N. and Adetunji, A.V. (2009). Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum*) supplementation. African Journal of Agricultural Research, 4: 511- 517.

Puvača, N.; Lukač, D.; Stanaćev, V.; Kostadinović, L.J.; Beuković, M.; Ljubojević, D. and Zec, S. (2014). Effect of spice herbs inbroiler chicken nutrition on productive performances. Proceedings of XVI International symposium, Feed Technology ", 28-30 October, Novi Sad, Serbia, 123-129.

Raeesi, M.; Hoeini-Aliabad, S.A.; Roofchaee, A.; Zare-Shahneh, A. and Pirali, S. (2010). Effect of periodically use of garlic (*Allium sativum*) power on performance and carcass characteristics in broiler chickens. World Academy of Science, Engineering and Technology, 68: 1213-1219.

Sallam, Kh. I.; Ishioroshi, M. and Samejima, K. (2004). Antioxidant and antimicrobial effect of garlic in chicken sausage. Lebensm.Wiss. Technol. 37: 849–855.

SAS. (2006). *Statistical Analysis System, SAS User's Guide.* Statistics SAS institute Inc., Cary, NC, USA.

Soheyla, H. and Foruhe, Z. (2013). Effect of Zeta Potential on the Properties of Nano-Drug Delivery Systems - A Review (Part 2). Tropical Journal of Pharmaceutical Research April 2013; 12 (2): 265-273.

Stanaćev, V.; Glamočić, D.; Milošević, N.; Puvača, N.; Stanaćev, V. and Plavša, N. (2011). Effect of garlic (*Allium sativum* L.) infattening chicks nutrition. African Journal of Agricultural Research, 6: 943-948.

Sun, Y.; Gates, B.; Mayers, B. and Xia, Y. (2002). Crystalline silver nanowires by soft solution processing. Nano Lett. 2: 165–168.

Tatara, M.R.; Sliwa, E.; Dudek, K.; Gawron, A. and Piersiak, T. (2008). Aged garlic extract and allicin improve performance and gastrointestinal tract development of piglets reared in artificial sow. Ann. Agric. Environ. Med., 15: 63-69.

Togashi, C.K.; Fonseca, J.B.; Soares, R.; Da Costa, A.P.D.; Ferreira, K.S. and Detmann, E. (2008). Use of garlic and copper in the feeding of broiler chicks. Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science, 37(6): 1036-1041.

Tollba, A.A.H. and Hassan, M.S.H. (2003). Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions. Black cumin (*Niglla sativa*) or garlic (*Allium sativum*). Egypt Poult. Sci. 23: 327–340.

Yalcin, S.; Onbaşilar, Ĺ.; Şehu, A. and Yalcin, S. (2007). The effects of dietary garlic powder on the performance, egg traits and bloodserum cholesterol of laying quails. Asian-australas. J. Anim. Sci., 20: 944–947.

Yugandhar, P. and Savithramma, N. (2013). Green synthesis of calcium carbonate nanoparticles and their effects on seed germination and seedling growth of *Vigna mungo* (L.). Hepper. International Journal of Advanced Research (2013), 1(8): 89-103.