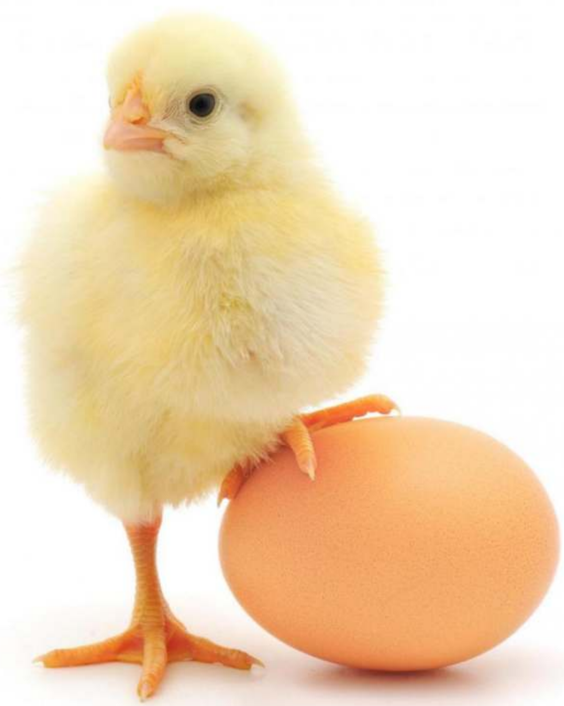
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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E-mail: editorijps@gmail.com

OPEN ACCESS International Journal of Poultry Science

ISSN 1682-8356
DOI: 10.3923/ijps.2019.122.128

Research Article
Role of Pomegranate Peels and Black Pepper Powder and Their Mixture in Alleviating the Oxidative Stress in Broiler Chickens

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Abstract
Background and Objective: The high level of oxidative stress (OS) is not only a compromise between welfare and health status but adversely affects survival, performance and the quality of final products. Antioxidants are defined as any substance which delays, prevents or removes overproduction of oxidative damage. The present study was conducted in order to investigate the efficacy of pomegranate peels (PPP) and black pepper (BPP) powders and their mixture added to diet of broiler chicks to alleviate the effect of oxidative stress. The stress is induced by H_2O_2 in drinking water. **Materials and Methods:** The sample encompassed 600 broiler chicks Ross 308. Birds were distributed randomly into the following 5 groups (4 replications in each): G1 (negative control) kept without any additives to feed or water, G2 (positive control) treated with 0.5% H_2O_2 additive to drinking water, G3-fed with 1% of PPP and 0.5% H_2O_2 additive to drinking water, G4-fed with 1% of BPP and 0.5% H_2O_2 additive to drinking water and G5-fed with 0.5% of PPP and 0.5% of BPP as well as 0.5% H_2O_2 additive to drinking water. The research was completed in the 6th week of birds' life. The productive characteristics, carcass traits and oxidation state in blood plasma were evaluated. **Results:** The results revealed that G3, G4 and G5 recorded a significant increase in body weight, feed and water intake, protein and energy efficiency ratio, production efficiency factor, carcass yield and breast muscle proportions compared to G2. It was noted that feed conversion ratio decreased considerably in experimental groups compared to G2. Groups G3, G4 and G5 achieved a significant increase in SOD, GPx and FRAP and in the CAT level in plasma with a decrease ($p<0.05$) in LOOH and MDA value compared to G2. **Conclusion:** It was concluded that the supplementation of pomegranate peels and black pepper powders or their mixture in birds' diet may alleviate the effect of oxidative stress by improving productive traits and oxidative parameters in blood plasma.

Key words: Black pepper, medicinal plants, oxidative stress, plant extracts, plasma parameters, pomegranate peels, productivity traits

Received: November 20, 2018 Accepted: January 01, 2019 Published: February 15, 2019

Citation: Karrar Imad Abdulsahib Al-Shammari, Justyna Batkowska and Sarah Jasim Zamil, 2019. Role of pomegranate peels and black pepper powder and their mixture in alleviating the oxidative stress in broiler chickens. Int. J. Poult. Sci., 18: 122-128.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Punica granatum peels constitutes a by-product and waste in the pomegranate industry. Peels are composed of an interior network of membranes that make up approximately 26-30% of the total fruit weight[1]. Pomegranate peels are rich in many bioactive antioxidants such as flavonoids (flavonols, flavanols, anthocyanins), condensated tannins (proanthocyanidins) and hydrolysable tannins (ellagitannins, gallotannins)[2]. Peels have a curative role in the treatment of various infections, diabetes, diarrhoea, dysentery, dental plaque and boost resistance against intestinal infections such as malarial parasites[1].

Piper nigrum L. is one of the most traditional spices. It has a pungent taste and was observed to stimulate digestibility, energy metabolism and improve public health[3]. It has been proven to be rich in certain active enzymes (glutathione peroxidise and glucose-6-phosphate dehydrogenase) which manifest benefits for metabolism and the inhibition of oxidation state. Additionally, black pepper is advantageous through its role as anticarcinogenic, anti-inflammatory and antimicrobial factor and by removing multiple-cardiopulmonary diseases[4]. Piperine is an active substance present in black pepper. It is an alkaline substance which modulates benzopyrene metabolism through the cytochrome P450 enzyme important in the metabolism and transfer of xenobiotic and metabolites[5]. Piperine can significantly increase the absorption of selenium, vitamin B complex, β-carotene and other nutritional ingredients[6,7].

H_2O_2 is one of the most important reactive oxygen species (ROS). It leads to oxidation when accumulated in high concentrations in living tissues. Numerous studies have indicated the role of H_2O_2 in the induction of the oxidative and toxic stress when supplemented to drinking water of chickens[8,9] and quails[10]. Previous studies did not employ pomegranate by-products or black pepper as a safe and non-conventional feed supplement under stress conditions in avian species. Therefore, the objective of the current study was to investigate the efficacy of pomegranate peels powder (PPP) and black pepper powder (BPP) against the oxidative stress (OS) in meat-type chickens via their reflective impact upon productive performance, carcass traits and certain oxidation parameters in blood plasma.

MATERIALS AND METHODS

This research was conducted in the Poultry Farm and Laboratories of Al-Musaib Technical College, Babylon, Iraq. The sample encompassed 600 unsexed broiler one-day-old Ross 308 chicks. The chicks were weighed individually at the hatchery and before reaching the farm. The mean weight of the chick was 42.5 g. The experiment started from the 1st day and ran until the 6th week of life. The chicks were divided into 5 groups. Each group included 4 replicates, 30 chicks/replicate. The birds were kept in 2×2 m^2 cages. The birds were fed ad libitum with isonitrogenous and isocaloric diets. All healthcare conditions required in the rearing period were ensured.

The OS was induced in birds by the application of a H_2O_2 solution (P.P.H Stanlab Sp.J/Poland) with a 30% concentration which was diluted to 0.5% in large plastic cans every second day to maintain its purity. The H_2O_2 was used as the oxidative factor and served daily in the drinking water of birds throughout the experimental period. In addition, pomegranate peels powder (PPP, Xi'an Tonking Biotech Co., Ltd., China) and black pepper powder (BPP, Bio Med Ingredients Co., Ltd., India) were used as dietary antioxidating substances. On the basis of the dry weight, PPP contains 17 g/100 g polyphenols, whereas BPP contains 7% piperine.

The experiment included 5 groups: G1 (negative control) kept without any additives to feed or water, G2 (positive control) treated with 0.5% H_2O_2 additive to drinking water, G3-fed with 1% of PPP and 0.5% H_2O_2 additive in drinking water, G4-fed with 1% of BPP and 0.5% H_2O_2 additive in drinking water and G5-fed with 0.5% of PPP and 0.5% of BPP as well as 0.5% H_2O_2 additive in drinking water.

The productive traits encompassed total mortality, feed intake, water intake, body weight, weight gain, feed conversion ratio, protein efficiency ratio and energy efficiency ratio. These were calculated weekly and accumulatively. The production efficiency factor was also calculated. After 6 weeks of rearing, 4 birds/replicate were randomly selected and slaughtered. Subsequently, a simplified dissection of their carcasses was conducted. During these procedures blood samples were drawn from the branchial vein. Blood was kept in K3-EDTA anticoagulant tube and then placed in a centrifuge at 3000 r.p.m for 15 min to separate plasma. In plasma, certain redox indicators were measured, for instance, hydroperoxide (LOOH) and malondialdehyde (MDA) according to Sodergren *et al.*[11] and Salih *et al.*[12]. With respect to antioxidating enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined on the basis of the method coined by Misra and Fridovich[13] and Aebi[14]. Moreover, the ferric-reducing ability of plasma (FRAP) was measured according to Benzie and Strain[15]. All plasma analyses were done with the use of kits provided by a research company (Sigma Aldrich, St. Louis, MO, USA).

The data were statistically analyzed by the General Linear Models procedure using the SAS software[16]. Statistically significant differences observed in the course of the study were isolated by means of the Duncan's multiple range test. The mortality of birds was verified by means of the nonparametrical χ^2 test. The probability level 5% was used.

RESULTS

Table 1 indicates that G2 (positive control) achieved the lowest mean for body weight at 3rd and 6th weeks and the smallest body weight gain and feed intake up to the 3rd week of research as well as during the whole period. There was also a significant increase in feed conversion ratio from day 1 up to 6th week in favour of G2. On the other hand, G1 (negative control), G4 and G5 exhibited a significant decrease in this trait followed by G3. At the same time, it was found that G4 and G5 significantly improved the majority of the mentioned traits followed by G3 and G1.

Table 2 revealed that water intake decreased considerably in favor of G2 compared to G5 up to the 3rd week, while during 6th weeks it was observed that G2 demonstrated a significant decrease in this trait compared to all other groups. Moreover, the lowest survivability of birds (92.5%) was registered in positive control (G2) and in G4 (97.5%). In the other groups no death cases were noted. This trait considerably depended upon the research group (p-value of χ^2 test = 0.001). G2 demonstrated a significant reduction in protein efficiency and energy efficiency ratios as well as production efficiency factor. On the other hand, G3, G4 and G5 achieved better values regarding these parameters compared with G2.

High value of dressing percentage was registered in G3, G4 and G5 followed by G1 compared to G2 (Table 3). In addition, all groups manifested a significant increase in the breast and femur ratio, compared with G2 (positive control,

Table 1: Effect of pomegranate peels and black pepper powders on body weight, weight gain, feed intake and feed conversion efficiency (Mean±standard error) of stressed broilers

Traits/ Groups	Live body weight (g) 3 weeks	Live body weight (g) 6 weeks	Weight gain (g) 1 day-3 weeks	Weight gain (g) 1 day-6 weeks	Feed intake (g) 1 day-3 weeks	Feed intake (g) 1 day-6 weeks	Feed conversion ratio 1 day-3 weeks	Feed conversion ratio 1 day-6 weeks
G1	726.5±23.87^{b}	2326±18.87^{ab}	684.4±42.87^{b}	2284±23.89^{b}	1287±38.98^{b}	3674±98.98^{b}	1.16±0.12	1.60±0.14^{c}
G2	675.1±21.64c	1474±16.98^{c}	632.9±39.98^{c}	1432±41.98^{c}	1122±45.86^{c}	3535±79.96^{c}	1.13±0.11	2.46±0.14^{a}
G3	732.5±11.98^{b}	2178±26.93^{b}	690.1±43.98^{b}	2135±35.98^{b}	1334±67.94^{ab}	3972±77.98^{a}	1.18±0.11	1.76±0.14^{b}
G4	828.4±17.8a	2430±19.82a	785.9±37.98^{a}	2388±38.98^{a}	1313±37.87^{ab}	39.23±88.90^{a}	1.02±0.02	1.64±0.12^{c}
G5	837.5±19.76a	2387±17.98a	795.3±36.98^{a}	2344±45.87^{a}	1473±36.83^{a}	3779±88.35^{a}	1.11±0.14	1.61±0.16^{a}

Groups; G1: Without additives (negative control), G2: (Positive control) 0.5% H_2O_2 in drinking water, G3: 1% pomegranate peels powder and 0.5% H_2O_2 in drinking water, G4: 1% black pepper powder and 0.5% H_2O_2 in drinking water, G5: 0.5% of pomegranate peels powder and 0.5% black pepper powder with 0.5% H_2O_2 in drinking water. ^{a,b}Means within columns with different letters differ significantly at $p \leq 0.05$

Table 2: Effect of pomegranate peels and black pepper powders on water intake, protein and energy efficiency ratio and production efficiency factor (Mean±standard error) of stressed broilers

Traits/groups	Water intake (mL day^{-1}) 3 weeks	Water intake (mL day^{-1}) 6 weeks	Protein efficiency ratio (g g^{-1} day^{-1}) 1 day-6 weeks	Energy efficiency ratio (g/100 kcal day^{-1}) 1 day-6 weeks	Production efficiency factor 1 day-6 weeks
G1	755±14.87^{a}	1295±55.76^{a}	2.88±0.43^{a}	20.42±0.76^{a}	344.8±37.96^{a}
G2	546±11.76^{b}	1003±36.98^{b}	1.87±0.37^{b}	13.34±0.48^{c}	120.9±46.90^{c}
G3	610±15.87^{ab}	1147±55.87^{a}	2.50±0.64^{a}	17.66±0.76^{b}	278.8±48.86^{b}
G4	678±14.98^{a}	1135±57.98^{a}	2.82±0.42^{a}	20.00±0.65^{a}	346.1±58.98^{a}
G5	646±19.12^{ab}	1148±59.93^{a}	2.88±0.52^{a}	20.30±0.95^{a}	352.7±54.97^{a}

Groups; G1: Without additives (negative control), G2: (Positive control) 0.5% H_2O_2 in drinking water, G3: 1% pomegranate peels powder and 0.5% H_2O_2 in drinking water, G4: 1% black pepper powder and 0.5% H_2O_2 in drinking water, G5: 0.5% of pomegranate peels powder and 0.5% black pepper powder with 0.5% H_2O_2 in drinking water. ^{a,b}Means within columns with different letters differ significantly at $p \leq 0.05$

Table 3: Effect of pomegranate peels and black pepper powder on carcass parts and giblets proportion (mean±standard error) of stressed broilers

Traits/ Groups	Carcass yield	Carcass parts: Breast	Drumstick	Thighs	Wings	Back	Neck	Giblets: Heart	Liver	Gizzard	Abdominal fat
G1	73.4±11.54^{b}	24.7±1.86^{b}	15.5±1.34^{a}	20.1±2.56^{b}	12.2±1.94^{a}	16.3±2.87	6.33±1.43	1.22±0.21	2.11±1.01	1.21±0.32	0.82±0.01^{b}
G2	71.1±12.87^{c}	22.6±2.98^{c}	14.2±1.87^{b}	19.2±5.98^{c}	11.8±4.87^{b}	16.8±3.97	6.71±2.87	1.03±0.36	2.62±1.27	1.79±0.23	2.53±0.02^{a}
G3	74.3±18.98^{a}	25.2±5.87^{a}	14.3±1.97^{b}	21.8±2.97^{a}	11.6±3.93^{b}	17.4±2.54	5.52±1.75	1.20±0.16	2.19±1.24	1.86±0.45	0.71±0.02^{b}
G4	74.5±17.98^{a}	26.4±4.54^{a}	15.2±1.46^{a}	20.6±4.97^{b}	11.3±3.86^{b}	16.8±2.97	6.41±1.47	1.40±0.11	2.12±1.17	1.46±0.34	0.84±0.05^{b}
G5	75.5±15.6^{a}	25.2±3.98^{a}	15.0±1.76^{a}	20.3±6.98^{b}	12.7±3.39^{a}	16.5±2.86	5.61±1.65	1.20±0.21	2.75±1.14	1.24±0.54	0.73±0.04^{b}

Groups; G1: without additives (negative control), G2: (Positive control) 0.5% H_2O_2 in drinking water, G3: 1% pomegranate peels powder and 0.5% H_2O_2 in drinking water, G4: 1% black pepper powder and 0.5% H_2O_2 in drinking water, G5: 0.5% of pomegranate peels powder and 0.5% black pepper powder with 0.5% H_2O_2 in drinking water. ^{a,b}Means within columns with different letters differ significantly at $p \leq 0.05$

Table 4: Effect of pomegranate peels and black pepper powder on oxidative stress indicators of blood plasma (mean±standard error) of stressed broilers

Traits/groups	LOOH (μmol L^{-1})	MDA (μmol L^{-1})	SOD (U mL^{-1})	CAT (U mL^{-1})	GPx (U L^{-1})	FRAP (μmol L^{-1})
G1	11.80±7.12[b]	9.81±6.97[c]	119.9±19.98[a]	5.81±1.86[a]	1.71±0.18[a]	720.1±41.98[b]
G2	17.87±10.97[a]	18.42±8.98[a]	90.9±15.87[b]	4.53±1.37[b]	1.22±0.13[b]	660.2±29.97[c]
G3	11.31±7.18[b]	10.49±6.98[c]	127.8±20.98[a]	6.55±1.88[a]	1.76±0.18[a]	845.2±48.97[a]
G4	10.93±7.82[b]	9.23±7.16[b]	117.9±20.98[a]	7.32±2.48[a]	1.91±0.24[a]	732.4±42.98[b]
G5	11.54±8.11[b]	10.35±6.98[c]	101.9±19.58[a]	6.83±1.84[a]	1.70±0.26[a]	822.1±49.9[a]

Groups; G1: Without additives (negative control), G2: (Positive control) 0.5% H_2O_2 in drinking water, G3: 1% pomegranate peels powder and 0.5% H_2O_2 in drinking water, G4: 1% black pepper powder and 0.5% H_2O_2 in drinking water, G5: 0.5% of pomegranate peels powder and 0.5% black pepper powder with 0.5% H_2O_2 in drinking water. [a,b]Means within columns with different letters differ significantly at $p \leq 0.05$

treated with oxidative stress). The highest ratio of drumstick and wings was in favor of G5 and G1. Low abdominal fat for all groups was achieved when compared with G2. There were no significant differences observed among the groups with respect to the parts of back and neck as well as heart, liver and gizzard.

According to Table 4, in G2 group, a significant increase in LOOH and high value of MDA was noted. At the same time, a significant decrease in SOD, GPx and FRAP and high significant decrease in CAT for G2 were observed. All of these traits have been significantly improved for G3, G4 and G5 groups.

DISCUSSION

Exposure of birds to OS generated by H_2O_2 led to lowered economic characteristics and oxidative properties of plasma. It is known that OS is caused by the imbalance between the production of antioxidants and oxidanting substances in blood. This causes the production of free radicals of oxygen, which in turn, leads to a significant damage of lipid membranes, carbohydrates, nucleic acids and proteins, thus altering their biological function[17]. Therefore, H_2O_2 supplemented water produces a series of reactions resulting in the emergence of OS by the increase of the amount of oxygen production in the stomach. Consequently, it permeate to blood and then to tissues, which increases lipid peroxidation in the central nervous system and in other vital organs[8-10].

High body weight and weight gain in the antioxidant groups (G3, G4 and G5) might be attributed to the role of these therapeutic and preventive plants and their extracts in improving immunological properties, activating enzymatic antioxidants and lowering the rate of free radical levels in blood under stress conditions[18,19]. This is reflected in the improved feed and water intake, total feed efficiency, carcass yield and production efficiency factor. Possibly, it is due to high intestinal digestion process and active feed utilization stimulated by extracts from phenols and tannins, such as proanthocyanidin. These compounds act against intestinal pathogens by inhibiting cellular exoenzymes and eliminating from substrate elements essential for bacterial growth, or by a direct intervention in microbial metabolism by inhibiting oxidative phosphorylation[20]. As a consequence, they increase the growth of beneficial intestinal microbes important in digestion. They also improve palatability[18]. This was confirmed by Sarica and Urkmez[20] in their previous study. The study revealed that birds fed with an extract of pomegranate peel (proanthocyanidin) at the concentration of 100 and 200 mg kg^{-1} in diet noted a substantial increase in Lactobacillus bacteria comparable to the decrease in the numbers of coliform and *E. coli* in the ileal part of broilers. Recently, it was also observed that adding 0.5 and 1% of dietary pomegranate by-products improved the microbial function of intestines, reduced the emissions of gases from excreta and lowered the pH of ileal digesta through increasing *Saccharomyces cerevisiae* and reducing *E. coli* and *Salmonella* spp. in the ileum, along with the increased Bacillus bacteria in caeca[21].

It has also been shown that a powerful component of black pepper (piperine), which has a pungent taste, plays a vital role in improving the flavour, digestion and in expediting absorptive metabolism with its various influences upon enteric nervous system[22]. The metabolic acceleration of active ingredients in black pepper reduces the risk of its aggregation in live tissue. Piperine stimulates dynamic changes and the permeability properties of membranes and increases the synthesis of proteins associated with cytoskeletal function and activation of the absorption area of the villi[23]. These results are consistent with those obtained by Saleh *et al.*[24] who stated that an addition of pomegranate peel extract at 0.2 g kg^{-1} resulted in improved feed conversion efficiency and no change in feed intake and weight gain. On the other hand, the 100, 200 and 300 mg of PPP kg^{-1} in diet resulted in the reduction of growth performance for broilers up to 42 days. Similarly, Bostami *et al.*[25] mentioned there emerged an increase in daily weight gain. The gain was observed without any change in feed intake and feed efficiency for broilers fed on diet containing 1 and 2% of fermented pomegranate by-products. Our results are convergent with the previous studies with respect to the impact of pomegranate peels upon weight gain, feed efficiency ratio as well as hot and cold carcass weight[20,21]. Our results were also similar to the findings of Abou-Elkhair *et al*.[26] who observed that diet supplemented with 0.5% BPP with or without the mixture of 2% coriander seeds or 0.5% turmeric powder has improved weight gain and cumulative feed conversion efficiency without the influence upon carcass yield. This might be due to the stimulative role of black pepper in intestinal absorption, activation of digestive enzymes, reduction of viscosity in intestines and the competitive exclusion of pathogenic bacteria in digestive system[27].

It was found that diet supplemented with 0.5 or 1% of black pepper, within 15-35 days or 36-42 days, resulted in only a numerical increase in body weight and improved the efficiency of feed conversion[4] with the increase of the whole carcass yield and drumstick, along with the reduction of abdominal fat proportion in broiler[27] when black pepper was incorporated in diet at 2%. Contradictory results were obtained by Sidhu *et al*.[28] who reported that 0.5% of black pepper powder in broiler diet during feed restriction stress (8-10 h day^{-1}) has reduced weight gain at 2nd week and reduced feed intake at 2nd and 3rd weeks without a significant influence upon feed efficiency. High mortality in OS group was probably due to the role of OS in inducting several pathological cases, such as cancer, neurological disorders, atherosclerosis, hypertension, ischemia, diabetes, acute respiratory distress, chronic obstructive pulmonary disease and asthma[29, 30].

Low number of dead birds in both PPP and BPP groups under stress conditions was related to the functional performance of plant materials in blood purification and metabolic inhibition of inflammatory prostaglandins via the materials' biological contents of phenolic substances, such as terpene and flavonoids. As indicated recently by Ahmadipour *et al*.[31], adding 0.75 and 1% of dietary PPP played a fundamental role in preventing pulmonary hypertension syndrome, decreasing heart weight and its right ventricle to total ventricle weight ratio through the high levels of plasma nitric oxide which acts as vasodilator and low level of hepatic endothelin-1 which acts as vasoconstrictor, thereby directly reducing the pulmonary vascular resistance and protecting heart from stresses. Moreover, PPP by-products increased the spleen and bursa of Fabricius weights with high levels of plasma immunoglobulins (IgA, IgG)[21]. In addition, Tajodini *et al*.[22] established that BPP performs pharmacological roles in the treatment of various infections related to bronchitis, asthma, rheumatic aches and enhances the bioavailability of certain drugs. It also acts as a vital preventive agent against carcinogens. Perhaps, not surprisingly, these benefits of BPP were associated with the contents of piperine, capsaicin and capsantine.

Stress exhibited by birds in productive traits was reflected in the deterioration of oxidative properties in blood plasma. H_2O_2 is one of the free radicals of oxygen and the harmful determinant resulting in changes in blood plasma, living body tissue, cell damage and lipid peroxidation associated with various diseases[30]. These changes are attributed to H_2O_2 activity elevating ROS production in blood and central nervous system[9]. This resulted in the reduction of antioxidating enzymes, such as GPx, SOD, CAT and FRAP, as well as the augmentation of lipid oxidation products (LOOH and MDA). CAT is one of the most important enzymes found in the peroxisome which acts in conjunction with SOD to detoxify H_2O_2 by catalyzing a reaction between two H_2O_2 molecules, resulting in the formation of H_2O and O_2[32,33]. The observed efficiency of both PPP and BPP groups in oxidation indices measured in plasma might be due to the role of the phenolic substances available in these plants in inhibiting the oxidation process. This depends upon the donation of the hydrogen atom in the phenolic hydroxyl group to the free radicals and thus inhibiting the free radicals propagation chain during the oxidation process[18,19,34]. This result was consistent with earlier results with respect to the role of H_2O_2 in the induction of OS in the blood and tissues of chickens and quails through measured oxidation indices[8,9,10].

In addition, results we obtained were convergent with Ahmadipour *et al*.[31] and Saleh *et al*.[24] regarding the effectiveness of PPP and its extract supplemented in diet to improve antioxidants state in blood plasma and breast meat of chickens. However, Imaseun and Ijeh[35] concluded that there emerged no change in total antioxidant capacity and MDA in chicken plasma of those fed a diet containing 0.5% BPP or the mixture of BPP and ginger powder of 0.25% each.

Due to numerous environmental stressors contributing to the emergence of OS in poultry, it is very important to find certain substances (especially natural, relatively cheap and available) which may alleviate the negative effect of OS and improve or maintain stable production performance of poultry. It seems that the evaluated additives (PPP and BPP) may be useful in this case.

CONCLUSION

On the basis of this research, it is concluded that the supplementation of pomegranate peels and black pepper powders or their mixture in chicken diet may alleviate the oxidative stress effect by improving the productive traits of birds and oxidative parameters in their blood plasma.

SIGNIFICANCE STATEMENT

This study examines the efficacy of dietary additives from plant origin (1% PPP or BPP both and their combination of 0.5% each) in broiler which can be beneficial for attenuating the OS incidence via their effect upon productive performance and oxidation indicators in blood plasma. Moreover, no instances of the application of pomegranate by-products or black pepper as a safe supplement under stress conditions in avian species were known.

This study will help the researcher to uncover the critical areas of using the optimal levels of these additives in diet, especially in case of chickens reared under stress. So far, many researchers were not able to explore the issue. As a consequence, a new theory based upon our data collected in the current study may be developed.

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