The Effect of Dry Yeast and Folic Acid Treatment on the Reproductive and Physiological Aspects of Quail Stressed with Hydrogen Peroxide

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Abstract. *This study aimed to assess the effect of prebiotics (dry yeast) and folacin (folic acid) on oxidative stress, performance parameters, and blood characteristics in Japanese quails. One-week-old, 180 sexed chicks were randomly distributed into 5 groups with 4 replicates (two replicates/sex). Birds were divided into 5 treatments: 1st – control basal diet (BD); 2nd –* H_2O_2 *1% in drinking water; 3rd –* H_2O_2 *+ folic acid (F) 5 mg/L in drinking water; 4th –* H_2O_2 *+ yeast (Y) 10% in a basal diet; and 5th –* H_2O_2*+F+Y. The results showed that hydrogen peroxide treatment caused a notable decrease in the body weight of both sexes, as well as in the relative weights of the female organs, while the weight of some of the male organs increased compared with the control, and all treatments with* H_2O_2 *caused an increase in the relative weights and the live body weight compared with the peroxide treatment. The peroxide treatment led to a significant decrease in the hematological parameters of both sexes in comparison with the control group, whereas all treatments improved the blood levels compared with the peroxide treatment. The biochemical parameters of both sexes increased with the administration of hydrogen peroxide, particularly at the MDA level, but they improved and returned to the normal condition in the various treatments with* H_2O_2*, especially in the levels of GSH as opposed to the peroxide treatment. These results indicate that the administration of yeast or folic acid alone or in combination reduces the harmful effects of oxidative stress and improves performance.*

Introduction

Stress increases free radicals by provoking lipid peroxidation in the cell membrane, which then directly releases glucose and lipid metabolism as well as protein catabolism via stress hormone release (Hosseini et al., 2010). Stress may influence negatively the chickens' performance by reducing feed intake, efficiency and weight gain (Odihambe et al., 2006). When oxidative stress occurs, the first line damaged is the intestine, leading to the improper digestion and malabsorption of nutrients, which causes illnesses and occasionally death (Bai et al., 2018).

The antioxidants involve two types: the natural ones such as medicinal grass and processed ones such as vitamins that have a protective role against oxidative free radicals (McDonald et al., 2010). There is worldwide interest in identifying compounds that have antioxidant properties and are pharmacologically effective with low side effects to use in the food industry and preventive medicine (Sati et al., 2010; Aziz et al., 2019).

Since 2006, the European Union Countries (EUC) have planned to use probiotics in broiler chicken rations as a performance enhancer (Castanon, 2007; Kabir, 2009).

Some live yeasts such as S. Cerevisiae and Kluyveromyces Marxianus are probiotics (Gaggia et al., 2010; Kasianenko et al., 2020). Folic acid, also called vitamin B9, is one of B the complex vitamins. The B complex vitamins that help the body convert food into fuel (energy) and folic acid are necessary to transfer donor or acceptor modification to one-carbon units for methylation or protein and DNA synthesis and gene expression. (Dean, 2007; Asaikutti et al., 2016). Folic acid is necessary for the biosynthesis of amino acids and deoxynucleotides required for DNA replication and repair, as well as the methylation of homocysteine to form methionine (Tapiero et al., 2001).

Quails are more resistant to pathogens and have recently attracted interest in the poultry production sector, so reducing stress in poultry remains a topic of concern among scientists and producers. *Coturnix coturnix, Japonica quails* become important livestock especially when it is used in embryological studies; furthermore, a large number of poultry can be kept in a narrow area, where they are easy to handle and have a small body. A limited number of parents can produce a large number of offspring; quails have high egg production; their eggs are also very protein-rich and a good source of riboflavin, iron, phosphorus, and selenium (Devarestti, 2016; Bing, 2022; Sree et

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al., 2016). Poor feed efficiency, feed intake and lower body gain often seen in meat poultry flocks are adverse effects of stress (Fellenberg & Speisky, 2006; Lyasere et al., 2021). The actual study aimed to determine which probiotic and tonic (Vit. B9) can improve the physiological and biochemical parameters in quail stressed with hydrogen peroxide.

Materials and Methods

This work was applied in the animal house of the Veterinary Medicine College at Mosul University. The chicks used were 7 days old, sexed, and then distributed into 5 groups with 4 replicates of 36 birds housed in cages under an artificial lighting program (16 hours / day) at 37° C. The treatments continued for 6 weeks, no vaccination was performed, and birds were fed a commercial ration containing 19.9% crude protein (CP) and 2878 kcal energy (McDonald et al., 2010).

Blood samples were obtained by jugular vein incision at the end of the period, and the collected samples were divided into 2 portions. The first one was kept in containers containing EDTA for blood pictures (RBC, WBC, Hb, PCV, MCV, MCH, and MCHC). The second one was separated directly by centrifuging at 3000 rpm / 10 min to get serum and then was kept at –20° C. After that, it was tested to determine some biochemical parameters, such as TC, TG, HDL, LDL, VLDL, TP and albumen. The GSH and MDA in the serum samples were determined by using commercial kits from the biodiagnostic company (Egypt) in a college research unit, and other parameters of the weight of the organs were obtained directly after bird slaughtering. Using the linear model method, the values were examined for variance (Al-Najjar et al., 2010), and the differences between treatment groups were determined using the Duncan multiple range (Steel et al., 1997).

Treatments were as follows:

1. Birds received a standard diet and were given tap water.
2. Birds received standard food and 1% H_2O_2/L drinking water.
3. Birds received regular food with 1% H_2O_2/L drinking water and 5 mg/L folic acid.
4. Birds received regular food that included dry yeast (10 g/kg ration) and 1% H_2O_2/L drinking water.
5. Birds received standard food of dry yeast (10 g/kg), folic acid (5 mg/L), and 1% H_2O_2/L in drinking water.

Results

Tables 1 and 2 show that administering hydrogen peroxide significantly reduced body weight in both male and female birds. Males also had a significant increase in the relative weight of pancreas and liver tissues, while females had a significant decrease in the relative weights of ovaries, heart, and kidneys compared to the control group ($P < 0.05$). All treatment groups with hydrogen peroxide led to a significant improvement in the weights of the relative organs of the testes, ovaries, kidneys, and heart in both sexes, compared with the hydrogen peroxide group ($P < 0.05$).

Table 1. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on relative organ weights of male Japanese quails

Traits / Treatments	BW g	R. Testis g/100 g BW	L. Testis g/100 g BW	Liver g/100 g BW	Kidney g/100 g BW	Heart g/100 g BW	Pancreas g/100 g BW
Control	207 ± 5.34 a	1.49 ± 0.05 b	1.6 ± 0.04 b	1.42 ± 0.04 c	0.45 ± 0.02 ab	0.83 ± 0.02 c	0.15 ± 0.01 d
H_2O_2	174 ± 1.34 c	1.52 ± 0.02 b	1.77 ± 0.03 b	2.36 ± 0.08 a	0.40 ± 0.01 b	0.85 ± 0.03 c	0.29 ± 0.01 a
H_2O_2+F	186 ± 3.36 b	1.53 ± 0.04 b	1.74 ± 0.04 b	1.65 ± 0.02 b	0.47 ± 0.02 a	1.00 ± 0.03 a	0.23 ± 0.01 b
H_2O_2+Y	191 ± 2.42 b	1.72 ± 0.06 a	2.42 ± 0.11 a	1.6 ± 0.04 b	0.44 ± 0.02 c	0.87 ± 0.02 ab	0.19 ± 0.01 c
H_2O_2+F+Y	206 ± 1.86 a	1.58 ± 0.07 ab	1.77 ± 0.04 B	1.59 ± 0.05 B	0.45 ± 0.01 ab	0.93 ± 0.02 b	0.26 ± 0.02 a

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Table 2. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on relative organ weights of female Japanese quails

Traits / Treatments	BW g	Ovary g/100 g BW	Oviduct g/100 g BW	Liver g/100 g BW	Kidney g/100 g BW	Heart g/100 g BW	Pancreas g/100 g BW
Control	233.6 ± 4.05 b	4.13 ± 0.11 b	3.23 ± 0.10 a	2.84 ± 0.08 bc	0.68 ± 0.02 a	0.89 ± 0.01 a	0.25 ± 0.01 ab
H_2O_2	206.5 ± 3.51 d	3.21 ± 0.12 d	3.21 ± 0.07 a	3.03 ± 0.011 ab	0.44 ± 0.03 c	0.81 ± 0.01 b	0.26 ± 0.01 ab
H_2O_2+F	237.6 ± 6.66 c	3.57 ± 0.09 c	3.09 ± 0.04 a	2.65 ± 0.02 cd	0.51 ± 0.01 b	0.81 ± 0.01 b	0.23 ± 0.01 b
H_2O_2+Y	225.0 ± 4.68 a	4.74 ± 0.12 a	3.29 ± 0.08 a	3.13 ± 0.08 a	0.56 ± 0.01 b	0.81 ± 0.02 b	0.28 ± 0.01 a
H_2O_2+F+Y	216.8 ± 5.61 cd	3.31 ± 0.13 cd	3.18 ± 0.01 a	2.47 ± 0.09 d	0.53 ± 0.01 b	0.74 ± 0.01 b	0.28 ± 0.01 a

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Tables 3 and 4 show the results, which demonstrate the impact on hematological characteristics. When compared with the hydrogen peroxide group, all treatment groups with peroxide improved all blood parameters in both sexes, males and females, whereas the treatment with peroxide significantly decreased all hematological parameters when compared with the control group ($P < 0.05$).

According to Tables 5 and 6, the hydrogen peroxide treatment significantly elevated all the assessed bio-

Table 3. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the blood parameters of male Japanese quails

Traits / Treatments	RBC Cell*10^6	WBC Cell*10^3	Hb g/dL	PCV %	MCH pg	MCHC g/100 mL	MCV fL
Control	4.59 ± 0.05 a	26.86 ± 0.27 c	21.78 ± 0.07 a	42.6 ± 0.48 Ab	47.24 ± 0.24 c	51.25 ± 0.52 a	92.86 ± 1.18 d
H_2O_2	4.00 ± 0.08 b	26.80 ± 0.28 c	17.24 ± 0.35 c	39.9 ± 0.81 c	42.01 ± 0.53 d	42.15 ± 0.67 c	99.76 ± 1.16 c
H_2O_2+F	3.91 ± 0.08 bc	30.08 ± 0.15 b	22.00 ± 0.21 a	43.6 ± 0.54 a	54.91 ± 0.89 b	50.82 ± 0.52 a	110.83 ± 1.45 b
H_2O_2+Y	3.23 ± 0.03 d	33.55 ± 0.21 a	19.71 ± 0.19 b	41.6 ± 0.56 b	60.97 ± 1.2 a	48.02 ± 0.41 b	124.40 ± 2.71 a
H_2O_2+F+Y	3.76 ± 0.6 c	29.39 ± 0.31 b	20.00 ± 0.39 b	41.5 ± 0.22 b	53.11 ± 0.26 b	48.59 ± 0.65 b	110.51 ± 1.84 b

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

chemical parameters in both sexes, especially the MDA levels compared with the control group ($P < 0.05$).

While albumin, total protein, and low-density lipoprotein (LDL) levels were all significantly decreased in all hydrogen peroxide treatment groups and increased significantly in the F+H_2O_2 and F+Y+H_2O_2 groups, respectively, when compared with the control group, all hydrogen peroxide treatment groups significantly increased GSH levels.

Table 4. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the blood parameters of female Japanese quails

Traits / Treatments	RBC Cell*10^6	WBC Cell*10^3	Hb g/dL	PCV %	MCH pg	MCHC g/100 mL	MCV fL
Control	4.41 ± 0.07 a	27.19 ± 0.52 c	20.72 ± 0.30 a	43.2 ± 0.64 a	41.76 ± 1.72 b	48.14 ± 1.39 a	86.46 ± 1.35 b
H_2O_2	3.67 ± 0.12 b	29.86 ± 0.48 b	18.18 ± 0.15 d	39.8 ± 0.41 b	49.57 ± 1.34 a	45.43 ± 0.36 b	109.17 ± 3.10 a
H_2O_2+F	3.89 ± 0.11 b	25.88 ± 0.32 d	19.2 ± 0.17 c	40.2 ± 0.29 b	49.60 ± 1.24 a	49.51 ± 0.64 a	104.24 ± 3.95 a
H_2O_2+Y	3.73 ± 0.11 b	32.30 ± 0.26 a	19.53 ± 0.17 bc	40.89 ± 0.34 b	52.58 ± 1.30 a	47.79 ± 0.59 a	110.35 ± 3.68 a
H_2O_2+F+Y	3.78 ± 0.05 b	28.26 ± 0.23 c	20.08 ± 0.12 b	40.3 ± 0.49 b	53.20 ± 0.67 a	49.88 ± 0.53 a	106.79 ± 1.78 a

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Table 5. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the biochemical parameters of male Japanese quails

Traits / Treatments	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	TP g/dL	Albumin g/dL	MDA nmol/mL	GSH nmol/mL
Control	281.7 ± 2.33 a	192.6 ± 1.52 b	63.9 ± 0.88 c	179.29 ± 2.09 a	38.52 ± 0.3 b	3.55 ± 0.06 c	1.24 ± 0.07 c	0.723 ± 0.003 b	1.491 ± 0.004 a
H_2O_2	253.8 ± 1.92 b	286.0 ± 2.3 a	57.4 ± 2.45 d	139.2 ± 3.12 b	57.2 ± 0.46 a	4.26 ± 0.02 a	1.65 ± 0.03 a	0.903 ± 0.002 a	1.027 ± 0.005 e
H_2O_2+F	172.00 ± 3.2 E	122.8 ± 4.0 e	105.6 ± 1.22 A	41.84 ± 2.23 E	24.56 ± 0.8 e	3.40 ± 0.01 d	1.24 ± 0.01 c	0.728 ± 0.002 b	1.397 ± 0.004 c
H_2O_2+Y	188.10 ± 3.3 d	132.6 ± 4.1 d	109.6 ± 1.57 a	51.98 ± 2.31 d	26.52 ± 0.82 d	3.85 ± 0.01 b	1.42 ± 0.03 b	0.713 ± 0.003 c	1.412 ± 0.004 b
H_2O_2+F+Y	238.4 ± 2.6 C	166.6 ± 1.6 c	74.7 ± 0.36 b	130.38 ± 2.53 c	33.32 ± 0.32 c	3.34 ± 0.03 d	1.20 ± 0.01 c	0.732 ± 0.002 b	1.384 ± 0.001 d

Data are shown as mean ± SE. n = 18.
Various letters denote significance in each column ($P < 0.05$).

Table 6. Effect of *saccharomyces cerevisiae* and folic acid with H_2O_2 on the biochemical parameters of female Japanese quails

Traits / Treatments	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	VLDL mg/dL	TP g/dL	Albumin g/dL	MDA nmol/mL	GSH nmol/mL
Control	118.3 ± 0.51 c	247.85 ± 0.83 a	65.60 ± 0.40 c	3.13 ± 0.20 bc	49.57 ± 0.16 a	4.10 ± 0.02 c	1.50 ± 0.02 b	0.757 ± 0.01 bc	1.567 ± 0.015 a
H_2O_2	130.6 ± 0.89 a	248.05 ± 0.85 a	79.00 ± 0.81 a	1.99 ± 0.23 b	49.61 ± 0.17 a	4.24 ± 0.06 c	1.51 ± 0.03 b	0.944 ± 0.006 a	1.011 ± 0.004 c
H_2O_2+F	109.7 ± 0.61 d	247.70 ± 0.80 a	54.80 ± 0.41 d	5.39 ± 0.59 a	49.51 ± 0.15 a	4.85 ± 0.11 a	1.75 ± 0.07 a	0.739 ± 0.002 c	1.500 ± 0.002 b
H_2O_2+Y	110.7 ± 1.08 d	249.4 ± 0.24 a	56.90 ± 0.97 d	3.92 ± 0.28 b	49.88 ± 0.04 a	3.42 ± 0.04 d	1.30 ± 0.02 c	0.755 ± 0.002 bc	1.495 ± 0.003 b
H_2H_2+F+Y	122.00 ± 1.24 b	248.00 ± 1.51 a	70.70 ± 1.15 b	2.39 ± 0.21 cd	49.6 ± 0.30 a	4.54 ± 0.08 b	1.74 ± 0.04 a	0.772 ± 0.003 b	1.493 ± 0.003 b

Data are shown as mean ± SE, n = 18.
Various letters denote significance in each column ($P < 0.05$).

Discussion

The results of treatment with hydrogen peroxide H_2O_2 showed a significant decrease in body weights for both sexes (Tables 1, 2), as well as a decrease in the relative weights of vital organs, especially among females (Table 2). This is consistent with what was indicated by Rajkumar et al. (2015) and Jiu et al. (2019) in the parents of broilers and may be attributed to stress providing an opportunity for effective oxygen species to attack the fats that form cell membranes and oxidize them to produce what is known as lipid peroxides, especially MDA as well as other compounds. This series of oxidative reactions negatively encouraged the ability of birds to construct GSH in the tissue, and due to the insufficient availability of the external source (low feed consumption) that promoted the construction of tissue GSH, the matter worsened on various other vital tissues, while the results of yeast and folic acid indicate their ability to provide tissue protection or reduce its action by promoting the antioxidant status and reducing the role of disruptive stress. It is believed that this is due to their role in promoting the construction of tissue glycogen by activating the pentose shunt, which is the main provider of NADPH needed to re-reduce the oxidized glutathione GSSG to a reduced form GSH, and inhibiting the process of gluconeogenesis that destroys body proteins; this is consistent with the results of El-Husseiny et al. (2007) and Li et al. (2021). They claimed that different levels of folic acid (0.5, 0.75, and 1.0 mg/kg) improved performance metrics in broiler chicks fed diets containing methionine (0.45%). Zhang et al. (2021) concluded that folic acid at different levels (0.75, 1.5, and 3 mg/kg) in the diet could significantly improve the average daily gain of broilers. The Association of Official Analysis of Chemists (AOAC, 2000) reported that folic acid is needed for animals with greater production rates or growth because it plays a role in amino acid and DNA metabolism and methylation of the homocysteine to form methionine plays a role in reducing corticosterone levels and then reducing stress (Shareef & Al-Dabbagh, 2009; Whisner & Castillo, 2018). Variations in gut flora and environmental factors may confuse the variable effect of yeast (SAC).

The results for both genders in Tables (Bai et al., 2018; McDonald et al., 2010) about hematological parameters are in line with those obtained by Rasheed and Al-Nuaimmi (2022) and Li and Gatlin (2003). When folic acid was used instead of the oxidative stress group, the adult quails' total WBC count increased significantly. This difference may be explained by the vitamin's increased antioxidant effect, which boosts the activity of phagocytes. However, it was discovered that certain hematological parameters were significantly improved by folic acid therapy (Mohamed et al., 2013). Leukocytosis may be indicative of higher activity of innate immune responses because yeast cell walls contain chitin manner and glucan that have triggered the immune stimulation, these findings are consistent with those of Reda et al. (2021) and Abdul-Majeed et al. (2021), who observed similar effects when yeast was added to the diet of Japanese quail.

The results provided in the Tables (Sati et al., 2010; Aziz et al., 2019) showing the role of hydrogen peroxide on the biochemical characteristics in both sexes indicate significantly rising levels of cholesterol and triglycerides. This increase in the above criteria may be attributed to the role of stress in encouraging the gluconeogenesis process, which works to liberate glucose from non-carbohydrate sources and raise fat levels through its role in the occurrence of disorders in the metabolic or digestive processes of fat in the stomach and intestines, which is in line with Mousa's (2021) findings, while other treatments with hydrogen peroxide showed an improvement in biochemical parameters, especially in folic acid, which is in line with the results obtained by Poonuru et al. (2011). In chicks, it plays a role in encouraging the methylation process necessary for the production of methionine, which has a role in activating the work of somatic cells, including pancreatic beta cells, which in turn stimulate the secretion of insulin, working indirectly through synthesis of the enzyme lipoprotein lipase (Abdul-Azeem, 2007).

The increase in anaerobic and cellulolytic bacteria that occurred when yeast was added to the experimental diet may have contributed to the positive effects of the dietary supplement on plasma total protein, albumin and total lipid. This in turn contributes to improved lactate utilization and a more stable PH of the gut, which improves nutrient digestibility and growth performance (Gao et al., 2008).

Conclusion

According to the study findings, giving dry yeast and/or folic acid to quails of both sexes that have been exposed to H_2O_2 stress has a positive impact on their hematological efficiency and aspects.

Highlights

1. The folic acid and dry yeast or a combination lessen the stress effects by improving the biochemical aspects in both sexes of quails.
2. Both above materials improved the body metabolism that reflected on body and vital organ weights by decreasing the harmful stress.

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Conflict of interest

There are no conflicts of interest involving the publication of this work, according to the authors.

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