

## Research Article

# The effect of supplementing different levels of fenugreek seeds (*Trigonella foenum – graecum*) on physiological performance of Japanese quail (*Coturnix-coturnix japonica*) exposed to oxidative stress induced by hydrogen peroxide

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**ABSTRACT: Background:** Fenugreek seed (*Trigonella foenum graecum*) was evaluated for its effects on physiological performance and antioxidant status in Japanese quail (*Coturnix-coturnix japonica*). In this trial 5 or 10 g of fenugreek seeds/kg diet were feed to birds in two replicated pens (five bird/cage) per treatment starting at 18 weeks of age. Two treatment were given standard diet and with or without H<sub>2</sub>O<sub>2</sub> in the drinking water (T1 and T2) Another two treatments were supplemented with 5 or 10 g fenugreek ground seed/kg diet (T3 and T4) and 0.5% H<sub>2</sub>O<sub>2</sub> in drinking water, A further two treatments were supplemented 5 or 10 g fenugreek grounded seeds/kg diet and without any addition to the drinking water (T5 and T6). The present study has shown that there was a significant decline in the malondialdihyde (MDA) and an increase in the glutathione (GSH) concentration in the liver tissue in the birds treatment with both ground fenugreek seed levels (5 and 10/kg diet). The fenugreek treated birds showed a significant difference to the control group ( $p < 0.05$ ) for the birds treated with H<sub>2</sub>O<sub>2</sub>. The results also indicated to enhanced antioxidants levels (as determined by GSH concentration) in birds treated with H<sub>2</sub>O<sub>2</sub> when supplemented with fenugreek seeds (5 or 10 g/kg diet).

We also report a significant ( $p < 0.05$ ) increase in RBC and Hb concentration and a difference to the control group decrease in the WBC, glucose, cholesterol levels as well as the and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes activities in blood serum in birds treated with both fenugreek seeds levels compared with control groups (T1 and T2). The results suggest that fenugreek seeds supplementation at 5 or 10 gm/kg diet may enhance antioxidant status in Japanese quail.

**KEYWORDS:** Fenugreek, Japanese quail, oxidative stress, hydrogen peroxide, enzymes

## INTRODUCTION

The overproduction of reactive oxygen species including hydroxyl radical, superoxide anion radical and hydrogen peroxide radical can contribute to oxidative stress.<sup>[1]</sup> Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases

including diabetes, hypertension, coronary heart disease, cancer etc.<sup>[2]</sup> Most of the reactive oxygen species are scavenged by endogenous defense systems. But, these systems may not be completely efficient, requiring them to depend on exogenous anti-oxidants from natural sources. Presently, there has been an increased interest worldwide to identify antioxidant compounds, which are pharmacologically effective, or have low or no side effects for use in preventive medicine and the food industry.<sup>[3]</sup> Several isolated plant constituents as well as crude extracts have been recognized to possess beneficial effects against free radicals in biological systems as anti-oxidants.<sup>[4]</sup>

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*Trigonella foenum graecum* L. commonly known as fenugreek belongs to the family Leguminosae which is an annual, herbaceous and aromatic plant. Fenugreek grows wild in India, the Mediterranean and North Africa. Fenugreek seeds are the small stony seeds from the pod of a bean-like plant, yellowish brown in colour and angular in shape.<sup>[5]</sup>

The active constituents in Fenugreek seeds are alkaloids, lysine and L-tryptophan. It also contains steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) and mucilaginous fiber which is believed to be responsible for many beneficial effects fenugreek exhibits such as having the ability to aid the digestive process.<sup>[6]</sup> Furthermore, fenugreek contains lecithin and choline that helps to dissolve cholesterol and fatty substances, minerals, B. complex vitamins, iron, phosphates, para-amino benzoic acid (PABA), and vitamins A and D. Moreover, fenugreek also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system.<sup>[6,7]</sup>

Fenugreek is also used as a supplement in poultry feed to lower the plasma total lipid and total cholesterol levels in Hubbard broiler chicks and to improve the antioxidant status and production performance in laying hens.<sup>[8]</sup>

The aim of this study was to induce oxidative stress by H<sub>2</sub>O<sub>2</sub>. Fenugreek seeds were used as an antioxidant material to study oxidative repair.

## MATERIALS AND METHODS

A total of sixty Japanese quail (*Coturnix-coturnix japonica*, eighteen weeks old) were used in this study. The birds included in this study were housed on twelve separated cages under artificial lighting program of (14 h light: 10 h dark per day) from the period from beginning of October to the end of November. For two months of experiment all birds were fed commercial ration for Japanese quail (*Coturnix-coturnix japonica*) breeding *ad libitum* which containing 2878 Kcal metabolisable energy and 19.9% crude protein. The birds were separated into six treatment groups consisted of 10 birds each. Treatments were as following:

Treatment (1): The birds were given standard diet + normal water.

Treatment (2): The birds were given the same ratio as treatment (1) + H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water.

Treatment (3): The birds were given a standard ratio + 5 g ground fenugreek/kg feed + H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water.

Treatment (4): The birds were given a standard ratio + 10 g ground fenugreek/kg diet + H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water.

Treatment (5): The birds were given a standard ratio + 5 g ground fenugreek/kg diet + normal water.

Treatment (6): The birds were given a standard ratio + 10 gm groundfenugreek/kg diet + normal water.

At the end of experiment, blood samples were obtained from all birds the jugular vein incision and the blood was carefully transferred to two type of collecting tubes: the first containing potassium EDTA (1.5 g/ml), and used for examination of total red blood cells count (RBC) and white blood cells count (WBC) and hemoglobin concentration (Hb) previously described.<sup>[9]</sup> The second type of tubes were without anti coagulant to produce serum. The blood serum was separated as soon as possible by centrifuging at 3000 g for 15 minutes stored at (-20 °C) until usage. All biochemical parameters were assayed with the commercial kits (Ranndox com.) and (Biolabo Reagents) as per the manufacturer's instructions.

Blood biochemical traits measured in this study were the concentrations of glucose, total cholesterol and the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). After the birds slaughter sacrificed, their livers excised and frozen on (-20 °C), Malondialdehyde (MDA) levels were measured according to previous methods.<sup>[10]</sup> Glutathione (GSH) levels were determined by standard assays.<sup>[11]</sup> The data was analyzed for variance using the general linear model method.<sup>[12]</sup> Testing for significance difference between different treatments was performed using the Duncan multiple range test.<sup>[13]</sup>

## RESULTS

Treatment of Japanese quail with different levels of fenugreek seed (T5, and T6) resulted in a significant ( $p < 0.05$ ) increase in RBC and significant ( $p < 0.05$ ) decrease in WBC, as well as blood serum glucose and cholesterol levels in comparison with the oxidative stress group (T2). The T5 group surpassed the other treatment groups (T1, T2 and T3) with regards to blood RBC, whilst there were no significant differences between T3 and T4 with relation to blood RBC, Hb and WBC. Moreover, T5 recorded the lowest blood WBC value compared with other treatments (Table 1). Our results (Table 1) also showed that there were significant differences ( $p < 0.05$ ) between treatment groups with respect to blood serum activities of AST and ALT enzymes. However, there was no significant difference between T3 and T4 with respect to this trait.

**Table 1: Effect of fenugreek seeds on some blood physiological characteristics of Japanese quails (*Coturnix-coturnix japonica*) exposed to oxidative stress induced by H<sub>2</sub>O<sub>2</sub> (Mean ± SE)**

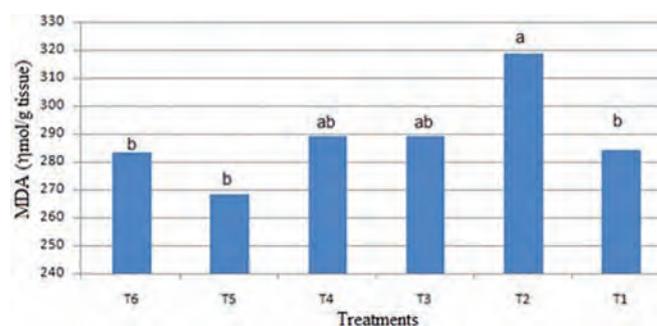
Treatments	RBC 1*10 <sup>6</sup>	HB (g/100 ml)	WBC/ml	Glucose (mg/100 ml)	Cholesterol (mg/100 ml)	AST (IU/L)	ALT (IU/L)
T1	3.84 ± 0.09ab*	14.5 ± 0.72a	25152 ± 2379ab	151.0 ± 1.12b	277.3 ± 4.68a	74.7 ± 1.41bc	11.6 ± .069ab
T2	3.51 ± 0.13d	13.1 ± 0.64b	26047 ± 1423a	165.5 ± 2.11a	262.5 ± 8.32ab	78.7 ± 2.00a	11.9 ± 0.78a
T3	3.63 ± 0.09dc	13.6 ± 0.78ab	24152 ± 14235ab	161.3 ± 2.84ab	277.3 ± 10.45a	76.0 ± 1.32ab	10.2 ± 0.78abc
T4	3.66 ± 0.12bcd	13.7 ± 0.82ab	23457 ± 17458ab	154.2 ± 1.98b	278.0 ± 9.38a	74.4 ± 1.12bc	9.4 ± 0.85bc
T5	3.91 ± 0.06a	14.7 ± 0.50a	19211 ± 13658c	142.7 ± 3.54c	237.8 ± 10.23b	70.7 ± 4.31c	9.8 ± .092abc
T6	3.78 ± 0.17abc	13.9 ± 1.28ab	22272 ± 17542b	146.3 ± 2.45bc	227.3 ± 11.34c	76.6 ± 2.62ab	8.4 ± .075c

\* different letters within the same column indicate a significant difference between treatments in the abstract level (p ≤ 0,05). T(1) control group. T(2) The birds were given the same ratio as treatment (1) + H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(3) The birds were given a standard ratio +5 g ground fenugreek/kg feed + H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(4) The birds were given a standard ratio +10 gm ground fenugreek/kg diet + H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(5) The birds were given a standard ratio +5 gm grounded fenugreek/kg diet + normal water. T(6) The birds were given a standard ratio +10 g ground fenugreek/kg diet + normal water.

Feeding Japanese quail with water containing 0.5% levels of H<sub>2</sub>O<sub>2</sub> (T2) resulted in a significant (p < 0.05) decrease blood RBC and HB levels (Table 1). However, (T2) recorded the highest mean WBC, serum total glucose, cholesterol and blood serum activities for AST and ALT enzymes in comparison with other treatments. Supplementation of Japanese quail feed with different levels of fenugreek seed (T3 and T4) resulted in a significant (p < 0.05) decrease in regards to blood serum glucose and blood serum activities of AST and ALT enzymes as compared with control group (T2). Furthermore, there were no significant differences with control group (T1).

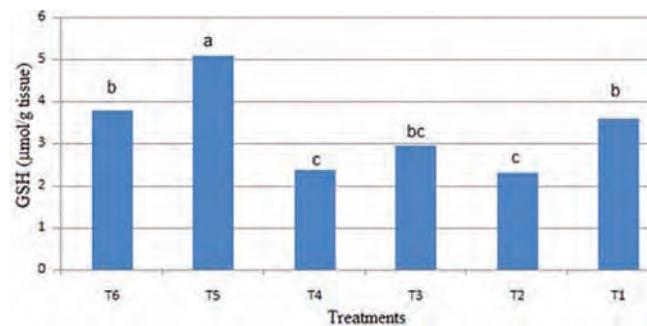
Results from Figure 1 also indicate that there were significant differences (p < 0.05) between treatment groups in MDA level in liver tissues (T2, T1, T5 and T6). However, there was no significant difference between (T2, T3 and T4) and (T3, T4, T5, T6 and T1) with respect to this trait.

Feeding Japanese quail with different levels of fenugreek seed (T3, T4, T5 and T6) resulted in a significant (p < 0.05) increase in GSH levels in liver tissue (Figure 2). (T5) recorded the highest means for this trait in comparison with the oxidative stress control group (T2) and with another treatments. Furthermore, there were no significant differences between groups (T1, T3 and T6) in relation to GSH treat.



**Figure 1.** Effect fenugreek seeds in Malondialdehyde (MDA) (nmol/gram tissue) level in liver tissue of Japanese quail (*Coturnix-coturnix japonica*) exposed to oxidative stress induced by H<sub>2</sub>O<sub>2</sub>.

\*different letters between columns indicate a significant difference between treatments in the abstract level (p ≤ 0,05). T(1) control group. T(2) The birds were given the same ratio as treatment (1) +H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(3) The birds were given a standard ratio +5 g ground fenugreek/kg feed +H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(4) The birds were given a standard ratio +10 g grounded fenugreek/kg diet +H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(5) The birds were given a standard ratio +5 g grounded fenugreek/kg diet + normal water. T(6) The birds were given a standard ratio +10 g grounded fenugreek/kg diet + normal water.



**Figure 2.** Effect fenugreek seeds in glutathione (GSH) (nmol/gram tissue) level in liver tissue of Japanese quail (*Coturnix-coturnix japonica*) exposed to oxidative stress induced by H<sub>2</sub>O<sub>2</sub>.

\*different letters between columns indicate a significant difference between treatments in the abstract level (p ≤ 0,05). T(1) control group. T(2) The birds were given the same ratio as treatment (1) +H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(3) The birds were given a standard ratio +5 g ground fenugreek/kg feed +H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(4) The birds were given a standard ratio +10 g grounded fenugreek/kg diet +H<sub>2</sub>O<sub>2</sub> (0.5%) in drinking water. T(5) The birds were given a standard ratio +5 g grounded fenugreek/kg diet + normal water. T(6) The birds were given a standard ratio +10 g grounded fenugreek/kg diet + normal water.

## DISCUSSION

In general, supplementing of fenugreek seeds to the diets of Japanese quail resulted in significant improvement of most the hematological traits studied. This improvement may be due to the fenugreek seeds contents of the active substances and the antioxidants which form a complex compounds with fatty acids in the cell membranes.<sup>[14,15]</sup> Noted that fenugreek seeds improve general health and immunity by increasing the blood globulin. The blood serum activities of AST and ALT enzymes is believed to be due to the fact that fenugreek seeds treatment improve the state of antioxidants and reduce oxidative stress.<sup>[16]</sup> A positive correlation was observed between of AST and ALT enzymes activities and the oxidative stress output. Besides, fenugreek seeds treatments were found to decrease the glucose and cholesterol concentrations. T5 and T6 were found to alter the oxidative stressful state of the bird compared with T3 and T4. This decline may be due to pectin fiber contents of the fenugreek seeds which result in decreasing the emptying process of the stomach which leading to a decrease of the absorption of the glucose from the intestines. It was reported<sup>[18]</sup> that the Dioxygenase enzymes contents of the fenugreek seeds contribute in forming 4-Hydroxyisoleucine that stimulate the secretion of insulin hormone, the antihypertensive effect and the concentration of the glucose in the blood.

The decrease of the RBC with H<sub>2</sub>O<sub>2</sub> treatment may be due to the ability of H<sub>2</sub>O<sub>2</sub> to induce the oxidative stress. Which increased with the oral administration that lead to rising oxygen pressure inside the stomach and tissues.<sup>[19]</sup> The increasing of the reactive oxygen could have an impact on the red blood cells formation process in the bone marrow thereby decreasing the total number of the red blood cells produced.<sup>[20]</sup> The low oxygen concentration could be considered a stimulating factor on red blood cells formation. Decreasing Hb concentration may be attributed to the positive correlation between the RBC and the Hb concentration.<sup>[15]</sup> This correlation supports the validity of the assumption that “free radicals owned the ability to destroy Hb and the sediments composition within the RBC (Heinz bodies) that have a role in dissolution of the RBC. The positive influence of MDA and GSH levels (Figures 1 and 2) that was obtained with the inclusion of Fenugreek seeds to the diet of Japanese quail may be attributed to its very high content of the antioxidant components.<sup>[22]</sup>

The improvement of the anti antioxidants activities could be achieved from the crushed fenugreek seeds in GSH

metabolism in liver tissues through stimulating building it from the oxidized GSH by supported insulin hormone.<sup>[23]</sup> Fenugreek seeds had the ability to stimulate the pancreatic beta cells to increase the secretion of insulin and stabilized the oxidation process in the cells, that's leads to increasing GSH level.<sup>[24]</sup> noted a reverse relationship between GSH level and oxidative stress, fenugreek seeds which may act to increasing the desire to eat food that's may be useful in preventing the reduction of GSH level but in contrast with the starvation state that lowers the GSH level.<sup>[25,26]</sup>

The significant increase and decrease of the MDA and GSH levels (Figures 1 and 2) with the H<sub>2</sub>O<sub>2</sub> treatment may be due the ability of H<sub>2</sub>O<sub>2</sub> to induce the oxidative stress state of Japanese quail. Because every 1% volume of H<sub>2</sub>O<sub>2</sub> equivalent to 3.3 volume of oxygen, so one ml with 0.5% of H<sub>2</sub>O<sub>2</sub> given orally may be leading to the generation of 6.1 ml of the molecular oxygen in the tissues reactions.<sup>[27]</sup> Supplementing H<sub>2</sub>O<sub>2</sub> orally leads to start the reaction chain leading to the oxidative stress through increasing the oxygen production in the stomach, which pass to the blood which increase tissues.

## CONCLUSIONS

In conclusion, it was found that supplementing the diet of Japanese quail exposed to oxidative stress with different levels of fenugreek seeds (5 or 10 g/d with or without H<sub>2</sub>O<sub>2</sub>) resulted in a significant improvement in most blood physiological and biochemical traits included in this study and enhanced antioxidant status. Therefore, fenugreek seeds may be useful as an efficient feed additive for enhancement general antioxidant status of birds.

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