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Effects of curcumin or nanocurcumin on blood biochemical parameters, intestinal morphology and microbial population of broiler chickens reared under normal and cold stress conditions

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ABSTRACT

This study was conducted to evaluate the effect of *curcumin*/*nanocurcumin* on blood parameters, intestinal morphology and microbial population in broiler chickens reared under normal and cold stress conditions. The experiment was designed with two identical houses; each consisted of five diets with 5 replicates of 10 birds each. The diets were (1) control; (2) and (3) Control + 200 or 400 mg/kg *curcumin*; (4) and (5) Control + 200 or 400 mg/kg *nanocurcumin*, respectively. Birds in both houses were reared under commercial temperatures until day 14. The temperature in the first house was maintained according to the commercial practices, whereas the temperature in the second house dropped to 15°C on day 14 and maintained between 13–15°C until day 42. Total weight gain was decreased, but plasma malondialdehyde (MDA), liver enzymes activities and heterophils/lymphocytes ratio were increased in cold-stressed birds compared to those that grew in normal temperature. Supplementation of *curcumin*/*nanocurcumin* in diet improved the weight gain and villus surface area of birds in concomitance to lower their plasma MDA, liver enzymes activities, caecal *E. coli* population compared to those fed control diet. It is concluded that the addition of 200 mg/kg *curcumin*/*nanocurcumin* to diet may improve the performance, antioxidant status and jejunal tissue health in broiler chickens.

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KEYWORDS
Curcumin; *nanocurcumin*; blood biochemical parameter; intestinal morphology; chickens

1. Introduction

Birds may suffer a variety of environmental stress such as cold stress, which affect animal health and welfare (Tsutsayeva & Sevryukova 2001). Cold stress is related to disease and tissue damage (Dhanalakshmi et al. 2007). Thermogenic mechanisms are based on the increased secretion of thyroid hormone and increased metabolic rate in birds (Guo et al. 2007). Increased metabolic rates lead to increased tissue requirements for oxygen (Currie 1999). An imbalance between oxygen demand and oxygen supply results in hypoxaemia (Hassanzadeh 2010). Hypoxia may increase the production of free radicals (Bottje & Wideman 1995). The increase in the *aspartate aminotransferase* and *lactate dehydrogenase* (AST/LDH) activities is an indicator of advanced liver cells damage followed by the increased production of free radicals (Arab et al. 2006). Antioxidants have a major role in protecting cells from the actions of reactive oxygen species (ROS) by reducing chemical radicals and disrupting the process of lipid peroxidation. Lipid peroxidation is an important feature of cellular damage due to free radicals attack (Ahmad et al. 2012). Using synthetic antioxidants, *butylated hydroxyl anisole* (BHA) and *butylated hydroxyl toluene* (BHT), have been restricted long ago, because of their possible carcinogenicity causing liver swelling and changing liver enzyme activities (Martin & Gilbert 1968). Recently, *curcumin*, the yellow pigment of *turmeric,* is considered. *Curcumin* is a compound that has a wide range of therapeutic activities including antioxidant (Sandur et al. 2007), free radical scavenging (Zhang et al. 2014), protection of biological membranes against peroxidative damage (Priyadarsini 1997), increased immuno function (Cleary 2004), antiviral and antibacterial (Singh et al. 2010) and *hypolipidemic* effect in rat (Rao et al. 1970). Poor bioavailability of *curcumin* due to low absorption, fast metabolism and fast systemic elimination from the body (Anand et al. 2007) has been a major issue. One of the suggestions to improve the bioavailability of *curcumin* is the use of *curcumin* nanoparticles which increases the oral absorption of *curcumin* (Nabavi et al. 2014). It has been demonstrated that *curcumin* in the form of nanoparticles (micellar *curcumin*) increased the in vivo bioavailability and tissue distribution and gave a 60-fold higher biological half-life (Ma et al. 2007) compared with the native *curcumin* treatment in a rat model. This study was conducted to compare the effect of *curcumin*/*nanocurcumin* on blood biochemical parameters, intestinal morphology and microbial population in broiler chickens reared under normal and cold stress conditions.

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2. Materials and methods

2.1. Birds, diets and housing

This experiment was conducted as a split plot, using two identical houses, each with 250 day-old Ross 308 male broiler chicks, allocated to 25 pens (mean body weight 43 ± 1.2) with five replicates of 10 birds each and fed five different diets. A mash-based corn-soybean meal diet for starter (1–10), grower (11–24) and finisher (25–42) periods was formulated according to Ross 308 nutrient recommendations (Aviagen 2014; Table 1). *Curcumin*/*nanocurcumin* was also added to the basal diet of each period to prepare five dietary treatments including (I) control (zero level of *curcumin*/*nanocurcumin*); (II) 200 mg/kg *curcumin*; (III) 400 mg/kg *curcumin*; (IV) 200 mg/kg *nanocurcumin* and (V) 400 mg/kg *nanocurcumin*. Diets and water were provided *ad libitum* throughout the experiment. The lighting programme was 23 h light and 1 h dark from day one to the end of the experiment. The temperature of both houses were set at 32°C for the first week and then reduced to 29°C in the second week. The temperature of one house was gradually decreased by approximately 0.5°C every other day from day 14 until it reached 22°C on 28 days of age and remained constant thereafter. The temperature in the second house gradually dropped to 15°C on day 14 and maintained between 13 and 15°C thereafter. The experimental protocol was reviewed and approved by the Animal Care Committee at the Ferdowsi University of Mashhad.

Table 1. The ingredients and composition of the basal diets.[a]

	Diets		
Ingredients %	Starter (0–10 days)	Grower (11–24 days)	Finisher (25–42 days)
Corn (8% CP)	47.53	51.63	57.56
Soybean meal (44%CP)	42.35	37.99	32.35
Soybean oil (9000 kcal/kg)	5.54	6.24	6.29
Limestone (38%Ca)	1.2	1.12	1.05
Dicalcium phosphate (21%Ca)	1.79	1.56	1.34
Vitamin premix[b]	0.25	0.25	0.25
Mineral premix[c]	0.25	0.25	0.25
NaCl	0.40	0.40	0.40
dl-Methionine (99%)	0.37	0.32	0.28
Lysine (78%)	0.28	0.22	0.22
Threonine (98.5%)	0.05	0.02	0.00
Calculated values[d]			
Metabolizable energy (kcal/kg)	2990	3082	3218
Crude protein (%)	23	21.3	19.3
Calcium (Ca) (%)	0.96	0.87	0.79
Available phosphorus (%)	0.456	0.409	0.361
Sodium (Na) (%)	0.16	0.16	0.16
Methionine (%)	0.71	0.64	0.58
Methionine_+ _Cystine (%)	1.07	0.89	0.89
Lysine (%)	1.46	1.3	1.17
Arginine (%)	1.56	1.45	1.3
Threonine (%)	0.96	0.87	0.78
Tryptophan (%)	0.35	0.32	0.29

[a]0, 200 and 400 mg *curcumin* or *nanocurcumin* were added per kg of starter, grower and finisher diets to provide five dietary treatments for each period.
[b]Vitamin concentrations per kilogram of diet: retinol 18 mg, cholecalciferol 4 mg, α-tocopherol acetate 36 mg, vitamin K3 2 mg, thiamine 1.75 mg, riboflavin 6.6 mg, niacin 9.8 mg, pantothenic acid 29.65 mg, pyridoxine 2.94 mg, folic acid 1 mg, vitamin B12 0.015 mg, biotin 0.1 mg, choline chloride 250 mg and ethoxyquin 1 mg.
[c]Mineral concentrations per kilogram of diet: Mn 99.2 mg, Fe 50 mg, Zn 84.7 mg, Cu 10 mg, I 0.99 mg, Se 0.2 mg.
[d]The values were calculated from Aviagen (2014).

2.2. Curcumin and nanocurcumin

Curcumin was obtained from Sami Labs Limited, Bangalore, India, and used without any treatment. The commercially available source of *curcumin* is usually composed of 77% *curcumin*, 18% *demethoxycurcumin* and 5% *bisdemethoxycurcumin* (Basnet et al. 2010). *Nanocurcumin*, used in our experiment, was a nanomicelle containing *curcumin* and registered as curcumin product (*SinaCurcumin*®) for human oral use which has been developed in the Nanotechnology Research Center of the Mashhad University of Medical Science and marketed by Exir NanoSina Company, Tehran, Iran (IRC:1228225765). *Nanocurcumin* is prepared from GRAS (generally recognized as safe) pharmaceutical excipients and C3-complex form of curcumin. The encapsulation of *curcumin* in this nanomicelle is near 100% and the sizes are around 10 nm. *Nanocurcumin* has a significantly higher bioavailability after oral use compared to *curcumin* powder (Rahimi et al. 2015).

2.3. Data collection

2.3.1. Performance

The pen weight of birds and feed intake were recorded and the corrected feed conversion ratio (FCR) was calculated from the replicate weight gain and feed intake corrected for mortality for the whole experimental period (1–42 days).

2.3.2. Blood parameters

One bird from each replicate pen was randomly selected at 42 day of age and blood sample was taken from wing vein, into a heparinized syringe. A drop of whole blood from each sample was used to prepare the smears on microscopic slides, dried and fixed with methanol. All smears were stained with May–Grunwald and Giemsa stains (Lucas & Jamroz 1961). In each slide, 100 white blood cells (WBC) were numerated and percent of *heterophils* (H), *lymphocytes* (L) and H/L ratio was recorded. Remaining portion of blood was centrifuged and the plasma samples stored at −20°C for later analysis (Tankson et al. 2002). Plasma total cholesterol, (*high density lipoprotein* (HDL), *low density lipoprotein* (LDL) cholesterol), triglyceride, *aspartate aminotransferase* (AST) and *lactate dehydrogenase* (LDH) activities were measured using commercial diagnostic kits (BioSystems S. A. Barcelona. Spain) by using an autoanalyser (Technicon RA1000, Bayer Diagnostics, Puteaux, France). Plasma *malondialdehyde* (MDA) was determined based on colorimetric assay of *thiobarbituricacid* reactive substances as described by Rao et al. (1989).

2.3.3. Intestinal histomorphology

The selected birds were slaughtered after the withdrawal of blood samples at 42 days of age. Samples of jejunum (2-cm segments) were obtained from the mid part of the jejunum and flushed by 0.9% saline, then immersed in a formalin solution (10%) for 72 h and processed according to the method described by Iji et al. (2001) and embedded in paraffin wax.

Processed sections were cut (2 µm) using a microtome, placed on a glass slide and then stained with haematoxylin–eosin. The samples were analysed under a light microscope to determine morphometric indices. The morphometric variables including villus height (VH), crypt depth (CD), VH/CD ratio and villus width (VW) were recorded and the villus surface area (VS) was calculated using the following formula: $(2\pi) \times (VW/2) \times (VH)$ (Sakamoto et al. 2000). There were three cross-sections per sample and the mean from five villi per cross section was used as the average value for analysis.

2.3.4. Bacterial populations

The same slaughtered birds were also used for the enumeration of microbial population. Caecal contents of each bird was separately collected by gently squeezing into a tube and stored at −80°C for the enumeration of microbial population. The sample tubes were later placed at room temperature until thawed and then 1 g from each sample was homogenized in 9 mL sterile water and serial dilutions were prepared. A volume of 100 µL from the concentration of 10^{-3} and 10^{-4}, which were determined as suitable density for *E. coli* and *Lactobacilli*, respectively, was separately removed and smeared onto plates containing Macconkey agar and Man–Rogosa–Sharpe (MRS) agar medium and completely distributed to all parts of each plate. The number of *Lactobacillus* on MRS agar and *E. coli* on Macconkey agar was counted after the plates were incubated at 37°C in an anaerobic chamber for 48 h and in an aerobic chamber for 24 h, respectively (Güban et al. 2006). Bacterial population were reported as logarithmic number of bacteria per 1 g of caecal sample.

2.5. Statistical analysis

The analysis of variance was performed using the general linear models procedure of SAS software based on a split plot design (SAS Institute Inc 2004). The significant difference between treatment means was determined by the Tukey test ($p < .05$). Moreover, orthogonal contrasts were used to compare mean response variables *curcumin/nanocurcumin* vs control diet and *curcumin* vs *nanocurcumin*.

3. Results

3.1. Performance

The interaction effect of temperature and diet *curcumin/nanocurcumin* was not significant for the feed intake and FCR for the whole experimental period. Feed intake and FCR significantly were higher in cold-stressed birds than in those reared under normal temperature (107.3 vs 102.3 g/b/day and 1.87 vs 1.71, respectively) for the 42-day experimental period. Supplementation of diet with *curcumin/nanocurcumin* did not affect birds' feed intake, but *nanocurcumin* at the rate of 200 mg/kg improved ($P < .05$) the FCR of birds compared to those fed control diet (1.73 vs 1.83) (data not presented).

The interaction effect of temperature and diet was significant on weight gain for the whole experimental period. Supplementation of diet with 400 mg *curcumin* or 200 mg *nanocurcumin* per kg in cold-stressed birds significantly improved weight gain compared with that in those fed control diet during the 42-day experimental period (58.1, 59.5 vs 55.7 g/b/day). Cold-stressed birds fed 200 mg/kg *nanocurcumin* had similar weight gain compared to those fed control diet and reared under normal temperature (59.5 vs 59.7 g/b/day). The weight gain of cold-stressed birds was significantly lower than that of those reared under normal temperature (57.3 vs 59.8 g/b/day).

3.2. Malondialdehyde and liver enzymes activities

Malondialdehyde (MDA), LDH and AST activities in birds fed diets supplemented with *curcumin/nanocurcumin* and reared under normal or cold environmental conditions are shown in Table 2. Plasma MDA, LDH and AST were increased in cold-stressed birds compared to that in those reared under normal conditions ($p < .05$). Using orthogonal contrast, birds fed diets supplemented with *curcumin/nanocurcumin* had lower plasma LDH activity and MDA than those fed control diet. Plasma MDA in birds was increased when *curcumin/nanocurcumin* supplementation increased from 200 to 400 mg/kg diet ($p < .05$). Using the orthogonal contrast, there was not a significant difference between plasma MDA in birds fed diet containing either *curcumin* or *nanocurcumin*. But, LDH/AST activities in birds fed diet supplemented with *nanocurcumin* were significantly higher than that in those fed *curcumin* diet.

Table 2. Effect of dietary supplementation of *curcumin* (Cur) and *nanocurcumin* (Nano) on MDA, LDH and AST in broiler chickens grown in normal and cold temperature conditions.

		Blood parameters		
Temperature (°C)	Diet supplementation (mg/kg)	MDA (nm/ml)	LDH (U/L)	AST (U/L)
Normal	Control	2.06	2038.05	347.8
	Cur (200)	1.87	1941.57	251.41
	Cur (400)	2.08	1996.9	270.59
	Nano (200)	1.78	2039.44	259.29
	Nano (400)	2.12	2097.57	320.74
Cold	Control	2.85	2375.74	347.8
	Cur (200)	2.51	2019.82	343.55
	Cur (400)	2.48	1908.98	296.59
	Nano (200)	2.31	2112.18	315.44
	Nano (400)	2.46	2247.12	366.95
Temperature				
Normal		1.98^{b}	2022.71^{b}	277.53^{b}
Cold		2.52^{a}	2132.77^{a}	334.06^{a}
Diet supplementation (mg/kg)				
Control		2.45^{a}	2206.90^{a}	316.73^{ab}
Cur (200)		2.19^{bc}	1980.70^{b}	297.47^{bc}
Cur (400)		2.28^{ab}	1952.94^{b}	283.58^{c}
Nano (200)		2.04^{c}	2075.81^{ab}	287.36^{c}
Nano (400)		2.29^{ab}	2172.35^{ab}	343.84^{a}
SEM		0.06	26.55	6.07
			p-value	
Temperature × Diet		.075	.065	.05
Temperature		.013	.032	.002
Diet		.001	.008	.001
Orthogonal contrasts:				
Control vs Cur/Nano		.001	.006	.099
Cur vs Nano		.478	.003	.002

[a-b]Means in the same column for each effect with no common superscript are significantly different.

3.3. Blood cholesterol

The effect of *curcumin*/*nanocurcumin* supplemented diet on *total cholesterol* (Tchol), HDL, LDL and *triglycerides* (TG) in plasma of birds reared in cold-stressed or normal environmental temperature is shown in Table 3. Cold stress increased Tchol, HDL, LDL and TG in the plasma of birds compared with that in those reared in normal environmental conditions ($p < .05$). Using orthogonal contrasts, the supplementation of diet with *curcumin*/*nanocurcumin* decreased Tchol, LDL and TG in the plasma of birds ($p < .05$) compared to that in those fed control diet. The amount of Tchol, HDL, LDL and TG in the plasma of birds fed diet containing *nanocurcumin* was significantly higher than that in those fed *curcumin* diet. Birds fed *curcumin* diet and exposed to cold stress had similar LDL compared to those fed control diet and grew under normal environmental temperature.

3.4. Immuno function

The effect of dietary *curcumin*/*nanocurcumin* supplementation on the number of WBC, H, L and H/L ratio in birds exposed to cold stress or normal temperature conditions is shown in Table 4. Cold stress significantly decreased the number of WBC, L and increased the number of H and H/L ratio compared to that of those reared at normal environmental temperature ($p < .05$). Using orthogonal contrasts, the supplementation of diet with *curcumin*/*nanocurcumin* significantly increased the number of WBC, L and decreased the number of H and H/L ratio in birds compared to that in those fed control diet ($p < .05$). The number of H and H/L ratio in birds fed diets supplemented with *nanocurcumin* was significantly higher than that in those fed *curcumin*-supplemented diet. Birds fed *curcumin* diet and exposed to cold stress had similar L and H/L ratio compared to those fed control diet and grew under normal environmental temperature.

3.5. Intestinal histomorphology

The effect of *curcumin*/*nanocurcumin* supplementation of diet on jejunal histomorphology of birds reared in normal or cold environmental conditions is shown in Table 5. Cold stress significantly decreased VH, VW and VS (figure 1). But there was no significant difference between CD or VH/CD in birds reared in normal condition compared to those reared in cold stress. Using orthogonal contrasts, VH, VH/CD and VS in birds fed *curcumin*/*nanocurcumin* supplementation were higher than that in those fed control diet. There was no significant difference between jejunal histomorphological parameters in birds fed diet containing either *curcumin* or *nanocurcumin*.

3.6. Bacterial populations

Caecal microbial population in birds fed diet supplemented with *curcumin*/*nanocurcumin* and reared in normal or cold environmental condition is shown in Table 6. Caecal *E. coli* in cold-stressed birds was higher than that in those reared in normal temperature condition ($p < .05$). However, the cold stress significantly decreased the caecal *lactobacilli* count. The

Table 3. Effect of dietary supplementation of *curcumin* (Cur) and *nanocurcumin* (Nano) on Tchol, HDL, LDL and TG in broiler chickens grown in normal and cold temperature conditions.

		Blood parameters			
Temperature (°C)	Diet supplementation	Tchol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
	Control	110.03^{c}	83.35^{e}	23.19^{c}	45.27^{de}
Normal	Cur (200)	94.202^{e}	86.17^{ed}	17.71^{e}	35.34^{g}
	Cur (400)	103.22^{d}	89.72^{d}	21.24^{d}	42.17^{ef}
	Nano (200)	108.94^{c}	107.40^{b}	19.96^{d}	40.52^{f}
	Nano (400)	111.67^{c}	96.80^{c}	20.37^{d}	46.26^{d}
	Control	135.32^{a}	127.37^{a}	26.08^{a}	71.52^{a}
Cold	Cur (200)	128.83^{b}	132.45^{a}	20.48^{d}	56.88^{c}
	Cur (400)	131.36^{ab}	132.28^{a}	24.28^{bc}	68.43^{a}
	Nano (200)	130.53^{ab}	129.76^{a}	26.48^{ab}	60.39^{bc}
	Nano (400)	133.78^{ab}	130.08^{a}	25.10^{ab}	61.67^{b}
Temperature					
Normal		105.61^{b}	92.68^{b}	20.49^{b}	41.91^{b}
Cold		131.96^{a}	130.38^{a}	24.32^{a}	63.78^{a}
Diet supplementation (mg/kg)					
Control		122.67^{a}	105.36^{d}	24.63^{a}	58.40^{a}
Cur (200)		111.51^{c}	109.31^{c}	19.09^{c}	46.11^{b}
Cur (400)		117.29^{b}	111.00^{bc}	22.76^{b}	55.30^{b}
Nano (200)		119.74^{ab}	118.58^{a}	22.82^{b}	50.45^{c}
Nano (400)		122.72^{a}	113.44^{b}	22.73^{b}	53.96^{b}
SEM		2.03	2.85	0.399	1.71
			p-value		
Temperature × Diet		.005	.001	.001	.001
Temperature		.001	.001	.001	.001
Diet		.001	.001	.001	.001
Orthogonal contrasts:					
Control vs Cur/Nano		.001	.001	.001	.001
Cur vs Nano		.001	.001	.001	.036

[a-b]Means in the same column for each effect with no common superscript are significantly different. Orthogonal contrast.

Table 4. Effect of dietary supplementation of *curcumin* (Cur) and *nanocurcumin* (Nano) on WBC, H, L and H/L in broiler chickens grown in normal and cold temperature conditions.

Temperature	Diet supplementation (mg/kg)	Blood parameters			
		WBC ($\times 10^3$/μl)	H (%)	L (%)	H/L
Normal	Control	19.3	36.98^{ab}	63.01^{bc}	0.58^{bc}
	Cur (200)	23.56	24.63^{c}	75.36^{a}	0.32^{d}
	Cur (400)	27.7	28.21^{c}	71.78^{a}	0.36^{d}
	Nano (200)	29.1	34.51^{b}	65.48^{b}	0.53^{c}
	Nano (400)	24.56	37.34^{ab}	62.65^{bc}	0.59^{abc}
Cold	Control	12.9	42.06^{a}	57.93^{c}	0.72^{a}
	Cur (200)	16.46	36.20^{ab}	63.79^{bc}	0.56^{bc}
	Cur (400)	23.16	37.37^{ab}	62.62^{bc}	0.59^{abc}
	Nano (200)	19.26	38.84^{ab}	61.15^{bc}	0.63^{abc}
	Nano (400)	21.66	40.35^{ab}	59.64^{bc}	0.67^{ab}
Temperature					
Normal		24.84^{a}	32.34^{b}	67.66^{a}	0.48^{b}
Cold		18.69^{b}	38.96^{a}	61.03^{b}	0.64^{a}
Diet supplementation (mg/kg)					
Control		16.10^{b}	39.52^{a}	60.47^{b}	0.65^{a}
Cur (200)		20.01^{ab}	30.41^{b}	69.58^{a}	0.44^{b}
Cur (400)		25.43^{a}	32.79^{b}	67.20^{a}	0.47^{b}
Nano (200)		24.18^{a}	36.68^{a}	63.31^{b}	0.58^{a}
Nano (400)		23.11^{a}	38.85^{a}	61.15^{b}	0.63^{a}
SEM		0.96	0.99	0.99	0.02
				p-value	
Temperature × Diet		.04	.007	.007	.321
Temperature		.021	.028	.028	.009
Diet		.001	.002	.002	.003
Orthogonal contrasts:					
Control vs Cur/Nano		.003	.001	.001	.005
Cur vs Nano		.001	.001	.001	.432

[a-b]Means in the same column for each effect with no common superscript are significantly different. Orthogonal contrast.

Table 5. Effect of dietary supplementation of *curcumin* (Cur) and *nanocurcumin* (Nano) on VH, CD, VH/CD, VW and VS in broiler chickens grown in normal and cold temperature conditions.

Temperature (°C)	Diet supplementation (mg/kg)	Morphological Parameters				
		VH (μm)	CD (μm)	VH/CD	VW (μm)	VS (mm^2)
Normal	Control	1242^{ab}	248	5.26	286	0.57
	Cur (200)	1684^{ab}	278	6.09	300	0.81
	Cur (400)	1634^{ab}	172	9.51	304	0.78
	Nano (200)	1724^{ab}	204	8.48	310	0.87
	Nano (400)	1886^{a}	228	8.91	230	0.66
Cold	Control	1152^{b}	210	5.9	212	0.37
	Cur (200)	1064^{b}	206	5.23	230	0.38
	Cur (400)	1526^{ab}	192	9.41	260	0.61
	Nano (200)	1626^{ab}	188	8.84	208	0.53
	Nano (400)	1152^{b}	200	6.19	294	0.54
Temperature						
Normal		1634^{a}	226	7.65	286^{a}	0.74^{a}
Cold		1304^{b}	199	7.11	240^{b}	0.48^{b}
Diet supplementation (mg/kg)						
Control		1197	229	5.58	249	0.47^{b}
Cur (200)		1374	242	5.66	265	0.59^{ab}
Cur (400)		1580	182	9.46	282	0.69^{a}
Nano (200)		1675	196	8.66	259	0.70^{a}
Nano (400)		1519	214	7.55	262	0.60^{ab}
SEM		58.39	6.81	0.434	12.21	0.027
				p-value		
Temperature × Diet		.035	.181	.625	.33	.114
Temperature		.008	.116	.445	.014	.001
Diet		.189	.052	.076	.886	.001
Orthogonal contrasts:						
Control vs Cur/Nano		.003	.162	.026	.584	.002
Cur vs Nano		.187	.584	.516	.658	.906

[a-b]Means in the same column for each effect with no common superscript are significantly different.

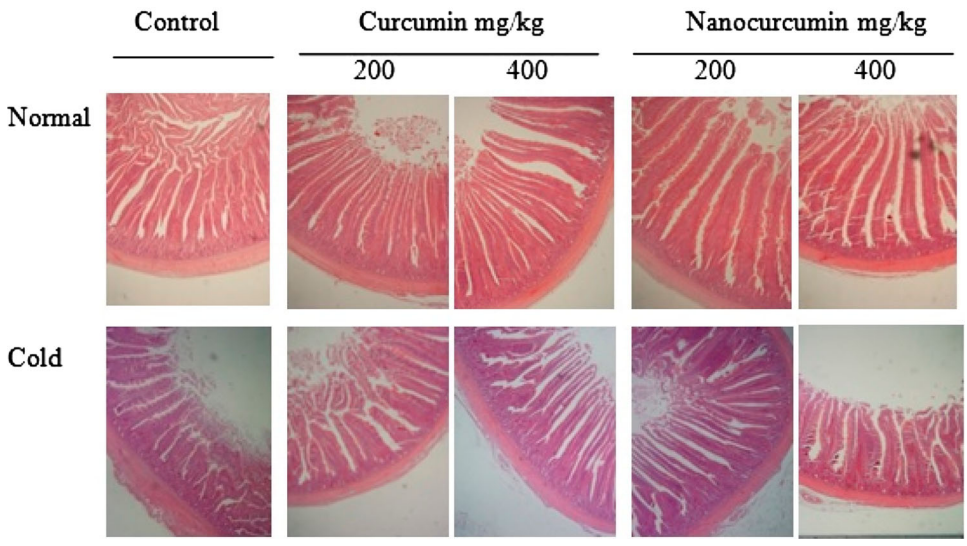

Figure 1. The effect of curcumin/nanocurcumin supplementation of diet on jejunal histomorphology of birds reared in normal or cold environmental conditions at day 42. The temperature of both houses were the same in the first two weeks; the temperature of one house was continued according to the recommended temperature, whereas the temperature in the second house was dropped to 15°C on day 14 and maintained between 13–15°C until day 42.

Table 6. Effect of dietary supplementation of *curcumin* (Cur) and *nanocurcumin* (Nano) on caecal bacterial population *E. coli* and *lactobacilli* in broiler chickens grown in normal and cold temperature conditions.

Temperature (°C)	Diet supplementation (mg/kg)	Caecal bacterial population	
		Lactobacilli Log CFU/(g. DM)[1]	*E. coli*
	Control	5.69^{d}	4.52^{cd}
Normal	Cur (200)	6.22^{ab}	4.86^{bc}
	Cur (400)	6.07^{d}	4.98^{b}
	Nano (200)	6.15^{ab}	4.78^{bcd}
	Nano (200)	6.17^{ab}	4.70^{bcd}
	Control	5.53^{dc}	5.56^{a}
Cold	Cur (200)	5.44^{d}	5.07^{b}
	Cur (400)	5.36^{d}	4.69^{bcd}
	Nano (200)	6.44^{a}	4.92^{b}
	Nano (400)	6.01^{bc}	4.44^{d}
Temperature			
Normal		6.03^{a}	4.77
Cold		5.79^{b}	4.94
Diet supplementation (mg/kg)			
Control		5.61^{c}	5.04^{a}
Cur (200)		5.83^{bc}	4.96^{a}
Cur (200)		5.72^{c}	4.84^{ab}
Nano (200)		6.30^{a}	4.85^{ab}
Nano (400)		6.09^{ab}	4.57^{b}
SEM		0.06	0.05
		p-value	
Temperature × Diet		.001	.001
Temperature		.104	.017
Diet		.005	.002
Orthogonal contrasts:			
Control vs Cur/Nano		.001	.001
Cur vs Nano		.001	.003

[a-b]Means in the same column for each effect with no common superscript are significantly different.
[1]CFU: Colony Forming Units.

use orthogonal contrast indicated that the number of *E. coli* and *lactobacilli* in birds fed *curcumin*-supplemented diet was significantly lower and higher than that in those fed control diet, respectively. *Nanocurcumin* supplementation decreased the number of *E. coli* and increased *lactobacilli* count as compared to those fed diet containing *curcumin* ($p < .05$).

4. Discussion

Supplementation of diet with *curcumin/nanocurcumin* increased weight gain in birds compared to those fed control diet and reduced the negative effect of cold stress on their performance. The positive effect of *curcumin* on growth performance and FCR of broiler chickens was also reported by others (Ahmadi et al. 2010; Nouzarian et al. 2011). *Curcumin* by improving the uptake of digested nutrients (Hernandez et al. 2004) results in better feed efficiency and growth performance. Under normal temperature, the increase in dietary *curcumin* from 200 to 400 mg/kg caused a significant reduction in the growth of birds during the whole experimental period (61.35 vs 58.83 g/b/day, respectively). But, the growth performance of cold-stressed birds fed diet containing 400 mg/kg *curcumin* was better than that of those fed 200 mg/kg *curcumin* (58.11 vs 57.76 g/b/day, respectively). It is proposed that the practical level of *curcumin* used for birds at normal temperature may not be adequate for cold-stressed birds. The negative effects of cold stress on birds' performance, as reported by others (Balog et al. 2003; Luger et al. 2001), may be due to the increase in metabolic rate to use more nutrients for the heat production to maintain body temperature (Ipek & Sahan, 2006). Therefore, cold stress causes an increase in oxygen requirements (up to 185%) (Gleeson 1986) and oxygen shortage may induce cellular hypoxia and increase the production of free radicals (Bottje & Wideman 1995) and subsequently increase tissue requirement of antioxidants in cold-stressed birds. The performance of cold-stressed birds fed diet containing 200 mg/kg of *nanocurcumin* was similar to that of those on diet with 400 mg/kg *curcumin* (59.47 vs 58.11 g/b/day, respectively), which may be due to more absorption or higher bioavailability of *nanocurcumin*. There is a water layer on the surface of intestinal epithelial cells and thus all types of substances should pass this barrier (Smithson et al. 1981). In fact, *curcumin* in the form of simple powder is lipophilic and thus insoluble in the water layer, whereas the

micellization form is hydrophilic and can easily be solved in the water layer.

Due to the presence of polyunsaturated fatty acids, the membrane lipids are highly sensitive to oxidative damage (lipid peroxidation) (Halliwell & Gutteridge 1985) and measuring the oxidative damage in cell membrane lipids is one of the best ways to measure the effects of free radicals (Lykkesfeldt & Svendsen 2007) produced by oxidative reactions. The involvement of oxidative stress in ascites in broilers has been reported (Bottje et al. 1995; Bottje & Wideman 1995). Cold stress-increased plasma MDA was similar to the results reported by Fathi et al. (2011). In our study, the high level of MDA in cold-stressed birds showed higher risk of ascites through oxidative damage. Similar to our results, Suvanated et al. (2003) reported that the use of *turmeric* in diet reduced TBA (*thiobarbituric acid*) indicator and improved antioxidant enzyme activity in broiler chickens compared with those fed control diet. Phenolic groups in the structure of *curcumin* have an important role in the prevention of lipid peroxidation. These groups can remove hydroxyl radical, superoxide ion and nitric oxides (Sreejayan & Rao 1996). *Curcumin* can reduce lipid peroxidation by the increase in antioxidants enzyme activity including glutathione peroxidase, superoxide dismutase or Catalase (Reddy & Lokesh 1996). But plasma MDA in birds was significantly increased when *curcumin*/*nanocurcumin* supplementation was increased from 200 to 400 mg/kg in diet. Donatus et al. (1990) reported that low concentration of *curcumin* can protect the liver against lipid peroxidation induced with paracetamol. But lipid peroxidation was increased when the concentration of used *curcumin* was increased to 100-fold because antioxidants may have the role of peroxidants depending on the dosage (Halliwell 2000). Therefore, the usage of high dose of *curcumin*/*nanocurcumin* results in induced lipid peroxidation and increased MDA.

Similarly, the effect of cold stress on increased AST/LDH activities in birds was reported by Fathi et al. (2011). Increased activities of AST/LDH is an indicator of advanced liver cells damage followed by increased production of free radicals resulting in the oxidative reactions chain in the liver and other organs (Arab et al. 2006). Increased production of free radicals during tissue hypoxia (Chen & Meyrick 2004) can negatively affect the activity of energy synthesis. In an anaerobic condition, LDH contributes to energy synthesis by anaerobic *glycolysis*. Hence, increased activity of LDH in cold-stressed birds is probably due to oxidative stress. On the contrary, dietary supplementation with *turmeric* increased AST and LDH activities in broiler chickens (Emadi & Kermanshahi 2007). Decreasing effects of *curcumin* on liver TBA indicated that *curcumin* can protect liver cells against free radicals attack through scavenging or neutralized free radicals (Akila et al. 1998). Therefore, decreased liver enzymes activities in this study may indicate improved liver function following the antioxidant effects of *curcumin* in the prevention of liver damage due to free radicals. In the present study, birds fed diet supplemented with *nanocurcumin* had higher liver enzymes activities (AST/LDH) than those fed *curcumin*-supplemented diet. It has been reported that *curcumin* in the form of nanoparticles (*micellarcurcumin*) has higher bioavailability, tissue distribution and biological half-life (Ma et al. 2007) than native *curcumin*. The high level of used *nanocurcumin* and its role as peroxidants (Halliwell 2000) result in induced liver cell injury and increased liver enzyme activities.

The effect of cold stress on increased plasma *cholesterol* in our study was in agreement with the results of Houshmand et al. (2012) who reported that stressors increased plasma cholesterol concentrations in broiler chickens. We observed that the supplementation of diet with *curcumin* significantly decreased Tchol, LDL and TG and increased HDL in the plasma of birds compared to that in those fed control diet. Different results on the effect of *curcumin* on Tchol/TG were reported by others, including a decrease in TG, Tchol and LDL in rat serum (Kim & Kim 2010), a decrease in liver TG without any effect on Tchol observed by the addition of 0.2% *curcumin* to diet (Manjunatha & srinivasan 2006), a decrease in TG with no effect on Tchol and LDL/HDL cholesterol (Nouzarian et al. 2011) or no change in Tchol, LDL and TG (Mehala & Moorthy 2008). The inconsistent results of *turmeric* on Tchol or TG may be attributed to the difference in the amount of *curcumin* in the used *turmeric* powder or the amount of *curcumin* may vary a lot between the various experiments. The conversion of *cholesterol* to bile acids in the liver is an important way to remove *cholesterol* from the body (Zhang et al. 2009). This conversion is a multistep process for which *cholesterol-7-alpha-hydroxylase* is the first stage in limiting the reaction rate. Because the animals fed diet supplemented with *curcumin* may have higher *cholesterol-7-alpha-hydroxylase* activity (Srinivasan & Sambaiah 1991), therefore decreasing the effects of *curcumin* on *cholesterol* may be through stimulating liver *cholesterol-7-hydroxylase* activity. However, the reducing effect of *curcumin* on TG may be due to *curcumin* binding with TG or through the disruption in the lipid micellation, absorption and finally their disposal (Kim & Kim 2010). It is proposed that if arterial LDL oxidation causes *atherosclerosis*, then co-antioxidants may be *anti-atherosclerotic* (Witting et al. 1999). It is reported that *turmeric* extract reduced the susceptibility of LDL to oxidation in rabbits (Ramírez-Tortosa et al. 1999). *Curcumin*, which has antioxidant effects in addition to *anti-hypercholestromic* effects, can prevent atherosclerosis by protecting LDL from oxidation. In our study, the amount of Tchol, TG and LDL in the plasma of birds fed diet supplemented with *nanocurcumin* was significantly higher than that in those fed *curcumin*. Ramírez-Tortosa et al. (1999) reported that rabbits fed with 1.6 mg/kg body weight of *turmeric* extract had lower LDL than the control group. But rabbits fed with 3.2 mg/kg body weight of turmeric extract (two fold) had higher TG/LDL than those fed with 1.6 mg/kg or the control group.

In the present study, H/L ratio significantly increased in cold-stressed birds. Under stress condition, the amount of *corticosterone* secretion increases from the adrenal glands, and is known to have a negative correlation between plasma *corticosterone* levels and lymphocyte proliferation (Hangalapura et al. 2004). But antioxidants prevent the proliferation of H, which are strong phagocytic cells, by a decrease in oxidative stress (Surai 2002). Similarly, the positive effect of *curcumin* in decreasing the number of H or H/L ratio was reported by Kijparkorn and Angkanaporn (2003). *Curcumin* increases the number of B and T *lymphocytes* and antibody production (Surh 1999; Gautam et al.

2007). Increased B and T cells in the intestine of rats fed with *curcumin* (1 g/kg) indicate that *curcumin* can improve immune function through *lymphocytes* proliferation (Churchill et al. 2000). The H/L ratio in birds fed diet supplemented with *nanocurcumin* was significantly higher than that in those fed *curcumin*. This may be related to the peroxidant activity of *curcumin* through phenoxyl radical production by the system of hydrogen peroxide peroxidase due to the oxidation of cellular glutathione or NADH and participation in the conversion of oxygen to ROS (Galati et al. 2002) which exacerbates the effect of stress on birds and increases H/L ratio.

Cold stress had a negative effect on jejunal histomorphological parameters (Figure 1). Stress condition increases the *corticosterone* secretion and thus dietary *corticosterone* supplementation (30 mg/kg diet) decreased the proliferation of jejunal epithelial cells and then decreased VH (Hu & Guo 2008). Our results showed that *curcumin*/*nanocurcumin* supplementation improved jejunal VH and VS. Similarly, the positive effect of *curcumin* on jejunal morphology was observed by Rajput et al. (2013) who reported that VH and VH/CD were improved in birds fed diet supplemented with *curcumin* (150 or 200 mg/kg) at 21 day of age compared to that in those fed 100 mg/kg or control diet. The epithelial cells of the intestine originate in the crypt and migrate to the villus tip (Potten 1998). The effect of *curcumin* on increased VH may be due to increased intestinal epithelial cell turnover, which is associated with activated cell mitosis (Samanya & Yamauchi 2002), and then longer villus, which can provide more VS and enhance nutrient absorption (Awad et al. 2008), may improve broiler chickens performance. In our study, there was no significant difference between the effect of *curcumin*/*nanocurcumin* on jejunal morphology parameters.

The number of *E. coli* in the caecum of cold-stressed birds was higher than that in those under normal condition. However, cold stress significantly decreased the number of *lactobacilli*. In stress condition, profitable microflora, especially *lactobacilli*, tends to decrease and harmful species have a tendency to increase. This phenomenon may reduce production parameters such as growth and feed efficiency (Fuller 2001). Dietary *curcumin* significantly decreased *E. coli* population in the caeca. *Curcumin* can increase bacterial cell membrane permeability. Thus, damage to the bacterial membrane is a key mechanism of *curcumin* in killing *E. coli* (Tyagi et al. 2015). Increased lactobacilli count in the caeca of birds fed diet supplemented with *curcumin*/*nanocurcumin* was observed in the present study. Viveros et al. (2011) reported that the effect of polyphenol compounds on the growth of some bacteria may be due to the ability of these bacteria to use phenolic compounds as food substrate. *Lactobacilli* can provide their energy requirements through the metabolism of phenolic compounds (Garcia-Ruiz et al. 2008). The effects of *nanocurcumin* on decreased *E. coli* population and increased lactobacilli population were higher than that of *curcumin* in our study. The higher activity of *nanocurcumin* compared to *curcumin* could be related to its particle size. The smaller particle size of *nanocurcumin* (2–40 nm) compared to *curcumin* (500–800 nm) is probably responsible for the better permeability and higher absorption into bacteria (Bhawana et al. 2011).

5. Conclusion

There was a beneficial effect of dietary supplementation of 200 mg/kg *curcumin*/*nanocurcumin* on chickens' growth performance at normal temperature. Unlike *curcumin*, cold-stressed birds fed 200 mg/kg *nanocurcumin* had similar weight gain compared to those fed control diet and reared under normal temperature (59.5 vs 59.7 g/b/day). Also, it is revealed that dietary *curcumin* supplementation reduced plasma MDA, Tchol and TG and improved liver enzyme activities, immune system, jejunal morphology and microbial population. The effect of *nanocurcumin* on different measured parameters was similar or lower than that of *curcumin* with the exception of microbial population, which might be due to the high dose of *nanocurcumin* used in our study. Future studies with less amount of *nanocurcumin* (<200 mg/kg) may be helpful to point out the suitable level of *nanocurcumin* for the poultry industry.

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