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The effects of different levels of *Curcuma longa* and zinc oxide nanoparticles on the quality traits of thigh and breast meat in broiler chickens

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Abstract

This research was conducted to investigate the effect of different levels of (nano-ZnO) and *Curcuma longa* (Cur-L) on the quality traits of thigh and breast meat in broiler chickens on d 42 ages. A total of 300 one-d-old male broiler (Ross-308) were randomly divided into 5 groups, including 60 birds with four replicates and 15 birds in each. The experimental groups were as follows: T1) basal diet, and T2-T5, basal diet supplemented with 50 mg of nano-ZnO with 2 and 4 grams Cur-L and 100 mg of nano-ZnO with 2 and 4 g Cur-L/kg of diet. On d 42, four birds from each treatment randomly selected, and then slaughtered. The breast and thigh muscle removed to measure of those quality parameters. The results showed that the effects of nano-ZnO and Cur-L on the humidity, dry matter, crude protein, ash, calcium and phosphor of breast muscles and humidity, dry matter, crude protein, calcium, phosphor and crude fat of thigh muscles were meaningful ($P<0.05$), But they had no significant effects on the breast crude fat and thigh ash ($P>0.05$). The lowest breast humidity and the highest breast dry matter observed in the diet including 100 mg of nano-ZnO and 2 grams of Cur-L per kilogram. The highest breast crude protein, ash and phosphor observed in basal diet (control). The breast Ca of the birds fed the diet inclusion of 100 mg of nano-ZnO and 2 g of Cur-L was the highest amount, but no significant difference with the control diet ($P>0.05$). The lowest thigh humidity and the highest thigh dry matter observed in the diet including 50 mg of nano-ZnO and 2g of Cur-L/kg. The highest thigh crude protein, calcium and phosphor numerically observed in the control diet. The thigh crude fat percentage in the diet including 100 mg of nano-ZnO and 4g of Cur-L/kg was significantly different when compared with other treatment ($P<0.05$). It was concluded that the blend of 50 mg of nano-ZnO and 2g of Cur-L/kg could be improved carcass quality. As well, the increase CP and the decrease the crude fat in the broiler's breast and thigh.

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Introduction

After the ban of antibiotics as growth promoter in the poultry nutrition, researchers have worked to find suitable nutritious alternatives that have positive features without any negative side effect. Nutrition experts have suggested options that have their natural origins in medicinal plants, biological probiotics, organic acids, *etc.* In recent years, researchers after using nanotechnology science have turned to use its products such as silver nanoparticles, selenium and zinc oxide as additive elements. The aim of his process was feeding broilers in order to reach a better and economic production. Nevertheless, available studies regarding the effect of nano-ZnO on economic performance and other parameters that have a great effect on the sufficiently breeding of poultry are limited (Ahmadi & Rahimi, 2010).

Zinc is one of the essential mineral elements that play a great role in the wide range of metabolic processes performed in the body. Physiological affairs relating to zinc are numerous and significant. This trace element act as cofactor activates more than 300 types of enzymes in the body (Karamouz *et al*, 2011; Sahin and Kucuk, 2003). Reduction of zinc levels in plasma causes physiological damages and various liver diseases such as cirrhosis and hepatitis (Halifeoglu *et al*, 2004; Cesur *et al*, 2005). Zinc exists in all the tissues of animal body and like other trace mineral has a tendency to be collected to a great extent in bone. The shortage of zinc in young chicken causes a reduction in the amount of many enzyme activities, growth delay, and reduction of feather growth, food consuming and reduction of appetite (Pourreza *et al*, 2006; Nik-khah and Amanlou, 2011).The using medicinal plant as group promoters after the ban of antibiotic had increased growth performance and improve health conditions.

Curcuma Longa is one of the alternatives that the main compounds of curcuma is yellow pigments that belong to Cur-Losides family. Araujo and Leon (2001) reported that there is information indicating a great variety of Cur-L pharmaceutical activities. Researchers believe that Cur-L is an insoluble phenolic antioxidant in water and its anti-oxidation performance is due to preventing from oxidation of membrane lipids (Emadi and Kermanshahi, 2007). Cur-L by increasing the activity of anti-oxidative enzymes like glutathione peroxidase, super oxide dismutase and catalase causes the reduction of lipid's oxidation. Also, this pharmaceutical plant by keeping acceptable levels of antioxidants in the body, protecting mitochondria against premature damage of oxidation through losing ATP and specializing cell performance prevents from cell degradation (Hosseinivashan *et al*, 2012). In this paper, The impact of different levels of nano-ZnO and Cur-L has been investigated on carcass quality traits on broilers with 42 days of age.

Materials and methods

Birds and housing

A total of three hundred one-d-old broilers (Ross 308) with an initial BW of 44.2g (±0.3) were purchased from a local commercial hatchery (Poultry Breeding Company, Kurdistan, Iran). The chickens were put in windowless houses and wire-floored pens (100cm×90cm×70cm). The house temperature was maintained at 33°C during the first 3 days of life and then was reduced gradually according to age until reaching 24°C at 42d. Chicks received a continuous lighting program (23D: 1L) throughout the study (42d). The birds had free access to feed and water throughout the trial. There were 5 dietary treatments consisting of four replicates and 15 birds in each pen. All chicks were inoculated based on the program of vaccination of local veterinary organization, Kurdistan, Iran (Newcastle and inactivated infectious bursal disease vaccine on 21 and 42d).

Basal diet zinc assay

The basal diet (table 1) was formulated to meet completely nutrient requirements of broiler based on NRC (1994) recommendations. The dietary treatments were based on corn gain, soybean meal and balanced with other nutrients. We have analyzed basal diet for zinc contained by atomic absorption spectrophotometer (Perkin Elmer, Precisely Analyst 200, Absorption spectrophotometer). Samples of

basal diets (four samples removed with high precision) were dried at 105°c for 12h; all samples were then dry-ashed at 550°C for 16h, solubilized in Hcl and filtered through 42 Whatman filter paper. Diets were analyzed for contents of zinc by means of AOAC 967.02 method (2000).

Experimental diets

The nano-ZnO provided by the US Research Nonmaterial's, Inc (Houston, TX 77084, USA). The product was a white powder with a measured ZONPs contented with purity of ≥99. 99% and size of nanoparticles was 35 to 45 nm (median size was 40 nm). To provide experimental diets and increased precision during mixing ZONPs and Cur-L in basal diet, first, we removed some amount of ZONPs and Cur-L based on experimental levels and then finely mixed with five kg from basal diet. Experimental diets were: T1) basal diet (control, without ZONPs and Cur-L), T2 and T3) basal diet with 50 mg/kg ZONPs and 2,4 g/kg Cur-L, T4 and T5) basal diet with 100 mg/kg ZONPs and 2,4 g/kg Cur-L, respectively.

Sampling collection

On d 42, four chickens were randomly selected and slaughtered for each replicate for determine carcass quality traits in order to investigate the effect of different levels of nano-ZnO and Cur-L. 50-100g of samples of selected chick's breast and thigh were separated and put in the zipped nylon bags and stored at 20°C until further analysis.

Statistical analysis

The data were subjected to one-way ANOVA as a completely randomized design using the general linear models (GLM) procedure of SAS software (SAS Institute, 2003). Significant differences among the means were determined by using Duncan's multiple-range test (1995) at $P<0.05$.

Results and discussion

Breast quality traits

The results related to the effect of nano-ZnO and Cur-L on breast quality parameters on d 42 are presented in table 2. Breast humidity in birds fed with T1 or control diet (75.39%) was significantly higher than those in T2, T3, T4 and T5 ($P<0.05$). The lowest breast humidity (71.12%) was observed in T4 treatment. Breast dry matter in birds fed with T1 or control diet (24.61%) was significantly lower than those in T2, T3, T4 and T5 ($P<0.05$). The highest breast dry matter (28.88%) was observed in T4 treatment. Results in the present study agree with reports of Bras (2010), Liu *et al.* (2011) and Tronina *et al.* (2007). They reported that amount of breast humidity was lower and the amount of breast dry matter of broilers fed with zinc was higher than those of the control group and carcass traits were not affected by zinc sources.

Breast crude protein in birds fed with T1 or control diet (92.65% of dry matter) was significantly higher than those in T2, T3 and T4 ($P<0.05$). The lowest breast crude protein (77.64%) was observed in T4 treatment. Results in the present study agree with reports of Tronina *et al.* (2007). They reported that amount of breast crude protein of broilers fed with zinc-glycine was lower than that in control treatment and broilers fed with zinc oxide. The findings of this study are in contrast to results of Al-Sultan (2003) that reported different levels of additive Cur-L (0, 0.25, 0.50 and 1 %) had no significant effect on the breast crude protein of broiler chickens.

Breast ash had significant difference among treatments ($P<0.05$). The lowest (3.44%) and the highest (5.50%) breast ash was observed in T4 and T1 (control), respectively. Results in the present study agree with the reports of Shyam-Sunder *et al.* (2008). They reported that amount of tibia bone ash in the different levels of zinc supplementation (0, 10, 20, 40, 80, 160 mg/kg of diet) of broilers had no significant difference, while significantly decreased at the 320 mg of zinc supplementation per each kg of diet. It may be symbol of the negative effect of high levels of zinc at the bone mineralization. The findings of this study are in contrast to results of Norouzi *et al.* (2014) and Ao *et al.* (2007). They had reported that supplemented diet with different levels of zinc had no effect on breast and thigh ash of broilers.

Breast calcium had significant difference among treatments (P<0.05). The lowest (0.47%) and the highest (0.60%) breast calcium were observed in T3 and T4, respectively. Breast phosphor had significant difference among treatments (P<0.05). The lowest (0.67%) and the highest (0.85%) breast phosphor were observed in T3 and T1 (control), respectively. Breast fat had no significant difference among treatments (P>0.05).

Table 1. Feed ingredients and nutrient composition of basal diets.

Ingredients	Starter (1-21d)	Grower (22-42d)
Corn	60.7	66.0
Soybean meal	30.0	24.0
Soybean oil	2.8	3.5
Corn gluten meal	2.5	3.0
Dicalcium phosphate	1.7	1.7
Limestone	1.2	1.2
Mineral and vitamin premix[1]	0.5	0.5
Sodium chloride	0.3	0.3
L- lysine	0.16	0.15
Dl- methionine	0.15	0.1
Analyzed chemical composition of experimental diet		
ME (kcal/kg)	3055	3120
Crude Protein (%)	21.92	19.65
Ether Extract (%)	5.48	6.13
Dry matter (%)	89.74	88.12
Methionine＋cystine* (%)	0.82	0.73
Available P* (%)	0.43	0.40

Calculated*

[1]Mineral premix provided per kilogram of diet: transretinyl acetate, 25 mg; cholecalciferol, 6 mg; menadione, 1.2 mg; thiamine, 2.3 mg; riboflavin, 8 mg; nicotinamide, 42 mg; choline chloride, 400 mg; calcium pantothenate, 10mg; pyridoxine HCl, 4mg; biotin, 0.04 mg; folic acid, 1mg; Cobalamin, 0.012 mg; Fe (from ferrous sulfate), 82mg; Cu (from copper sulfate), 7.5 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 64 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.28 mg.

Thigh quality traits

The results related to the effect of ZONPs and Cur-L on thigh quality parameters at 42d are presented in Table 3. Thigh humidity in birds fed with T1 or control diet (78.62%) was significantly higher than that in T2, T3, T4 and T5 (P<0.05). The lower the humidity (69.44%) was observed in T2 treatment. Thigh dry matter in birds fed with T1 or control diet (21.39%) was significantly lower than that in T2, T3, T4 and T5 (P<0.05). The highest thigh dry matter (30.56%) was observed in T2 treatment. Results in the present study agree with reports of Liu *et al.* (2011) and Tronina *et al.* (2007). They reported that amount of thigh humidity decreased (2.84%) and the amount of thigh dry matter of broilers fed with zinc-glycine increased (2.84%) compared with broilers fed with zinc oxide and control diet. The findings of this study were in contrast to results of Norouzi *et al.* (2014) that reported supplemented diet of different levels of zinc and magnesium had no effect on thigh humidity and dry matter of broilers.

The thigh crude protein had significant difference among treatments (P<0.05). The lowest (75.35%) and the highest thigh crude protein (90.12%) were observed in T5 and T1 (control) respectively. Results in the present study were in contrast to results of Tronina *et al.* (2007), Norouzi *et al.* (2014). They reported that different levels of zinc caused significant increase for protein of broiler chickens. The increase in the crude protein in the birds may be caused by the improvement of nutrient digestibility, especially proteins, and higher performance especially increased feed intake caused by zinc

supplementation. Al-Sultan (2003) reported that the percentage of thigh crude protein in birds fed with different levels of Cur-L (0, 0.25, 0.50 and 1%) had no significant difference (P>0.05).

Thigh ash had no significant difference among treatments (P>0.05). Thigh calcium in birds fed with T1 or control diet (0.62%) was significantly higher than that in T2, T3, T4 and T5 (P<0.05). The lowest thigh calcium (0.43%) was observed in T5 treatment. Results in the present study were in contrast with the reports of Shyam-Sunder *et al.* (2008). They reported that calcium absorption in the bone significantly was lower compared with that in other treatments in the broilers fed with the control diet (without zinc supplementation) and by increasing the zinc level in the diet calcium absorption improved. Thigh phosphor had significant difference among treatments (P<0.05). The lowest (0.59%) and the highest (0.68%) thigh phosphor were observed in T5 and T1 (control), respectively. Results in the present study were in contrast with the reports of Shyam-Sunder *et al.* (2008). They reported that phosphor absorption in the tibia bone significantly was higher in the diet supplemented with 40 and 80 mg/kg of zinc compared with that in other levels of zinc and this indicates the importance of zinc existence in the bone for the optimized level of phosphor.

Table 2. The effect of experimental diets on quality traits of broiler breast meat in 42d.

Traits (%)	Experimental diets						
	T1	T2	T3	T4	T5	SEM	P-Value
	Control	50 mg nano-ZnO+ 2g Cur-L	50 mg nano-ZnO+ 4g Cur-L	100 mg nano-ZnO+ 2g Cur-L	100 mg nano-ZnO+ 4g Cur-L		
Moisture	75.3^{a}	73.0^{b}	71.1^{c}	71.1^{c}	73.2^{b}	3.52	0.003
DM	24.6^{c}	26.9^{b}	28.8^{a}	28.8^{a}	26.7^{b}	1.52	0.001
CP	92.6^{a}	82.9^{b}	83.2^{b}	77.6^{c}	90.8^{a}	4.94	0.004
Ash	5.50^{a}	5.03^{a}	4.95^{a}	3.44^{b}	4.05^{b}	0.28	0.082
Ca	0.52^{ab}	0.52^{ab}	0.47^{b}	0.60^{a}	0.54^{ab}	0.04	0.015
P	0.85^{a}	0.81^{ab}	0.67^{c}	0.74 ^{bc}	0.69^{c}	0.04	0.013
Crude fat	3.38	2.88	3.84	3.48	3.38	0.33	0.405

The numbers of each columns that don't have similar letters, have meaningful difference (P<0.05).
The above values for crude protein, ash, calcium, phosphor and crude fat are the percent of dry matter.

Table 3. The effect of experimental diets on quality traits of broiler thigh meat in 42d.

	Experimental diets						
Traits (%)	T1	T2	T3	T4	T5	SEM	P-value
	Control	50 mg nano-ZnO +2g Cur-L	50 mg nano-ZnO +4g Cur-L	100 mg nano-ZnO +2g Cur-L	100 mg nano-ZnO+ 4g Cur-L		
Moisture	78.6^{a}	69.44^{c}	71.0^{bc}	73.3^{b}	70.4^{bc}	5.13	0.025
DM	21.3^{c}	30.5^{a}	28.9^{ab}	26.6^{b}	29.5^{ab}	2.11	0.046
CP	90.1^{a}	82.6^{bc}	83.7^{ab}	86.0^{ab}	75.3^{c}	4.42	0.012
Ash	3.7	4.0	3.9	4.0	3.7	0.15	0.383
Ca	0.62^{a}	0.49^{bc}	0.46^{bc}	0.52^{b}	0.43^{c}	0.02	0.039
P	0.68^{a}	0.62 ^{ab}	0.62 ^{ab}	0.63 ^{ab}	0.59^{b}	0.02	0.035
Crude fat	20.9^{b}	18.6^{b}	19.8^{b}	18.8^{b}	25.2^{a}	2.29	0.029

$^{a-c}$ Mean values within the same row sharing a common superscript letter are not statistically different at P< 0.05.
The above values for crude protein, ash, calcium, phosphor and crude fat calculated based on the percent of dry matter.

Thigh fat had significant difference among treatments (P<0.05). The lowest (18.61%) and the highest (25.2%) thigh fat were observed in T2 and T5, respectively. However, in numerical comparison among T_1 with T_2, T_3 and T_4 less thigh fat was observed. Results in the present study agree with reports of Karamouz *et al.* (2010). They reported that different levels of zinc oxide in the diet of the broilers

significantly decreased amount of cholesterol and MDA of thigh muscles. It can be due to zinc element of diet that acts as an antioxidant. In the other researches, they reported that different levels of zinc oxide decrease MDA and also increase all antioxidants in the serum of broilers that is due to zinc role in the formation of super oxide dismutase enzyme that decreases fat peroxidation and zinc oxide supplementation improved meat quality by decreasing the amount of muscle oxidation. Al-Sultan (2003) reported that the lower thigh fat percentage was recorded in the carcass of broilers fed diet consisting of 0.5% and then 0.25 and 1% Cur-L and control group. It was in agreement with previous observation by Osawa *et al.* (1995). On the other hand, findings of Norouzi *et al.* (2014), Tronina *et al.* (2007) and Liu *et al.* (2011) were not in agreement with the results of this study. Norouzi *et al.* (2014) reported that supplemented diet with different levels of zinc and magnesium had no effect on thigh fat of broilers. Tronina *et al.* (2007) and Liu *et al.* (2011) reported that the amount of thigh fat in birds fed diet with zinc showed a greater tendency to increase compared with that of birds fed with control diets.

Conclusion

One possible explanation for these differences may be related to new physicochemical properties of nano-ZnO, animal species and zinc sources. According to the results of this research, one can infer that the use of 50 mg of nano-ZnO and 2 g of Cur-L per kg of diet, causes the improvement of carcass quality and the increase in the crude protein and the decrease in the crude fat in the broiler's breast and thigh.

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