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Effects of curcumin and nanocurcumin on growth performance, blood gas indices and ascites mortalities of broiler chickens reared under normal and cold stress conditions

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ABSTRACT

The aim of this study was to evaluate the effect of curcumin/nanocurcumin on performance, blood gases and ascites mortality in broiler chickens reared under normal and cold stress conditions. This experimental design was a split plot with 500 Ross 308 male chicks. Plots were two identical houses; each consisted of five diets (as sub-plots) with 5 replicates of 10 birds each. The diets were: (1) control; (2) control+ 200 mg/kg curcumin; (3) control+ 400 mg/kg curcumin; (4) control+ 200 mg/kg nanocurcumin; (5) control+ 400 mg/kg nanocurcumin. Birds in both houses reared under recommended temperatures until 14 d, when the temperature was dropped in one house from 28.5 to 13–15 °C and maintained at this level to induce ascites until 42 d. Whereas, in the second house the temperature was maintained according to the hybrid production guidelines. Weight gain was reduced and FCR was increased in birds reared under cold temperature compared to those in normal temperature condition. Cold stress increased blood pCO₂, HTC and decreased pO₂ and O₂ saturation ($p < .05$) in birds at 42 d of age. Birds fed diets containing 200 mg/kg curcumin/nanocurcumin had higher weight gain compared to those fed control diet ($p < .05$). Moreover, supplementation of diets with curcumin/nanocurcumin alleviated the adverse effect of cold stress as reflected by a reduction in HTC and an increased O₂ saturation at 42 d of age. It is concluded that the addition of curcumin/nanocurcumin to diet might be an effective feed additive to alleviate the adverse effect of cold stress on performance and ascites syndrome.

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Introduction

Broiler chickens have high metabolic rate because they have been genetically selected for high growth rate and feed efficiency (Julian 1993; Hassanzadeh 2010). This high metabolic rate makes broilers (especially males) highly susceptible to metabolic disorders (Druyan et al. 2008). Ascites syndrome is a prevalent cardiovascular-related metabolic disorder characterised by accumulation of fluid in the abdominal cavity and around the heart (Hassanzadeh 2010). Ascites can cause a mortality of up to 8% in broiler flocks or 20–30% in heavy flocks (Pakdel et al. 2002). It occurs at the end of the growing period, which is a very important economic loss (Pakdel et al. 2002; Druyan et al. 2008). It has been assumed that low temperatures increases the secretion of thyroid hormone and increase the conversion of T₄ to T₃ to increase the

birds' metabolic rate as they adapt to cold stress conditions (Guo et al. 2007). Increased basal metabolic rates lead to increased tissue requirements for oxygen that cannot be supplied by immature cardiopulmonary systems of modern broilers (Currie 1999). Cold stress induces the ascites by increase of oxygen requirements up to 185% (Gleeson 1986). In fact, the ascites involve an imbalance between oxygen demand and oxygen supply, which results in hypoxaemia (Hassanzadeh 2010). Hypoxemia increases pulmonary arterial pressure and then causes enlargement of the right ventricle (RV) (Wideman et al. 2010). It is suggested that the systemic hypoxia may induce cellular hypoxia and increase production of free radicals (Bottje & Wideman 1995). Oxygen-derived free radicals play an important role in the genesis of tissue damage during inflammatory reaction (Halliwell & Gutteridge 1990). It is proposed that

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ascites syndrome might be associated with oxidative stress and lipid peroxidation induced by reactive oxygen species (ROS) (Bottje & Wideman 1995). The incidence of ascites may be resulting in part from free radical generation in birds, with subsequent depletion of tissue antioxidants (Bottje et al. 1995). There are many reports that test the preventive effect of commonly used antioxidants on ascites incidence in broilers. These studies were limited to vitamins C and E, coenzyme Q10, L-carnitine, uric acid, selenium and turmeric powder (Bottje et al. 1995; Daneshyar et al. 2009). However, no information is available regarding the effect of dietary pure curcumin supplementation on ascitic birds induced by cold stress. Curcumin or diferuloylmethane is an active ingredient in the rhizome of turmeric (Rahimi & Kazemi-Oskuee 2014). Curcumin has protective effects against oxidative stress by decreasing the lipid peroxidation and developing antioxidant status, thus can be considered an effective antioxidant (Rukkumani et al. 2004). Other activities of curcumin including: protection of biological membranes against oxidative damage (Indira Priyadarsini 1997), prevention of cardiovascular disease (Lopez-Lazaro 2008) and the improvement of endogenous digestive enzymes secretion (Toghyani et al. 2011). It is also used in respiratory disorders (Anwarul et al. 2006). But, the low oral bioavailability of curcumin has been a major issue (Yu & Huang 2012). Reasons for poor bioavailability of this substance are related to low absorption, fast metabolism and fast systemic elimination in the body (Anand et al. 2007). In this regard, Wahlstrom and Blennow showed that administration of 1 g/kg curcumin orally to rat resulted in rapid excretion of 75% curcumin in the faeces along with a marginal amount in urine and blood plasma (Wahlstrom & Blennow 1978). Many scientists have focussed on the improvement of curcumin bioavailability with different strategies. One of these strategies includes the use of curcumin in the form of nanoparticles which increases oral absorption of curcumin (Hani & Shivakumar 2014). Nanoformulations increased solubilisation of curcumin and protect curcumin against inactivation by hydrolysis (Kurita & Makino 2013). It has been demonstrated that curcumin in the form of nanoparticles (micellar curcumin) increased *in vivo* bioavailability and tissue distribution (Ma et al. 2007), and increased the half-life of curcumin and higher amount of it was found to be distributed in lung and brain (Song et al. 2011) compared with the native curcumin treatment in rat models. This study was conducted to compare the effect of curcumin/nanocurcumin on performance, blood parameters and

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

	Diets		
	Starter (0–10 d)	Grower (11–24 d)	Finisher (25–42 d)
Ingredients, %			
Maize, 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	1.20	1.12	1.05
Di-calcium 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			
Metabolisable 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 + cysteine, %	1.07	0.89	0.89
Lysine, %	1.46	1.30	1.17
Arginine, %	1.56	1.45	1.30
Threonine, %	0.96	0.87	0.78
Thryptophan, %	0.35	0.32	0.29

^a0, 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.

^bVitamin 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.

^cMineral 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.

^dThe values were calculated from Aviagen (2014).

ascites mortality of broiler chickens reared under normal and cold stress conditions.

Materials and methods

Birds, diets and housing

This experiment was conducted in two identical houses in the poultry research station at the Ferdowsi University of Mashhad, Iran. The experiment was conducted as a split plot. The plots were the two identical houses, in each, 250 d-old Ross 308 male broiler chicks were equally allocated to 25 pens (similar mean body weight 43 ± 1.2) and randomly assigned to five different diets, with five replicates. 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 and nanocurcumin were added to the basal diet to prepare the 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; (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 on day one and then reduce half degree Celsius every other day to reach 28.5 °C on day 14. The temperature of one house was gradually decreased at the same trend to reach 22 °C by day 28 and remained constant thereafter. The temperature in the 2nd house gradually dropped to 15 °C on day 14 and maintained between 13 °C and 15 °C thereafter, to induce ascites. The experimental protocol was reviewed and approved by the Animal Care Committee at the Ferdowsi University of Mashhad.

Curcumin and nanocurcumin

Curcumin was obtained from (Sami Labs Limited, Bengaluru, India) and used without any treatment. It should be noted that commercially available sources of curcumin usually is composed of 77% curcumin I, 18% curcumin II (demethoxycurcumin) and 5% curcumin III (bisdemethoxycurcumin) (Basnet et al. 2010).

Nanocurcumin, a nanomicelle containing curcumin is a registered curcumin product (SinaCurcumin[®]) for oral use which has been developed in Nanotechnology Research Center of Mashhad University of Medical Science and marketed by Exir Nano Sina Company in Tehran, Iran (IRC:1228225765). Nanocurcumin is prepared from GRAS (generally recognised as safe) pharmaceutical excipients and C3-complex form of curcumin. The percent of encapsulation of curcumin in this nano-micelle is near to 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).

Data collection

The body weight of each pen at the end of the study and feed intake on a per pen basis (in the whole experimental period) were recorded. Weight gain and feed intake were calculated for all replicate of each treatment for the whole experimental period and corrected FCR were calculated by corrected data of total weight gain and feed intake for mortality for all replicate of each treatment for the whole experimental period. Ascites mortalities were recorded daily as recognised by the accumulation of fluid in the abdominal cavity, pericardium and RV/TV ratio of equal or larger than 0.25 throughout the study (Varmaghany et al. 2013). One bird from each replicate pen was randomly selected at 14, 28 and 42 d of age and blood sample

was taken from wing vein into a heparinised syringe, kept in ice, and analysed within two hours by pH/ Blood Gas Analyzer (ABL 50, Radiometere, Copenhagen, Denmark). Five chickens from each replicate (pen) were randomly selected, weighed, slaughtered and the eviscerated carcass, heart, spleen, bursa, were weighed, and the right ventricle and total ventricles from each heart were also weighed to determine the ascites index at the end of the experiment (42 d).

Statistical analysis

The analysis of variance was performed using the MIXED procedure of SAS software (SAS Institute 2004) based on a split plot design with temperature as main plot and diet as sub-plot. All data were tested for normality and the significant difference between treatment means were determined by Tukey's test ($p < .05$). Moreover, orthogonal contrasts were used to compare mean response variables to curcumin/nanocurcumin vs control diet and curcumin vs nanocurcumin.

Results

Growth performance

The effect of dietary curcumin/nanocurcumin supplementation on performance of birds reared under normal or cold temperature conditions is shown in Table 2.

Birds fed control diet and reared under cold stress grew significantly less than their counterpart grew under normal environmental condition in the whole experimental period (2409 vs 2512 g) ($p < .05$). In general, the cold stress reduced weight gain of birds by about 4.1% during the whole experimental period. The orthogonal contrast showed that the supplementation of diet with either curcumin/nanocurcumin improved weight gain of birds ($p < .05$) compared to those fed control diet, but there was no significant difference between weight gain of birds fed diet containing curcumin compared with those fed nanocurcumin diet.

Body weight gain of birds were significantly reduced when curcumin/nanocurcumin supplementation increased from 200 to 400 mg/kg diet. In addition, the interaction effect of environmental temperature and dietary treatments was significant for weight gain. Birds fed 200 mg/kg nanocurcumin supplemented diets and exposed to cold stress had similar weight gain compared with those fed control diet and grew under normal environmental temperature.

Cold stress significantly increased feed intake of birds compared with those reared in normal

Table 2. Effect of dietary supplementation of curcumin (Cur) and nanocurcumin (Nano) on performance in broiler chickens grown in normal and cold temperature conditions at 0–42 day of age.

	Diet supplementation, mg/kg	Weight gain, g/b	Feed intake, g/b	Feed conversion ratio
Normal temperature	Control	2508 ^{ab}	4347	1.73
	Cur (200)	2577 ^a	4318	1.67
	Cur (400)	2471 ^{bc}	4333	1.75
	Nano (200)	2542 ^{ab}	4196	1.65
	Nano (400)	2461 ^{bc}	4280	1.73
Cold temperature	Control	2340 ^d	4537	1.93
	Cur (200)	2426 ^{cd}	4492	1.85
	Cur (400)	2441 ^c	4514	1.84
	Nano (200)	2498 ^{abc}	4544	1.81
	Nano (400)	2337 ^d	4449	1.90
Temperature	Normal	2512 ^a	4295 ^b	1.71 ^b
	Cold	2409 ^b	4507 ^a	1.87 ^a
Diet supplementation, mg/kg	Control	2424 ^c	4442	1.83 ^a
	Cur (200)	2502 ^{ab}	4405	1.76 ^{ab}
	Cur (400)	2456 ^{bc}	4424	1.80 ^{ab}
	Nano (200)	2520 ^a	4370	1.73 ^b
	Nano (400)	2399 ^c	4364	1.82 ^{ab}
SEM		12.19	27.74	0.01
			<i>p</i> -Value	
Temperature × Diet		.04	.649	.539
Temperature		.002	.027	.002
Diet		.001	.869	.047
Orthogonal contrasts	Control vs Cur/Nano	.034	.353	.044
	Cur vs Nano	.289	.338	.891

^{a-d}Means in the same column for each effect with no common superscript are significantly different. 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 2nd house was dropped to 15°C on d14 and maintained between 13°C and 15°C until day 42.

Table 3. Average pCO₂, pO₂, O₂ saturation, HTC, Hb, HCO₃ and pH in broiler chickens grown in normal and cold temperature conditions at 14 and 28d.

Temperature	Blood gas indices						
	pCO ₂ , mmHg	pO ₂ , mmHg	O ₂ Sat, %	HTC, %	Hb, g/dl	HCO ₃ , mmol/L	pH
14 days of age							
Normal	38.97	75.42	74.35	19.23	6.26	24.9	7.36
Cold	39.05	74.28	75.66	19.12	6.2	25	7.37
<i>p</i> -Value	0.929	0.842	0.052	0.803	0.321	0.809	0.896
SEM	0.52	0.76	0.62	0.31	0.14	0.39	0.02
28 days of age							
Normal	42.95 ^b	59.36	72.51	26.04 ^b	7.83 ^b	27.94	7.42
Cold	48.23 ^a	56.7	71.44	29.10 ^a	8.97 ^a	29.52	7.39
<i>p</i> -Value	.034	.283	.09	.001	.001	.186	.075
SEM	0.65	1.5	0.24	0.36	0.1	0.53	0.01

pCO₂: partial pressure of CO₂; pO₂: partial pressure of O₂ in venous blood; O₂sat: oxygen saturation in venous blood; HTC: haematocrit; Hb: haemoglobin.

^{a-b}Means in the same column for each effect with no common superscript are significantly different. The effect of dietary supplementation and temperature × diet interaction were not significant for different parameters at 14 and 28 days of age.

temperature condition ($p < .05$). Different levels of curcumin/nanocurcumin did not have a significant effect on feed intake ($p > .05$). The interaction of temperature and diet was not significant for feed intake ($p > .05$). Feed conversion ratio was significantly more efficient in birds reared under normal environmental temperature than those exposed to cold stress. The orthogonal contrast showed that the supplementation of diet with curcumin/nanocurcumin significantly improved FCR compared to control birds, but there was no significant difference between FCR of birds fed diet

containing curcumin compared to those fed nanocurcumin diet.

Blood gas indices

The effect of dietary curcumin/nanocurcumin supplementation on blood gas indices in birds exposed to cold stress or normal temperature conditions at 14, 28 and 42 days of age are shown in Tables 3 and 4, respectively. There was no significant difference in the partial pressure of carbon dioxide (pCO₂), partial

Table 4. Effect of dietary supplementation of curcumin (Cur) and nanocurcumin (Nano) on average pCO₂, pO₂, HTC, Hb, O₂ saturation, HCO₃ and pH in broiler chickens grown in normal and cold temperature conditions at 42 day of age.

	Diet supplementation, mg/kg	Blood gas indices (42d)						
		pCO ₂ , mmHg	pO ₂ , mmHg	HTC, %	Hb, g/dl	O ₂ Sat, %	HCO ₃ , mmol/L	PH
Normal temperature	Control	36.95	59.66	34.26 ^{cd}	11.3	64.90 ^{ab}	29.05	7.39
	Cur (200)	38.40	62.80	33.16 ^d	11.27	65.00 ^a	26.7	7.46
	Cur (400)	35.56	53.53	34.03 ^{cd}	11.23	65.06 ^a	27.2	7.4
	Nano (200)	39.80	61.53	34.23 ^{cd}	11.29	65.73 ^a	24.76	7.44
	Nano (400)	42.03	58.66	33.90 ^{cd}	11.18	64.03 ^{ab}	29.7	7.36
Cold temperature	Control	44.23	54.40	39.13 ^a	12.13	57.23 ^{cd}	30.06	7.45
	Cur (200)	51.76	52.40	35.66 ^c	12.12	62.26 ^{abc}	28.96	7.44
	Cur (400)	46.00	52.50	36.20 ^b	12.3	59.53 ^{bcd}	30.66	7.36
	Nano (200)	50.70	56.10	35.10 ^{cd}	12.28	63.63 ^{ab}	30	7.43
	Nano (400)	44.76	51.26	38.30 ^{ab}	12.25	56.02 ^d	29.43	7.38
Temperature	Normal	38.55 ^b	59.24 ^a	33.92 ^b	11.25 ^b	64.94 ^a	27.49	7.41
	Cold	47.49 ^a	53.33 ^b	36.88 ^a	12.22 ^a	59.75 ^b	29.82	7.42
Diet supplementation, mg/kg	Control	40.59	57.03	36.70 ^a	11.71	61.06 ^{bc}	29.55	7.45
	Cur (200)	45.08	57.60	34.41 ^c	11.70	63.63 ^{ab}	27.83	7.42
	Cur (400)	40.78	53.01	35.16 ^{bc}	11.76	62.30 ^{abc}	28.93	7.41
	Nano (200)	45.25	58.81	34.66 ^c	11.79	64.73 ^a	27.38	7.39
	Nano (400)	43.40	54.96	36.10 ^{ab}	11.72	60.03 ^c	29.58	7.43
SEM		1.12	0.85	0.36	0.09	0.68	0.47	0.02
				<i>p</i> -Value				
Temperature × Diet		.197	.295	.017	.888	.022	.792	.907
Temperature		.033	.006	.013	.014	.037	.139	.755
Diet		.134	.052	.002	.955	.035	.829	.749
Orthogonal contrasts								
Control vs Cur/Nano		.101	.569	.003	.842	.040	.561	.608
Cur vs Nano		.371	.293	.112	.861	.337	.953	.808

pCO₂: partial pressure of CO₂; pO₂: partial pressure of O₂ in venous blood; O₂sat: oxygen saturation; HTC: haematocrit; Hb: Haemoglobin in venous blood.

^{a-d}Means in the same column for each effect with no common superscript are significantly.

pressure of oxygen (pO₂), O₂ saturation, haematocrit (HTC), haemoglobin (Hb), HCO₃ and pH of blood in 14d chicks reared in both houses prior to the implementation of cold stress in one house.

The pCO₂ in birds exposed to cold stress was significantly higher than those reared under normal environmental temperature at 28 day, and this trend continued until 42 d of age. The pO₂ in cold stressed birds decreased, but only the difference was significant between the birds reared at two temperature conditions at 42 day of age ($p < .05$). The HTC and Hb in cold-stressed birds were significantly higher than those reared under normal temperature at 28 and 42 days of age ($p < .05$). Oxygen saturation in blood of cold stressed birds was significantly lower than those reared under normal environmental temperature at 42 days of age. The orthogonal contrast showed that the O₂ saturation in birds fed diet supplemented with curcumin/nanocurcumin were significantly higher than those fed control diet (42d). Blood bicarbonate level in the cold-stressed birds was higher than those reared under normal environmental temperature condition. The blood pH in birds was not influenced by cold stress or the age of birds ($p > .05$). The orthogonal contrast showed that there were no significant differences between blood gases in birds fed diet

containing curcumin compare with those fed nanocurcumin diet during each periods.

Ascites index

Effect of dietary supplementation of curcumin/nanocurcumin on ascites index, ascites mortality, relative weight of carcass and internal organs such as heart, spleen, bursa of Fabricius in broiler chickens reared under normal and cold environmental temperature at 42 day of age are shown in Table 5. The ascites index in cold-stressed birds was significantly higher than those reared under normal environmental temperature ($p < .05$). The orthogonal contrast showed that the ascites index in birds fed diet supplemented with curcumin/nanocurcumin were significantly lower than those fed control diet. The mortality due to ascites in cold-stressed birds was significantly higher than those reared in normal environmental temperature. The relative weight of carcass in cold-stressed birds fed diet supplemented with curcumin/nanocurcumin was similar to those reared under normal temperature. The relative weight of heart, spleen and bursa of Fabricius in birds reared under cold stress was significantly higher than those exposed to normal environmental temperature. The orthogonal contrast showed that

Table 5. Effect of dietary supplementation of curcumin (Cur) and nanocurcumin (Nano) on ascites index, ascites mortality, relative weight of the carcass and internal organs (heart, spleen, bursa of Fabricius) in broiler chickens grown in normal and cold temperature conditions at 42 day of age.

	Diet supplementation, mg/kg	Ascites* Index	Ascites mortality	Relative weights (%)			
				Heart	Carcass	Spleen	Bursa of Fabricius
Normal temperature	Control	0.24 ^{bc}	0.02	0.513	72.34 ^a	0.095	0.115
	Cur (200)	0.24 ^{bc}	0.00	0.513	72.42 ^a	0.093	0.115
	Cur (400)	0.22 ^c	0.02	0.532	71.90 ^{ab}	0.083	0.122
	Nano (200)	0.23 ^{bc}	0.00	0.523	72.00 ^{ab}	0.096	0.114
	Nano (400)	0.22 ^c	0.02	0.542	71.30 ^{abc}	0.107	0.125
Cold temperature	Control	0.27 ^{ab}	0.16	0.635	69.06 ^c	0.124	0.147
	Cur (200)	0.26 ^{abc}	0.08	0.641	70.28 ^{abc}	0.125	0.129
	Cur (400)	0.25 ^{abc}	0.06	0.639	70.54 ^{abc}	0.138	0.132
	Nano (200)	0.24 ^{bc}	0.10	0.630	71.66 ^{ab}	0.111	0.139
	Nano (400)	0.28 ^{ab}	0.18	0.646	69.86 ^{bc}	0.150	0.157
Temperature	Normal	0.23 ^b	0.012 ^b	0.529 ^b	72.00 ^a	0.095 ^b	0.118 ^b
	Cold	0.26 ^a	0.116 ^a	0.638 ^a	70.28 ^b	0.130 ^a	0.141 ^a
Diet supplementation (mg/kg)	Control	0.26	0.09	0.585	70.70	0.109	0.131
	Cur (200)	0.25	0.02	0.577	71.35	0.109	0.122
	Cur (400)	0.23	0.04	0.585	71.22	0.110	0.128
	Nano (200)	0.24	0.05	0.577	71.83	0.104	0.127
	Nano (400)	0.25	0.10	0.594	70.58	0.129	0.141
	SEM		0.002	0.012	0.008	0.208	0.006
				p-Value			
Temperature × Diet		.010	.356	.763	.032	.907	.753
Temperature		.001	.003	.002	.013	.041	.028
Diet		.049	.240	.653	.210	.835	.278
Orthogonal contrasts							
Control vs Cur/Nano		.029	.204	.893	.115	.822	.866
Cur vs Nano		.672	.13	.597	.799	.669	.238

*Ascites Index = ratio of right to total ventricular weight (RV/TV);

^{a-c}Means in the same column for each effect with no common superscript are significantly different.

there were no significant differences between ascites index, ascites mortality, relative weight of carcass and relative weight of heart, spleen and bursa of Fabricius of birds fed diets containing curcumin than those fed nanocurcumin diet.

Discussion

Similar to our study, the negative effects of cold stress on WG and FCR were reported by others (Balog et al. 2003) and it is suggested that this result may be due to the increase in metabolic rate and use of more nutrients for the heat production to maintain body temperature of birds under cold stress (Ipek & Şahan 2006). There was a beneficial effect of dietary curcumin/nanocurcumin supplementation on chicken's growth at both environmental temperature. The use of curcumin/nanocurcumin in diet significantly reduced the negative effect of cold stress on body weight gain and FCR compared with those fed control diet. Similar to our results, the growth performance and FCR of broiler chickens were improved with supplementation of turmeric powder at 5 g/kg diet (Durrani et al. 2006) and 7 g/kg diet (Salih 2013). Ahmadi (2010) also reported that the addition of 0.3 or 0.6 g/kg turmeric to diet contaminated with aflatoxin improved weight gain and FCR, but did not have an effect on feed

intake. On the contrary, dietary supplementation with different levels of turmeric powder did not affect weight gain (Nouzarian et al. 2011) or weight gain and FCR (Emadi & Kermanshahi 2006).

Due to the genetic diversity, soil characteristics (nutrients and acidity) (Sasikumar 2005) and growth stage at harvest time of turmeric, the content of curcumin can vary more than 10 times (0.25–2.7%) (Asghari et al. 2009). Therefore, inconsistent results reported on the effect of turmeric on broiler growth performance may be attributed to the difference in the amount of curcumin in the used turmeric powder.

The positive effects of curcumin to prevent the growth reduction of birds exposed to cold stress may be due to antioxidant properties and better activity of the thyroid gland (Durrani et al. 2006). Curcumin may also cause an increase in bile production and consequently enhance the digestion of fat (Al-Sultan & Gameel 2004), lipase, amylase and protease secretions that plays an important role in accelerating their substrates digestion (Platel & Srinivasan 2000), by improving consumption of digestion products (Hernandez et al. 2004) and resulting better feed efficiency and growth performance.

The increase in dietary curcumin/nanocurcumin from 200 to 400 mg/kg caused a significant reduction in growth of birds during the whole experimental

period. This may be related to the prooxidant 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 the ROS (Galati et al. 2002).

A high positive correlation has been reported between the $p\text{CO}_2$ and susceptibility to ascites (Scheele et al. 2005). In our study the birds exposed to cold stress were more prone to right ventricular hypertrophy and ascites which was correlated with higher blood $p\text{CO}_2$. Higher feed intake and a higher metabolism in cold-stressed birds, increased $p\text{CO}_2$ that was followed by a decrease in $p\text{O}_2$. The mean $p\text{CO}_2$ and $p\text{O}_2$ (48.2 mmHg and 56.7 mmHg; respectively) in the cold-stressed birds in our study were higher than those reported by Closter et al. (2009) (45.4 mmHg and 52.46 mmHg). It should be noticed that only the male broilers were used in our study, but average of male and female were used in the study of Closter et al. (2009). Scheele et al. (2005) showed that there were much differences between male and female in the ascites sensitivity. Due to the lower growth rate, females are less susceptible to ascites and change in their $p\text{CO}_2$ and $p\text{O}_2$ (Closter et al. 2009).

Similar to our results, an increase in haematocrit and a decrease in oxygen saturation of haemoglobin in cold-stressed birds were reported by others (Luger et al. 2003; Ipek & Şahan 2006). Scheele et al. (2005) reported that hyperkepnia, followed by hypoxia induced red blood cells proliferation. Stress can also induce corticosterone hormone secretion, which can have a negative effect on the maturation of red blood cells, so that the oxygen transport capability of red blood cells decreases (Luger et al. 2003). Similarly, Luger et al. (2003) showed a haemoglobin content of red blood cells in ascitics chickens significantly reduced compared to control birds. Therefore, a decrease in oxygen saturation in cold-stressed birds compared to those reared under normal environmental temperature, may be explained by a reduction in haemoglobin to haematocrit ratio. Hassanzadeh et al. (1997) did not find a difference in O_2 saturation between ascitics (induced by T3 administration) and normal birds. The method of induction of ascites and intensity of its induction may affect the observations. Due to the effects of curcumin and its derivatives on haemoglobin protection from oxidation (Unnikrishnan & Rao 1995), supplementation of diet with curcumin/nanocurcumin improved the oxygen saturation of haemoglobin in cold-stressed birds compared to those fed control diet. High HTC is an important indicator of hypoxaemia (Bautista-Ortega et al. 2014), therefore,

lower HTC in birds fed curcumin/nanocurcumin diet compared to those fed control diet may be due to the effect of curcumin on improved oxygenation. The similarity of blood pH of cold-stressed and normal birds in our study was in agreement with the report by Tekeli (2014).

In our study, the ascites index in birds fed control diet and exposed to cold stress was 0.27, which indicates that ascites had been successfully induced. Supplementation of diets with different levels of curcumin/nanocurcumin alleviated the adverse effect of cold stress as reflected by reduction in the RV/TV ratio. It has been demonstrated that turmeric powder lowers the arterial blood pressure in rats, which is probably due to the blockage of extracellular Ca^{2+} influx and the inhibition of intracellular Ca^{2+} release from inositol-1,4,5-triphosphate sensitive stores (Adaramoye 2008). Therefore, anti-hypertensive effect of curcumin on blood pressure may partially explain the observed decrease in RV/TV in these birds. Similarly, the rise in percentage of mortality in cold-stressed birds reported by others (Ipek & Şahan 2006; Daneshyar et al. 2007).

Similar to our results, the increase in the relative weight of heart in cold-stressed birds was shown by Balog et al. (2001) who indicated that the relative weight of heart in the susceptible line to ascites was higher than the resistant line. Similarly, the effect of turmeric powder on lowering the relative weight of heart was reported by Emadi and Kermanshahi (2006). The lack of effect of curcumin on mortality and relative weight of internal organs such as heart, spleen and bursa of Fabricius in the present study agrees with the results of other investigators (Mehala & Moorthy 2008; Nouzarian et al. 2011), who revealed that the different levels of turmeric in diet did not affect the relative weight of bursa of Fabricius and spleen in birds.

Conclusions

The results of this study revealed the beneficial effect of dietary curcumin/nanocurcumin supplementation on broiler chicken's performance. Curcumin/nanocurcumin might be an effective feed additive to alleviate the adverse effect of cold stress on ascites through a reduction in HTC and RV/TV ratio and an increase in blood O_2 saturation. We assumed that bioavailability of nanocurcumin could be more than curcumin and thus it could be more effective to alleviate ascites in birds. However, this was not shown in our study. In general, there was not a difference between the effect of curcumin or nanocurcumin on performance, blood gas indices and ascites in broiler chickens, which might be due to high dose of curcumin/nanocurcumin

used in our study. Further studies with less amount of curcumin/nanocurcumin may be helpful to clarify the importance of nanoparticles in broiler chickens health.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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