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INFLUENCE OF GINGER AS AN ANTIOXIDANT ON THE PHYSIOLOGICAL PERFORMANCE OF MALE QUAIL STRESSED BY HYDROGEN PEROXIDE

Abdullah F. Abdul-Majeed , **Hassan A. Al-krad**

Department of Physiology, College of Vet. Medicine, Hama, Syria.

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Correspondence Email:

abdullahfathi@yahoo.com

ABSTRACT

This research was carried out to find out the ability of crushed dry ginger (*Zingiber officinale*) to recover or prevent the impacts of oxidative stress that is induced by hydrogen peroxide by studying some hematological and productive traits of quail. One hundred fifty sexed male quail (21 days old) were randomly distributed into five groups of 30 birds each (3 replicates). G_1: control; G_2: H_2O_2 group; G_3: ginger group; G_4: H_2O_2+ginger from the beginning of the experiment; G_5: H_2O_2+ginger after the 2^{nd} week from the beginning of the experiment. Blood samples were collected at the age of 56 days, and the results showed that the addition of H_2O_2 led to a significantly increased ($p \leq 0.05$) feed consumption compared with other groups and enhanced significantly the feed conversion ratio compared with ginger and the control. Also, H_2O_2 caused a significant increase in blood glucose levels compared to the ginger group and a significant elevation in cholesterol and triglycerides compared with other groups, while PCV%, hemoglobin, and lymphocytes decreased significantly, which led to a significant elevation in the stress index in comparison to the ginger group. As for the impact of ginger, it was opposite to the effect of hydrogen peroxide, as it significantly reduced feed consumption and lowered blood glucose, cholesterol, and triglycerides, which led to an improvement in the blood picture and a decrease in the value of the stress index for the birds that ate ginger alone or the birds in the groups that ate ginger with hydrogen peroxide.

INTRODUCTION

The quail bird has been known in Arab countries for thousands of years, as have the areas of its presence and spread (Abdul-Majeed, 2013). The sexual maturity of the quail occurs between 35 and 42 days of age, and the duration of its one generation is approximately 3 to 4 cycles per year. The hatching period is between 16 and 18 days, and its modern breeds lay eggs in abundance between 200 and 300 eggs/year (François *et al.*, 2021). Quail is a dual-purpose bird, as it produces eggs and meat (Arthur and Bejaei, 2017).

God Almighty endowed many plants and herbs, including medicinal ones. Centuries ago, human knew about these plants while searching for a way to get rid of the pains and diseases he suffers from (Abdul-Majeed, 2013), especially those plants that contain some antioxidants that have a role in preventing the oxidative stress in many pathological conditions and thus elevating body performance (Arcusa *et al.*, 2022). It has been noted that among those medicinal plants that have positive antioxidant effects and improve the physiological functioning of the body is the ginger plant (*Zingiber officinale*) (Mbaveng and Kuete, 2017; Mohammad and Hamed, 2012).

Ginger is grown by tubers under the soil, and these roots (tubers or rhizomes) are the most used part of the plant, as they contain volatile oils that reflect the pungent smell and pungent taste of ginger (Mao *et al.*, 2019). In Qin and Xu (2008), it was stated that ginger contains volatile oils at 2.5–3%, phenolic compounds, vitamin C, vitamin B_6, resins, potassium, magnesium, phosphorus, calcium, and linoleic acid; it also contains gingerols (which account for the sharp, pungent taste of ginger), in addition to the group of shogaol ginger and a high content of starch (40–60%) (Godiyal *et al.*, 2021). Murakami *et al.* (2000) reported that ginger has antioxidant capabilities comparable to or greater than those of vitamin E or C when used in the same quantities. Also, Al-Khalaifah *et al.* (2022) revealed that ginger protects oxidatively stressed cells, which can occur in many pathological conditions and experimentally by many chemicals, including hydrogen peroxide (Abdul-Majeed and Abdul-Rahman, 2022).

According to Abdul-Majeed *et al.* (2012), the addition of 0.5% hydrogen peroxide to drinking water caused oxidative stress in quail, and Abdul-Majeed (2013) stated that the indicator of the occurrence of lipid peroxidation associated with oxidative stress is a considerable rise in malondialdehyde level and a significant drop in glutathione level.

The aim of this study was to investigate the impact of ginger as an antioxidant, its ability to protect against oxidative stress caused by hydrogen peroxide or prevent its effect, as well as its reflection on some blood traits and the male quail's productivity.

MATERIALS AND METHODS

This study is part of a Ph.D. dissertation submitted by the first author in the college of veterinary medicine in Hama, Syria, in which one hundred fifty male quail (*Coturnix coturnix*) were randomly distributed in floor cages into five groups (30 chicks/group, with three replications) and raised from 21 to 56 days of age. The requirements of quail raising (lighting, ventilation, and temperatures) according to their age were considered, and the feed and water were supplied *ad libitum* until the study's conclusion. The birds were fed starter and grower rations (24.65% and 22% crude protein and 2838 and 2942 kcal/kg, respectively) that met nutritional requirements according to the poultry nutrition requirements (Dale, 1994).

Crushed dry ginger rhizomes (*Zingiber officinale*) were added to the basic diet (1000 mg/kg diet) and 0.5% hydrogen peroxide to the drinking water as described in the experimental groups, which were as follows:

- G_1 (Control): Birds were given drinking water + basic ration.
- G_2 (0.5% hydrogen peroxide): Birds were given drinking water containing 0.5% hydrogen peroxide + basic ration.
- G_3 (ginger): Birds were given drinking water + basic ration with 1000 mg of crushed ginger/kg of ration.

G_4 (Hydrogen peroxide + ginger, which were added at the beginning of the experiment): Birds were administered drinking water containing 0.5% hydrogen peroxide + basic ration with 1000 mg of crushed ginger/kg of ration added at the beginning of the experiment (Prevention).

G_5 (hydrogen peroxide + ginger, which were added 2 weeks after the experiment began): Birds were administered drinking water containing 0.5% hydrogen peroxide + basic ration with 1000 mg of crushed ginger/kg of ration added two weeks after the experiment began (Recovery).

Body weight (BW) was determined at the end of the experiment; body weight gain (BWG) was determined by subtracting the initial weight from the final weight; feed conception (FC) was determined for the total period; and then feed conversion ratio (FCR) was determined from FC/BWG.

At the age of 56 days, blood samples were collected from the slaughtered birds (6 birds/group) in 2 types of laboratory tubes: The first contains anticoagulants (EDTA) for blood tests that were carried out according to the techniques mentioned in Campbell (1995) and were the following: red blood cell count, packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). Giemsa stain was used to determine the differential leukocyte count on blood smears (DLC), also according to Campbell (1995). The other type of tube (plain tubes without anticoagulant) is used to collect blood serum for biochemical tests, which include estimating the concentration of glucose, cholesterol, and triglycerides using the Biosystems kit (made in Spain).

Statistical Analysis

The data were statistically analyzed using the one-way analysis of variance procedure, according to the complete randomized design (CRD), by using a statistical software program (SAS, 2009). The means were compared using Duncan's multiple range test at $P \leq 0.05$, as described by Steel and Torrie (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

We note from Table 1 that adding hydrogen peroxide to drinking water (G_2) significantly increased the amount of feed consumption and deteriorated the feed conversion ratio compared to the control group at $P \leq 0.05$. This result is consistent with the results of Abdul-Majeed (2013). Also, we note from the same table that the addition of ginger (G_3) had no effect on final body weight, weight gain, or the amount of feed consumed at $P \leq 0.05$. This result is consistent with Ademola *et al.* (2009), who added 1.5% ginger to the diet and did not observe significant variations in final body weight or weight gain of broiler chickens during the 4 weeks.

Table (1): Effects of hydrogen peroxide and ginger on stressed and unstressed male quail body weight, feed consumption, weight gain, and feed conversion ratio.

Groups	Parameters				
	Initial body weight (on day 21) (g)	Final body weight (on day 56) (g)	Total weight gain (g)	Total feed consumption (g)	Feed conversion ratio
G_1	61.40±2.85 a	189.47±3.81 a	128.08±6.65 a	596.28±8.88 b	4.67±0.18 bc
G_2	61.84±2.86 a	187.75±2.43 a	125.92±0.43 a	658.61±5.40 a	5.23±0.06 a
G_3	61.33±2.39 a	196.92±1.38 a	135.59±3.76 a	607.11±6.15 b	4.48±0.08 c
G_4	61.83±1.49 a	193.73±2.52 a	131.90±1.03 a	641.45±5.47 a	4.87±0.01 abc
G_5	61.67±1.88 a	191.05±2.18 a	129.38±4.06 a	648.92±8.08 a	5.02±0.22 ab

- Values with different letters vertically show a significant difference at P≤0.05.
- G_1=Control, G_2= 0.5%H_2O_2, G_3= 1000 mg ginger/kg ration, G_4= H_2O_2 + Ginger added from the beginning, G_5= H_2O_2 + Ginger added after 2 weeks from the beginning.

From Table 1, we also observe that giving ginger + hydrogen peroxide from the beginning of the experiment (G_4) or after two weeks from the beginning of the experiment (G_5) prevented or reduced the negative effects of H_2O_2, which led to an improvement in the values of the feed conversion ratio and were close to the values of the control group, despite the significant increase in feed consumption in comparison to the control group at P≤0.05. This might be attributed to the fact that ginger possesses effective antioxidants that improve these traits (Al-Khalaifah *et al.*, 2022) or because the ginger stimulates the secretion of digestive enzymes, which leads to improved digestion and an increase in the good food conversion rate (Duwa *et al.*, 2020; Nemati *et al.*, 2021).

Table 2 showed that ginger addition resulted in a significant reduction (P≤0.05) in quail blood glucose and cholesterol levels in comparison to the other groups and a significant reduction in triglycerides in comparison with the treatments in G_2 and G_5. Perhaps this is because ginger has phenolic compounds that are classified as hypoglycemic compounds (Shahrajabian *et al.*, 2019), or maybe it is a result of the presence of substances that promote insulin production, which in turn enhances the cellular uptake of glucose and hence lowers blood glucose levels (Mao *et al.*, 2019). These findings are consistent with those of Mohamed *et al.* (2012), who reported ginger has a significant lowering effect on blood glucose in broiler chickens when compared to a control group.

As for the ability of the ginger plant to lower the level of cholesterol and triglycerides in the blood, it is consistent with Han *et al.* (2005), who stated that it may be due to the inhibition of fat absorption in the intestine because the ginger plant contains a high percentage of fiber, as well as due to the presence of oleo-resin compounds (shogaol, zingiberole) (Sharma, 2017). Oleo-resin inhibits the absorption of bile salts in the gut and encourages the liver to produce new bile salts from cholesterol, lowering the amount of cholesterol in the blood (Al-Khalaifah *et al.*, 2022). Furthermore, Herve *et al.* (2019) found that certain ginger components decreased the intestinal reabsorption of biliary cholesterol in laying quail.

Table (2): Effects of hydrogen peroxide and ginger on the biochemical parameters of stressed and unstressed male quail.

Groups	Parameters		
	Glucose mg/dl	Cholesterol mg/dl	Triglycerides mg/dl
G_1	320.00±4.84 ab	208.35±8.75 b	163.62±08.31 bc
G_2	338.73±4.90 a	247.68±7.49 a	220.25±20.70 a
G_3	278.05±12.3 c	166.80±8.25 c	122.55±12.21 c
G_4	308.88±4.75 b	221.62±6.12 b	147.72±13.53 bc
G_5	319.60±3.26 ab	215.95±8.84 b	172.25±9.28 b

- Values with different letters vertically show a significant difference at $P \leq 0.05$.
- G_1=Control, G_2= 0.5%H_2O_2, G_3= 1000 mg ginger/kg ration, G_4= H_2O_2 + Ginger added from the beginning, G_5= H_2O_2 + Ginger added after 2 weeks from the beginning.

Also, when given ginger with hydrogen peroxide, it prevents or reduces its negative effect, which may be due to the effect of camphene, shogaols, and zingiberol, which are the basis of the action of ginger as an antioxidant (Wen *et al.*, 2019).

Table 3 showed that H_2O_2 caused a deterioration in the values of packed cell volume and hemoglobin concentration, and this deterioration was significant in the packed cell volume value compared to the control group at $P \leq 0.05$, and when ginger was given with H_2O_2 (G_4 and G_5), the blood picture improved and its values were close to the control group values.

Table (3): Effects of hydrogen peroxide and ginger on the blood picture of stressed and unstressed male quail.

Groups	Traits					
	PCV %	HB g/dl	RBCs $10^6/mm^3$	MCV fl/cell	MCH pg/cell	MCHC g/dl
G_1	47.50±0.67 ab	11.70±0.40 ab	3.15±0.16 a	152.58±7.77 a	37.57±2.31 a	24.63±0.92 a
G_2	45.50±0.43 c	10.86±0.34 b	2.94±0.21 a	158.88±11.0 a	38.08±3.30 a	23.87±0.78 a
G_3	48.50±0.22 a	12.57±0.49 a	3.30±0.07 a	147.27±3.64 a	38.06±1.13 a	25.94±1.06 a
G_4	47.33±0.49 ab	11.73±0.43 ab	3.11±0.10 a	153.18±6.54 a	37.73±0.97 a	24.83±1.12 a
G_5	46.33±0.56 bc	11.82±0.48 ab	2.93±0.12 a	159.84±7.94 a	40.92±3.00 a	25.50±0.96 a

- Values with different letters vertically show a significant difference at $P \leq 0.05$.
- G_1=Control, G_2= 0.5%H_2O_2, G_3= 1000 mg ginger/kg ration, G_4= H_2O_2 + Ginger added from the beginning, G_5= H_2O_2 + Ginger added after 2 weeks from the beginning.

Our results agree with Salah (2008), who reported that the treatment of broiler breeder males with hydrogen peroxide significantly decreased the PCV% and hemoglobin concentration due to oxidation that occurred with H_2O_2. On the contrary, the groups that were given ginger with H_2O_2 from the beginning of the experiment (G_4) had a significantly higher PCV% than the H_2O_2 group at $P \leq 0.05$. That indicates an improvement in blood characteristics compared with the H_2O_2 group, due to the ginger antioxidant components, which help to strengthen the blood cell membranes and thus maintain the levels of these blood characteristics within normal levels (Abdul-Majeed and Abdul-Rahman, 2022; Ogbuewu *et al.*, 2019).

Our findings supported those of Abu Taleb *et al.* (2008), which found that adding 0.3% ginger to the diet for six weeks significantly improved the blood characteristics of Japanese quail when compared to the control group.

As shown in Table 3, the red blood cell counts, MCV, MCH, and MCHC did not significantly differ between the groups. Our results are consistent with the findings of Onu (2010), which indicated there was no statistically significant change ($P \leq 0.05$) in each of the RBC counts, PCV%, hemoglobin, MCV, MCH, and MCHC when ginger was added at 0.25% to the final ration of broiler chickens in comparison to the control group.

It was observed in Table 4 that H_2O_2 (which induced oxidative stress) significantly reduced the lymphocyte percentage, in contrast to ginger, which preserved the percentage of lymphocytes and significantly reduced the heterophils' percentage in comparison to the control group. Moreover, it resulted in an improvement in the stress index value (heterophils/lymphocytes ratio), and was better than its value in the stress group (G_2).

Table (4): Effects of hydrogen peroxide and ginger on the differential leukocytes of stressed and unstressed male quail.

Groups	Traits					
	Lymphocytes %	Monocytes %	Heterophils %	Eosinophils %	Basophils %	H/L
G_1	67.17±2.66 ab	2.33±0.33 a	29.00±2.54 a	1.17±0.40 a	0.33±0.21 a	0.44±0.06 ab
G_2	57.33±3.75 b	3.50±0.89 a	36.83±4.00 a	1.33±0.42 a	1.00±0.52 a	0.69±0.14 a
G_3	75.83±1.14 a	2.33±0.61 a	18.83±1.38 b	1.50±0.22 a	0.50±0.34 a	0.25±0.02 b
G_4	64.33±5.31 b	2.17±0.79 a	31.50±4.58 a	1.00±0.26 a	1.00±0.45 a	0.55±0.15 ab
G_5	60.33±2.46 b	2.83±0.31 a	34.83±1.89 a	1.00±0.37 a	1.00±0.26 a	0.59±0.06 a

- Values with different letters vertically show a significant difference at $P \leq 0.05$.
- G_1=Control, G_2= 0.5%H_2O_2, G_3= 1000 mg ginger/kg ration, G_4= H_2O_2 + Ginger added from the beginning, G_5= H_2O_2 + Ginger added after 2 weeks from the beginning.

It is believed that was done through ginger's improvement of the cellular antioxidant status, and thus the cells maintained these physiological characteristics in normal proportions, in contrast to the H_2O_2 group, in which the percentage of lymphocytes decreased significantly and the heterophils/lymphocytes ratio increased compared to the ginger group, indicating the occurrence of oxidative stress (Abdul-Majeed, 2013).

Scanes (2016) observed that exposure of birds to stressful conditions increases the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland and corticosterone from the adrenal cortex, which leads to a rise in the total number of white blood cells, heterophils' percentage, and a decrease in the lymphocytes' percentage, and as a consequence, the stress index value (heterophils/lymphocytes), is elevated, and this was observed and reported in the H_2O_2 group.

This result agreed with Al-Khalaifah *et al.* (2022), who stated that adding 5, 10, or 15 g/kg of ginger to the broiler chicks' diets enhanced the white blood cell count, and the birds that were given ginger at 10 or 15 g/kg of feed led to a significant increase in the heterotrophile cells compared to the control group. However, I didn't concur with Shewita and Taha (2018), who did not record significant differences in differential leukocyte counts between the groups that given ginger and the control group.

Ginger with it contains proteins, fats, carbohydrates, minerals, vitamins, and trace elements (capsaicin, curcumin, limonene, and proteolytic enzymes) makes it one of the herbs that improve digestion and absorption (Belewu *et al.,* 2009), and this leads to an improvement in the cellular antioxidant status and preservation of the physiological properties of cellular membranes, and thus the proportions of blood cells remain at a normal level (Mao *et al.,* 2019).

CONCLUSIONS

1- Ginger played a significant role in preventing or removing the effects of oxidative stress caused by hydrogen peroxide through its positive effects on blood traits and productivity, and thus the functional efficiency of those birds exposed to oxidative stress improved.
2- Ginger reduced the level of serum glucose, cholesterol, and triglycerides and improved the PCV% and hemoglobin concentration of the birds.
3- Ginger prevented the effect of hydrogen peroxide in many indicators, including raising the percentage of lymphocytes and decreasing the percentage of heterophils, and these effects returned the ratio H/L (stress index) to its level in the control group when given at the same time, and thus ginger prevented the effect of hydrogen peroxide stress.
4- Hydrogen peroxides had a negative effect on the blood parameters and productivity of quails during the experimental period.

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CONFLICT TO INTEREST

There are no conflicts of interest among the researchers in publishing this study.

تأثير الزنجبيل كمضاد للأكسدة في الأداء الفسيولوجي لذكور السمان المجهدة ببيروكسيد الهيدروجين

عبدالله فتحي عبالمجيد   حسن عطية الكراد

قسم وظائف الاعضاء، كلية الطب البيطري في حماه، سوريا

الخلاصة

أجري هذا البحث لمعرفة قدرة مجروش الزنجبيل الجاف *Zingiber officinale* في علاج أو منع تأثيرات الإجهاد التأكسدي الناجم عن بيروكسيد الهيدروجين وذلك بدراسة بعض الصفات الدموية والإنتاجية للسمان. وزع عشوائياً مائة وخمسين طائراً من ذكور السمان بعمر 21 يوماً في خمس مجموعات كل منها 30 طائراً (3 مكررات). المجموعة الاولى: مجموعة سيطرة؛ المجموعة الثانية: مجموعة بيروكسيد الهيدروجين؛ المجموعة الثالثة: مجموعة الزنجبيل؛ المجموعة الرابعة: مجموعة بيروكسيد الهيدروجين + زنجبيل مضاف من بداية التجربة؛ المجموعة الخامسة: مجموعة بيروكسيد الهيدروجين + زنجبيل مضاف بعد اسبوعين من بداية التجربة. جمعت عينات الدم عند عمر 56 يوماً، وتبين من النتائج أن إضافة بيروكسيد الهيدروجين أدت إلى زيادة معنوية ($p\leq0.05$) في كمية العلف المستهلكة مقارنة بالمجموعات الأخرى وتحسن معنوي لمعامل التحويل الغذائي مقارنة بمجموعة الزنجبيل والسيطرة. كما تسبب بيروكسيد الهيدروجين في زيادة معنوية في مستوى كلوكوز الدم مقارنة بمجموعة الزنجبيل وارتفاع معنوي في الكوليسترول والدهون الثلاثية مقارنة بالمجموعات الأخرى، في حين انخفض معنوياً كل من حجم خلايا الدم المرصوصة وخضاب الدم والخلايا الليمفاوية، مما أدى إلى ارتفاع معنوي في مؤشر الإجهاد مقارنة بمجموعة الزنجبيل. أما بالنسبة لتأثير الزنجبيل فقد كان معاكساً لتأثير بيروكسيد الهيدروجين، إذ قلل بشكل معنوي من كمية العلف المستهلكة وخفض مستوى الكلوكوز والكوليسترول والدهون الثلاثية في الدم، مما أدى إلى تحسن صورة الدم وانخفاض قيمة مؤشر الاجهاد في الطيور التي تناولت الزنجبيل بمفرده أو الطيور في المجموعات التي تناولت الزنجبيل مع بيروكسيد الهيدروجين.

الكلمات الدالة: بيروكسيد الهيدروجين، الصفات الدموية، الإجهاد التأكسدي، *Zingiber officinale*

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