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Effect of Environmental Heat Stress on Oxidative Stress Genes in Liver and Intestine of Broilers Fed with Silver Nanoparticles Coated on Zeolite and Organic Acids

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Abstract

Background and aim: The effect of external heat stress on the oxidative stress genes in the liver and intestine of broiler chickens fed with silver nanoparticles coated on zeolite and organic acid was investigated.
Materials and Methods: 450 one-day-old Cobb chicks were divided into 5 groups of 15, with 6 replicates of each group and kept for 42 days in the same rearing conditions. 1) Control (basal diet), 2) A base diet with 1% zeolite, 3) A basic diet supplemented with 0.5% nanosilver-coated clinoptilolite at 1%, 4) A base diet with 0.15% organic acid, and 5) A base diet with 1% zeolite covered in 0.5% nanosilver and 0.15% organic acid. The heat stress (34±1˚C temperature for 6 hours per day) was provided from 35th to 42th days. To examine antioxidant enzymes gene expression on day 42, liver and intestinal samples were taken.
Results: In treated groups, the relative expression of the liver SOD gene was not significantly different compared with control group ($P>0.05$), whereas in all treatment groups SOD and glutathione peroxidase (GPX) expression had shown decrease in the intestine compare to control group ($P<0.05$). In group treated with zeolite coated with nanosilver and an organic acid, the relative expression of the catalase (CAT) gene showed decrease while it increased in group treated with a basal diet.
Conclusion: It was observed that the intestine has higher levels of antioxidant enzymes activity compared to the liver. Also, to increase the growth efficiency, it is preferred to use the combination of silver nanoparticles with organic acids.

Keywords: *Antioxidant, Broiler, GPX, Heat stress, Nanosilver*

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Introduction

Numerous factors such as extreme heat, temperature instabilities, and increased moisture levels cause heat stress in poultry. Heat stress is one of the most important environmental stressors in the poultry industry, especially in tropical regions. In poultry, heat stress can cause physiological system disorders, including a variety of homeostasis changes, immune system suppression, alkalosis, and hormone imbalance (Liu *et al.,* 2020). Heat stress may lead to oxidative stress by affecting mitochondrial function and changing reactive oxygen species (ROS) levels, thereby mitochondrial ROS generation, damage to proteins and lipids, and changes in the levels of oxidative stress markers, such as malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) (Emami *et al.,* 2021; Hosseini-Vashan *et al.,* 2016).

On the other hand, antibiotics used as growth promoters (AGPs) in feed have been banned in the European Union on January 1, 2006. Therefore, the animal and poultry industry are looking for effective and real alternatives. Organic acids, enzymes, probiotics, prebiotics, and herbal plants could serve as an alternate source to antibiotics (Hashemi *et al.,* 2012; Papatisiros *et al.,* 2016). Organic acids are among the most seriously researched alternatives to antibiotics. However, acidic compounds consisting of organic acids show promise as antibiotic alternatives (Papatisiros *et al.,* 2016; Hashemi *et al.,* 2014 b). Weak organic acids (C_1-C_7) with a pK_a between 3 and 5 are explicitly used for their antimicrobial activity (Papatisiros *et al.,* 2016). Organic acids are nutrients with acidifying effects that have demonstrated the capability to enhance poultry performance by altering the pH of the gastrointestinal tract (GIT) and consequently changing the composition of the microbiome. In addition, organic acids, by altering the composition of the microbiome, protect poultry from pH-sensitive pathogens such as Salmonella, so they can improve birds' health and performance (Hashemi *et al.,* 2014b).

Also, recent advances in nanotechnology radically changed the way we diagnose, treat, and prevent various diseases in all aspects of human and animal life. Silver nanoparticles are one of the most vital and attractive nanomaterials among several metallic nanoparticles that are involved in biomedical applications. Silver nanoparticles play an important role in nanotechnology, particularly in nanomedicine. The capacity of nanosilver to destroy infectious micro-organisms makes it one of the most powerful antimicrobial agents. Silver nanoparticles have various applications in poultry production, for example, they are used in disinfectant preparations in hatcheries (Banach *et al.,* 2016) and they are subjugated for their antibacterial effect on many gram-negative and gram-positive bacteria (Ahmed *et al.,* 2016; Farouk *et al.,* 2020). Moreover, they exhibit antibacterial activity against *Campylobacter jejuni, Escherichia coli, Bacillus* spp., *Klebsiella pneumoniae, Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Saraniya Devi *et al.* 2014; Vadalasetty *et al.,* 2018). Silver nanoparticles have also been used as a growth promoter for broiler chickens and have been reported to improve their health and performance (Bolandi *et al.,* 2012; Hashemi *et al.,* 2014 a).

Recently, silver nanoparticles have been reported to exert toxicity through an increase in ROS production. However, this is still subject to debate. The induction of ROS by silver nanoparticles is certainly a primary mechanism of toxicity. Research on mouse fibroblasts and human hepatocytes reveals that an increase in ROS levels induced by nanosilver is accompanied by a reduction of mitochondrial membrane potential, release of cytochrome C into the cytosol and translocation of BAX gene to mitochondria (Hsin *et al.,* 2008; Piao *et al.,* 2011). The antiapoptotic protein Bcl_2 is expressed at high levels by human HCT116 colon cancer cells that are resistant to nanosilver (Hsin *et al.,* 2008). Further, silver nanoparticles and silver cation (Ag^+) mediate ROS generation, including superoxide, hydroxyl radicals and hydrogen peroxide in cell-free systems (He *et al.,* 2011; He *et al.,* 2012 a). Whether the toxicity of nanosilver toxicity is caused by Ag^+, the nanoparticle structure or other factors is unknown.

There is informal evidence that combinations of antibiotic alternatives can be effective even if the bacteria are resistant and the best results can be achieved in the combination of these feed additives under good environmental conditions. Hence, research was conducted to investigate the effect of environmental heat stress on oxidative stress genes (SOD, GPX, and CAT) in the liver and intestine of broilers fed silver nanoparticles coated on zeolite and organic acid.

Materials and Methods

Diet and husbandry

A total of 450 one-day-old Cobb 500 chicks (mixed sex) were divided into 5 and subdivided into 6 replicates with 15 chicks per each and were kept for 42 days in the same rearing conditions in the form of a completely random design (CRD). The basal diet without any feed additive and antibiotic served as control and one of the following additives. Experimental diets were: 1) Control or basal diet (C), 2) Basal diet supplemented by 1% zeolite (Z), 3) Basal diet supplemented by 1% zeolite coated with 0.5% nanosilver (NS), 4) Basal diet supplemented by 0.15% organic acid (OA), and 5) Basal diet supplemented by

1% zeolite coated with 0.5% nanosilver and 0.15% organic acid (NSOA). Isoprotein and isocaloric diets were formulated according to Cobb 500 recommendations using the user-friendly feed formulation (UFFDA) software (Table 1). To create a heat stress environment, birds were subjected to a temperature of 34°C from d 35 to d 42. This heat-stress environment was prolonged for 6 hours each day. All diets were fed in mash form and birds had free access to feed and water for 42 days and during heat stress conditions. Temperature and relative humidity were recorded every hour during the acute heat stress period.

Ingredients (%)	Starter (21-1)	Grower
Yellow corn	51.6	(42-22)
Soybean meal (44% CP)	39.95	57.84
Soybean oil	3.69	33.68
Dicalcium phosphate (DCP)	1.47	4.11
Limestone	1.18	1.09
Salt	0.43	1.28
Vitamin premix	0.25	0.32
Mineral premix2	0.25	0.25
DL-Methionine	0.13	0.25
L-Lysine	0.05	0.05
Chemical analysis		0.13
ME (Kcal/kg)	2950	
CP (%)	21.2	3050
Ca (%)	0.92	19.06
P (%)	0.41	0.86
Na (%)	0.18	0.33
Lys (%)	1.01	0.14
Met (%)	0.47	0.95
Cys (%)	0.36	0.36
Arg (%)	1.45	0.37
Thr (%)	0.84	1.27

Table 1. Composition and analysis of the basal diet (as fed basis). 1 The diet is prepared based on the guidance of the Cobb 500 strain. 2 Each kilogram of feed contains: vitamin A, 1500 IU; cholecalciferol, 200 IU; Vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; Niacin 30 mg; choline chloride, 1000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine, 1.5 mg; pyridoxine, 0.3 mg; iron, 80 mg; zinc, 40 mg; Manganese, 60 mg; Iodine, 0.18 mg; copper, 8 mg; Selenium, 0.15 mg; cobalamin, 15 µg.

The organic acid (Biotronic® SE forte, BIOMIN, Austria) was a kind gift from Dr. A. Khosravi. The organic acid contains formic and propionic acids on a special carrier that is able to protect acids and release them sequentially in the intestinal tract.

The zeolite used in this experiment was supplied by the Afrand Tooska Company from the region of Semnan province, Iran. Zeolitic rock was pulverized and sieved to make particles in the size of 1-2 mm. distilled water was used to wash and remove contaminations from the particles and subsequently dried at 105 °C all over the night in the oven. The chemical formula of pure zeolite was (K_2, Na_2, Ca and Mg) $_3$ Al_6 $Si_{30}O_{72}.24H_2O$ (Table 2).

Semnan natural zeolite-rich tuffs	
Constituents	% by weight
SiO2	68.95
Al2O3	11.14
Fe2O3	0.97
CaO	4.83
Na2O	0.95
K2O	0.90
MgO	0.79
TiO2	0.201
MnO	0.011
P2O5	0.012
SO3	0.068
L.O.l2	10.64
Si/Al	4.81
Ag3	< 5ppm

Table 2. The summarized chemical composition of the used zeolite utilizing the X-ray Fluorescence (XRF)1 technique. [1] XRF PHILIPS PW1480 XRF spectrometer with Rh tube. [2] LOI: loss on ignition. [3] The silver content was measured by a graphite furnace atomic absorption spectros-copy (GFAAS).

Nanosized silver particles (Ag-NPs) with a maximum diameter of 50 nm were coated on zeolite and received as a gift from Dr. D. Davoodi at the Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran. Briefly, the preparation of the coating method on zeolite with desired properties was that the zeolite was first stirred in distilled water with a stirrer, and then, the prepared nanosilver (20-50 nm) with the desired percentage was added to the mixture after adjusting the pH and stirring was continued for a further 30 min at 15 ^{o}C. The stabilizers were gradually added to the mixture and stirred until the resulting mixture color became brown. After settling the precipitate dried at ambient temperature.

Zeolites were analyzed by X-ray Fluorescence (XRF) technique to determine their chemical composition and the XRF data was collected on a PHILIPSPW1480 XRF spectrometer with a Rh tube (Nikpey *et al.* 2013) (Table 1). The morphology was studied by field emission scanning electron microscopy (FESEM, Mira, 3-XMU) and the materials composition was investigated by energy-dispersive X-ray spectroscopy (EDX) and elemental mapping analyses at Razi Metallurgical Research Centre, Iran. To measure the Ag content of zeolite we used the method described by Kulthong *et al*. (2010). Briefly, 0.2-0.3 g of sample was digested in 5 mL of 14.4 M HNO_3 by a microwave digestion system to dissolve all the silver content. The microwave irradiation cycles were 250 W (5 min), 400 W (5 min), and 600 W (5 min). Then the digested sample was cooled and diluted up to 25 mL with deionized water to enable quantification of silver by a graphite furnace atomic absorption spectroscopy or GFAAS (Perkin Elmer Analyst 300, Waltham, MA).

Sample collection and bioinformatic analysis

At the end of the heat stress condition on d 42, six birds from each treatment were randomly selected and slaughtered. The liver and intestine (duodenum) were collected aseptically and immediately stored in liquid nitrogen at -196 °C for subsequent RNA analysis. Total RNA of individual samples was extracted using a pure RNA kit (Sinaclon Bioscience Co, Tehran, Iran) following manufacturer recommendations. The purity and integrity of the total RNA bands were evaluated by loading 200 ng of RNA samples on 1% agarose gel stained with GelRed®.and pictured under UV light in GEL DOC XR + and CHEMIDOC XRS + imaging Systems containing the IMAGE LAB software (Biorad, CA, USA). Total RNA was subjected to reverse transcript (RT)-PCR as previously described (Taouis *et al.,* 1996; Raimbault *et al.,* 2001) Qualitative and quantitative assessments of the isolated RNA were carried out on an ND-1000 NanoDrop (NanoDrop Technologies, USA) spectrophotometer. Only RNA with an absorbance ratio (A260/A280) greater than 1.8 was used for the synthesis of cDNA. The RNA was reverse transcribed into cDNA with the transcription first strand cDNA synthesis kit (one-step RT-PCR kit, Qiagen, USA)

The levels of SOD, CAT, GPX and β-actin transcripts were determined. In all cases, Ct values were determined based on three biological replicates each with two technical replicates. RT-qPCR was performed with a reaction mixture containing 8 µl of SYBR Green master mixture (Applied Biosystems SYBR Green, Carlsbad, CA, USA). To normalize data in a qRT-PCR experiment, β-actin was used as an endogenous reference gene. Specific primers of SOD, CAT, GPX and GAPDH were designed using Oligo7 software on the sequence of genes on the NCBI database and their synthesis was done by Pishgaman (Pishgaman Co, Tehran, Iran). Primers for housekeeping genes (GAPDH) and target genes are listed in Table 3. The relative gene expression in target and reference samples was analyzed using the $2^{-\triangle\triangle Ct}$ method (Livak *et al.,* 2001). Details of all primers and housekeeping genes used are provided in Table 3.

Primer name	Orientation	Primer sequence (5′–3′)	TM ^{o}C
SOD	Forward	TGGTGTAATTGGAATAGC	55
	Reverse	AGTAGTAATGAGATTAAGTGAT	
GPX	Forward	GTGAGTGTGGGTTTACAGATAGT	60
	Reverse	CTTTGTTGGTGTCTGGTTCTTG	
CAT	Forward	TTACTTTCCCTCTTCCCTTACCA	60
	Reverse	GCTTCTTCCAATCCAACTGCTA	
GAPDH	Forward	GGTGTTAAGGTGGTGGCTGT	59
	Reverse	GGGCTCTTTGCACTGGTAGA	

Table 3. The primer sequences used in relative quantitative real-time PCR (q-PCR).

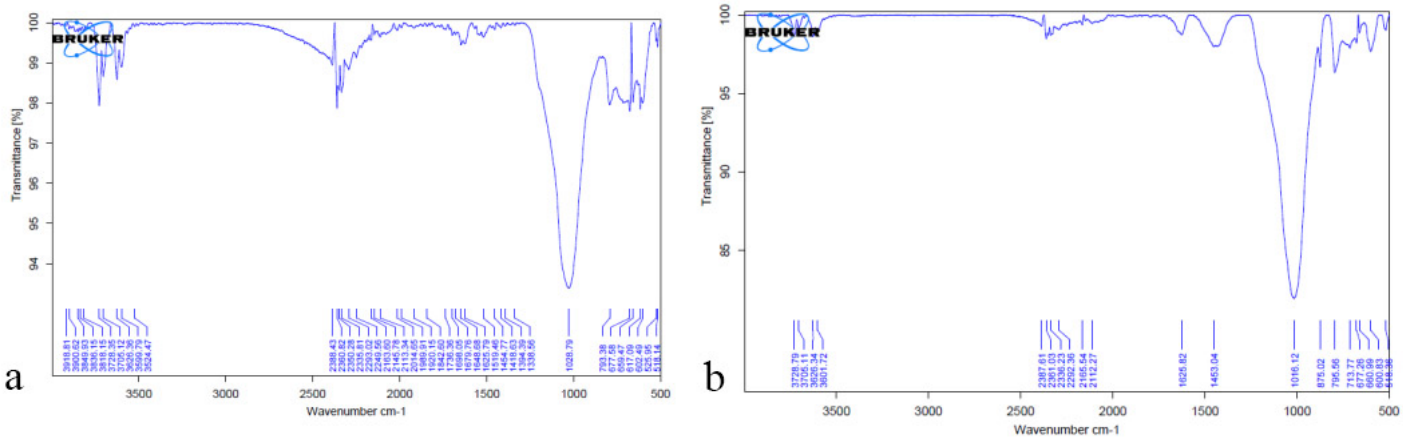
Statistical analysis

All data were initially checked for normality and homogeneity of variance using Bartlett and Kolmogorov–Smirnov tests. All data were subjected to analysis of variance as a completely randomized design, using the general linear model's procedure of

SAS (SAS Institute Inc., Cary, NC) (SAS 2008). Tukey's honestly significant difference test was used to separate means with significance levels. Statistical significance was set at P≤0.05.

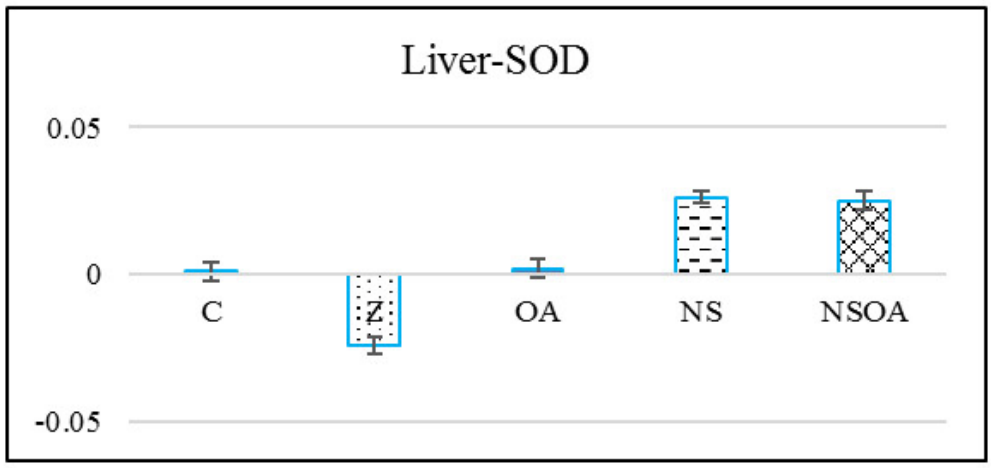
Results

Silver nanoparticles coated on zeolite were investigated using XRF and FTIR techniques, and the desired silver nanoparticle synthesis was confirmed (Graph 1).

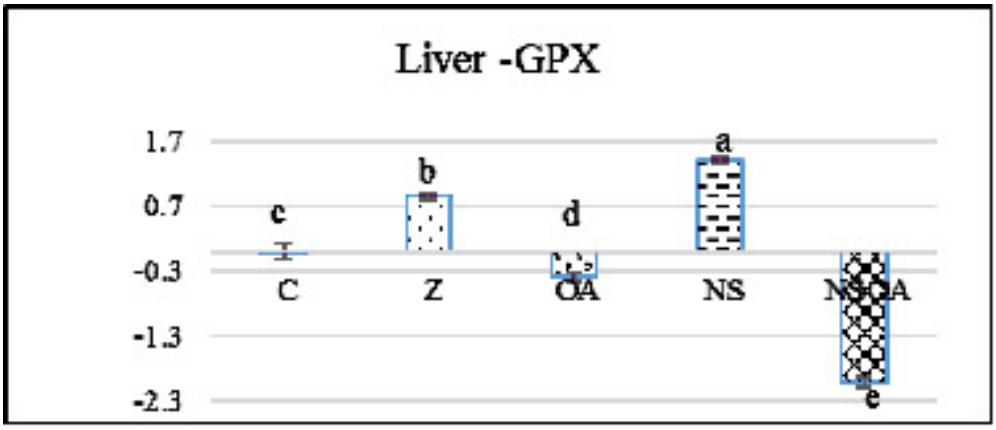


Graph 1. Fourier transforms infrared spectra (FTIR) of zeolite (a) and silver nanoparticles coated on zeolite (b).

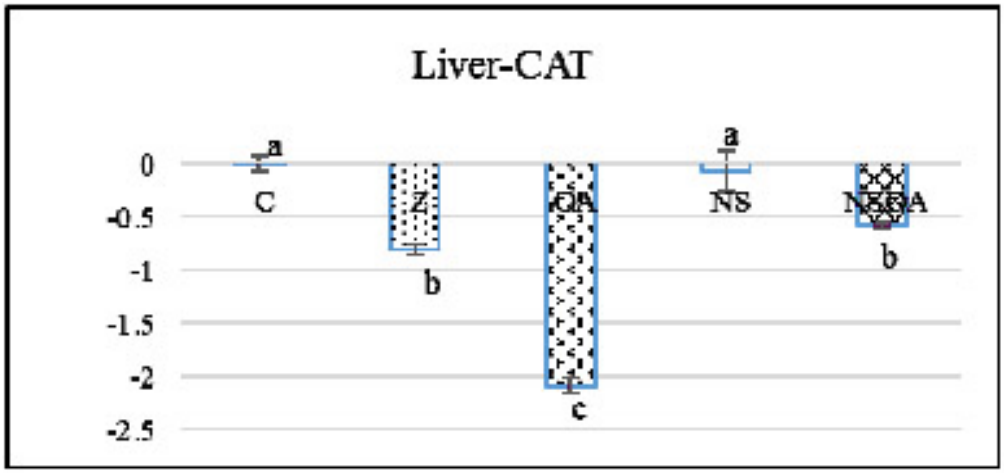
The effects of experimental treatments on relative gene expression (SOD, GPX and CAT) of liver tissue on d 42 are shown in graphs 2 to 4. Statistically, there was no significant difference between the experimental treatment and control groups in the relative expression of the SOD gene (Graph 2) (P>0.05). A significant difference was observed in the relative expression of the GPX gene in all treatment groups compared to control (P<0.05). This significant difference was increased in NS and Z treatments and decreased in OA and NSOA treatments (Graph 3). In the Z, OA and NSOA groups, the relative expression level of CAT gene was significantly difference compared to the control group (P<0.05, Graph 4) and the lowest level of expression was related to OA group. A decrease in the relative expression of the CAT gene was observed in all treatment gruops.



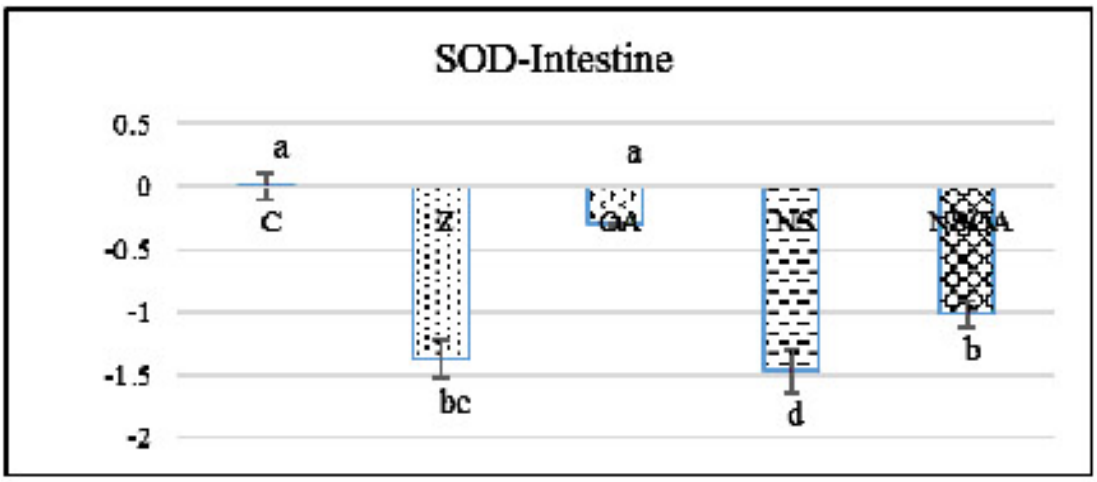
Graph 2. Relative expression of SOD gene in liver tissue under heat stress conditions on d 42.



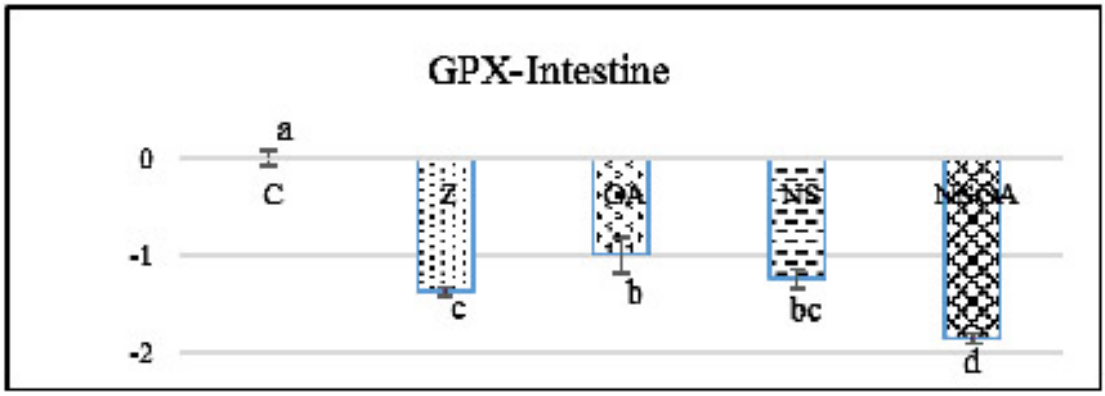
Graph 3. Relative expression of GPX gene in liver tissue under heat stress conditions on d 42



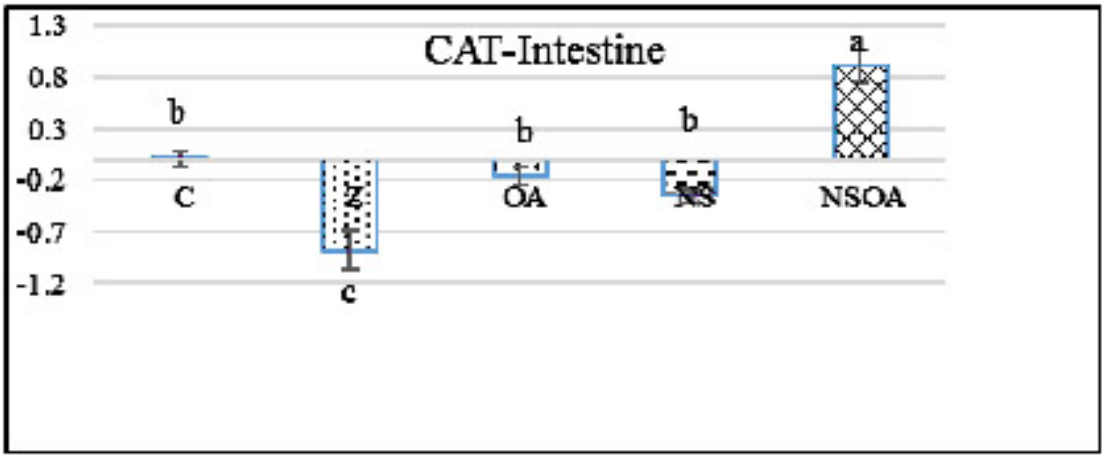
Graph 4. Relative expression of CAT gene in liver tissue under heat stress conditions on d 42.
C: control or basal diet; OA: basal diet supplemented by organic acid; Z: basal diet supplemented by zeolite; NS: basal diet supplemented by zeolite coated with nanosilver; NSOA: basal diet supplemented by zeolite coated with nanosilver and organic acid. [a,b,c,d] Values with different superscripts for each figure are significantly different (P<0.05).



Graph 5. Relative expression of SOD gene in intestine tissue under heat stress conditions on d 42.



Graph 6. Relative expression of GPX gene in intestine tissue under heat stress conditions on d 42.



Graph 7. Relative expression of CAT gene in intestine tissue under heat stress conditions on d 42. C: control or basal diet; OA: basal diet supplemented by organic acid; Z: basal diet supplemented by zeolite; NS: basal diet supplemented by zeolite coated with nanosilver; NSOA: basal diet supplemented by zeolite coated with nanosilver and organic acid. [a-e] Values with different superscripts for each figure are significantly different (P<0.05).

Relative expression of SOD, GPX and CAT genes in the intestinal tissue of 42-d-old broilers are shown in graphs 5 to 7. The relative expression of the SOD gene in all treatments was lower than in the control group (Graph 6). Z, NSOA and NS treatments showed a significant difference compared to the control group (P<0.05). No significant difference was observed between OA treatment and control birds (P<0.05). Graph 7 shows the relative expression of the GPX gene in the intestinal tissue. All treatments showed lower expression than the control group. The difference between the treatments and the control group was significant (P<0.05). The lowest relative expression of GPX gene was related to NSOA treatment. Figure 8 shows the relative expression of the CAT gene and NSOA and Z treatments showed a significant difference compared to the control group (P<0.05). The relative expression of CAT gene increased in NSOA group and decreased in Z group. No significant difference was observed between NS and OA treatments with the control treatment (P<0.05).

Discussion

FTIR analysis is used for the identification of organic, inorganic, and polymeric materials utilizing infrared light for scanning the samples. Figure 2 shows the compared structure of zeolite and silver nanoparticles coated on zeolite. FTIR spectrum of zeolite showed bands at 1028/cm for H-O-H bending. A large peak at 1016/cm was seen for silver nanoparticles coated on zeolite, which was due to van der Waals interaction between hydroxyl groups in the zeolite structure related to H2O and partial positive charge on the surface of silver nanoparticles (Shameli *et al.,* 2011).

In our previous research, it was shown that nanosilver at the level of 50 ppm can improve growth performance of broiler chickens (Esmaili *et al.,* 2016; Aliabadi *et al.,* 2021) and the histological studies of the liver and kidney tissues was revealed no adverse

impact on kidney and hepatic tissue when broiler fed nanosilver at the level of 50 ppm (Tavakoli *et al.,* 2020). Also, our previous study showed that the use of nanosilver in the diet of broiler chickens under normal conditions does not have any adverse effect on the immune system (Abbasi *et al.,* 2021), but it can suppress the immune system of broilers in heat stress conditions (Bolandi *et al.,* 2012). Our findings also showed that nanosilver can cause changes oxidative enzymes and increase superoxide dismutase (Esmaili *et al.,* 2017) and showed that nanosilver increases the expression of the NF-kB gene as a marker of inflammation (Arabiyan *et al.,* 2019) and increases the expression of cytochrome P450 as a marker of xenobiotic Contamination (Arabiyan *et al.,* 2020). Meanwhile there was no comprehensive research on the safety of using nanosilver in the broiler diet at the molecular cellular level, there is a question that needs to be asked: what is the effect of nanosilver on the gene expression of oxidative enzymes in heat stress conditions and what is the reaction of the body of broiler chickens to the use of nanosilver in these conditions?

As a nanomaterial with broad-spectrum bactericidal and virucidal effects, silver nanoparticles have been extensively studied as antimicrobial materials. However, concerns about the potential toxicological effects of nanoparticles remain (Song *et al.,* 2012). Despite, the widespread use of silver nanoparticles, relatively few studies have been undertaken to determine the cytotoxic effects of silver nanoparticles. Silver nanoparticles have a higher tendency to generate excessive amounts of ROS. Due to the strong oxidation potential, the excess ROS induced by nanoparticles can result in the damage of biomolecules and organelle structures and lead to protein oxidative carbonylation, lipid peroxidation, DNA/RNA breakage, and membrane structure destruction, which further cause necrosis, apoptosis, or even mutagenesis (Yu *et al.,* 2020).

In our study, the effects of experimental treatments on liver SOD gene expression indicated that there was no significant difference between the experimental groups and the control group ($P>0.05$). Also, the expression of the SOD gene in the intestine in all treatment groups was lower than in the control group. Animals are well-equipped with basic anti-ROS defense systems to maintain ROS homeostasis. Superoxide dismutase is an essential antioxidant enzyme that catalyzes of oxygen superoxide anion to H_2O_2 and oxygen and the removal of O^{-2} from the mitochondrial membrane and mitochondrial matrix, often regarded as an indicator of the ability to respond to oxidative stress.

Our results in intestinal tissue showed that silver nanoparticle treatments (NS) have an indirect effect on the increase of superoxide and decrease in the expression of the SOD expression gene. Superoxide, an anion radical, is produced by the one-electron reduction of molecular oxygen. Superoxide is a primary ROS that is produced via multiple enzymatic and non-enzymatic reactions that use oxygen molecules as an electron acceptor (Fridovich *et al.,* 1995). Enzymatic pathways for superoxide production are classified into two categories. One class of enzymes produces superoxide and, the other class occasionally produces superoxide as a byproduct, a process that depends on environmental conditions. SOD plays a primary role in the cellular defense against oxidative insults by ROS.

The lack of a significant modification in hepatic SOD compared to the intestine may be due to the fact that organic acids and silver nanoparticles are more active in the digestive tract than in the liver, or that the dose of the treatments used in this experiment is insufficient to produce superoxide anion in the liver and stimulate SOD gene expression. In line with these results, our previous study in 2020 showed that nanoparticles coated on zeolite and with organic acid don't have any adverse effect on the histopathological liver and kidney in broiler chickens (Tavakoli *et al.,* 2020).

The results of the present study showed that NS significantly increased the relative expression of the GPX gene in the liver tissue under the condition of heat stress. Also, the treatments containing organic acid (OA and NSOA) caused a significant decrease in the relative expression of GPX and CAT genes.

GPX, a selenium-containing antioxidant enzyme, that effectively reduces H_2O_2 and lipid peroxides to water and lipid alcohols, respectively, and in turn, oxidizes glutathione to glutathione disulfide. CAT is an antioxidant enzyme expressed by the CAT gene family and exists in almost all aerobic organisms. The release of ions by silver nanoparticles may be accompanied by oxidation-reduction reaction cycle (oxidation-reduction) and chemical catalysis through Fenton reaction or Fenton-like reaction [$Ag^+ H_2O_2+H^+ = Ag^+ + \bullet OH + H_2O$] (Nel *et al.,* 2006; He *et al.,* 2012 b; Li *et al.,* 2017). Enzyme deactivation due to metal-ion dissociation (*i.e.,* Ag^+), membrane structure disruption (Carlson *et al.,* 2008; Jiang *et al.,* 2014), disturbed electron-shuttling process, redox potential levels depletion, decreased mitochondrial membrane potentials (MMP) (Kang *et al.* 2012) and further enhances the accumulation of intracellular ROS. Nanosilver has been also reported to promote intracellular ROS accumulation by disturbing the electron transfer process (Wang *et al.,* 2017; Giorgio *et al.,* 2005), increasing the $NADP^+$/NADPH ratio (Lee *et al.,* 2018), and interfering with mitochondrial function (Asha Rani *et al.,* 2009). Silver nanoparticle

further interferes with the expression of oxidative stress-related genes, such as soxS, soxR, oxyR, and ahpC (Zhang *et al.,* 2018); antioxidant genes, like sod1 and GPX 1 (Lee *et al.,* 2002; Yamada *et al.,* 1999); and the NADPH production-related gene met9 (Lee *et al.,* 2018). The instability in the expression of oxidative and antioxidant genes caused by silver nanoparticles accelerates intracellular ROS accumulation.

Antioxidant enzymes have been documented as important modulators in silver nanoparticle-induced oxidative stress. CAT and SOD are prominent enzymes for maintaining the level of ROS in organisms. In agreement with our results, the activities of SOD and CAT were upregulated when *Drosophila melanogaster* was exposed to nanosilver. Exposure to nanosilver resulted in inhibition (Huang *et al.,* 2018; Buffet *et al.,* 2013) or activation (Ali *et al.,* 2014; Jiang *et al.,* 2008) of SOD and CAT activities in different aquatic organisms. Studies on the aquatic plant *Spirodela polyrhiza* reported that the activity of SOD displayed a dose-dependent increase, but the activity of CAT was not affected by exposure to nanosilver (Jiang *et al.,* 2008). It is currently accepted that the alteration of the enzyme activity might be due to either regulation of genes or to direct surface interaction of the enzymes with nanosilver. The underlying molecular mechanisms, and how the interaction of nanosilver with CAT or SOD can be responsible for redox state disruption, are not fully elucidated (Pudlarz *et al.,* 2019).

Also, our results generally provide evidence that organic acid treatment has a reducing effect on the expression of oxidative stress genes in intestinal and liver tissue. So far, no articles have been published about the relationship between organic acid and oxidative stress enzymes in poultry and the underlying mechanism remains unclear. However, limited literature has focused on the effects of organic acid in diets on antioxidant properties, as well as oxidative stress enzymes. Organic acids play a crucial role in numerous metabolic processes accompanied by the transfer of electrons and protons and are linked to the reduction/oxidation of major redox couples in cells, such as NAD, NADP, glutathione, and ascorbate (Igamberdiev *et al.,* 2018). Weak organic acids are shown to exert a strong pro-oxidant action on aerobic yeast cells (Piper *et al.,* 1999).

Conclusions

The liver and kidney are the main target organs for the distribution of silver nanoparticles following systemic availability, although multiple review studies have described the toxicity of nanosilver to specific key organs. According to our research, nanosilver may cause ROS production and be more active in the gut than in the liver. Additionally, in chickens fed xenobiotics, enzymes involved in the antioxidant defense system are more active in the gut than in the liver. Additionally, it is preferable to combine organic acids with silver nanoparticles when using them as a growth promoter in chicken feed to reduce the risk of free radical formation and other negative effects.

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Conflict of interest

There is no conflict of interest between the authors.

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Institutional review board statement

This study obtained ethical clearance (Approval No: 97/20/1655) from the institutional animal care and use committee of Gorgan University of Agricultural Sciences and Natural Resources. Poultry husbandry and care were under the management of the Faculty of Animal Science farm where the research took place.

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تاثیر تنش گرمایی خارجی بر ژن‌های استرس اکسیداتیو در کبد و روده جوجه‌های گوشتی تغذیه شده با نانوذرات نقره پوشش داده شده روی زئولیت و اسید آلی

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چکیده

زمینه و هدف: تاثیر تنش گرمایی خارجی بر ژن‌های استرس اکسیداتیو در کبد و روده جوجه‌های گوشتی تغذیه‌شده با نانوذرات نقره پوشش داده شده روی زئولیت و اسید آلی مورد بررسی قرار گرفت.

مواد و روش‌ها: در قالب طرح کاملاً تصادفی ۴۵۰ قطعه جوجه کاب یک روزه در ۵ تیمار و ۶ تکرار تقسیم شدند؛ در هر واحد آزمایشی ۱۵ قطعه قرار گرفت و به مدت ۴۲ روز در شرایط پرورش یکسان نگهداری شدند. ۱) رژیم های کنترل یا پایه، ۲) جیره پایه با ۱٪ زئولیت، ۳) جیره پایه مکمل با ۰/۵٪ کلینوپتیلولیت پوشش داده شده با ۰/۱٪ نانو نقره، ۴) یک جیره پایه با ۰/۱۵٪ اسید آلی و ۵) جیره پایه با ۱٪ زئولیت پوشیده شده در ۰/۵٪ نانو نقره و ۰/۱۵٪ اسید آلی. استرس گرمایی (دمای ۱±۳۴ درجه سانتیگراد به مدت ۶ ساعت در روز) از روز ۳۵ تا ۴۲ ارائه شد. در روز ۴۲، نمونه‌های کبد و روده گرفته شد و بیان ژن آنزیم های سوپراکسید دیسموتاز (SOD)، گلوتاتیون پراکسیداز (GPX) و کاتالاز (CAT) مورد بررسی قرار گرفت.

یافته‌ها: بیان نسبی ژن SOD کبدی در گروه‌های تیمار شده در مقایسه با گروه کنترل تفاوت معنی‌داری نداشت (P۰/۰۵) کاهش نشان دادند. در گروه های تیمار شده با زئولیت پوشش داده شده با نانو نقره و اسید آلی، بیان نسبی ژن CAT در مقایسه با گروه کنترل کاهش یافت، در حالی که در گروه تیمار شده با جیره پایه افزایش یافت.

نتیجه گیری: روده دارای سطوح بالاتری از فعالیت آنزیم های آنتی اکسیدانی در مقایسه با کبد است. همچنین، برای افزایش راندمان رشد ترجیح داده می شود که از ترکیب نانوذرات نقره همراه با اسیدهای آلی استفاده شود.

واژه‌های کلیدی: *آنتی اکسیدان، جوجه های گوشتی، گلوتاتیون پراکسیداز، استرس گرمایی، نانو نقره*

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