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The moderating effect of *Panax ginseng* roots on the male reproductive system and heat shock protein 70 in heat-stressed Japanese quails

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Abstract

This research aims to investigate if ginseng may aid in the reduction of the harmful influence of heat stress on the male reproductive system and the level of heat shock protein70. Eighty mature quails were randomly distributed to four equal groups: the control one reared under normal temperature, the heat-stressed group reared under the temperature of 39±1°C for 4 hrs/day, the heat-stressed group treated with ginseng 500 mg/kg diet, and a group reared under normal temperature and treated with ginseng 500 mg/kg diet. The results showed a significant decrease in red blood cells, white blood cells, lymphocytes, hemoglobin, packed cell volume, total antioxidant capacity, body weight, testis weight, the total number of sperm, and percentage of living sperm, accompanied by a significant increase in MCV, MCHC, heterophil, H/L ratio, heat shock protein70, percentage of dead and malformed sperm in quails exposed to heat stress compared with control. Dietary supplementation of ginseng to birds exposed to heat stress significantly increased the studied parameters in comparison to the group under stress. While, the addition of the nutritional supplement ginseng alone led to a significant increase in RBC, Hb, PCV, MCV, TAC, low percentage of monocytes, H/L ratio, and heat shock protein70, compared to the control. We conclude that the administration of ginseng as a diet additive can ameliorate the effects caused by heat stress in quail, which is attributed to the antioxidant effect of Panax ginseng and its ability for scavenging free radicals.

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Introduction

Poultry is one of the most important sources of food in the world, especially the third-world countries. The world has recently shifted to domesticating quail and using it for human nutrition due to its high nutritional value and because quail is characterized by their low resistance to heat stress, which represents a major problem for birds, where stress leads to a lack of feed intake and a decrease in the efficiency of Nutritional conversion and consequently a decrease in live weight gain, as well as physiological changes, immune and vital changes in birds (1). The increase in ambient temperature is sufficient to increase the body temperature of birds, which leads to changes in blood components, including a change in the percentage of white blood cells, which leads to an increase in the H/L stress index (2). Heat shock protein 70 is present inside cells and prevents the cell and its proteins from being damaged while exposed to active oxygen species. Heat shock protein 70 works to adequately regulate proteins. This name is because it increases with body temperature and is affected by other stress factors. As the gene expression of this protein increases with the increase in cellular stress, this is another vital indicator of cell damage (3). Heat shock protein 70 regulates the proliferation of sperm cells and prevents their death, according to researchers (4), because the death of sperm cells is linked to the formation of heat shock protein 70 inside the sperm cell as a result of exposure to the hazards of the

environment, like heat, radiation, chemicals (5), and as mentioned earlier, when cells are exposed to a variety of stresses, heat shock protein formation rises, heat stress has been shown to play a significant role in the genesis of oxidative-stress in all regions of the body. Heat stress causes a disturbance in the release of FSH and LH, which affects the efficiency of the male reproductive system. It also leads to harmful changes in the structure and function of the testis (6). The ginseng plant is of medical and therapeutic importance because it contains antioxidants: ginsenoside and flavonoids, which are the most effective group of phenolic compounds, and saponin (7). Also, Kumar *et al*. (8) indicated a positive effect on the acid phosphatase enzyme activity of testicular tissue, which is an indicator of cell membranes' protection by reducing lipid peroxidation stimulating the defense systems in the cell.

There are a few studies on *Panax ginseng* in our country/Iraq, thus the goal of our research was to check into the effects of diet supplements of *Panax ginseng* roots on the male reproductive system, blood, and heat shock protein 70 concentration in quail.

Materials and methods

This research was carried out at the College of Veterinary Medicine, University of Mosul, Iraq in the animal house from 22/2/2021 to 1/4/2021.

Animals and experimental design

After one week of acclimation in controlled environment, eighty male quail, two months old and weighing 150-180 grams, were randomly divided into four groups, each with 20 birds: The first group was the control group, in which the birds were reared at in a temperature-controlled environment with a constant temperature of 22 ±1ºC for the length of the experiment. The second group consisted of birds subjected to heat stress at 39±1 ºC for four hours/day (9:00 am –to 1:00 pm). The third group was Supplemented with ginseng roots 500 mg/kg of diet (9) and was subjected to heat stress at 39±1ºC for four hours/day (9:00 am –1:00 pm). In the fourth group, which was not exposed to heat, ginseng was added to the ration at a dose of 500 mg/kg diet. Throughout the rearing period (6 weeks), the birds were kept in (1mx2m) floor pens with 16 hours of continuous light and ad libitum clean tap water for drinking. The nutritional composition of the feed was as suggested by the National Research Council (NRC) (10). Dried ginseng roots were obtained from local markets, grinded and kept in sealed bags until being used. The plant was classified at the College of Agriculture and Forestry at Mosul University.

Sample collection and laboratory analysis

Blood was obtained for hematology examination using special tubes containing (EDTA), and red blood cells and white blood cells were counted using Natt and Herrick solution (11), packed cell volume (percent), and concentration of hemoglobin (Hb g/100ml) were estimated (12). Blood smears were made from fresh blood and stained with Giemsa staining to calculate the differential leucocyte count (DLC) and determine the stress index heterophil /lymphocytes.

Blood serum is obtained using other tubes that do not contain an anticoagulant and is kept frozen at -20°C until biochemical studies, such as the measurement using an HSP70 chicken ELISA kit to detect heat shock protein 70 and the total antioxidant capacity (TAC) using a TAC chicken spectrophotometer kit, are performed. After sacrificing the birds, the weights of the male reproductive organs as relative weight /100 grams of body weight were recorded. Semen was obtained according to the method described in (13) for measurement of total sperm count, life/ dead sperm ratio, and the ratio of deformities in sperms.

Statistical analysis

The SPSS program 10.00 pack was used to conduct a one-way analysis of variance between groups, and Duncan's Multiple Range Test at $P<0.05$ was used to define significant differences (14).

Results

In comparison to the control, heat stress-induced a significantly decrease ($P<0.05$) in RBC, WBC, PCV, and Hb in birds (Table 1). When ginseng was administered to the group of heat stress, there was an increase significantly ($P<0.05$) in RBC, Hb, PCV, and WBC compared to the heat stress group. When birds were given ginseng alone, their RBC, Hb, and PCV levels increased significantly ($P<0.05$), but their WBC levels remained unchanged compared to the control.

Table 2 shows an increase of a significant ($P<0.05$) in the value of MCV and MCH in birds exposed to heat stress in comparison with the control, whereas adding ginseng to the stressed birds' diet caused a significant decline in the value of MCV and MCH respectively, but increase significantly ($P<0.05$) in the value of MCHC when compared to the group exposed to heat stress. When ginseng was given alone, MCH and MCHC levels increased significantly ($P<0.05$) compared with the control.

Table 3 demonstrates the effect of the exposure to heat stress which showed a considerable rise in heterophil cells and a significant decrease ($P<0.05$) in lymphocytes, with no significant changes in eosinophils, basophils, or monocytes when compared with the control group. When ginseng was added to the diet of heat-stressed birds, it caused a significantly decrease ($P<0.05$) in heterophil cells and a significantly increase ($P<0.05$) in lymphocytes, but no changes in eosinophils, basophils, or monocytes. When

compared with control, the ginseng alone group exhibited a significantly decrease (P<0.05) in monocytes.

The values in the heat-stress group indicated a significant increase (P<0.05) in the ratio of heterophil/lymphocytes (H/L) in comparing with control (Table 4). In comparison to the stressed group, adding ginseng to the diet reduced the H/L value significantly (P<0.05). As ginseng was added alone, a non-significant reduction in H/L was seen in comparison with the control.

Heat stress at 39±1℃ for four hours a day for six weeks resulted in a significant increase in the heat shock protein 70 value compared to the control. On the contrary, giving ginseng to this group resulted in a non-significantly decreased (P>0.05) in the level of heat shock protein 70 compared to the heat stress group, but adding ginseng alone to the group not exposed to heat-stress resulted in a significantly decrease (P<0.05) in comparison to the control.

Compared to the control group, the total antioxidant capacity of the group exposed to heat stress decreased. The total antioxidant capacity of the group subjected to heat stress and treated with ginseng showed a non-significant increase (P<0.05) compared to the group exposed to heat stress alone. When compared to the control, there was a significant rise (P<0.05) in the ginseng-treated group that was not exposed to heat-stress in total antioxidant capacity (Table 4).

Table 1: The effect of *Panax ginseng* on certain blood parameters in heat-stressed quails

Groups	Means ± Standard Error			
	RBC (10^6 cell/cm^3)	Hb (g/100ml)	PCV (%)	WBC (10^3/cm^3)
1st group	3.80± 0.05 b	13.53 ±0.13 c	48.48±0.29 c	14.92±0.25 a
2nd group	2.012 ± 0.06 c	12.67 ± 0.09 d	43.50±0.49 d	4.76±0.27 c
3rd group	4.88± 0.13 a	20.48± 0.13 b	50.80±0.39 b	8.33±0.27 b
4th group	4.42± 0.03 a	21.44± 0.11 a	55.20±1.06 a	14.26±0.62 a

Values in the different letter columns are different significantly at P<0.05.

Table 2: The effect of *Panax ginseng* on blood indices in heat-stressed quails

Groups	Means ± Standard Error		
	MCV (fl)	MCH (Pg)	MCHC (g/dl)
1st group	127.38±1.21 b	35.54±0.37 c	27.90±0.11 c
2nd group	217.31±4.64 a	63.42±1.75 a	29.18±0.49 c
3rd group	113.94±2.97 c	45.92±1.12 b	40.33±0.27 a
4th group	124.38±2.86 b	48.28±0.31 b	38.96±0.75 b

Values in the different letter columns are different significantly at P<0.05.

Table 3: The effect of *Panax ginseng* on differential leucocytes number in heat-stressed quails

Groups	Means (%) ± Standard Error				
	Heterophil	Lymphocyte	Eosinophil	Basophil	Monocyte
1st group	23.40±2.02 b	70.40±1.97 a	2.40±0.75 a	1.20±0.39 a	2.60±0.64 a
2nd group	36.80±2.19 a	59.40±1.92 b	1.00±0.30 a	1.00±0.67 a	1.80±0.53 ab
3rd group	22.80±1.25 b	71.50±1.80 a	1.40±0.54 a	1.20±0.39 a	3.10±0.84 a
4th group	23.20±1.72 b	74.40±1.78 a	1.00±0.30 a	0.70±0.30 a	0.70±0.42 b

Values in the different letter columns are different significantly at P<0.05.

Table 4: The effect of *Panax ginseng* on stress index, Hsp70, and total antioxidant capacity in heat-stressed quails

Groups	Means ± Standard Error		
	H/L ratio (%)	Hsp70 (ng/ml)	Total antioxidant capacity (U/ml)
1st group	0.34±0.052 b	41.17±1.88 b	10.02±2.06 b
2nd group	0.63±0.041 a	49.74±0.83 a	4.07±1.29 c
3rd group	0.32±0.024 b	46.56±0.80 a	6.98±0.80 bc
4th group	0.32±0.032 b	36.68±1.73 c	14.67±1.82 a

Values in the different letter columns are different significantly at P<0.05.

Between groups, there was no significant difference in the weight of the right and left testis (Table 5). In the heat-stressed group of birds, the vas deferens and body weight were significantly decreased (P<0.05) compared to those in the control. As ginseng was given with the diet of heat stressed birds, there was a non-significant increase (P>0.05) in vas differences weight, but a significant increase (P<0.05) in body weight when compared to the heat stress group. When comparing the ginseng group to the control, there was a non-significant increase (P>0.05) in vas differences weight but no change in body weight.

Heat stress resulted in a significant decrease (P<0.05) in total count of the sperm and the count of the live sperm, as well as a significantly increase (P<0.05) in dead and abnormal sperm counts, as compared to a control group (Table 6). Adding ginseng to the stressed birds' diet resulted in a non-significant increase (P>0.05) in total sperm count, a significant increase (P<0.05) in the number of live sperms, and a significant decrease (P<0.05) in the number of dead and abnormal sperm (Figure 1) as compared to the heat stress group. When ginseng is used alone, it results in a significant increase (P<0.05) in the total count of sperm and the count of live sperm, as well as a significantly decrease (P<0.05) in the count of dead and abnormal sperm when compared to a control group.

Table 5: The effect of *Panax ginseng* on body weight, vas deference, and relative testis weight in heat-stressed quails

Groups	Means (g/100g BW) ± Standard Error			
	Right testis weight	Left testis weight	Vas difference	Body Weight (g)
1st group	1.72±0.036 a	1.61±0.079 a	0.0810±0.004 ab	196.20±4.414 a
2nd group	1.69±0.060 a	1.63±0.065 a	0.0426±0.004 b	174.00±5.565 b
3rd group	1.51±0.040 a	1.61±0.064 a	0.0562±0.010 ab	192.80±5.748 a
4th group	1.57±0.120 a	1.51±0.101 a	0.1220±0.045 a	204.80±5.531 a

Values in the different letter columns are different significantly at P<0.05.

Table 6: The effect of *Panax ginseng* on physical characteristics of semen in heat-stressed quails

Groups	Means ± Standard Error			
	Sperm count (10^6 /mm^3)	Live sperm (%)	Dead sperm (%)	Abnormal Spermatozoa (%)
1st group	2.41±1.392 a	70.80±0.57 c	29.20±0.57 b	41.80±0.61 c
2nd group	1.51±0.963 b	58.40±1.20 d	41.60±1.20 a	75.60±1.22 a
3rd group	1.57 ±0.8 b	74.20±0.98 b	25.80±0.98 c	53.60±0.81 b
4th group	2.21±1.183 a	80.80±0.65 a	19.20±0.65 d	33.00±0.92 d

Values in the different letter columns are different significantly at P<0.05.

Figure 1: Abnormal and dead sperms were detected in the group exposed to heat.

Discussion

Heat stress resulted in a large decline in RBC, Hb, and PCV and increased MCV and MCH, according to the current study. Our findings are matched to Hassan and Al-Ma'atheedi (12) in adult roosters exposed to oxidative stress and Hassan and Asim (15) in broilers exposed to heat-stress.

Quails subjected to a temperature of 40°C had a significantly lower number of RBCs, suggesting that heat stress can reduce the overall quantity of RBCs by decreasing the lifespan of existing RBCs and preventing new RBCs from forming in the bone marrow (2). RBCs are very sensitive to oxidative damage due to their high oxygen content could be the cause of such an outcome. RBCs are assumed to be the first cells impacted by oxidative stress because hemoglobin is an effective stimulator of the oxidative stress process (16). Heat stress causes hemoglobin to deplete, which is eventually transformed into methemoglobin (metHb), which is unable to bind or transport oxygen. Hemoglobin oxidation also causes the production of Heinz bodies by creating disulfide cross-links between globin chains. The reticuloendothelial macrophage system may be able to remove this membrane-bound denatured protein (Heinz bodies), but more serious cellular alterations result in RBC hemolysis (17).

The results showed a significant increase in blood parameters in groups G3 and G4, these results are consistent with Simsek *et al*. (18), and the reason may be attributed to the fact that ginseng may stimulate the activity of bone marrow stem cells and thus strengthen the systemic cellular defenses and immunity in particular of the organism. S*pirulina platensis* and *Panax ginseng* supplements may benefit humans and animals' anemic or immunocompromised (18).

A decrease in white blood cells was also seen in group G2, and this result conforms to (2). These findings could be linked to the shrinkage of all lymphatic organs (thymus, bursa, spleen, and liver) as a result of heat stress, resulting in dramatically lower weights due to reduced food intake (19). Heat stress has been connected to leucocyte response because of its linkage to circulating ACTH hormone, affecting corticosterone levels by lowering serum levels, resulting in a fall in white blood cell counts (20). In hot weather, the high temperature is adequate to raise the temperature of the body and affect the components of circulating leucocytes in chickens.

When ginseng was given to the diet of animals subjected to heat stress and ginseng was put into the diet of birds not subjected to heat-stress, a significantly rise in the number of WBC was observed. Panax ginseng enhances bone marrow and stem cell activity, which raises the organism's systemic cellular defenses and, in particular, immunity. Ginseng has been shown to interfere positively with bone marrow cell production and the cellular immune response, implying that it could be utilized as adjuvant therapy for anemia or immunodeficiency, according to Simsek *et al.* (18).

Heat stress caused a large increase in heterophil cells, which was countered by a considerable decrease in lymphocytes, resulting in a rise in the H/L ratio compared to the control treatment. ACTH reduces corticosterone levels in the blood serum, reducing the WBC count (20). In hens, heat stress results in an increase in body temperature as well as alterations in the components of circulating WBC. In birds, the H/L cell ratio predicts physiological stress (20).

When ginseng was added to birds' feed exposed to heat stress, the H/L index decreased significantly. Because ginseng has been shown to modify immunity, the roots, stems, and leaves of ginseng were employed to maintain the immunological balance of birds exposed to heat stress. In addition, ginseng's saponins and polysaccharides can help with immunological regulation, antioxidant activity, and overall wellness (21).

Current results showed a significant elevation in heat shock protein 70 in birds exposed to heat stress compared to the control, and these findings were similar to those in (15). The increase in serum Hsp70 levels is due to an increase in Hsp70 genes expression in the liver of the chicken during a change in ambient temperature, and the Hsp70 gene is highly susceptible to environmental changes. The data demonstrate that increased Hsp70 expression in chicken tissues is one of the essential protective mechanisms to prevent harmful protein structure and function changes due to various types of stress (22).

Heat-stressed birds have a lower total antioxidant capacity (TAC) than control birds. When stressed birds were given ginseng, there was a slight rise, but not enough to make a significant difference. When birds who had never been exposed to heat stress were given ginseng, their total antioxidant capacity increased significantly. This activity is reflected in a considerable increase in TAC glutathione (GSH) levels, convincing evidence of ginseng root powder's antioxidant action since it lowers lipid oxidation and restores antioxidant capacity by suppressing oxidative stress (23).

In comparison to the control group, a significantly decrease in the total number of sperms and the percentage of live sperms was observed in the heat stress group, offset by a significant increase in the percentage of dead and distorted sperms, indicating that heat stress, which causes an increase in temperature, is the mechanism responsible for this deterioration.

The results of this study clearly show the impact of heat stress on the male reproductive system. Because the testicle is one of the components of the male reproductive system that is impacted by high temperatures, because of the increased heat inside the testicle, increased metabolic activity induces lipid peroxidation as well as a rise in reactive oxygen species generation. Consequently, causes spermatogenetic cell apoptosis, as well as disrupting cell proliferation and differentiation. Since of their high quantities of fatty acids, spermatozoa are particularly vulnerable to oxidative damage. Furthermore, the quantity of antioxidants in sperm decreases as the cytoplasm in the sperm decreases (24). Adding ginseng to the diet of stressed and non-stressed birds resulted in a large increase in the proportion of live sperms, countered by a significantly drop in the percentage of dead and deformed sperms compared to the heat stressed group and the control one. Ginseng root has the capacity to enhance sperm sensitivity of the hormone androgen, as the ginseng root's potential to promote protein creation in testicular tissue and DNA protection in their cells (25).

Conclusion

We conclude that ginseng root can lessen the impacts of oxidative stress while improving the activity of male quail's reproductive system.

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Conflict of interest

Authors state that the publication of this manuscript does not include any conflicts of interest.

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التأثير المعدل لجذور البانكس جنسنك في الجهاز التناسلي الذكري وبروتين الصدمة الحرارية ٧٠ في السلوى الياباني المجهد حرارياً

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الخلاصة

تهدف الدراسة الحالية الى معرفة تأثير الجينسنغ في تقليل آثار الإجهاد الحراري على الجهاز التناسلي الذكري ومستوى بروتين الصدمة الحرارية ٧٠. تم توزيع ثمانون طائرا من السمان البالغ الى اربعة مجاميع متساوية: مجموعة السيطرة تمت التربية تحت درجة حرارة طبيعية، مجموعة الاجهاد الحراري تمت التربية تحت درجة حرارة ٣٩±١ درجة مئوية لمدة أربع ساعات لكل يوم، مجموعة الإجهاد الحراري والمعاملة بالجينسنغ ٥٠٠ملغم/كغم علف، والمجموعة الغير مجهدة والمعاملة بالجينسنغ ٥٠٠ملغم/كغم علف. أظهرت النتائج انخفاضًا معنويًا في عدد خلايا الدم الحمر، خلايا الدم البيض، الخلايا اللمفية، تركيز الهيموكلوبين، حجم الخلايا المرصوصة، السعة الكلية المضادة للأكسدة، وزن الجسم،

وزن الخصى، عدد النطف ونسبة النطف الحية، مصحوبة بزيادة معنوية في معدل حجم الخلية ومعدل هيموكلوبين الخلية ونسبة الخلايا المغايرة ونسبة الخلايا المغايرة/الخلايا اللمفية، بروتين الصدمة الحرارية ٧٠، النسبة المئوية للنطف الميتة والمشوهة في طيور السمان المعرضة للإجهاد الحراري مقارنة بمجموعة السيطرة. اضافة الجينسنغ الغذائي الى عليقة الطيور المعرضة للإجهاد الحراري ادت الى زيادة معنوية في القياسات المدروسة مقارنة بالمجموعة المجهدة. بينما ادت إضافة الجينسنغ الغذائي لوحده الى حدوث زيادة معنوية في عدد خلايا الدم الحمر وهيموكلوبين الدم وحجم الخلايا المرصوصة ومعدل حجم الخلية والسعة الكلية المضادة للأكسدة، وانخفاض في نسبة الخلايا أحادية النواة، نسبة الخلايا المغايرة /الخلايا اللمفاوية وبروتين الصدمة الحرارية ٧٠ بالمقارنةً بمجموعة السيطرة. نستنتج من النتائج أعلاه أن اعطاء الجينسنغ كإضافة علفية له القدرة على تخفيف الآثار الناجمة عن الإجهاد الحراري في السمان، والذي يُعزى إلى التأثير المضاد للأكسدة له وقدرته على كبح الجذور الحرة.