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# Effect of Dietary Humic Acid on the Oxidative Status in Liver, Meat and Eggs of Quails

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**Abstract:** This study was carried out to examine the influence of dietary humic acid on the oxidative status in liver, meat and eggs of quail. A total of 260 Japanese quails, one week of age, were randomly assigned to one control and three experimental groups. The quails were fed with a basal diet (H0), the basal diet supplemented with 360 mg $kg^{-1}$ (H1), 480 mg $kg^{-1}$ (H2) or 600 mg $kg^{-1}$ (H3) humic acid. After 9 weeks of feeding, the all quails were slaughtered. Liver, meat and eggs were analyzed for antioxidant and oxidant parameters. Liver samples of H1, H2 and H3 groups showed a higher degree of oxidant parameters (indicated by a lower antioxidative capacity) than those of H0 samples. However, the antioxidant and oxidant parameters in the meat and eggs samples were not affected by adding humic acid. Our findings indicated safety of humic acid for meat and eggs of quail and support the use of humic acid as a food additive.

**Key words:** Quail, humic acid, oxidant, antioxidant, parameters, additive

## INTRODUCTION

In recent years, it has been observed that humates included in feed and water of poultry promote growth. Humic acids stabilize the intestinal flora and thus ensure an improved utilization of nutrients in animal feed. This leads to an increase in live weight of the animal without increasing the amount of feed given to the animal (Bailey *et al*., 1996; Shermer *et al*., 1998). However, humic acid is also believed to be a semiquinonetype radical (Rex, 1960). In addition, humic acid has been shown to be a toxic factor for many mammalian cells, including chondrocytes and erythrocytes (Liang *et al*., 1998, 1999; Cheng *et al*., 1999). However, to our knowledge, effect of humic acid on the oxidant and antioxidant potential of liver, meat and eggs of quails have not been studied. The objective of the present study was to investigate the effect of supplementation of humic acid at different levels on antioxidant-oxidant status in the liver, meat and eggs of quails.

## MATERIALS AND METHODS

**Quails, diets and slaughter:** In this research, a total of 260 Japanese quails (*Coturnix coturnix japonica*) at one weeks of age were used. The birds were randomly assigned to one control and three experimental groups based on their initial body weight, comprising five replicates of 13 birds each. They were fed a basal diet (H0), the basal diet supplemented with either 360 (H1), 480 (H2) or 600 (H3) mg $kg^{-1}$ of humic acid. Ingredients and chemical compositions of the basal diets are shown in Table 1. Chemical compositions of the diets were analyzed using the international procedures of AOAC (1994). The study was lasted 9 weeks. At the end of the feeding period, 15 birds were selected at random from each treatment group. Prior to slaughtering, the birds were held without feed for 10 h and then they were slaughtered by a neck cut. The birds were eviscerated manually, washed and allowed to drain 10 min. After eviscerating, carcasses were frozen at -20°C until further use. After eggs were collected daily, whole eggs were wrapped in Parafilm® and stored at 4°C for later analysis.

**Measurement of antioxidative status:** Total Antioxidant Capacity (TAC) levels were determined using a novel automated colorimetric measurement method developed by Erel (2004). Catalase (CAT) activity was assayed using a method described by Goth (1991).

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Table 1: Ingredients and chemical analyses of diets fed to quails (g $kg^{-1}$)

| Ingredients | H0 | H1 | H2 | H3 | | H0 | H1 | H2 | H3 |
|---|---|---|---|---|---|---|---|---|---|
| Maize | 554.50 | 553.55 | 552.00 | 550.95 | Chemical analysis | | | | |
| Soybean meal | 364.00 | 363.00 | 363.50 | 364.00 | Crude protein | 241 | 240 | 240 | 241 |
| Fish meal | 51.00 | 51.00 | 51.00 | 51.00 | Calcium | 8.1 | 8.0 | 8.1 | 8.1 |
| Vegetable oil | 11.00 | 11.00 | 11.00 | 11.00 | Total phosphorus | 7.2 | 7.1 | 7.2 | 7.1 |
| DCP | 3.50 | 3.50 | 3.50 | 3.50 | Calculated values | | | | |
| Ca | 11.00 | 10.70 | 11.00 | 10.80 | ME (MJ $kg^{-1}$) | 12.129 | 12.121 | 12.129 | 12.155 |
| Salt | 2.50 | 2.50 | 2.50 | 2.50 | Lysine | 13.8 | 13.8 | 13.8 | 13.8 |
| Vit and Min[a] | 2.50 | 2.50 | 2.50 | 2.50 | Met+cys | 8.0 | 8.0 | 8.0 | 8.0 |
| Humic acid (16%) | -- | 2.25 | 3.00 | 3.75 | | | | | |
| Total | 1000 | 1000 | 1000 | 1000 | | | | | |

[a]Vitamin and mineral premix provided the following per kg diet: Vitamin A, 12500 IU; Vitamin D3, 1500 IU; Vitamin E, 31.25 mg; Vitamin K3, 3.75 mg; Vitamin B1, 2.5 mg; Vitamin B2, 7.5 mg; Niacin 25 mg; Cal. D-pantothenate 10 mg; Vitamin B6, 5mg; Vitamin B12, 0.019 mg; Folic acid 1 mg; Choline chloride 250 mg; Mn 100 mg; Fe 75 mg; Zn 75 mg; Cu 6.25 mg; Co 0.25 mg; I, 1.25 mg; Se 0.19 mg

Table 2: Effect of humic acid on the oxidative status in the liver and meat*

| | Liver | | | | Meat | | | |
|---|---|---|---|---|---|---|---|---|
| | TAC | LPO | TOS | CAT | TAC | LPO | TOS | CAT |
| H0 | 1.20±0.04[a] | 1.49±0.44[bc] | 1.54±0.30[c] | 106.42±4.12 | 0.71±0.03 | 0.55±0.18 | 0.79±0.14 | 11.38±03.36 |
| H1 | 1.03±0.12[ab] | 1.40±0.29[c] | 2.30±0.14[b] | 98.72±4.23 | 0.65±0.10 | 0.56±0.15 | 0.88±0.32 | 10.57±01.49 |
| H2 | 1.02±0.21[ab] | 2.01±0.52[b] | 2.82±0.71[b] | 94.12±5.90 | 0.67±0.08 | 0.60±0.20 | 0.85±0.04 | 08.31±01.89 |
| H3 | 0.96±0.13[b] | 2.56±0.30[a] | 3.61±0.28[a] | 95.60±8.00 | 0.59±0.05 | 0.79±0.18 | 0.91±0.10 | 08.21±03.57 |
| P | * | ** | ** | * | ns | ns | ns | ns |

* TAC: mmol Trolox Eqv./L; LPO: µmol $H_2O_2$ Eqiv/L; TOS: µmol $H_2O_2$ Eqiv/L; CAT: U/L

Table 3: Effect of humic acid on the oxidative status in the eggs*

| | Egg white | | | Egg yolk | | |
|---|---|---|---|---|---|---|
| | TAC | LPO | TOS | TAC | LPO | TOS |
| H0 | 0.06±0.04 | 0.16±0.04 | 0.32±0.07 | 0.35±0.07 | 1.51±0.17 | 2.50±0.21 |
| H1 | 0.10±0.02 | 0.22±0.04 | 0.28±0.11 | 0.34±0.09 | 1.41±0.21 | 2.86±0.29 |
| H2 | 0.11±0.03 | 0.20±0.07 | 0.33±0.10 | 0.39±0.09 | 1.60±0.17 | 2.66±0.15 |
| H3 | 0.13±0.02 | 0.25±0.04 | 0.40±0.10 | 0.40±0.05 | 1.43±0.28 | 2.87±0.42 |
| P | ns | ns | ns | ns | ns | ns |

* TAC: mmol Trolox Eqv./L; LPO: µmol $H_2O_2$ Eqiv/L; TOS: µmol $H_2O_2$ Eqiv/L

**Measurement of oxidative status:** Total Oxidant Status (TOS) were determined by using a new automated colorimetric method developed by Erel (2005). Levels of Lipid Peroxidation (LPO) were determined using an automated method described by Khelifa and Steghens (Khelifa and Steghens, 2004).

## RESULT AND DISCUSSION

The oxidant and antioxidant status in liver and meat samples are shown in Table 2. Humic acid supplementation significantly affected the levels of oxidant ($p<0.01$) and antioxidant ($p<0.05$) parameters in livers. The levels of TOS and LPO in livers were highest in the H3 samples, followed by the H2, H1 and H0 samples. The TAC and CAT values of H1, H2 and H3 samples were significantly higher than H0 samples liver tissue. Oxidant levels and antioxidant enzyme activities have been evaluated in several studies. Although conflicting results have been reported (Ramachandran and Iyer, 1984; Jansson *et al*., 1985), it is generally accepted that oxidative status is increased through increase in oxidant levels and/or decrease in antioxidant enzyme capacities. In our study we indicated that antioxidant levels decreased when oxidant levels increased in liver. On the other hand, the levels of TOS, LPO, TAC and CAT in meat and eggs from H1, H2 and H3 groups were not different compared with those from H0 group (Table 2 and 3).

There is limited information about the effect of dietary humic acid on oxidant and antioxidant status in meat and eggs for discussion of the results. In only one study, Aksu *et al.* (2005) reported there is the effect of dietary humate on the lipid oxidation in broilers meat. In our study, meat and eggs of quails were not affected by humic acid supplementation. Ho *et al.* (2003) reported that the increase in lipid peroxidation has an almost linear relationship with the increase in humic acid concentration from 0 to 50 µg $mL^{-1}$ in liver. Humic acid concentrations in the tissues were not determined in this study. However, it might be suggested that humic acid-fed quails were unable to absorb and transfer more humic acid in their meat and eggs than in their liver.

## CONCLUSION

The results of our study indicate safety of humic acid for meat and eggs and support the use of humic acid as a food additive. However, further studies are required to determine the humic acid concentration in tissue from humic acid-fed quails for oxidant status.

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