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EFFECT OF DIETARY CURCUMIN AND CURCUMIN NANOPARTICLES SUPPLEMENTATION ON GROWTH PERFORMANCE, IMMUNE RESPONSE AND ANTIOXIDANT OF BROILERS CHICKENS

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ABSTRACT: The current study evaluated the effect of dietary inclusion of curcumin (Cur) or curcumin nanoparticles (CurNPs) on growth performance, blood metabolites, antioxidant status and humoral immunity of broiler chicks. In a completely randomized block design, a total of 504 one-day-old Ross-308 broiler chicks were randomly allocated to seven experimental groups with eight replications of nine birds each. The first (control) group received the basal diets without supplements while the 2nd, 3rd and 4th groups were fed diets supplemented with 25, 50 and 100 mg.kg^{-1} of Cur and the 5th, 6th and 7th groups were fed the same levels but from CurNPs, respectively. Results showed that the addition of 50 and 100 mg.kg^{-1} of Cur, irrespective of the form used, improved body weight, weight gain, feed conversion ratio and reduced feed consumption. The same doses increased relative weights of liver, thymus and bursa of Fabricius. Hypocholestrolimic impact of Cur and CurNPs was remarkably observed in treated birds. Regardless Cur form, lipid peroxidation was reduced while glutathione peroxidase and superoxide dismutase activities were enhanced in groups treated with 50 and 100 mg.kg^{-1}. Both Cur forms exhibited immunomodulatory effect as total serum antibody titer against SRBCs, IgG and IgM were elevated at 21 and 35 day of age. In conclusion, supplementation of Cur and CurNPs to broiler diets, particularly at levels of 50 and 100 mg.kg^{-1}, improved growth performance, reduced blood cholesterol, and enhanced redox status and humoral immune response of broiler chickens.

Keywords: Growth performance; antioxidant; hypolipidemic; immunomodulatory; curcumin nanoparticles; broiler.

INTRODUCTION

The sub-therapeutic use of antibiotic growth promoters as disease prevention practice in poultry is in increasingly opposed in many countries around the world and has been banned in the European Union. According to the latest Veterinary Feed Direction, the United States will soon pursue the suit with the US Food and Drug Administration. These antibiotic growth promoters include a spectrum of antibiotics used for human whose indiscriminate and flagrant use in poultry may lead to the emergence of antibiotic-resistant strains and increase the gravity of human infections (Abdel-Moneim *et al*., 2019a, Abdel-Moneim *et al*., 2019b). Thus, there is an urgent need to develop and adopt newer and safer growth promoters and disease preventers. Several researches have looked forward to find such alternative solutions but most of them focused on the use of feed additives and gut conditioners including probiotics, prebiotics, exogenous enzymes, essential oils, antioxidants and herbal extract (Abd El-Moneim and Sabic, 2019, Abd El-Moneim *et al*., 2019, Zeng *et al*., 2015).

In many countries, the use of intact botanicals, mostly herbs and spices, is a widely accepted practice in traditional veterinary and human medications. Phytogenic as natural products, dried powder, extracts or phytochemicals have a significant bioactivities e.g. antioxidant, antimicrobial, immune-modulatory, anti-inflammatory, appetizing and gastroprotective (Johannah *et al*., 2018). The pharmacological activities of phytogenics are able to optimize poultry growth performance and profitability through its ability to promote intestinal health and tackle microbial threats. Various culinary species have been used as feed additives in poultry diets including ginger, black cumin, thyme, garlic, coriander and turmeric (Murugesan *et al*., 2015). The yellow dye in turmeric namely "curcumin; Cur" or diferuloylmethane has been recognized as the prime bioactive constituent in turmeric with a multitude of impacts including antimicrobial, anti-inflammatory, antioxidant, antiproliferative, gastroprotective, antiarthritic and neuroprotective activities (Prasad *et al*., 2014). It has been reported that addition of Cur in animal diets may improve nutrient digestibility and metabolism by stimulating the bile acids secretion and activating digestion enzymes activities (Chattopadhyay *et al*., 2004, Platel and Srinivasan, 2000). Furthermore, Cur can enhance hepatic function biomarkers, reduce blood cholesterol and modulate the architecture of small intestine (Gandhi *et al*., 2011, Seo *et al*., 2008, Viveros *et al.*, 2011). However, Cur has been characterized by its poor bioavailability that related to low absorption rate, rapid metabolism and fast systemic removal from the body (Anand *et al*., 2007, Yu and Huang, 2012). Nevertheless, many possible ways could be used to overcome these problems. Adjuvants, liposomes, phospholipids complexes, micelles and nanoparticles are promising formulations that appear to prolong circulation time, increase permeability and enhance resistance to metabolic processes (Anand *et al*., 2007, Prasad *et al*., 2014, Yu and Huang, 2012). Bioavailability of curcumin nanoparticles (CurNPs) has been reported to be higher than Cur because nano-formulations protect Cur against inactivation by hydrolysis and increase its solubilization (Kurita and Makino, 2013, Rahmani *et al*., 2017). The present study aimed to

compare the effect of dietary supplementation of Cur or CurNPs on growth performance, carcass traits, blood parameters, redox status and humoral immune response of broiler chickens.

MATERIALS AND METHODS

Preparation and characterization of curcumin nanoparticles

Curcumin [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] powder (95% purity) was purchased from Shaanxi Ming Chemical Technology Co. Ltd, China. Synthesis of CurNPs was performed according to the procedure described by Pandit *et al*. (2015). Briefly, 100 mg of Cur was dissolved in 20 ml dichloromethane and then one ml of this solution was added in drop-wise manner to 50 ml of boiling water. After ultrasonication (50 kHz) for 30 min, the mixture was stirred for 20 min at 800 rpm till obtaining the orange colored precipitate. Subsequently, the supernatant was removed and the pellet was collected to use as a dietary supplement.

Size and topology of synthesized CurNPs were determined using transmission electron microscopic analysis (TEM; Figure 1 A). In brief, a drop of CurNPs solution was located on carbon-coated copper grids and spotted with infrared light until get dried. Powdered CurNPs was loaded on specimen holder and TEM micrograph was taken by HRTEM-EDS; JEOL-JEM- 1200. Furthermore, crystalline structure of synthesized CurNPs was characterized using X-ray diffraction method (XRD; Figure 1 B). The diffraction patterns of CurNPs dried powder were recorded by Europe 600 GNR bench top X-Ray Diffractometer.

Birds and experimental design

The experiment was conducted at the Poultry Production Research Unit, Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority under the protocol approval of Local Animal Experimentation Ethics Committee in ARC. A total of 504 one day old Ross-308 broiler chicks were allocated equally to seven experimental groups with eight replications of nine birds each, and were caged in galvanized metal cages (98 cm length × 65 cm width × 55 cm height). All birds had free access to water and feed and were subjected to the same management and environmental conditions. The light schedule throughout the experimental period was artificially elevated to 23 h per day. The basal diets were formulated to meet NRC recommendations (NRC, 1994) and their chemical composition are presented in Table 1. The control diets were supplemented with two forms of Cur (native Cur and CurNPs) and three levels of each (25, 50 and 100 $mg.kg^{-1}$ diet) to obtain seven experimental groups that received basal diet without supplementations (Control); basal diet plus 25 Cur $mg.kg^{-1}$ diet (25 Cur); basal diet plus 50 Cur $mg.kg^{-1}$ diet (50 Cur); basal diet plus 100 Cur $mg.kg^{-1}$ diet (100 Cur); basal diet plus 25 CurNPs $mg.kg^{-1}$ diet (25 CurNPs); basal diet plus 50 CurNPs $mg.kg^{-1}$ diet (50 CurNPs); basal diet plus 100 CurNPs $mg.kg^{-1}$ diet (100 CurNPs). For the homogeneous distribution of Cur and CurNPs, they were first mixed with a small amount of basal diet and subsequently were blended with a gradual feed amounts until homogeneous mixing of the respective diets was achieved.

Growth performance

At the end of 21 and 35 days of age, cumulative body weight (BW), weight gain (WG) and feed consumption (FC)

were recorded on a replicate basis weekly and feed conversion ratio (FCR) was consequently calculated. Mortality was also monitored daily in each pen. One bird/replicate was randomly chosen and killed by cervical dislocation at the end of the experiment. Birds were de-feathered, eviscerated, dressed and then hot carcass, liver, heart, spleen, gizzard, proventriculus, thymus and bursa of Fabricius were collected, weighed and their relative weights to live body weight were calculated. Carcass yield was calculated according to the equation described in Abd El-Moneim *et al*. (2019).

Blood samples collection and biochemical analysis

Blood samples were collected at slaughtering time (35 days of age), kept to clot and centrifuged at 3000 × g for 15 min. Sera samples were aliquoted into 5-mL vials and stored at -24°C until the biochemical analysis. Serum concentrations of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total protein (TP), albumin (ALB), glucose (GLU), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were analyzed using commercial kits (Spinreact Co., Spain) according to the manufacturer's instructions using spectrophotometer (Milton Roy Spectronic 1201, USA). Serum superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and malondialdehyde (MDA) content were determined using commercial kits (Cell Biolabs Inc., USA).

Concerning the humoral immune response against sheep red blood cells (SRBCs), eight birds were randomly selected from each group, individually caged and intravenously injected in the left brachial vein with 0.5 ml of 25% SRBCs suspension at 14 and 28 days of age. Blood samples were collected seven days post-immunization, kept to clot and centrifuged at 3000 × g for 10 min to obtain sera which were stored at -20 ºC. Determination of total antibodies against SRBCs (Total anti-SRBCs), immunoglobulin G (IgG) and M (IgM) as described by Nelson *et al.* (1995).

Statistical analysis

Data were statistically analyzed as a randomized block design. Each replicate pen was considered as an experimental unit for performance parameters while the experimental unit for the other parameters was individual birds. All data were subjected to two-way analysis of variance by the General Linear Model procedure using the SPSS 18.00 statistical package (SPSS Ltd., Surrey, UK) to examine the following effects: a) main effect of Cur form (native Cur and CurNPs) b) main effect of Cur level (25, 50 and 100 $mg.kg^{-1}$ diet) and c) interaction between Cur forms × Cur level. Results were expressed as mean values with a pooled standard error of the mean (SEM). Tukey's multiple comparison test was performed to define the significant differences of main or interaction effects at $P<0.05$.

RESULTS

Growth performance and carcass characteristics

Compared with the control group, the addition of 50 and 100 $mg.kg^{-1}$ of both Cur and CurNPs improved BW and WG ($P<0.001$, both) during starter (1-21 d), grower (22-35 d) and overall (1-35 d) periods (Table 2). Feed consumption was decreased by dietary supplementation of Cur and CurNPs at level 50 $mg.kg^{-1}$ during starter ($P=0.032$) and overall

(P=0.023) periods and at 50 and 100 mg.kg^{-1} during the grower (P=0.036) one. Irrespective of Cur form, the addition of Cur in levels 50 and 100 mg.kg^{-1} enhanced FCR during starter (P=0.004), grower and overall (P<0.001) periods. As presented in Table 3, all dietary levels of Cur in both forms increased liver and thymus weights (P<0.001), except 25 mg Cur.kg^{-1} for liver weight, and at the levels 50 and 100 mg.kg^{-1} of Cur and CurNPs resulted in an increase in bursa weight (P<0.001) relative to the control. The interaction between Cur form and level did not statistically affect all growth and carcass traits except the relative weight of liver.

Blood biochemistry

The inclusion of Cur and CurNPs in the amount of 50 and 100 mg.kg^{-1} elevated (P<0.001) serum level of TP while globulin level was increased (P=0.001) only in birds fed 100 mg.kg^{-1} of both forms of Cur (Table 4). Dietary levels of 100 mg Cur.kg^{-1} and 50 and 100 mg CurNPs.kg^{-1} were able to reduce (P=0.023) serum AST level. Serum concentrations of ALB, GLU and ALT were not significantly affected by all levels and forms of Cur. Both Cur forms observed a remarkable hypocholesterolemic effect when fed to broiler chickens. The addition of Cur to broiler diets at levels 50 and 100 mg.kg^{-1} caused an increase in HDL-C and decrease in TG levels, while TC, LDL-C and HDL-C: LDL-C were decreased (P<0.001) regardless of dietary Cur level. The interaction between Cur forms × Cur levels did not affect all blood biochemical parameters.

Redox parameters

Antioxidant enhancing effect of Cur and CurNPs are observed as presented in Figure 2. Serum concentration of MDA, as a biomarker of lipid peroxidation, was reduced (P<0.001) in birds fed all CurNPs levels and 50 and 100 mg Cur.kg^{-1}. Both forms of Cur at dietary levels of 50 and 100 mg.kg^{-1} elevated (P<0.001) serum activity of GPx while SOD activity was enhanced (P<0.001) at dietary Cur level of 100 mg.kg^{-1} and by the addition of CurNPs in the amount of 50 and 100 mg.kg^{-1} diet.

Humoral immunity

Immunomodulatory effect of Cur and CurNPs is presented in Table 5. Total serum antibody titer against SRBCs, IgG and IgM were significantly affected by Cur forms and levels while not affected by the interaction between them. At 21 day of age, compared with the control group, all dietary levels of Cur and CurNPs increased serum levels of total anti-SRBCs, IgG and IgM, except level of 25 mg CurNPs.kg^{-1} for total anti-SRBCs. Moreover, at 35 day, total anti-SRBCs and IgM were increased in groups fed all levels of Cur and CurNPs, however IgG level was significantly elevated only in groups received 50 mg.kg^{-1} of both Cur forms. Birds fed diets supplemented with 50 mg.kg^{-1} of Cur or CurNPs recorded the highest antibody titers at 21 and 35 day of age.

DISCUSSION

Dietary supplementation of Cur or CurNPs increased BW and WG and reduced FCR of birds compared to those of control birds. Dietary supplementation of Cur or CurNPs also increased liver, thymus and bursa weights. The positive impact of Cur on growth performance of broiler chicks was also reported by several researchers (Nouzarian *et al*., 2011, Rahmani *et al*., 2017, Rahmani *et al*., 2018, Rajput *et al*., 2012). Rahmani *et al*. (2018) and Rahmani *et al*. (2017) revealed that 200 mg.kg^{-1} from Cur or

CurNPs improved WG and FCR in broiler chicks while FC was not affected. Furthermore, Rajput *et al*. (2012) observed significant increase in BW and decrease in FCR of broilers fed diets supplemented with 200 ppm of Cur during the periods 22-42 d and 1-42 d. Johannah *et al*. (2018) reported significant elevation in BW and improvement in FCR when treated with Cur at 1% (w/w) in chicks. The authors also noticed significant increase in liver, gizzard, heart, pancreas and intestine weight at 42 d in birds received diets included with Cur at 1% (w/w). Growth promotion and better feed efficiency achieved by feeding on Cur might be attributed to anti-inflammatory, antibacterial and antioxidant properties (Chattopadhyay *et al*., 2004, Durrani *et al*., 2006), better activity of thyroid gland (Durrani et al., 2006), stimulating the secretion of digestive enzymes (Platel and Srinivasan, 2000), increase bile production and secretion (Al-Sultan and Gameel, 2004), improving the consumption of digested nutrients (Hernandez *et al*., 2004), and/or its probiotic like effects (Niamsa and Sittiwet, 2009).

As shown in our study, the inclusion of Cur and CurNPs to broiler diets enhanced hepatic function as serum TP and GLO were elevated and AST and ALT activities were reduced. The reduction in hepatic enzyme activity reflects enhancement in liver function and the hepatoprotective effect of Cur against the toxicity of many toxins that might be found in the diets. The beneficial effects of Cur on hepatic function could be attributed to its pleiotropic anti-oxidant activity (Kim *et al*., 2013b). It restrains formation and scavenges reactive nitrogen species (Kim et al., 2003) and reactive oxygen species (ROS) (Ak and Gülçin, 2008). Moreover, Cur has been shown to stimulate many enzymatic antioxidants, like catalase, heme-oxygenase-1 and glutathione transferase (GST) (Iqbal *et al*., 2003).

Furthermore, dietary Cur in both forms remarkably reduced serum cholesterols and triglycerides. Several previous investigations also have been reported the hypocholesterolaemic action of Cur (Pornanek and Phoemchalard, 2019, Rajput *et al*., 2012, Xie *et al*., 2018). However, other researchers reported different results, including a decrease in TG without affecting TC and LDL-C:HDL:C ratio (Nouzarian *et al*., 2011), a decrease in liver TG with no effect on TC (Manjunatha and Srinivasan, 2006) or no changes in TG, TC and LDL-C levels (Mehala and Moorthy, 2008). These conflicting results may be due to the difference in Cur amount used in each experiment. The hypocholesterolaemic effect of Cur might be due to the mobilization of cholesterols from extrahepatic tissues in order to catabolize it in the liver, the stimulation of cholesterol-to-bile acid conversion through activating cholesterol-7-a-hydroxylase which presents the rate limiting step in cholesterol catabolism and decreasing cholesterol absorption from the intestine (Akila *et al*., 1998). Curcumin may also regulate lipid metabolism by inhibiting the activities of some hepatic enzymes, e.g. acyl CoA cholesteryl acyltransferase (ACAT) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which have important role in decreasing esterification and oxidation of fatty acids and cholesterol synthesis in the liver (Pornanek and Phoemchalard, 2019).

The reduction of serum MDA content and elevation in both GPx and SOD activities in birds fed Cur and CurNPs confirms curcumin's ability to reduce lipid peroxidation and improve antioxidant status of treated birds. These results are similar to those obtained by Rahmani *et al*. (2018) who reported significant decrease in serum MDA level in broilers received diet supplemented Cur and CurNPs at level 200 $mg.kg^{-1}$. Furthermore, Yarru *et al*. (2009) noticed that feeding on 0.5% turmeric powder (74 mg of total curcuminoids/kg diet) enhanced overall antioxidant protection in broiler chickens fed diets with aflatoxin B1 by upregulating the gene expression of SOD, GPx and catalase. Phenolic groups in Cur structure prevents lipid peroxidation by restrain formation and scavenges reactive nitrogen species and ROS (Ak and Gülçin, 2008, Kim *et al*., 2003). Furthermore, Cur can increase the activities of several enzymatic antioxidants, like SOD, GPx, GST, heme-oxygenase-1 and catalase (Iqbal *et al*., 2003). NF-kB-mediated expression of inducible nitric oxide synthase (iNOS) and activation of pro-inflammatory cytokines, NF-kB by ROS and protein kinase C are also inhibited by Cur (Nanji *et al*., 2003).

Immunomodulatory effect of Cur and CurNPs are greatly observed in the present study since birds fed diets supplemented of Cur or CurNPs particularly at level 50 $mg.kg^{-1}$ exhibited higher total antibody titers against SRBCs, IgG and IgM at 21 and 35 day of age. In agreement with our results, Rajput *et al*. (2013) reported significant elevation in serum antibody titers against Newcastle disease virus (NDV) and Avian influenza (AI) of broilers supplemented with Cur at 20 and 30 d. Moreover, Kim *et al*. (2013a) showed that systemic humoral immune responses of chickens fed turmeric-supplemented diets was enhanced compared with control birds. Additionally, IgG level was increased in rats received dietary Cur at 40 $mg.kg^{-1}$ for 5 weeks (South *et al.*, 1997). Contrarily, Nouzarian *et al*. (2011) reported that the treatment with turmeric powder up to 1% failed to induce any effect on antibody production against NDV and AI at 25 and 45 d. Arshami *et al*. (2013) also did not observe any changes in antibody titers against SRBCs or IgG and IgM levels in laying hens supplemented with Cur powder up to 2.5% at 64 and 66 weeks of age. The immunomodulatory effect of Cur could be attributed to its pharmacological properties, including antimicrobial, antioxidant and anti-inflammatory activities. Furthermore, Cur can induce T_3 and T_4 secretion that enhance lymphocyte production which play a vital role in stimulating antibody production in chickens (Rajput *et al*., 2012, Rajput *et al*., 2013). The proliferative response of lymphocytes is commonly used to evaluate the immunomodulatory effects of potential therapeutic agents. It has been reported that Cur reasonably boosts the proliferation and activation of B and T lymphocytes without changing phagocytic macrophages count (Gautam *et al*., 2007). These explanations suggest that, in the present study, the immune cells were probably preactivated after immunization with SRBCs and produce higher antibodies in birds treated with Cur or CurNPs.

CONCLUSION

Results of the present study concluded that dietary inclusion of curcumin or curcumin nanoparticles has positive effects on growth performance, hepatic

function, antioxidant status and humoral immune response of broiler chicks. Hypocholesterolemic effect of Cur and CurNPs were remarkably observed in treated birds compared to the control. Although the bioavailability of CurNPs has been reported to be much higher than those of native Cur, no significant alterations were noticed in most of studied parameters between these forms of Cur. The level of 50 mg.kg^{-1} from both supplement forms recorded the best observations followed by 100 mg.kg^{-1}.

Table (1): Ingredients and calculated chemical composition of the basal diet**.**

Ingredients	**Starter (1 to 21 d)**	**Finisher (22 to 35 d)**
Yellow corn	59.50	62.5
Soybean meal (44%)	26.00	23.94
Maize gluten meal (62%)	9.00	7.00
Vegetable oil	1.50	2.50
Limestone	1.12	1.23
Di-Calcium Phosphate	1.75	1.70
Premix[1]	0.30	0.30
NaCl (salt)	0.30	0.30
L-lysine	0.36	0.36
DL-Methionine	0.17	0.17
Total	100	100
Calculated composition[2]		
ME (kcal kg^{-1})	3055.00	3120
Crude protein	22.10	20.20
Calcium	0.93	0.95
Non phytate phosphorus	0.46	0.45
Methionine	0.3	0.3
Lysine	1.28	1.2
TSAA	0.98	0.90

[1]Provides each Kg of diet: Vit. A: 12000 IU, Vit. D_3: 5000 IU, Vit. E: 40.0 mg, Vit. K_3:3.605 mg, Vit. B_1:3.0 mg, Vit. B_2:8.0 mg, Vit. B_6:4.95 mg, Vit. B_{12}: 0.17 mg, Niacin:60.0 mg, Folic acid:2.083 mg, D-Biotin:200.0 mg, calcium D-Pantothenate: 18.333 mg, Copper:80 mg, Iodine:2.0 mg, Selenium:150.0 mg, Iron:80.0 mg, Manganese:100.0 mg, Zinc:80.0 mg, Cobalt 500.0 mg.
[2]Calculated according to (NRC, 1994).

Table (2): Effect of different dietary curcumin forms and levels on growth performance of broiler chickens

Indices	Treatment groups[1]							SEM[2]	Significance levels		
	C	25 Cur	50 Cur	100 Cur	25 CurNPs	50 CurNPs	100 CurNPs		Level effect (L)	From effect (F)	L × F interaction
Body weight, g											
Initial	40.98	39.95	40.57	39.78	40.01	40.32	40.23	0.294	0.988	0.840	0.962
21 day	830.56^{c}	831.43^{c}	837.85^{b}	846.75^{a}	834.03^{c}	848.97^{b}	863.14^{a}	2.368	<0.001	0.048	0.386
35 day	1734.8^{b}	1740.2^{b}	1781.2^{a}	1801.1^{a}	1751.5^{b}	1815.5^{a}	1832.2^{a}	6.470	<0.001	0.040	0.500
Body weight gain, g											
Days 1-21	37.60^{c}	37.69^{c}	37.97^{b}	38.43^{a}	37.81^{c}	38.51^{b}	39.19^{a}	0.117	<0.001	0.049	0.387
Days 22-35	64.59^{b}	64.91^{b}	67.38^{a}	68.17^{a}	65.53^{b}	69.04^{a}	69.22^{a}	0.440	<0.001	0.262	0.878
Days 1-35	48.39^{b}	48.58^{b}	49.73^{a}	50.32^{a}	48.90^{b}	50.72^{a}	51.20^{a}	0.187	<0.001	0.040	0.500
Feed consumption, g/bird/day											
Days 1-21	54.18^{a}	53.30^{a}	50.85^{b}	52.96^{ab}	53.09^{a}	49.51^{b}	52.48^{ab}	0.452	0.032	0.600	0.963
Days 22-35	127.73^{a}	124.16^{ab}	123.19^{b}	123.38^{b}	124.19^{ab}	120.50^{b}	122.19^{b}	0.741	0.036	0.520	0.906
Days 1-35	83.98^{a}	81.64^{ab}	79.39^{b}	81.12^{ab}	81.53^{ab}	77.69^{b}	80.36^{ab}	0.563	0.023	0.600	0.959
Feed conversion ratio, g feed/g gain											
Days 1-21	1.44^{a}	1.41^{ab}	1.34^{c}	1.38^{bc}	1.40^{ab}	1.29^{c}	1.34^{bc}	0.013	0.004	0.310	0.865
Days 22-35	1.98^{a}	1.92^{a}	1.83^{b}	1.81^{b}	1.90^{a}	1.75^{b}	1.77^{b}	0.018	<0.001	0.226	0.786
Days 1-35	1.74^{a}	1.68^{a}	1.60^{b}	1.61^{b}	1.67^{a}	1.53^{b}	1.57^{b}	0.014	<0.001	0.252	0.821

[1]treatment groups: C – corn-based diet, 25 Cur, 50 Cur and 100 Cur – 25, 50 and 100 mg/kg curcumin, respectively, 25 CurNPs, 50 CurNPs and 100 CurNPs – 25, 50 and 100 mg/kg curcumin nanoparticles, respectively; [2]SEM – standard error of means; a–d– means with different superscripts are significantly different.

Table (3): Effect of different dietary curcumin forms and levels on carcass traits and lymphoid organs indices of broiler chickens at 35 day of age

Indices (%)	Treatment groups[1]							SEM[2]	Significance levels		
	C	25 Cur	50 Cur	100 Cur	25 CurNPs	50 CurNPs	100 CurNPs		Level effect (L)	From effect (F)	L × F interaction
Dressing	70.78	68.65	71.34	71.73	70.83	73.47	72.97	0.584	0.291	0.229	0.887
Liver	2.387^{c}	2.380^{bc}	2.413^{a}	2.387^{b}	2.403^{b}	2.453^{a}	2.410^{b}	0.005	<0.001	<0.001	0.004
Heart	0.386	0.403	0. 366	0.399	0.381	0.371	0.409	0.006	0.105	0.856	0.656
Proventriculus	0.403	0.430	0.407	0.423	0.410	0.427	0.436	0.004	0.145	0.675	0.299
Gizzard	1.467	1.560	1.540	1.593	1.563	1.560	1.597	0.016	0.058	0.821	0.995
Thymus	0.307^{c}	0.337^{b}	0.390^{a}	0.373^{a}	0.343^{b}	0.380^{a}	0.373^{a}	0.008	<0.001	0.932	0.943
Spleen	0.117	0.110	0.107	0.120	0.113	0.117	0.113	0.003	0.819	0.753	0.725
Bursa of Fabricius	0.100^{b}	0.110^{b}	0.187^{a}	0.200^{a}	0.157^{b}	0.193^{a}	0.210^{a}	0.011	<0.001	0.208	0.536
Carcass yield	75.02	72.99	75.72	76.11	75.18	77.92	77.39	0.604	0. 275	0.222	0.883

[1]treatment groups: C – corn-based diet, 25 Cur, 50 Cur and 100 Cur – 25, 50 and 100 mg/kg curcumin, respectively, 25 CurNPs, 50 CurNPs and 100 CurNPs – 25, 50 and 100 mg/kg curcumin nanoparticles, respectively; [2]SEM – standard error of means; a–c– means with different superscripts are significantly different.

Table (4): Effect of different dietary curcumin forms and levels on blood metabolites of broiler chickens at 35 day of age

Indices	Treatment groups[1]							SEM[2]	Significance levels		
	C	25 Cur	50 Cur	100 Cur	25 CurNPs	50 CurNPs	100 CurNPs		Level effect (L)	From effect (F)	L × F interaction
Protein fractions, g/dl											
total protein	3.85^{b}	3.86^{b}	4.09^{a}	4.25^{a}	3.95^{b}	4.30^{a}	4.41^{a}	0.057	<0.001	0.116	0.716
albumin	2.07	2.12	2.19	2.21	2.14	2.35	2.24	0.048	0.435	0.584	0.933
globulin	1.78^{b}	1.74^{b}	1.89^{b}	2.04^{a}	1.80^{b}	1.95^{b}	2.17^{a}	0.040	0.001	0.231	0.838
A/G ratio	1.16	1.22	1.16	1.09	1.20	1.24	1.04	0.038	0.506	0.974	0.922
Glucose, mg/dl	222.2	224.5	225.3	229.0	219.5	225.1	231.4	1.702	0.302	0.869	0.873
Enzymes activity, U/L											
AST	129.3^{a}	123.5^{ab}	129.9^{a}	114.8^{b}	128.8^{a}	111.9^{b}	112.7^{b}	2.231	0.023	0.277	0.120
ALT	46.04	43.76	46.04	47.01	47.10	42.27	42.38	1.161	0.940	0.588	0.597
Lipid profile, mg/dl											
TC	212.5^{a}	204.9^{b}	188.8^{c}	185.9^{c}	191.9^{b}	177.1^{c}	181.9^{c}	2.926	<0.001	0.020	0.323
TG	202.3^{a}	199.7^{ab}	188.8^{c}	195.7^{b}	198.9^{ab}	182.0^{c}	189.6^{c}	1.798	<0.001	0.143	0.604
HDL-C	38.90^{b}	40.91^{b}	50.98^{a}	55.84^{a}	43.57^{b}	60.94^{a}	54.54^{a}	1.913	<0.001	0.148	0.229
LDL-C	133.1^{a}	124.1^{b}	100.0^{c}	90.9^{c}	108.5^{b}	79.77^{c}	89.46^{c}	4.409	<0.001	0.033	0.183
HDL-C:LDL-C	3.48^{a}	3.09^{b}	1.97^{c}	1.63^{c}	2.52^{b}	1.33^{c}	1.65^{c}	0.187	<0.001	0.151	0.493

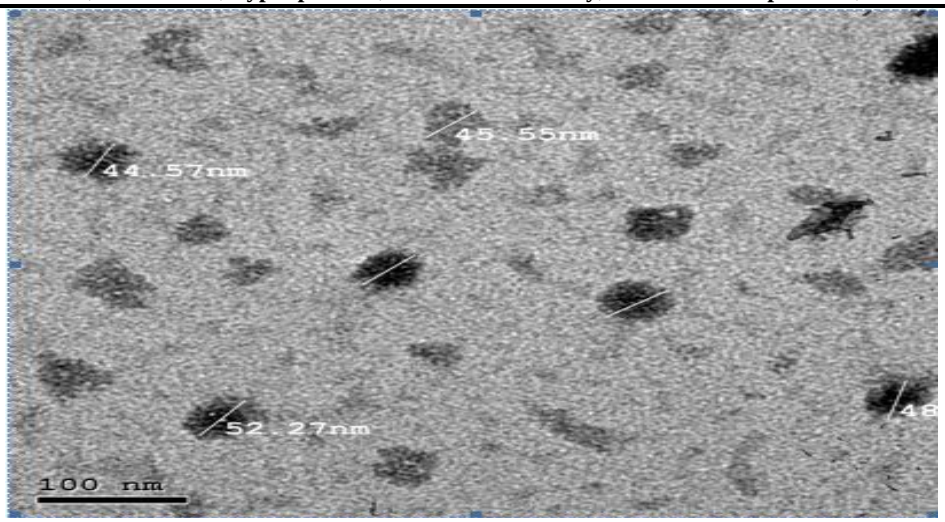
[1]treatment groups: C – corn-based diet, 25 Cur, 50 Cur and 100 Cur – 25, 50 and 100 mg/kg curcumin, respectively, 25 CurNPs, 50 CurNPs and 100 CurNPs – 25, 50 and 100 mg/kg curcumin nanoparticles, respectively, A/G ratio – albumin:globulin ratio, AST – aspartate aminotransferase, ALT – alanine aminotransferase, TC – total cholesterol, TG – triglycerides, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol; [2]SEM – standard error of means; a–e– means with different superscripts are significantly different.

Table (5): Effect of different dietary curcumin forms and levels on anti-SRBCs (total), IgG and IgM titers of broiler chickens at 21 and 35 days of age

Indices	Treatment groups[1]							SEM[2]	Significance levels		
	C	25 Cur	50 Cur	100 Cur	25 CurNPs	50 CurNPs	100 CurNPs		Level effect (L)	From effect (F)	L × F interaction
Total anti-SRBCs[3]											
Day 21	3.80^{c}	4.25^{b}	5.23^{a}	4.37^{b}	4.57^{b}	5.81^{a}	4.76^{b}	0.146	<0.001	0.006	0.274
Day 35	5.81^{d}	5.91^{c}	7.53^{a}	6.44^{b}	6.39^{c}	7.76^{a}	6.60^{b}	0.162	<0.001	0.047	0.478
IgG[4]											
Day 21	2.01^{c}	2.34^{b}	3.02^{a}	2.31^{b}	2.57^{b}	3.31^{a}	2.56^{b}	0.096	<0.001	0.004	0.310
Day 35	3.79^{b}	3.70^{b}	4.31^{a}	3.73^{b}	3.88^{b}	4.40^{a}	3.92^{b}	0.062	<0.001	0.018	0.424
IgM[5]											
Day 21	1.79^{d}	1.91^{c}	2.21^{a}	2.07^{b}	2.00^{c}	2.50^{a}	2.20^{b}	0.052	<0.001	0.012	0.190
Day 35	2.02^{d}	2.22^{c}	3.22^{a}	2.71^{b}	2.51^{c}	3.36^{a}	2.68^{b}	0.105	<0.001	0.031	0.287

[1]treatment groups: C – corn-based diet, 25 Cur, 50 Cur and 100 Cur – 25, 50 and 100 mg/kg curcumin, respectively, 25 CurNPs, 50 CurNPs and 100 CurNPs – 25, 50 and 100 mg/kg curcumin nanoparticles, respectively; [2]SEM – standard error of means; [3]Total anti-SRBCs – total antibody titers against sheep red blood cells; [4]IgG – Immunoglobulin G; [5]IgM – Immunoglobulin M; a–d– means with different superscripts are significantly different.

A



B

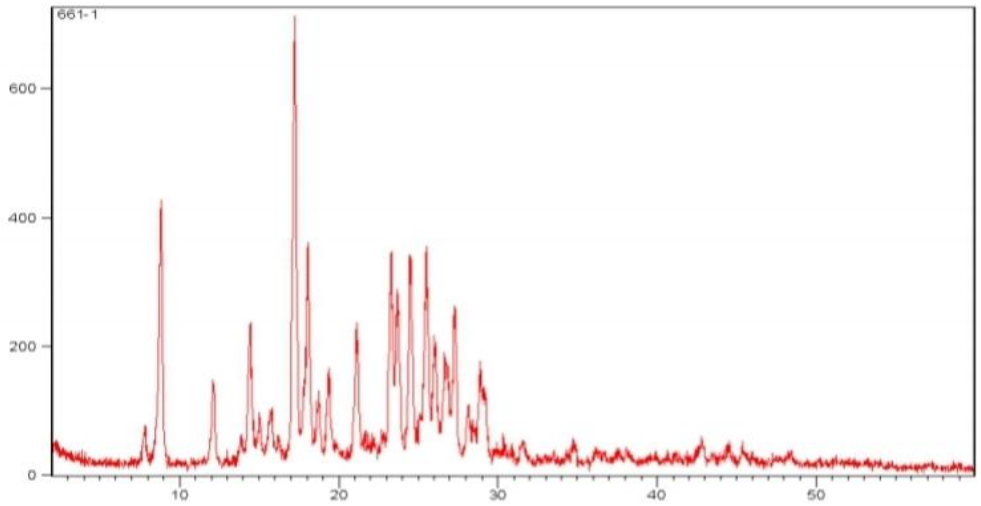


Figure (1): Characterization of curcumin nanoparticles. **A** Transmission electron microscope image of CurNPs showing its size is about 50 nm. **B** X-ray diffraction pattern of synthesized CurNPs.

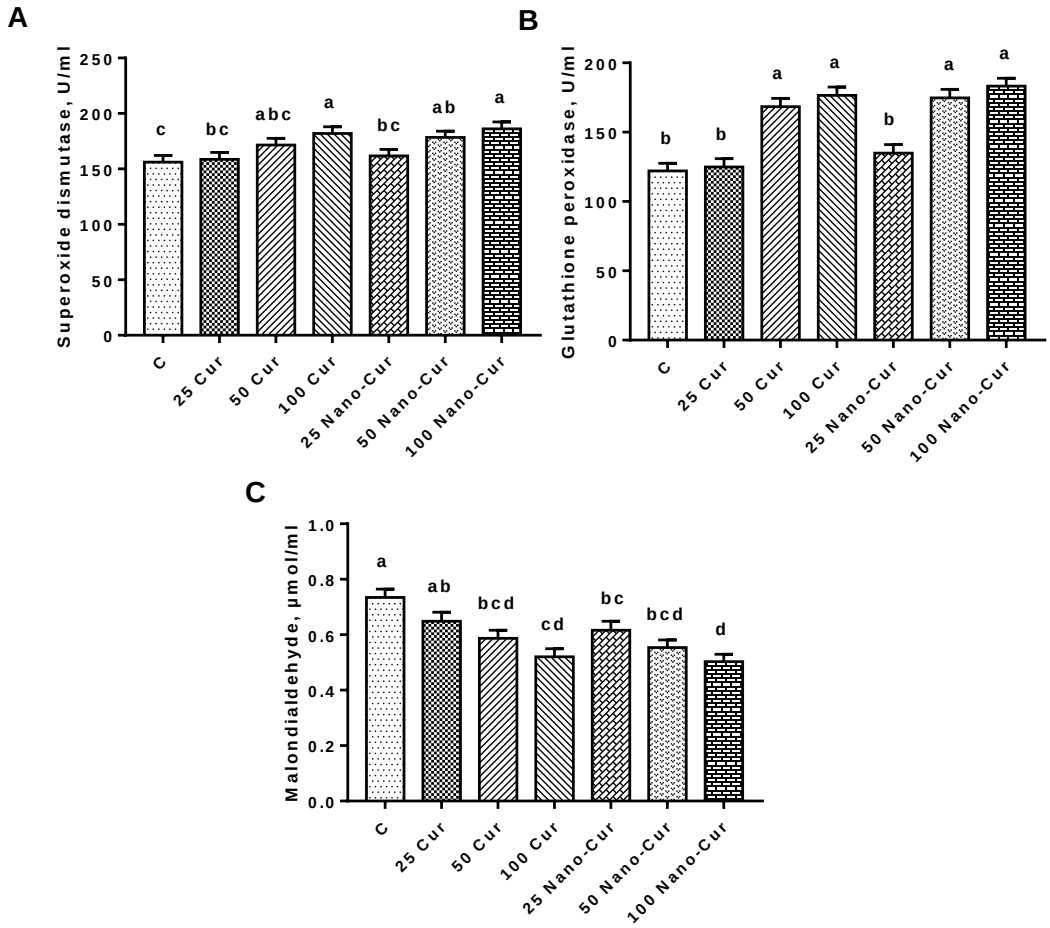


Figure (2): Effect of different dietary curcumin forms and levels on oxidative status A SOD, B GPx and C MDA in the serum of broiler chickens at 35 day of age.
Treatment groups: C – corn-based diet, 25 Cur, 50 Cur and 100 Cur – 25, 50 and 100 mg/kg curcumin, respectively, 25 CurNPs, 50 CurNPs and 100 CurNPs – 25, 50 and 100 mg/kg curcumin nanoparticles, respectively. Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P<0.05$).

REFERENCES

Abd El-Moneim, A.E. and Sabic, E.M. 2019. Beneficial effect of feeding olive pulp and *Aspergillus awamori* on productive performance, egg quality, serum/yolk cholesterol and oxidative status in laying Japanese quails. Journal of Animal and Feed Sciences 28: 52-61

Abd El-Moneim, E.A.; El-Wardany, I.; Abu-Taleb, A.M.; Wakwak, M.M., Ebeid, T.A. and Saleh, A.A. 2019. Assessment of in ovo administration of *Bifidobacterium bifidum* and *Bifidobacterium longum* on performance, ileal histomorphometry, blood hematological, and biochemical parameters of broilers. Probiotics and Antimicrobial Proteins, In Press

Abdel-Moneim, A.E., Elbaz, A.M., Khidr, R.E. and Badri, F.B., 2019a. Effect of in ovo inoculation of *Bifidobacterium* spp. on growth performance, thyroid activity, ileum histomorphometry and microbial enumeration of broilers. Probiotics and Antimicrobial Proteins, In Press

Abdel-Moneim, A.E.; Selim, D.A.; Basuony, H.A.; Sabic, E.M.; Saleh, A.A. and Ebeid, T.A. 2019b. Effect of dietary supplementation of *Bacillus subtilis* spores on growth performance, oxidative status and digestive enzyme activities in Japanese quail birds. Tropical animal health and production, In Press

Ak, T. and Gülçin, İ. 2008. Antioxidant and radical scavenging properties of curcumin. Chemico-biological interactions, 174: 27-37

Akila, G.; Rajakrishnan, V.; Viswanathan, P.; Rajashekaran, K. and Menon, V.P. 1998. Effects of curcumin on lipid profile and lipid peroxidation status in experimental hepatic fibrosis. Hepatology research, 11: 147-157

Al-Sultan, S. and Gameel, A. 2004. Histopathological changes in the livers of broiler chicken supplemented with turmeric (Curcuma longa). International Journal of Poultry Science, 3: 333-336

Anand, P.; Kunnumakkara, A.B.; Newman, R.A. and Aggarwal, B.B. 2007. Bioavailability of curcumin: problems and promises. Molecular pharmaceutics, 4: 807-818

Arshami, J.; Pilevar, M.; Azghadi, M.A. and Raji, A.R. 2013. Hypolipidemic and antioxidative effects of curcumin on blood parameters, humoral immunity, and jejunum histology in Hy-line hens. Avicenna journal of phytomedicine, 3: 178

Chattopadhyay, I.; Biswas, K.; Bandyopadhyay, U. and Banerjee, R.K. 2004. Turmeric and curcumin: Biological actions and medicinal applications. Current Science-Bangalore, 87: 44-53

Durrani, F.; Ismail, M.; Sultan, A.; Suhail, S.; Chand, N. and Durrani, Z. 2006. Effect of different levels of feed added turmeric (Curcuma longa) on the performance of broiler chicks. Journal of Agricultural and Biological Science, 1: 9-11

Gandhi, P., Khan, Z. and Chakraverty, N., 2011. Soluble curcumin: a promising oral supplement for health management, Journal of applied pharmaceutical science, 1, 1-7

Gautam, S.C., Gao, X. and Dulchavsky, S. 2007. Immunomodulation by curcumin. The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease.Springer, 321-341

Hernandez, F.; Madrid, J.; Garcia, V.; Orengo, J. and Megias, M. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Science, 83: 169-174

Iqbal, M.; Sharma, S.D.; Okazaki, Y.; Fujisawa, M. and Okada, S. 2003. Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity. Pharmacology & toxicology, 92: 33-38

Johannah, N.; Joseph, A.; Maliakel, B. and Krishnakumar, I. 2018. Dietary addition of a standardized extract of turmeric (TurmaFEED TM) improves growth performance and carcass quality of broilers. Journal of animal science and technology, 60: 8

Kim, D.K.; Lillehoj, H.S.; Lee, S.H.; Jang, S.I.; Lillehoj, E.P. and Bravo, D. 2013a. Dietary Curcuma longa enhances resistance against Eimeria maxima and Eimeria tenella infections in chickens. Poultry Science, 92: 2635-2643

Kim, J.E.; Kim, A.R.; Chung, H.Y.; Han, S.Y.; Kim, B.S. and Choi, J.S. 2003. In vitro peroxynitrite scavenging activity of diarylheptanoids from Curcuma longa. Phytotherapy Research, 17: 481-484

Kim, S.W.; Ha, K.C.; Choi, E.K.; Jung, S.Y.; Kim, M.G.; Kwons, D.Y.; Yang, H.J.; Kim, M.J.; Kang, H.J.; Back, H.Y.; Kim, S.Y.; Park, S.H.; Baek, H.Y.; Kim, Y.J.; Lee, J.Y and Chae, S.W. 2013b. The effectiveness of fermented turmeric powder in subjects with elevated alanine transaminase levels: a randomised controlled study, BMC complementary and alternative medicine, 13: 58-63

Kurita, T. and Makino, Y. 2013. Novel curcumin oral delivery systems. Anticancer research, 33: 2807-2821

Manjunatha, H. and Srinivasan, K. 2006. Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. The FEBS journal, 273: 4528-4537

Mehala, C. and Moorthy, M. 2008. Effect of Aloe vera and Curcuma longa (Turmeric) on carcass characteristics and biochemical parameters of broilers. Int. J. Poult. Sci, 7: 857-861

Murugesan, G.R.; Syed, B.; Haldar, S. and Pender, C. 2015. Phytogenic feed additives as an alternative to antibiotic growth promoters in broiler chickens. Frontiers in veterinary science, 2: 21

Nanji, A.A.; Jokelainen, K.; Tipoe, G.L.; Rahemtulla, A.; Thomas, P. and Dannenberg, A.J. 2003. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-κB-dependent genes. American Journal of Physiology-Gastrointestinal and Liver Physiology, 284: G321-G327

Nelson, N.; Lakshmanan, N. and Lamont, S. 1995. Sheep red blood cell and Brucella abortus antibody responses in chickens selected for multitrait immunocompetence. Poultry Science, 74: 1603-1609

Niamsa, N. and Sittiwet, C. 2009. Antimicrobial activity of Curcuma longa aqueous extract. Journal of Pharmacology and Toxicology, 4: 173-177

Nouzarian, R.; Tabeidian, S.; Toghyani, M.; Ghalamkari, G. and Toghyani, M. 2011. Effect of turmeric powder on performance, carcass traits, humoral immune responses, and serum metabolites in broiler chickens. Journal of Animal and Feed Sciences, 20,

NRC, 1994. Nutrition Requirements of Poultry, (National Academy Press, Washington, DC)

Pandit, R.S.; Gaikwad, S.C.; Agarkar, G.A.; Gade, A.K. and Rai, M. 2015. Curcumin nanoparticles: physico-chemical fabrication and its in vitro efficacy against human pathogens. Biotech, 5: 991-997

Platel, K. and Srinivasan, K. 1996. Influence of dietary spices or their active principles on digestive enzymes of small intestinal mucosa in rats. International journal of food sciences and nutrition, 47: 55-59

Platel, K. and Srinivasan, K. 2000. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Food/Nahrung, 44: 42-46

Pornanek, P. and Phoemchalard, C. 2019. Feed added curcumin with increased solubility on plasma lipoprotein, meat quality, and fat content in broiler chicks. Tropical animal health and production, 1-6

Prasad, S.; Tyagi, A.K. and Aggarwal, B.B. 2014. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. Cancer research and treatment, 46: 2-18

Rahmani, M.; Golian, A.; Kermanshahi, H. and Bassami, M.R. 2017. Effects of curcumin and nanocurcumin on growth performance, blood gas indices and ascites mortalities of broiler chickens reared under normal and cold stress conditions. Italian Journal of Animal Science, 16: 438-446

Rahmani, M.; Golian, A.; Kermanshahi, H. and Reza Bassami, M. 2018. Effects of curcumin or nanocurcumin on blood biochemical parameters, intestinal morphology and microbial population of broiler chickens reared under normal and cold stress conditions. Journal of applied animal research, 46; 200-209

Rajput, N.; Muhammah, N.; Yan, R.; Zhong, X. and Wang, T. 2012. Effect of dietary supplementation of curcumin on growth performance, intestinal morphology and nutrients utilization of broiler chicks. The Journal of Poultry Science, 50: 44-52

Rajput, N.; Naeem, M.; Ali, S.; Zhang, J.; Zhang, L. and Wang, T. 2013. The effect of dietary supplementation with the natural carotenoids curcumin and lutein on broiler pigmentation and immunity. Poultry Science, 92: 1177-1185

Seo, K.I.; Choi, M.S.; Jung, U.J.; Kim, H.J.; Yeo, J.; Jeon, S.M. and Lee, M.K. 2008. Effect of curcumin supplementation on blood glucose, plasma insulin, and glucose homeostasis related enzyme activities in diabetic db/db mice. Molecular nutrition & food research, 52: 995-1004

South, E.; Exon, J. and Hendrix, K. 1997. Dietary curcumin enhances antibody response in rats. Immunopharmacology and immunotoxicology, 19: 105-119

Viveros, A.; Chamorro, S.; Pizarro, M.; Arija, I.; Centeno, C. and Brenes, A. 2011. Effects of dietary

polyphenol-rich grape products on intestinal microflora and gut morphology in broiler chicks. Poultry Science, 90: 566-578

Xie, Z.; Shen, G.; Wang, Y. and Wu, C. 2018. Curcumin supplementation regulates lipid metabolism in broiler chickens. Poultry Science, 98: 422-429

Yarru, L.; Settivari, R.; Gowda, N.; Antoniou, E.; Ledoux, D. and Rottinghaus, G. 2009. Effects of turmeric (Curcuma longa) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. Poultry Science, 88: 2620-2627

Yu, H. and Huang, Q. 2012. Improving the oral bioavailability of curcumin using novel organogel-based nanoemulsions. Journal of agricultural and food chemistry, 60: 5373-5379

Zeng, Z.; Zhang, S.; Wang, H. and Piao, X. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of animal science and biotechnology, 6, 7

الملخص العربى

تأثير الاضافة الغذائية للكركمين وجزيئات الكركمين النانوية على أداء النمو و الاستجابة المناعية ومضادات الأكسدة لبداري التسمين

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تم اجراء هذه التجربه بوحدة بحوث انتاج الدواجن بقسم التطبيقات البيولوجية – مركز البحوث النووية – هيئة الطاقة الذرية بهدف دراسه تاثير الاضافة الغذائية للكركمين وجزيئات الكركمين النانوية على اداء النمو وصورة الدهون و حالة مضادات الاكسده والاستجابة المناعية المنسابة لبداري التسمين. تم استخدام عدد 504 كتكوت تسيمين عمر يوم من سلالة روس-308 وتم توزيعهم عشوائيا على 7 مجاميع تجريبه (كل منها مقسمة الي 8 مكررات يضم كل مكرر 9 كتاكيت) كالتالى: الاولى تعتبر مجموعة الكنترول و تغذت على عليقة اساسية بدون اضافات وتغذت المجموعة الثانيه و الثالثة والرابعة على العليقة الاساسية مضاف اليها 25 و 50 و 100 مجم/كم عليقة من الكركمين في حين تغذت باقي المجاميع على نفس العليقة السابقة مضاف اليها نفس الجرعات السابقة ولكن من النانوكركمين. اوضحت النتائج ان صفات وزن الجسم و وزن الجسم المكتسب و معامل التحويل الغذائي قد تحسنوا بصوره معنويه في المجاميع المعامله وخاصه المجاميع المغذاه على مستويات 50 و 100 مجم/ كجم عليقه من الكركمين والنانوكركمين بينما انخفض استهلاك العليقة. كما ادي استخدام جرعات 50 و 100 مجم/ كجم عليقه من الكركمين والنانوكركمين الي زيادة في الوزن النسبي لكل من للكبد والبيرسا والغدة التيموسيه. واوضحت النتائج وضوح التاثير الخافض لليبيدات للكركمين والنانو كركمين حيث لوحظ انخفاض في مستوي الدم من الكولستيرول والجلسريدات الثلاثية و LDL في حين زادت قيمه ال HDL تقريبا في جميع المجاميع المعامله خاصة مستويات 50 و 100 مجم/ كجم عليقه من الكركمين والنانوكركمين. كذلك لوحظ تحسن في الاستجابه المناعية المنسابة حيث لوحظ زيادة تتر الاجسام المضادة الكليه ضد كرات الدم الحمراء للغنم وكذلك مستوي الجلوبيولينات المنعايه من النوعين M و G. كما لوحظ ارتفاع في قيم SOD و GPx و MDA في السيرم وخاصه في المجاميع المغذاه على مستويات 50 و 100 مجم/كجم من كل من الكركمين و النانوكركمين. **و توصي الدراسة باضافة الكركمين والنانو كركمين بمستويات 50 أو 100 مجم/كجم عليقة لتحسين اداء النمو و حالة مضادات الاكسدة والاستجابة المناعية لبداري التسمين.**