

Effect of dietary supplementation of nanocurcumin on oxidant stability of broiler chicken breast meat infected with *Eimeria* species

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Abstract

Poultry meat is very susceptible to oxidation because of the high concentration of polyunsaturated fatty acids, which negatively affects the quality and nutritional values of chicken meat. Coccidiosis is the most common parasitic disease of poultry. Intending to limit anti-parasites usage in poultry feed and also because of the concerns about antibiotic resistance and residues in poultry products, there is a need for research to discover natural alternatives. The effect of nanocurcumin on antioxidant profile (carotenoid and vitamin E contents, lipid oxidation and antioxidant capacity) and pH of broiler chicken breast meat infected with *Eimeria* species was investigated. Fifty, one-day-old male Ross 308 broiler chickens were assigned to five treatments including non-infected and non-medicated control (NNC), infected non-supplemented control (INC), infected and medicated with nanocurcumin 300 mg kg⁻¹ feed (NCRM1), infected and medicated with nanocurcumin 400 mg kg⁻¹ feed (NCRM2) and infected and antibiotic medicated group. Infection with *Eimeria acervulina*, *E. maxima*, and *E. tenella* decreased vitamin E and carotenoid contents of chicken breast meat significantly. The NCRM2 had significantly enhanced carotenoid and vitamin E levels in chicken breast meat, so there was no significant difference between NCRM2 and NNC group. No significant change was observed in pH value among groups. Malondialdehyde value of breast meat was significantly lower in NCRM1 and NCRM2 than the INC group. The NCRM2 and NCRM1 showed the best antioxidant capacity even better than NNC. In conclusion, nanocurcumin could be a potential feed additive that can increase oxidant stability of broiler chicken breast meat.

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Introduction

Broiler chickens are the fastest-growing and the most efficient species among meat-producing animals.¹ Consumers prefer poultry meat due to its desirable nutritional characteristics. However, poultry meat is very susceptible to oxidation because of high concentration of polyunsaturated fatty acids, which negatively affects quality and nutritional values of meat.¹ Dietary manipulation such as antioxidant supplementation is a common way to enhance the antioxidant profile of tissues to improve oxidant stability of meat products.² Beside feeding method, other factors such as bird genotype, slaughter age and illness can affect the quality and composition of poultry meat.³

Coccidiosis is the most common parasitic disease of poultry causing economic damage not only by mortality but also by growth constraint, body weight loss, and negative effects on the product's yield and quality.⁴ Control of coccidiosis is primarily based on the use of anticoccidial drugs including sulphonamides which is costly and increases the risk of drug-resistance.⁵ Consequently, to limit antibiotics usage in poultry feed and also because of the concerns about antibiotic resistance and residues in poultry products, there is a need for research to discover natural alternatives that can improve the nutritional value and organoleptic properties of the meat.⁶ The ability of phytochemicals for dietary immunomodulation of gut immunity in broiler chickens makes them promising substitutes for antibiotics that can be applied to control

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many infectious diseases, without the limitations of traditional methods.⁷

Curcumin is a xanthophyll carotenoid isolated from a spice known as turmeric.⁸ This natural carotenoid is one of the most commonly used and accepted natural dietary carotenoids in commercial poultry feed and has a wide range of therapeutic activities including antioxidative,⁹ antibacterial,¹⁰ immunomodulatory,¹¹ anti-inflammatory,¹² anti-mutagenic¹³ and anti-cancer properties.¹⁴ Effects of curcumin against oxidative stress by decreasing lipid peroxidation and developing anti-oxidant status, makes it a potentially effective antioxidant.¹⁵ An issue about curcumin is low bio-availability because of its low absorption, fast metabolism and fast systemic elimination from the body.¹⁶ Using curcumin nanoparticle is one of the suggestions for improving the bioavailability of curcumin which can increase the oral absorption of curcumin. Shaikh *et al.* have reported that nanoparticle encapsulation causes an at least 9-fold increase in the oral bioavailability of curcumin compared to curcumin administered with piperine as an absorption enhancer.¹⁷

The present study was designed to investigate the effect of nanocurcumin on antioxidant profile (carotenoid content, vitamin E content, lipid oxidation, and antioxidant capacity) and pH of broiler chicken breast meat infected with *E. acervulina*, *E. maxima*, and *E. tenella*.

Materials and Methods

Reagents and chemicals. Nanocurcumin was purchased from Exir Nano Sina Company (Tehran, Iran). All other chemicals used in the present study were obtained from Merck (Darmstadt, Germany).

Birds, diets, and housing. The experimental protocol was approved by the Animal Care Committee of Amol University of Special Modern Technologies, Amol, Iran (ir.ausmt.rec.1397.03.11). Fifty, one-day-old male Ross 308 broiler chickens were purchased from a local hatchery and randomly assigned to five treatments including non-infected and non-medicated control (NNC), infected non-supplemented control (INC), infected and medicated with nanocurcumin 300 mg kg⁻¹ feed (NCRM1), infected and medicated with nanocurcumin 400 mg kg⁻¹ feed (NCRM2) and infected and antibiotic medicated (AMG) groups. The chickens were reared up to 42 days of age in wire-floored cages with free access to water and feed according to the latest catalog of Ross 308.¹⁸ All birds were fed with the basal diet (Table 1) for starter (1-10), grower (11-24) and finisher (25-42) periods according to Ross 308 nutrient recommendations.¹⁸ Chickens from NCRM1 and NCRM2 groups received nanocurcumin with a certain amount in the diet from the first day of the breeding. Chickens were challenged with a mixture of *Eimeria* species (*E. acervulina*, *E. maxima* and *E. tenella*) oocysts (5.00×10^5) at 18 days of age. Administration of Coxidine® (25.60 mg mL⁻¹

sulfaquinoxaline and 6.40 mg mL⁻¹ diaveridine) was also started in the AMG group on the same day. A solution containing 3.00 mL of Coxidine® in each liter of drinking water was offered to AMG group *ad libitum* for three days and then the treatment was stopped, drinking water was given for two days and the treatment was resumed for another three days. At the end of the experiment, three chickens from each group were slaughtered and breast meat samples were excised and stored in polyethylene bags at refrigerator temperature till the day of analysis.

Total carotenoid assessment. The homogenization of breast meat was conducted by Teflon homogenizer (Omni International, Kennesaw, USA). The homogenized meat was used to determine the carotenoids level. It was calculated based on standard curve of β -carotene and at 470 nm using spectrophotometer (T80 UV/VIS; PG Instruments Ltd., Leicestershire, UK).^{19,20} The concentration of carotenoid pigments was calculated using the standard curve obtained by a commercial carotene reagent.

Vitamin E content assessment. After homogenizing 10.00 mg of breast meat, samples were exposed to Fe³ solution, 2,4,6-Tripyridyl-s-triazine, and acetate buffer (pH = 4). The standard curve was prepared with appropriate vitamin E concentrations. The absorbance of samples was read at 595 nm.^{20,21}

pH. The pH value of the meat samples was determined using a digital pH meter (EIL7020; Kent Industrial Measurement Ltd., Surrey, UK) using 10.00 g of the breast meat blended with 50.00 mL distilled water. Means of three replicates were reported for each treatment.

Table 1. The Composition of the basal diet.

Ingredients (%)*	Starter	Grower	Finisher
Corn	55.40	59.20	64.50
Soybean meal	39.00	34.00	28.00
Vegetable oil	1.20	3.00	3.70
Oyster shell	1.10	1.10	1.05
Dicalcium phosphate	2.00	1.50	1.55
Common salt	0.30	0.35	0.35
L-Lysine HCL	0.15	0.10	0.10
DL-Methionine	0.25	0.15	0.15
Vitamin E	0.10	0.10	0.10
Vitamin and mineral Premix	0.50	0.50	0.50
Calculated contents (%)			
Metabolizable Energy (kcal kg⁻¹)	2851	3000	3094
Crude protein	21.00	19.17	17.07
Energy: Protein	135.70	156.40	181.20
Calcium	0.97	0.93	0.86
Available phosphorus	0.48	0.43	0.35
Sodium	0.16	0.17	0.17
Lysine	1.38	1.15	1.01
Methionine	0.70	0.55	0.48
Methionine + Cystine	1.03	0.86	0.78

* Vitamin and mineral premix supplied per kg of diet including Vitamin A: 10,000 IU; Vitamin D3: 9,800 IU; Vitamin E: 121 IU; B12: 20.00 μ g; Riboflavin: 4.40 mg; Calcium pantothenate: 40.00 mg; Niacin: 22.00 mg; Choline: 840 mg; Biotin: 30.00 μ g; Thiamin: 4.00 mg; Zinc sulfate: 60.00 mg; and Manganese oxide: 60.00 mg.

Lipid peroxidation. Lipid peroxidation of breast meat was assessed by the formation of thiobarbituric acid in breast meat samples according to an original method.²² Briefly, the breast meat samples were homogenated before the experiment, mixed with 20% trichloroacetic acid and centrifuged. Then, the supernatant was mixed with thiobarbituric acid and heated. The data were obtained by measuring the absorbance of each sample at 532 nm. Consequently, they were shown using a molar extinction coefficient of 1.56×10^5 M per cm and expressed as nmol of malondialdehyde (MDA) 0.50 g^{-1} of meat.

Antioxidant activity. The cupric ion reducing antioxidant capacity assay was used for antioxidant activity screening.^{23,24} The method is based on the reduction of Cu (II) to Cu (I). The samples were mixed with solutions of CuCl_2 and neocuproine reagent in ammonium acetate buffer. The resulting absorbance at 450 nm is recorded directly after incubation at $50.00 \text{ }^\circ\text{C}$ for 20 min.

Statistical analysis. The data were analyzed by analysis of variance (ANOVA) and significant differences were assessed by Duncan's multiple range test using SPSS statistical package (version 22.0; SPSS Inc., Chicago, USA). Data are presented as means \pm standard deviations. For all analyses, $p < 0.05$ was considered statistically significant.

Results

Results given in Table 2 showed that vitamin E and carotenoid contents were significantly ($p < 0.05$) lower in the INC group versus NNC group. The NCRM2 group had significantly ($p < 0.05$) enhanced carotenoid and vitamin E levels, so there was no significant difference between the NCRM2 group and the NNC group ($p > 0.05$). Although supplementation with antibiotics and nanocurcumin did not completely compensate for the reduced vitamin E in coccidiosis infection, the NCRM2 group showed the best results among supplemented groups ($p < 0.05$). No significant change was observed in pH value among groups ($p > 0.05$).

Table 2. Effect of dietary supplementation with different concentrations of nanocurcumin and antibiotic on vitamin E, carotenoid content, and pH values of broiler chicken breast meat infected with Eimeria species. Data are presented as mean \pm SD.

Groups	Carotenoid (ppm)	Vitamin E (ppb)	pH
NNC	0.04 ± 0.007^b	7.16 ± 0.923^c	5.86 ± 0.208
INC	0.006 ± 0.002^a	1.86 ± 0.862^a	6.00 ± 0.173
AMG	0.01 ± 0.007^a	2.06 ± 0.750^a	5.80 ± 0.100
NCRM1	0.02 ± 0.007^a	3.73 ± 1.625^{ab}	5.78 ± 0.028
NCRM2	0.04 ± 0.01^b	5.60 ± 2.424^{bc}	5.90 ± 0.173

NNC: Non-infected, non-medicated control; INC: Infected, non-medicated control; AMG: Antibiotic medicated group; NCRM1: Nanocurcumin (300 mg kg^{-1}) medicated group; NCRM2: Nanocurcumin (400 mg kg^{-1}) medicated group.

abc Means with different superscripts within each column are significantly different ($p < 0.05$).

Infection with Eimeria species induced an increase of MDA value in comparison with the uninfected control group ($p < 0.05$). The results of the breast meat lipid oxidation analysis (Fig. 1) showed that the MDA value of breast meat was significantly lower in curcumin supplemented broilers than infected non-supplemented broilers ($p < 0.05$). Between the two concentrations of nanocurcumin, lipid peroxidation in the NCRM2 group was lower than the NCRM1 group, but the difference was not significant ($p > 0.05$). Coccidiosis infection decreased total antioxidant capacity in the INC group compared to the NNC group (Fig. 2), but it was not significant ($p > 0.05$).

The NCRM2 and NCRM1 groups showed the best antioxidant capacity even better than the healthy group ($p < 0.05$), while the AMG group did not show any significant differences in comparison with the INC group in any of the analyzed parameters ($p > 0.05$).

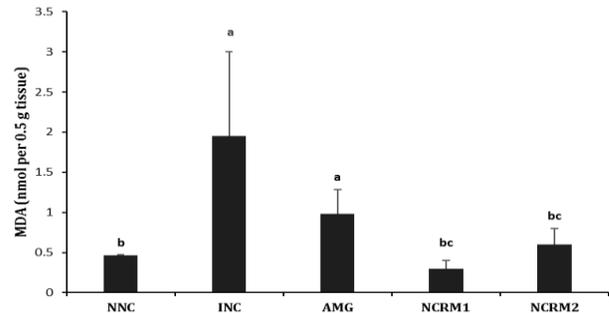


Fig. 1. Effect of dietary supplementation with different concentrations of nanocurcumin and antibiotic on lipid peroxidation of coccidiosis infected broiler chicken meat. NNC: Non-infected, non-medicated control; INC: Infected, non-medicated control; AMG: Antibiotic medicated group; NCRM1: Nanocurcumin (300 mg kg^{-1}) medicated group; NCRM2: Nanocurcumin (400 mg kg^{-1}) medicated group; MDA: Malondialdehyde. abc Means with different superscripts within each column are significantly different ($p < 0.05$).

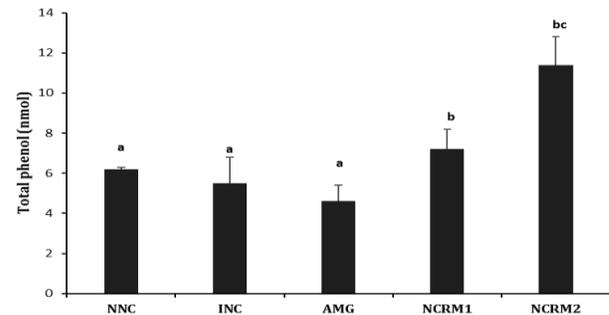


Fig. 2. Effect of dietary supplementation with different concentrations of nanocurcumin and antibiotic on antioxidant capacity of coccidiosis infected broiler chicken meat. NNC: Non-infected, non-medicated control; INC: Infected, non-medicated control; AMG: Antibiotic medicated group; NCRM1: Nanocurcumin (300 mg kg^{-1}) medicated group; NCRM2: Nanocurcumin (400 mg kg^{-1}) medicated group.

abc Means with different superscripts within each column are significantly different ($p < 0.05$).

Discussion

The absorption of vitamin E and carotenoids particularly decreases during enteric infections. Sepp *et al.* have reported that nitric oxide produced by macrophages in coccidiosis infection decreases the absorption of fat-soluble antioxidants and causes oxidative damage in greenfinches.²⁵ Infection with *Eimeria* species in the present research decreased broilers' breast meat vitamin E and carotenoid contents significantly. According to the results, vitamin E and carotenoid contents were significantly higher in the NCRM2 group compared to INC and AMG groups. This can be explained by dietary supplementation with curcumin that is a naturally produced xanthophyll carotenoid that can enhance the carotenoid content of chicken breast meat.²⁵ Curcumin may compensate for reduced absorption of vitamin E and carotenoids in enteric infections and protect them against oxidation. It was reported that dietary supplementation of turmeric enhances the growth of broiler chicks and improves feed conversion ratio.²⁶ This can be attributed to the effect of turmeric on digestion improvement due to increase in the length and width of intestinal villi.²⁶ Lee *et al.* have reported that dietary supplementation of turmeric, hot pepper, and shiitake mixture significantly improves local innate immunity against infection with *E. acervulina*.²⁷ Coccidiostatic effect of turmeric has been also proved in other studies.²⁸ Carotenoid accumulation in muscles increases the quality of poultry meat by inducing yellow color and flavor improvement.²⁹ Vitamin E improves the color and lipid stability of meat by delaying oxidation of phospholipids.³⁰ The lipid and pigment oxidations in meat are closely associated and supplementation with antioxidants can increase the color stability of the meat.³¹ The results of the present research are in good agreement with Rajput *et al.*⁴ and Sepp *et al.*²⁵ They have reported that dietary supplementation with the natural carotenoids, curcumin, and lutein enhances color stability as well as antioxidant capacity of meat from broilers infected with *Eimeria* species.

Becoming infected with *Eimeria* species increased the MDA level in broilers breast meat. The main reason for meat quality deterioration after slaughter is lipid oxidation caused by high levels of free radicals.³² The high concentration of polyunsaturated fatty acids in poultry meat makes it sensitive to free radical attack and oxidative spoilage.^{1,4} In the current study, lipid oxidation was significantly lower in the meat of nanocurcumin supplemented broilers compared to the infected groups.

Antioxidant capacity of broilers breast meat was significantly higher in those treated with nanocurcumin in both concentrations even higher than the NNC group. This can be due to the unique conjugated structure of curcumin that shows typical radical-trapping ability as a chain-breaking antioxidant and also curcumin acts as a

scavenger of oxygen-free radicals.³³ Zhang *et al.* have examined the effect of curcumin on the antioxidant profile of breast muscle in broilers and concluded that curcumin improves oxidant stability of muscle in broilers.¹ In this regard, Baghban Kanani *et al.* have reported that turmeric and cinnamon powders reduce thiobarbituric acid reactive substances and free radicals scavenging activity of broiler chicken under heat stress condition and consequently decrease lipid peroxidation.³⁴ Rajput *et al.* have concluded that curcumin individually or combined with lutein act as a natural carotenoid improving oxidative stability of broiler chicken under heat stress condition.⁴ Mancini *et al.* have reported that turmeric powder has an antioxidant activity similar to ascorbic acid in stored and fresh rabbit burgers.³⁵

In conclusion, the results of the present study demonstrated that nanocurcumin could be a potential feed additive that can increase the quality and oxidant stability of broiler chickens breast meat.

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Conflict of interest

The authors declare that there is no conflict of interest.

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