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Meat Quality Attributes of Broiler Chickens Fed Diets Supplemented with Silver Nanoparticles Coated on Zeolite

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Abstract

The aim of this study was to assess the meat quality attributes of broiler chickens fed diets supplemented with silver nanoparticles coated on zeolite. A total of 375 one-day-old broilers was assigned in a completely randomized design to 1 of 5 treatment groups including: basal diet, basal diet supplemented with 1% zeolite, and basal diet supplemented with one of the three levels of zeolite coated with 25, 50 and 75 ppm nanosilver. On d 42, five birds per treatment were slaughtered to assess the meat quality attributes of breast and thigh. Breast meat quality attributes were not influenced by the dietary treatment. Birds fed basal diet supplemented with 50 and 75 ppm nanosilver had higher levels of water-holding capacity than those fed on diets containing 1% zeolite ($P<0.05$). Thigh muscle of birds fed basal diet supplemented with 50 and 75 ppm nanosilver had a higher L* value than the control diet ($P<0.05$). For b* values, thigh muscle of birds fed the control diet were significantly higher than those of birds fed zeolite coated with 75 ppm nanosilver diet. The highest value of hardness, gumminess, and chewiness for breast meat were recorded by birds fed zeolite coated with 75 ppm nanosilver diet. The highest values of gumminess and chewiness of broiler chickens thigh muscle were observed in the group of birds receiving zeolite diets without nanosilver supplementation ($P<0.01$). In conclusion, broiler diets supplemented with silver nanoparticles coated on zeolite improved water-holding capacity of thigh muscle; although, further studies are needed to provide strong evidences to the exact mechanisms of action for silver nanoparticles coated on zeolite.

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Introduction

The world's population will grow to an estimated 8 billion people by 2025 and 9 billion by 2050, and it is widely recognized that global agricultural efficiency must increase to feed a quickly growing world population (FAO, 2006). In developing and non-developing countries, the poultry industry is the most successful application in agriculture because of the rapid adoption of new technologies, the successful achievement of the new products and processes into daily operations (Wicker, 2001). Nanotechnology is one of the most important tools and focuses on nanosized particles with unique properties in modern animal productivity, and nanoagriculture is expected to become a driving economic force in the near future. Silver (Ag) has been known to be a strong antibacterial, antifungal and antiviral agent, but recently, the use of silver as a biocide especially in nano-particulate format has experienced a dramatic revival (Batsmanova *et al*., 2013; Scott and Chen, 2002). As a result of the silver properties at the nano scale, nanosilver is currently recommended to be used in animal industry and medical products.

The extremely strong antimicrobial activity is the most important explanation for the widespread use of nanosilver. On the other hand, zeolites are aluminosilicates with physicochemical properties such as permitting ion exchange, molecular sieving, absorption, diffusion, dehydration, reversible dehydration and catalysis that encourage the use of these products in animal nutrition (Bish and Ming, 2001; Eleroğlu and Yalçin, 2005). Zeolite could be used as an antimicrobial agent (Haile and Nakhla, 2010). Furthermore, zeolites have been suggested to be highly effective in the metabolic utilization of nitrogen in poultry and pigs (Shurson *et al*., 1984; Strakova *et al*., 2008). Mallek *et al*. (2012) found that supplementing of zeolite to broiler diets had a positive effect on performance, organoleptic parameters and mainly increased level of Omega 3 fatty acid. Although many studies have been done to evaluate the impact of nanosilver in birds, the effects of silver-nanoparticles coated on zeolite on meat quality attributes of broiler chickens as the main protein sources for human have not been examined.

Materials and Methods

Animals and treatments

Commencing from day one, 375 one-day-old broilers (Ross 308) were randomly assigned in a completely randomized design (CRD) to 1 of 5 treatment groups including 75 chicks and each dietary treatment was replicated five times with 15 birds per pen. The experimental diets were as follow:

1. Corn-soy diet without nanosilver and zeolite (Control diet) (C)
2. Corn-soy diet containing 1% zeolite (Z)
3. Corn-soy diet containing 1% zeolite coated with 25ppm nanosilver (NZ25)
4. Corn-soy diet containing 1% zeolite coated with 50ppm nanosilver (NZ50)
5. Corn-soy diet containing 1% zeolite coated with 75ppm nanosilver (NZ75)

Natural zeolite used in this research was a clinoptilolite, prepared from well-defined zeolitic stratigraphic units from Semnan province region, Iran. Zeolitic rock was pulverized and sieved to give a particle size of 1-2 mm and then washed

with distilled water to remove all the soluble impurities, and then dried in the oven overnight. The chemical formula of pure clinoptilolite was $(K_2,Na_2,Ca,Mg)_3 Al_6Si_{30}O_{72}. 24H_2O$. Zeolites coated with silver nanoparticles were prepared by Nano Nasb Pars Company (Tehran, Iran) and the silver nanoparticles size was max 50 nm.

Diets were formulated to meet broiler nutrient requirements according to the Ross 308 Management Guideline (Avigen, 2009) and proximate analyses confirmed formulated values for all critical nutrients in the diets fed. Birds had free access to water and feed. The lighting schadual was according to a 23L/1D program. The ingredients and chemical composition of experimental diets are presented in Table 1. All chicks were vaccinated against Newcastle disease (ND) on seven and 17 days, by eye-drop and also were vaccinated with infectious bursal disease vaccine (IBDV) via eye-dropping at 14 and 28 d of age. Vaccination was carried out according to the regional vaccination program routine. All experimental protocols were reviewed and approved by the Animal Care Committee of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Table 1. Composition and analysis of the basal diet (as fed basis)

Ingredients (%)	Control diet		Zeoilte diets	
	Starter (1-21)	Grower (22-42)	Starter (1-21)	Grower (22-42)
Yellow corn	53.70	59.96	51.60	57.84
Soybean meal (44% CP)	39.52	33.25	39.95	33.68
Soybean oil	3.00	3.41	3.69	4.11
Zeolite (Coated zeolite)	0	0	1.00	1.00
Dicalcium phosphate	1.47	1.09	1.47	1.09
Limestone	1.19	1.29	1.18	1.28
Salt	0.43	0.32	0.43	0.32
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL-Methionine	0.13	0.05	0.13	0.05
L-Lysine	0.06	0.13	0.05	0.13
Chemical analysis				
ME (Kcal/kg)	2950	3050	2950	3050
CP (%)	21.2	19.06	21.2	19.06
Ca (%)	0.92	0.86	0.92	0.86
P_a (%)	0.41	0.33	0.41	0.33
Na (%)	0.18	0.14	0.18	0.14
Lys (%)	1.01	0.95	1.01	0.95
Met (%)	0.47	0.36	0.47	0.36
Met + Cys (%)	0.36	0.37	0.36	0.37

[1]Supplied per Kg of diet: Vitamin A, 1,500 IU; Cholecalciferol, 200 IU; Vitamin E, 10 IU; Riboflavin, 3.5 mg; Pantothenic acid, 10 mg; Niacin, 30 mg; Cobalamin, 10 µg; Choline chloride, 1,000 mg; Biotin, 0.15 mg; Folic acid, 0.5 mg; Thiamine 1.5 mg; Pyridoxine 3.0 mg.

[2]Supplied per Kg of diet: Iron, 80 mg; Zinc, 40 mg; Manganese, 60 mg; Iodine, 0.18 mg; Copper, 8 mg; Selenium, 0.15 mg.

Meat quality attributes

On d 42, five birds from each treatments were slaughtered, plucked, and kept in a chiller approximately for 30-45 min until the internal temperature of the birds reach 0-2°C and then, eviscerated and cut in parts and the breast muscles (*Pectoralis major*) and right thigh were collected and kept in refrigerator for the accomplishment of meat quality attributes after slaughter.

The pH

The pH was measured immediately after slaughter with a portable pH-meter (Model pH 211; Hanna Instruments, Woonsocket, RI, USA) equipped with a spear-tipped glass electrode pH probe. Measurements were performed by directly inserting the probe approximately 1 cm into the right thigh and 2 cm below the right portion of the breast muscle (pectoralis major) at approximately 2 cm from the top of the breast and 2 cm from the breast bone. The pH meter was calibrated by measuring buffer solutions (pH=4 and pH=7) after every 5 observations.

Moisture

Moisture contents of chicken thighs and breast muscle were determined according to AOAC (1999), by drying about 10 g of the sample at 105°C in the oven until a constant weight was recorded.

Oxidative stability

Oxidative stability in the meat samples is based on the reaction of one molecule of malondialdehyde (MDA) with 2 molecules of thiobarbituric acid (TBA). The color of the final complex is pink and the absorbance of the complex is measured spectrophotometrically. MDA is the major degradation product of oxidation of polyunsaturated fatty acids. Evaluation of TBA was performed according to Narciso-Gaytan *et al.* (2010).Briefly 30 gram of meat, in duplicate, was added with 15 mL of EDTA:propyl gallate solution (Sigma Aldrich, St. Louis, MO, USA), blended for two min, and 2 subsamples were added with 30 g of two mL of 4 N HCl and boiled in the Kjeldahl apparatus, 50 mL of MDA was distilled and five mL of it was added to five mL of TBA solution. The mixture was boiled in a water bath for 30 mins, followed by cooling in water at room temperature for 10 min. Thiobarbituric acid reactive substances values were measured in a spectrophotometer (Brite, UV/Vis Spectrometer BT 600) at 532 nm. Values were multiplied by a correction factor (7.8) and lipid oxidation results were provided in milligrams of MDA /Kg of sample (Narciso-Gaytan *et al.*, 2010).

Water holding capacity (WHC)

Water-holding capacity (WHC) was assessed by the method described by Jang *et al.* (2008). The breast meat of each broiler was ground in a food processor. Approximately two gram of minced meat was placed into a small polyethylene

bag with small holes. The polyethylene bag with meat sample in was then fitted into a 5-mL glasses tube. The tube was centrifuged at 1500× *g* for 5 mins. After centrifugation, the remained water was measured by drying the samples overnight at 70˚C (Jang *et al*., 2008).

Texture profile analysis (TPA) and color

Texture profile analyses (TPA) was assessed using a texture analyzer (Brookfield, LFRA. 4500 Texture Analyser, USA) as described by Santhi and Kalaikannan (2014). Briefly, samples were allowed to equilibrate at room temperature for 20 mins and then cut into uniformly sized cubes of 1 cm (width) × 1 cm (thickness) × 1 cm (length). Each sample was compressed twice to 80% of the original height using a compression probe (TA11/1000, 20mm). A crosshead speed of 10 mm/s was used. The values were recorded based on the software available in the instrument. The variables determined were: hardness (the maximum force needed to compress the sample), cohesiveness (a ratio between the total energy required for the first and second compression), springiness (the ability of a sample to recover to its original form after removing of the compressing force), and chewiness (a resultant of springiness × hardness × cohesiveness) (Al-Owaimer *et al*., 2014).

For the color analysis, lightness (L*), redness (a*), and yellowness (b*) were measured, using a colorimeter (Lovibond CAM-system 500). Briefly, after exiting samples from chiller, thigh and breast were taken out of the bags and rinsed thoroughly with tap water. Breast and thigh skins were raised up carefully and kept in room temperatures for 30 min. Then, the surfaces of meat samples (thigh and breast) were photographed from the similar sections. A Hunter Lab spectrophotometer model Color Quest II was used, calibrated with white standard (C6299 - Date 03/96 - X 77.46 - Y 82.08 - Z 88.38) and gray standard (C6299G - Date 03/96- X 47.71 - Y 50.83 - Z 54.94).

Statistical analysis

A completely randomized design (CRD) with 5 treatments and 5 replicates of 15 birds was employed. Statistical analyses were performed using GLM procedure of SAS software (SAS, 2005). Significant differences were further separated using Duncan's multiple range test. Statistical significance was considered at $P<0.05$.

Results and Discussion

The effects of dietary treatments on the breast meat quality of broiler chickens are shown in Table 2. There were no significant differences between treatments in selected quality traits. The meat color parameters such as L* (lightness), a* (yellowness) and b* (redness) values of breast muscle did not differ significantly. The effects of dietary treatments on the thigh meat quality of broiler chickens are summarized in Table 3. Dietary supplementation with 1% zeolite coated with 50 ppm nanosilver (NZ50) and 75 ppm nanosilver (NZ75) resulted in higher levels of WHC than those from the birds fed diets containing 1% zeolite (Z) ($P<0.05$).

Table 2. Selected quality of broiler breast muscle fed different treatment diets

Selected quality traits	Dietary treatments					
	Control	Z[1]	NZ25[2]	NZ50[3]	NZ75[4]	*SEM*[5]
Moisture (%)	69.2	68.5	70.3	71.4	68.6	3.10
pH	5.57	5.58	5.78	5.38	5.59	0.11
WHC[6] (%)	72.2	70.6	73.1	67.1	70.8	2.14
TBA-RS[7] (mg MDA/Kg)	2.35	2.58	2.80	2.84	3.07	0.26
Color parameters:						
L*(Lightness)	58.22	57.14	58.98	63.2 0	65.52	2.90
a* (Yellowness)	11.08	11.52	11.24	12.02	11.7	0.79
b* (Redness)	6.02	5.48	5.78	5.94	4.84	0.52

[1]Diet containing 1% Zeolite; [2]Diet containing 1% Zeolite coated with 25 ppm nanosilver; [3]Diet containing 1% Zeolite coated with 50 ppm nanosilver; [4]Diet containing 1% Zeolite coated with 75 ppm nanosilver; [5]Standard error of means; [6]Water holding capacity; [7]Thiobarbituric acid reactive substances. No significant difference was observed between treatments in each trait at $P>0.05$.

Table 3. Selected quality of broiler thigh muscle fed different treatment diets

Selected quality traits	Dietary treatments					
	Control	Z[1]	NZ25[2]	NZ50[3]	NZ75[4]	*SEM*[5]
Moisture (%)	74.33	74.02	73.51	69.20	68.02	2.89
pH	5.71	5.98	5.70	5.66	5.76	0.10
WHC[6] (%)	70.06^{ab}	64.51^{b}	73.21^{ab}	74.32^{a}	74.11^{a}	4.30
TBA-RS[7] (mg MDA/Kg)	2.35	2.81	2.80	2.91	2.79	0.18
Color parameters:						
L*(Lightness)	54.04^{b}	57.8^{ab}	56.18^{ab}	61.42^{a}	60.56^{a}	1.60
a* (Yellowness)	12.62	12.56	13.62	14.18	13.62	1.2
b* (Redness)	7.02^{a}	6.72^{ab}	6.08^{ab}	6.06^{ab}	5.46^{b}	0.48

[1]Diet containing 1% zeolite; [2]Diet containing 1% zeolite coated with 25 ppm nanosilver; [3]Diet containing 1% zeolite coated with 50 ppm nanosilver; [4]Diet containing 1% zeolite coated with 75 ppm nanosilver; [5]Standard error of means; [6]Water holding capacity; [7]Thiobarbituric acid reactive substances.
[a,b] mean values within a row with different superscripts differ significantly at $P<0.05$.

Although the exact mechanisms for the relation between nanosilver and meat quality have not yet been published and are still unclear, but Huff-Lonergan and Lonergan (2005) reported that status of antioxidant defense system would affect calpain activity, proteolysis, and thus water-holding capacity characteristics could be influenced by proteolysis. Myofibrils make up a large proportion of the muscle

cell. These organelles constitute as much as 82–87% of the volume of the muscle cell. Also, much of the water inside the living muscle cells is located within the myofibril. In fact, it is estimated that as much as 85% of the water in a muscle cell is held in the myofibrils. Much of that water is held by capillary forces arising from the arrangement of the thick and thin filaments within the myofibril (Huff-Lonergan and Lonergan, 2005). The endogenous calpain system plays a major role in regulating proteolysis of muscle proteins under postmortem conditions. It has been shown that the presence of oxidizing species have significantly impeded the ability of calpains to degrade their substrates. Since rapid proteolysis of intermediate filament proteins (like desmin) in meat has been associated with the improved water-holding capacity.

In our study, although MDA concentration was not significantly different among treatments, but a decreasing trend in MDA levels with an increase in nanosilver supplementation levels was observed. Protein oxidation can lead to the production of intermolecular bonds including disulfide, dityrosine, and other intermolecular bridges to form protein aggregation and polymerization (Morzel *et al*., 2006). Physical and chemical properties of proteins including solubility, hydrophobicity, WHC, and even the nutritional value can be modified by protein oxidation (Zhang *et al*., 2013). In postmortem muscle, protein oxidation has been become gradually recognized as an important factor for meat quality. During postmortem storage, muscle has a decreased ability to maintain its antioxidant defense system and this can cause the increased accumulation of reactive oxygen and nitrogen species (Renerre *et al*., 1996). The improved antioxidant status may promote the maintenance of cell membrane integrity (Cheah *et al*., 1995; Cai *et al*., 2012) which could be explained by the water holding capacity results in our study. Therefore, high WHC in the birds fed diets NZ50 and NZ75 resulted in higher levels of WHC may be due to the low level of protein oxidation.

Color values of broiler thigh muscle for lightness (L*) and redness (b*) were affected by dietary treatments ($P<0.05$). Birds fed NZ50 and NZ75 diets had a significantly higher L* value than the control diet. The b* values for thigh muscle of birds fed the control diet were significantly higher than those of birds fed NZ75 diets. Color variations and color defects of raw poultry meat and their related problems occuring in the poultry industry, have been a problem for many years. It has been reported that lightness (L*) had the highest correlation of the L*, a*, b* color values with the pale, soft, and exudative (PSE) like condition (Barbut, 1993). Several researchers have also demonstrated that a significant negative correlation exists between breast meat lightness color values and breast meat pH (Allen *et al*., 1997). According to Sams and Mills (1993), normal pH values at the end of the postmorten process are between 5.60 to 5.80 and 5.78 to 5.86, respectively. There are some evidences that there are positive correlations between WHC and pH and a negative correlation between WHC and moisture (Qiao *et al*., 2001). In our study, no differences were observed in pH values after slaughter between control birds

and the birds fed treatments diets. Indeed, Young *et al*., (2003) explained that there is no good relative correlation between pH and water-holding capacity and the overall lower final pH did not result in an overall decrease in water-holding capacity. Lightness color values in the birds were fed diets NZ50 and NZ75 may be explained by the fact that higher levels of the WHC were in the NZ50 and NZ75 diets. There are, however, other possible explanations. The color and antibacterial properties of nanosilver may be given possible a lighter meat that may not have any relation to pH value.

The effects of dietary treatment on the texture profile of broiler chickens breast meat are presented in Table 4. The hardness, cohesiveness, gumminess and chewiness values were influenced by treatment, while there were no significant differences between treatments in adhesiveness and springiness. The highest values of hardness, gumminess and chewiness were recorded by birds fed NZ75.

Table 4. Effect of treatments on texture profile analyses (TPA) of broiler chicken breast muscles

Dietary treatments	TPA					
	Hardness (g)	Adhesiveness	Springiness (cm)	Cohesiveness (ratio)[1]	Gumminess[2]	Chewiness[3]
Control	1361.50^{ab}	-36.23	1.05	0.49^{b}	667.14^{ab}	700.26^{ab}
Z[4]	1309.50^{b}	-30.92	1.04	0.48^{b}	628.56^{ab}	653.71^{ab}
NZ25[5]	1370.52^{ab}	-42.02	0.97	0.42^{b}	575.62^{b}	558.35^{b}
NZ50[6]	1410.47^{ab}	-32.48	1.13	0.56^{a}	789.78^{ab}	892.55^{a}
NZ75[7]	1560.32^{a}	-43.90	1.09	0.53^{a}	826.97^{a}	901.40^{a}
SEM	62.54	7.72	0.03	0.01	43.22	49.62

[1]Cohesiveness was calculated as area under second curve/area under first curve, [2]Gumminess was calculated as hardness × cohesiveness, [3]Chewiness was calculated as hardness × springiness × cohesiveness, [4]Diet containing 1% Zeolite; [5]Diet containing 1% Zeolite coated with 25 ppm nanosilver; [6]Diet containing 1% Zeolite coated with 50 ppm nanosilver; [7]Diet containing 1% Zeolite coated with 75 ppm nanosilver.

^{a,b} mean values within a rows with different superscripts differ significantly at $P<0.05$.

Texture profile analyses of broiler chickens thigh muscle are offered in Table 5. Hardness, adhesiveness and cohesiveness were not influenced by dietary treatment. There was a significant difference between control and other treatment groups in springiness ($P<0.05$). The highest values of gumminess and chewiness were observed in the group of birds receiving zeolite diets without nanosilver supplementation (Z) ($P<0.05$). However, since no article in relation to the meat quality and nanosilver has been published. it is difficult to explain this result, but it might be related to significant differences in water-holding capacity between treatments. WHC is a quality parameter closely correlated with the process of meat tenderness, which is a determinant qualitative factor and one of the most important sensory characteristics of the meat (Koohmaraie *et al*., 1990).

Table 5. Effect of treatments on texture profile analyses (TPA) of broiler chicken thigh muscles

Dietary treatments	TPA					
	Hardness (g)	Adhesiveness	Springiness (cm)	Cohesiveness (ratio)[1]	Gumminess[2]	Chewiness[3]
Control	1419.00	-29.21	1.17 [a]	0.48	681.12 [b]	796.91 [ab]
Z[4]	1622.50	-30.37	0.97 [b]	0.56	908.60 [a]	881.35 [a]
NZ25[5]	1470.00	-29.23	0.94 [b]	0.51	749.70 [b]	704.72 [ab]
NZ50[6]	1620.50	-31.68	0.97 [b]	0.54	874.80 [a]	848.82 [ab]
NZ75[7]	1556.32	-42.50	0.96 [b]	0.49	762.44 [ab]	732.10 [b]
SEM	58.37	6.51	0.02	0.03	38.22	39.61

[1]Cohesiveness was calculated as area under second curve/area under first curve, [2]Gumminess was calculated as hardness × cohesiveness, [3]Chewiness was calculated as hardness × springiness × cohesiveness, [4]Diet containing 1% Zeolite; [5]Diet containing 1% Zeolite coated with 25 ppm nanosilver; [6]Diet containing 1% Zeolite coated with 50 ppm nanosilver; [7]Diet containing 1% Zeolite coated with 75 ppm nanosilver.

[a,b] mean values within a rows with different superscripts differ significantly at $P<0.05$.

Conclusion

The present study was a preliminary one investigating the possible impact of nanosilver on meat quality. Furthermore, this study is helpful for poultry nutritionist and physiologist and the sector, although, more information and detailed studies needed to define the exact mechanisms for these biological effects.

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