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Decreasing the Effects of Aflatoxins on Color and Oxidative Stability of Broiler Meats using Nanozeolite

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ABSTRACT

This study was carried out to evaluate how various amounts of nanozeolite decrease the effects of Aflatoxins (AF) on color and oxidative stability of the broiler thigh meat. Three hundred and thirty six one-day-old Ross 308 male broiler chickens were divided into six groups including a control group, which received diet without AF and nanozeolite, and five treatment groups that received diet with 0.5 ppm of AF, as well as 0, 0.25, 0.5, 0.75, and 1% of nanozeolite. Results showed that broilers fed by AF contaminated diet without nanozeolite had the highest rate of *L and *a** values and the lowest rate of *b** value (P> 0.05). AF contaminated diet without nanozeolite increased ThioBarbituric Acid Reactive Substances (TBA-RS) significantly, compared to the control group and the treatment groups that received AF and nanozeolite (P< 0.05). Results suggest that nanozeolite (especially 0.75 and 1%) is able to reduce the adverse effects of AF on meat quality, especially lipid oxidation.**

Keywords: Lipid oxidation, Meat quality, Thigh, Thiobarbituric acid reactive substances.

INTRODUCTION

Aflatoxins (AF), a group of closely related extremely toxic chemicals, are produced by *Aspergillus flavus* and *A. parasiticus* and can occur as natural contaminants of poultry foods. Aflatoxicosis is an important disease of the livestock and poultry (Kaya *et al*., 1990; Leeson *et al*., 1995; Manafi, 2012). In poultry, aflatoxicosis causes listlessness, anorexia with lowered growth rate, poor feed utilization, decreased weight gain, decreased egg production, increased susceptibility to environmental and microbial stresses, and increased mortality (Bailey *et al*., 1998; Kubena *et al*., 1998). Various methods, such as physical separation, thermal inactivation, irradiation, microbial degradation and treatment with a variety of chemicals have been used for the detoxification or inactivation of AF-contaminated feedstuff (Goldblatt and Dollear, 1979; Anderson, 1983). One of the most promising approaches is the use of sorbents in diets that adsorbs AF in gastrointestinal tract of animals and reduces bioavailability and toxicity. Zeolite is one of these sorbent agents. The basic mechanism of action of zeolites is binding to AF in food irreversibly; thus, limiting AF absorption in digestive tract (Harvey *et al*., 1993; Kececi *et al*., 1998; Rosa *et al*., 2001; Mallek *et al*., 2012; Fendri *et al*., 2012).

Nowadays, nanotechnology shows promising solutions in food and pharmaceutical industries (Ahmadi and Hafsi Kurdestany, 2010). One of the substances used in nano formulations is silver. Silver-

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containing compounds used as antimicrobial agents in medicine have been applied in nano formulations by several methods (Russell and Hugo, 1994). The introduction of a single nanoparticle into other substrates results in novel systems with new applications. These systems are called nanocomposites (Evanoff and Chumanov, 2005). Nanocomposite properties depend on the properties of single constituents, particle size, shape, and interaction of surfaces. Zeolite as a porous solid material is a good candidate for this purpose (Talebi *et al*., 2010). The introduction of silver into the zeolite needs a good control of the particle size and a uniform distribution of silver metals on the internal and external surfaces (Talebi *et al*., 2010). Silver nanoparticles can be used as additive to replace supplemental antibiotics due to their good antibacterial effects and appropriate adaption to biological systems (Ahmadi and Hafsi Kurdestany, 2010). Nanosilver has been reported to inhibit growth of fungus and production of fungal mycotoxins (Egger *et al*., 2009; Gajbhiye *et al*., 2009).

It has been shown that addition of sorbents to diets can reduce the adsorption of AF in the gastrointestinal tract and reduce AF toxicity and, therefore, improve performance in broilers (Shi *et al*., 2006; Pasha *et al*., 2007). However, no report has been published on the effects of additive sorbents on chicken meat oxidation, color, water holding capacity (WHC), and pH in avian aflatoxicosis. Therefore, the current research was carried out to decrease the effects of AF on color and oxidative stability of broiler meats using various amounts of nanozeolite.

MATERIALS AND METHODS

Animals and Experimental Treatments

This experiment was carried out at Poultry Research Center, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Three hundred and thirty six male Ross 308 broiler chickens were randomly divided into six experimental groups, including a control group, which received diet without AF and nanozeolite, and five treatment groups that received diet contaminated with 0.5 ppm of AF, as well as 0, 0.25, 0.5, 0.75, and 1% of nanozeolite. Four replications including 14 birds were allocated to each group. The experimental treatments consisted of corn-soy basal diets and were prepared with the same value of energy and protein in the starting (3,000 kcal kg^{-1} ME and 215.6 g kg^{-1} CP) and growing (3,100 kcal kg^{-1} ME and 193 g kg^{-1} CP) periods according to NRC (1994) recommendations (Shabani *et al*., 2010).

AF Production and Nanozeolite Preparation

AF was produced by growing *A. Parasiticus*, IRCD 50 strain, based on the method described originally by Shotwel *et al*. (1996). Sterile soybean oil was used as vehicle for the incorporation of AF in diets (Del Bianchi *et al*., 2005). Then, diets oil was replaced with AF contained oil in treatments and mixed well. To verify the existence of AF in the final mixtures, AF concentration of 100 g sample from all treatments was assessed by HPLC (AOAC, 2005). The AF concentration in contaminated diets was 0.5 ppm. Nanozeolite PZE-105 supplements consisted of 1.5% silver and 98.5% zeolite (Pars Nano Nasb Company, IRAN).

Sample Collection

At day 42 of age, eight broilers of each treatment group were slaughtered. After slaughtering and dressing, thigh meats were packaged and stored at -20°C until use.

Meat Quality Analysis

Meat quality analysis was carried out after 1, 30, 90, and 180 days of storage in freezer.

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This included colorimetry and measuring of ThioBarbituric Acid Reactive Substances (TBA-RS), WHC, pH, moisture and lipid values, immediately after defreezing of meats.

Meat Color Analysis

Color measures were carried out with a Lovibond Tintometer Cam-System 500 (Amesbury, UK) using the colored tile provided by the manufacturer as the internal standard and set to use Illuminant D65 and CIE 10° standard observer. Four measurements were taken on the biceps femoris muscle. The measurements were expressed as *L*a*b** (L*: Relative lightness; a*: Relative redness, and b*: Relative yellowness). Higher *L** values indicated paler meats and higher *a** and *b** values indicated more red and yellow meats, respectively.

Lipid Oxidation Analysis

Lipid oxidation was measured by the 2-thiobarbituric acid distillation method (Haghparast *et al*., 2011). Briefly, 10 g of meat sample was transferred to a distillation flask and a few drops of silicone anti-foaming agent, then, 2.5 mL HCl (4 mol L^{-1}) and 97.5 mL distilled water were added. The sample was distilled until 50 mL of distillate had been collected. Then, 5 mL of the distillate were added to 5 mL of 0.02 mol L^{-1} thiobarbituric acid and heated in boiling water for 35 minutes. The solution was cooled under running tap water for 10 minutes and the absorbance was determined at 538 nm. Values for ThioBarbiturate Acid Reducing Substances (TBA-RS) were obtained by multiplying the absorbance by 7.8. Oxidative products were quantified as milligrams malondialdehyde per kilogram of meat (mg MDA kg^{-1} meat).

Analysis of WHC, pH, Moisture and Lipid

WHC of 1 g thigh meat sample was measured by centrifuging at 1500 g for four min and drying at 70°C (Castellini *et al*., 2002). pH of the samples was measured using pH meter. Moisture of the samples was measured for 12-16 hours after weighing 1 g of each sample and drying it in oven at 80°C. Lipid content of the samples was measured using Soxhlet device (AOAC, 2005).

Statistical Analysis

Data were analyzed by a repeated measurements design using SAS software (SAS, 2003). The model included treatments, times of storage in freezer (1, 30, 90 and 180 days), and the interactions (Treatment × Time) as fixed factors. The model used was as follows:

$$Y_{ijk}= \mu+T_i+t_k+(T\times t)_{ik}+\delta_{ij}+\varepsilon_{ijk} \quad (1)$$

Where, y_{ijk} represents each of the dependent variable; μ is the overall mean; T_i is the fixed treatment effect; t_k is the time effect; $(T \times t)_{ik}$ is the effect due to the interaction of treatment and time; δ_{ij} is the between-subject error term within a treatment, and ε_{ijk} is the random residual effect. Tukey-Kramer test at 5% probability level was used for the comparison of least squares means. Differences at $P< 0.05$ were considered significant.

RESULTS

pH

Treatments had no significant effects on pH of the meats (Table 1). After one day of storage, pH was significantly greater than that of other storage times ($P< 0.05$). No significant differences were seen using other storage times. Interaction of various amounts of nanozeolite and storage times

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Table 1. Mean comparisons of the effects of various treatments and storage times on pH in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments
	1	30	90	180	
Control	6.24 ±0.03	6.18 ±0.03	6.17 ±0.03	6.17 ±0.04	6.19 ±0.02
AF [a]	6.17 ±0.03	6.14 ±0.03	6.13 ±0.03	6.13 ±0.04	6.14 ±0.02
AF+0.25% NZ [b]	6.23 ±0.03	6.17 ±0.03	6.16 ±0.03	6.16 ±0.04	6.17 ±0.02
AF+0.5% NZ	6.23 ±0.03	6.17 ±0.03	6.16 ±0.03	6.16 ±0.04	6.18 ±0.02
AF+0.75% NZ	6.24 ±0.03	6.17 ±0.03	6.17 ±0.03	6.17 ±0.04	6.19 ±0.02
AF+1% NZ	6.25 ±0.03	6.18 ±0.03	6.18 ±0.03	6.17 ±0.04	6.20 ±0.02
Mean storage times [c]	6.21 ±0.01^{x}	6.17 ±0.01^{y}	6.16 ±0.01^{y}	6.16 ±0.01^{y}	

[a] Aflatoxin, [b] Nano Zeolite. [c] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

and the pH was not statistically significant (P> 0.05).

Color

Tables 2, 3, and 4 show mean comparisons of various levels of nanozeolite and storage times for the meat color coordinates (L*, a* and b* values). Broilers fed by AF contaminated diet without nanozeolite had the highest rate of *L** value, but with no significant difference (P> 0.05). Storage of the meat for 90 days caused a significant increase in *L** value, compared to that of meats with one and 30 days of storage (P< 0.05). Storage of the meat for 180 days caused a significant increase in *L** value, compared to that of meats with 1, 30, and 90 days of storage (P< 0.05). No significant differences were seen in *L** values between 1 and 30 days of storage. Interaction of various treatments and storage times and the *L** value was not significant. Results showed that the *L** value increased by increasing the storage time.

The *a** value indicates redness of the meat. The highest rate of *a** value belonged to broilers fed by AF contaminated diets without nanozeolite. Effects of various treatments on the *a** value were not significant. Storage of meats for 180 days resulted in significantly increased *a** value, compared to that of meats with 1, 30, and 90

Table 2. Mean comparisons of the effects of various treatments and storage times on color lightness (L*) value distribution in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments
	1	30	90	180	
Control	47.87 ±1.10	47.97 ±1.05	49.02 ±1.05	50.30 ±0.88	48.79 ±0.94
AF [a]	50.92 ±1.10	51.17 ±1.05	51.92 ±1.05	52.42 ±0.88	51.61 ±0.94
AF+0.25% NZ [b]	48.52 ±1.10	48.85 ±1.05	49.55 ±1.05	50.80 ±0.88	49.43 ±0.94
AF+0.5% NZ	48.05 ±1.10	48.42 ±1.05	49.30 ±1.05	50.52 ±0.88	49.07 ±0.94
AF+0.75% NZ	47.92 ±1.10	48.17 ±1.05	49.17 ±1.05	50.42 ±0.88	48.92 ±0.94
AF+1% NZ	47.65 ±1.10	48.05 ±1.05	49.05 ±1.05	50.30 ±0.88	48.76 ±0.94
Mean storage times [c]	48.49 ±0.45^{x}	48.77 ±0.43^{x}	49.67 ±0.43^{y}	50.79 ±0.36^{z}	

[a] Aflatoxin, [b] Nano Zeolite, [c] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

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Table 3. Mean comparisons of the effects of various treatments and storage times on color redness (a*) value distribution in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments
	1	30	90	180	
Control	11.00 ±0.74	11.15 ±0.89	11.65 ±0.89	12.32 ±0.89	11.53 ±0.89
AF [a]	11.77 ±0.74	11.97 ±0.89	12.57 ±0.89	13.22 ±0.89	12.39 ±0.89
AF+0.25% NZ [b]	11.27 ±0.74	11.52 ±0.89	12.27 ±0.89	12.85 ±0.89	11.98 ±0.89
AF+0.5% NZ	11.27 ±0.74	11.51 ±0.89	12.01 ±0.89	12.62 ±0.89	11.86 ±0.89
AF+0.75% NZ	11.02 ±0.74	11.37 ±0.89	11.97 ±0.89	12.60 ±0.89	11.74 ±0.89
AF+1% NZ	10.92 ±0.74	11.07 ±0.89	11.57 ±0.89	12.30 ±0.89	11.47 ±0.89
Mean storage times [c]	11.21 ±0.30^{x}	11.43 ±0.36^{x}	12.01 ±0.36^{x}	12.65 ±0.36^{y}	

[a] Aflatoxin, [b] Nano Zeolite. [c] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

Table 4. Mean comparisons of the effects of various treatments and storage times on color yellowness (b*) value distribution in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments
	1	30	90	180	
Control	1.50 ±0.79	1.11 ±0.76	0.86 ±0.65	0.41 ±0.53	0.97 ±0.36
AF [a]	1.42 ±0.79	1.02 ±0.76	0.52 ±0.65	-0.10 ±0.53	0.72 ±0.36
AF+0.25% NZ [b]	1.50 ±0.79	1.05 ±0.76	0.80 ±0.65	0.40 ±0.53	0.94 ±0.36
AF+0.5% NZ	1.52 ±0.79	1.10 ±0.76	0.85 ±0.65	0.41 ±0.53	0.97 ±0.36
AF+0.75% NZ	1.52 ±0.79	1.11 ±0.76	0.86 ±0.65	0.41 ±0.53	0.98 ±0.36
AF+1% NZ	1.52 ±0.79	1.13 ±0.76	0.86 ±0.65	0.41 ±0.53	0.98 ±0.36
Mean storage times [c]	1.50 ±0.32^{x}	1.09 ±0.31^{xy}	0.79 ±0.26^{y}	0.32 ±0.22^{z}	

[a] Aflatoxin, [b] Nano Zeolite.[c] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

days of storage (P< 0.05). No significant differences were seen between *a** values after 1, 30, and 90 days of storage. Interaction of various treatments and storage times and the *a** value was not significant.

The *b** value indicates yellowness of the meat. Broilers fed by AF contaminated diets without nanozeolite had the lowest rate of *b** value (P> 0.05). Storage of meats for 90 days caused a significant increase in *b** value, compared to that of meats with 1 day of storage (P< 0.05). Storage of meats for 180 days resulted in significantly increased *b** value, compared to that of meats with 1, 30, and 90 days of storage (P< 0.05). No significant differences were seen between *b** values after 30 and 90 days of storage. Interaction of various treatments and storage times and the b* value was not significant (P> 0.05).

Water Holding Capacity (WHC)

Data show that the meat of boilers fed by AF contaminated diets without nanozeolite had the lowest rate of WHC (Table 5). However, no significant differences were seen between various treatments and WHC (P> 0.05). WHC decreased by increasing the storage time, but this reduction was not statistically significant. Interaction between

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Table 5. Mean comparisons of the effects of various treatments and storage times on WHC in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments
	1	30	90	180	
Control	58.98 ±1.69	58.14 ±1.69	57.15 ±1.69	55.32 ±1.69	57.40 ±0.92
AF [a]	55.00 ±1.69	54.40 ±1.69	54.28 ±1.69	53.16 ±1.69	54.21 ±0.92
AF+0.25% NZ [b]	57.05 ±1.69	56.93 ±1.69	56.54 ±1.69	54.27 ±1.69	56.20 ±0.92
AF+0.5% NZ	58.74 ±1.69	56.24 ±1.69	56.17 ±1.69	54.81 ±1.69	56.50 ±0.92
AF+0.75% NZ	57.59 ±1.69	57.14 ±1.69	57.03 ±1.69	54.34 ±1.69	56.53 ±0.92
AF+1% NZ	58.85 ±1.69	56.88 ±1.69	56.31 ±1.69	55.11 ±1.69	56.80 ±0.92
Mean storage times [c]	57.71^{x} ±0.69	56.62^{xy} ±0.69	56.24^{xy} ±0.69	54.50^{xy} ±0.69	

[a] Aflatoxin, NZ: NanoZeolite. [c] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

various treatments and storage times was not significant (P> 0.05).

Moisture

Moisture of the meat was not affected by various treatments and storage times (Table 6).

TBA-RS

Effects of various values of nanozeolite and storage times on the oxidative stability of meats are shown in Table 7. AF contaminated diets without nanozeolite resulted in a significant increase in the rate of meat spoilage, compared to that of the control group and groups that received AF and nanozeolite (P< 0.05). No significant differences were found using other treatments. Storage of meats for 90 days caused a significant increase in malondialdehyde (MDA) levels, compared to that of meats with 1 and 30 days of storage ($P < 0.05$). Storage of meats for 180 days resulted in significantly increased MDA levels, compared to that of meats with 1, 30, and 90 days of storage (P< 0.05). This indicates an increased meat spoilage by increasing the storage time. No significant differences were seen between MDA levels of meat after 1 and 30 days of storage. Interaction between various treatments and storage times was not significant (P> 0.05).

Table 6. Mean comparisons of the effects of various treatments and storage times on moisture (%) in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments
	1	30	90	180	
Control	72.12 ±0.69	72.21 ±2.65	72.65 ±0.56	73.04 ±0.73	72.50 ±0.72
AF [a]	72.54 ±0.69	72.68 ±2.65	72.79 ±0.56	73.12 ±0.73	72.78 ±0.72
AF+0.25% NZ [b]	72.21 ±0.69	72.31 ±2.65	72.70 ±0.56	72.90 ±0.73	72.53 ±0.72
AF+0.5% NZ	72.19 ±0.69	72.27 ±2.65	72.67 ±0.56	72.90 ±0.73	72.50 ±0.72
AF+0.75% NZ	72.17 ±0.69	72.20 ±2.65	72.67 ±0.56	72.93 ±0.73	74.49 ±0.72
AF+1% NZ	71.96 ±0.69	72.11 ±2.65	72.60 ±0.56	72.83 ±0.73	71.37 ±0.72
Mean storage times	72.20 ±0.28	72.30 ±1.08	72.68 ±0.23	72.95 ±0.29	

[a] Aflatoxin, [b] NanoZeolite.

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Table 7. Mean comparisons of the effects of various treatments and storage times on TBA–RS in broiler thigh meats.

Treatment	Storage time (Day)				Mean treatments [d]
	1	30	90	180	
Control	0.44 ±0.05	0.49 ±0.04	0.55 ±0.04	0.92 ±0.11	0.60 ±0.03[b]
AF [a]	0.53 ±0.05	0.63 ±0.04	0.83 ±0.04	1.25 ±0.11	0.81 ±0.03[a]
AF+0.25% NZ [b]	0.44 ±0.05	0.49 ±0.04	0.54 ±0.04	0.92 ±0.11	0.60 ±0.03[b]
AF+0.5% NZ	0.43 ±0.05	0.48 ±0.04	0.54 ±0.04	0.90 ±0.11	0.59 ±0.03[b]
AF+0.75% NZ	0.43 ±0.05	0.48 ±0.04	0.53 ±0.04	0.89 ±0.11	0.58 ±0.03[b]
AF+1% NZ	0.43 ±0.05	0.47 ±0.04	0.52 ±0.04	0.86 ±0.11	0.57 ±0.03[b]
Mean storage times [c]	0.45 ±0.02[z]	0.51 ±0.02[z]	0.58 ±0.02[y]	0.95 ±0.04[x]	

[a] Aflatoxin, [b] NanoZeolite. [c] Superscripts indicate significant differences (P< 0.05) when treatments are compared. [d] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

Lipid Content

As shown in Table 8, broilers fed with AF contaminated diets without nanozeolite had the lowest lipid content of the meat (P< 0.05). No significant differences were seen in lipid content of meats between other treatments. Storage of meats for 90 days caused a significant reduction in lipid content of the meat, compared to that of treatments with 1 and 30 days of storage (P< 0.05). Storage of meats for 180 days resulted in significantly decreased lipid content, compared to that of meats with 1, 30, and 90 days of storage (P< 0.05). No significant differences were seen between lipid contents after 1 and 30 days of storage. Interaction between various treatments and storage times was not significant (P> 0.05).

DISCUSSION

pH, Color and WHC

Important meat quality parameters include pH, color, WHC and tenderness. After slaughter, pH of the meat is affected

Table 8. Mean comparisons of the effects of various treatments and storage times on lipid content (%) in broiler thigh meats.

Treatment [a]	Storage time (day)				Mean treatments [d]
	1	30	90	180	
Control	29.65 ±1.35	29.20 ±1.20	28.03 ±0.85	26.78 ±1.18	28.41 ±0.85[a]
AF [a]	25.64 ±1.35	24.80 ±1.20	22.53 ±0.85	20.28 ±1.18	23.31 ±0.85[b]
AF+0.25% NZ [b]	29.68 ±1.35	29.22 ±1.20	28.14 ±0.85	26.72 ±1.18	28.44 ±0.85[a]
AF+0.5% NZ	29.71 ±1.35	29.26 ±1.20	28.19 ±0.85	26.83 ±1.18	28.50 ±0.85[a]
AF+0.75% NZ	29.71 ±1.35	29.26 ±1.20	28.18 ±0.85	26.95 ±1.18	28.53 ±0.85[a]
AF+1% NZ	29.74 ±1.35	29.29 ±1.20	28.24 ±0.85	27.21 ±1.18	28.62 ±0.85[a]
Mean storage times [c]	29.02 ±0.65[x]	28.50 ±0.65[x]	27.22 ±0.65[y]	25.80 ±0.65[z]	

[a] Aflatoxin, [b] NanoZeolite, [c] Superscripts indicate significant differences (P< 0.05) when treatments are compared. [d] Superscripts indicate significant differences (P< 0.05) when storage times are compared.

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primarily by the postmortem conversion of muscle glycogen stores to lactic acid that accumulates in muscles (Monin and Ouali, 1992). Lactic acid accumulates in tissues and gradually lowers the pH from near neutral to mild acidic (Coetzee and Hoffman, 2001). pH of the meat is fixed by increasing the storage time. Then, post mortem glycolysis is inhibited even in presence of residual glycogen, depending on species and muscle type. The mechanisms of action have been discussed by Filgueras *et al*. (2010).The lack of Adenosine MonoPhosphate (AMP), a cofactor of glycogenolytic and glycolytic enzymes, in postmortem possibly explains the stability of pH in the presence of glycogen.

In the current study, meat color was changed during storage. Meat color is an important factor for the consumers (Clydesdale, 1991). It depends on the concentration and oxidation rate of myoglobin and the meat structure (Chasco *et al*., 1995). Changes in muscle pH also directly affect color and WHC due to the effect on protein structure and subsequent hydration properties of the meat proteins (Monin and Ouali, 1992). Muscle pH and meat color are highly related. Low pH generally produces a pale meat colour and high pH results in a much darker color (Fletcher, 2002). In 2010, Filgueras *et al*. reported that the decrease in pH resulted in denaturation of sarcoplasmic proteins and, therefore, increasing light scattering and meat paleness. Similarly, Owens *et al*. (2000) indicated that lower ultimate muscle pH is associated with higher *L** values. Furthermore, pH is suggested to effect via influencing various haem reactions in muscles that are pH dependant. Redness (a*) is the most important color parameter to evaluate meat oxidation. Amount of myoglobin, specifically the balance between the various myoglobin complexes, influences redness of the meat. For example, oxymyoglobin produces a bright red color characteristic whereas metmyoglobin forms a browner color (Fletcher, 2002). In 2006, Seydim *et al*. reported that decrease in redness was due to myoglobin oxidation, especially when meat pH was higher than 6. At high pH values, mitochondrial enzyme systems (cytochrome, succinate and pyruvate-malate oxidase) do not shut down and are able to utilize the available oxygen (Lawrie, 1998). Filgueras *et al*. (2010) reported that oxygen consumption in high-pH muscles is higher than that in muscles with normal pH. Hence, prolonged storage induces the transformation of oxymyoglobin (bright red color) into brown metmyoglobin (Renerre, 2000). Similar to findings of the present study, Wang *et al*. (2006) observed that feeding the contaminated diets resulted in a decrease in *b** value, which suggested more fat and pigment oxidation; for example, less pigment retention.

WHC is an important quality characteristic of the meat. About 88 to 95% of water in the muscle is held intracellularly within the space between actin and myosin filaments (Ranken, 1976; Offer and Knight, 1988). WHC determines juiciness, flavor, and tenderness of the meat (Wood, 1993). Lactic acid accumulation and pH decline in postmortem result in protein denaturation and an overall decrease in muscle WHC. During the rigor mortis period, divalent cations in sarcoplasm bind to the reactive groups on adjacent protein chains, reducing the electrostatic repulsion between negatively charged groups that maintain their separation (Wismer-Perdersen, 1986). This reduces the space available for water to be retained intramuscularly.

TBA-RS and Lipid Content

Lipid oxidation is linked to adverse changes in appearance, flavor and texture of the poultry meat (Jensen *et al*., 1998). Muscle contains several endogenous pro-oxidants such as myoglobin and ionic irons, which are strongly regulated by various endogenous antioxidant factors, including reducing compounds (e.g. ascorbic acid), natural antioxidants (carnosine, anserine and α-tocopherol) and antioxidant enzymes

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(catalase and superoxide dismutase) (Chan and Decker, 1994; Min and Ahn, 2005). However, regulation of these pro-oxidants may be decreased fast when muscles are processed to meat (Morrissey *et al*., 1998). Generally, lipid oxidation in muscles is believed to initiate in highly unsaturated phospholipid fraction of the subcellular membranes (Gray and Pearson, 1987). The autocatalytic peroxidation process possibly starts immediately after slaughter and depends on various factors. One of the most important factors is the amount of PolyUnsaturated Fatty Acids (PUFA) (Allen and Foegeding, 1981). Phospholipids of the subcellular membranes (e.g. mitochondria and microsomes) are found extensively in PUFA (Gray and Pearson, 1987), hence, the susceptibility of membranes to peroxidation is increased because of the close proximity of a range of pro-oxidants. The oxidation rate of meats is generally assessed by measuring the content of secondary degradation products that arise from oxidation of PUFA. Analytical methods use substances that react with TBA-RS as a determinant of the lipid oxidation rate. TBA-RS values are usually reported in MDA equivalents, a compound that results from the decomposition of PUFA lipid peroxides. A higher TBA-RS value indicates a greater degree of lipid oxidation (Berges, 1999).

AF is known as a free radical creator. Free radicals are very active and unstable. MDA is the main index of lipid peroxidation made by free radicals. MDA are one of the final products of peroxidation or promote the peroxidation process (Dinc *et al*., 2006). *In vivo* and *in vitro* studies have shown that AF cause cellular damages. Free radicals are formed in the damaged cells (Decoudu, *et al*., 1992; Souza, *et al*., 1999). They react with the complex of cellular membrane and cause peroxidation of lipid of membrane (Kohen and Nyska, 2002; Eraslan *et al*., 2005). As mentioned earlier, AF stops protein synthesis which causes reduction in concentration of synthesized ceruloplasmin and transferrin in liver. This reduction causes increased free copper and iron ions in organs that subsequently results in changes in immune system against lipid peroxidation. Iron plays an important role in response to fentons, a lipid preoxidation phase (Agil *et al*., 1995; Kohen and Nyska, 2002). Banding iron from outside and inside absorption causes reduction of free iron levels, which acts as a mechanism to prevent lipid peroxidation (Comporti, 1993). Eraslan *et al*. (2005) have found that AF possibly causes interference in compounding iron and internal absorbers. Therefore, liver damages and protein synthesis failures result in increased free iron ions.

Results of the current study show the increase of MDA levels, which has been confirmed previously by Dinc *et al*. (2006). They found that quails fed with contaminated diets by 2.5 ppm of AF had significantly more plasma MDA levels. Eraslan *et al*. (2005) reported MDA as an index of cell wall damages caused by AF. They showed a significant increase in erythrocyte MDA levels during a study on the effects of 0.05, 0.1, 0.5 and 1 ppm of AF on the oxidative stress in broilers. A similar study was carried out by Verma and Nair (2001) on the MDA level in mouse testicles. These researchers have also reported that AF causes increased MDA levels and reduces the activity of superoxide dismutase, catalase and glutathione peroxidase enzymes in testicle tissues.

Furthermore, AF makes cellular damages in digestive system which cause6+ reduction of nourishments such as vitamins. Hence, the antioxidant defense mechanism will become weak in the body. Vitamin A, which is stored in liver, plays a role in prevention of lipid peroxidation (Decoudu *et al*., 1992). Reduction in vitamin levels, especially vitamin A, can be one of the reasons for the increase of MDA levels. Eraslan *et al*. (2005) reported that 0.5 and 1 ppm of AF caused reduction of enzyme activities of SuperOxide Dismutase (SOD), catalase, Glutathione Peroxidase (GSH-Px) and Glucose-6-Phosphate Dehydrogenase (G6PD). This reduction may either be related to inhibitory effect of AF, similar to

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the case valid for SOD, or to consumption during the breakdown of high levels of H_2O_2 inside the cell. Another reason that may lead to decrease in GSH-Px activity is loss of liver glutathione stocks and weakening of glutathione synthesis by AF. Decrease in activity of G6PD can also be an indicator of the inhibitory effect of AF. Changes in the enzyme activity indirectly cause changes in the levels of MDA (Eraslan *et al*., 2005). Therefore, it can be concluded that AF and/or prolonged storage cause increased lipid oxidation. Furthermore, reduction in lipid content due to feeding broilers with AF contaminated diets can be linked to inhibitory effects of AF on lipid synthesis, transferring lipid from liver, and increasing peroxidation of lipids.

CONCLUSIONS

The present research seems to be the first study on the effects of nanozeolite on the quality of thigh meats in broilers fed with AF contaminated diets. According to the findings of this study, it can be concluded that use of nanozeolite (especially 0.75 and 1%) in AF contaminated diets can reduce the adverse effects of AF on the meat quality by limiting AF absorption from the gastrointestinal tract. Furthermore, nanozeolite can be used to improve the oxidative stability of the meat. It is worth to note that the reduction of pH during the storage resulted in decreased meat color, WHC, and lipid contents and increased MDA levels (A higher MDA level indicates a greater rate of oxidation in meats).

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کاهش اثرات آفلاتوکسین بر رنگ و پایداری اکسیداتیو گوشت جوجه های گوشتی با استفاده از نانوزئولیت

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چکیده

این مطالعه به منظور بررسی تاثیر مقادیر مختلف نانوزئولیت بر کاهش اثرات آفلاتوکسین بر رنگ و پایداری اکسیداتیو گوشت ران جوجه های گوشتی انجام شد 336 .جوجه خروس یکروزه راس ۳۰۸ در شش گروه شامل گروه شاهد (دریافت کننده جیره غذایی بدون آفلاتوکسین و نانوزئولیت) و پنج گروه دریافت کننده جیره غذایی حاوی ۰/۵ پی پی ام آفلاتوکسین و همچنین سطوح صفر، ۰/۲۵، ۰/۵، ۰/۷۵ و ۱ درصد نانوزئولیت توزیع شدند. نتایج نشان داد که جوجه های گوشتی تغذیه شده با جیره غذایی آلوده به آفلاتوکسین و فاقد نانوزئولیت بالاترین میزان شاخص L^*و پایین ترین میزان از شاخص b^*را داشتند (۰/۰۵ .(<Pجیره غذایی آلوده به آفلاتوکسین و فاقد نانوزئولیت بطور قابل توجهی سبب افزایش TBA-RS در مقایسه با گروه شاهد و تیمارهای دریافت کننده آفلاتوکسین و نانوزئولیت شد .(05/0P<)نتایج نشان می دهد که نانوزئولیت قادر به کاهش اثرات سوء آفلاتوکسین بر کیفیت گوشت، به ویژه اکسیداسیون چربی است.

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