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# Effects of Fennel Extract on Egg Production, Antioxidant Status and Bone Attributes of Laying Hens Administered Carbon Tetrachloride

Hadavi A, Kermanshahi H, Nassiri Moghaddam H & Golian A

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

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**Corresponding author**
Hassan Kermanshahi
kermansh@um.ac.ir

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**Abstract**

This study was conducted to investigate the effects of fennel ethanol extract on egg production, serum parameters, and bone attributes of laying hens administered carbon tetrachloride (CCl4). A total of 192 Hy-Line W-36 laying hens were assigned to four treatments of four replications in a completely randomized design for 9 weeks (36-44 weeks of age). Experimental groups consisted of 1) positive control (PC) diet (basal diet only), 2), negative control (NC) diet (basal diet + 30 mL CCl4/kg diet), 3) NC diet plus 50 mg fennel extract/kg diet (50F), 4) NC diet plus 100 mg fennel extract/kg diet (100F). Egg production and feed intake were recorded weekly. At the end of the experiment, blood samples were collected and bone mineralization and strength were measured. The higher dose of fennel extract (100 mg) increased ($P < 0.05$) egg production and feed intake, and decreased ($P < 0.05$) cracked egg percentage compared to positive control group. Serum concentrations of Ca and P were also higher in this group ($P < 0.05$) while the lipid levels and activities of hepatic enzymes in the serum were lower ($P < 0.05$). This group also had improved ($P < 0.05$) Ca and P retention in tibia bone, and also greater tibia shear force and stiffness. However, these improvements did not restore the state to the same level as that observed in chickens in the positive control. In conclusion, supplementing 100 mg of fennel extract to diets of layers administered with CCl4 partially ameliorates the detrimental effects of CCl4, and also could improve egg production, serum parameters, and bone attributes.

## Introduction

Farm animals encounter various stressors in modern farming including environmental, nutritional and management stressors, all of which could decrease resistance to diseases and tolerance of other challenges. Higher metabolic rate in chickens can consider as a stressor, which tend to increase the production of free radicals and reactive oxygen species (ROS) (Brunet-Rossinni, 2004). Innate defense systems usually balance oxidant production with removal by the antioxidant systems (Sies, 1991) to prevent oxidative stress (Botsoglou *et al*., 2009). Oxidative stress can include lipid peroxidation which has been associated with negative effects on growth performance (Christaki, 2012). Supplementing diets with exogenous antioxidants may assist in cellular protection against oxidants (Christaki, 2012).

There are many investigations that have demonstrated the positive effects of phytogenics on broilers and layers, including improvements to performance, gut morphology, and immune functions (Williams and Losa, 2001; Sonkusale *et al*., 2011). Fennel (*Foeniculum vulgaris*) is one of

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the most common herbs in the world that is used as food, feed additive, and herbal remedy. The major component of fennel extract is anethole, a phytosterol with antioxidant effects (Stashenko *et al*., 2002).

Carbon tetrachloride ($CCl_4$) is used as a liver toxin and works by increasing the formation of reactive free radicals which can bind to cellular macromolecules and form adducts of nucleic acid, protein and lipid, which may inhibit protein synthesis (Manibusan *et al*., 2007). Our previous study (Hadavi *et al*., 2015) showed that 30 mL $CCl_4$/kg of diet could negatively affect liver function and performance in laying hen. To the best of our knowledge, there has yet to be a study that investigated the effects of fennel extract on various parameters of laying hens under stressful conditions. Therefore, the purpose of this experiment was to investigate the effects of fennel extract as a popular antioxidant on egg production, serum profile and bone characteristics in Hy-Line W-36 laying hens administered $CCl_4$.

## Materials and Methods

### Laying hens, diets and management

A total of 192 36-wk Hy-Line (W-36) laying hens with similar body weights (1650 ± 34 g) and egg production (85 ± 2.8%) were randomly allocated to four treatments with four replicates. Each replicate contained three cages of four birds (48 birds/treatment). Hens were housed in 3-layer cages of similar size (41 × 46 cm). The feeder troughs were filled twice daily at 0600 and 1400 h and birds had free access to feed. Layers were fed to meet the nutrient requirements recommended by the Hy-Line W-36 recommendations (Hy-Line International, 2005). The formulated basal diet and its chemical analysis are shown in Table 1.

**Table 1.** Ingredients and chemical composition of the basal diet

| Ingredients | g/kg |
|---|---|
| Corn | 500 |
| Wheat | 181 |
| Soybean meal (440 g/kg protein) | 190 |
| Soya oil | 10 |
| $CaCo_3$ | 94 |
| Di-calcium phosphate | 15 |
| Salt | 3 |
| Vitamin premix[1] | 2.5 |
| Mineral premix[2] | 2.5 |
| DL- Methionine | 2 |
| *Calculated nutrients and energy* | |
| ME (Kcal/kg) | 2750 |
| Crude Protein (g/kg) | 153.0 |
| Methionine (g/kg) | 3.4 |
| Methionine + Cysteine (g/kg) | 6.0 |
| Lysine (g/kg) | 6.6 |
| Threonine (g/kg) | 5.0 |
| Available phosphorous (g/kg) | 4.0 |
| Calcium (g/kg) | 39.0 |
| Sodium (g/kg) | 1.8 |

[1] Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin $D_3$, 9790 IU; vitamin E, 121 IU; vitamin $K_2$, 2 mg; vitamin $B_{12}$, 0.02 mg; thiamin, 4 mg; riboflavin, 4.4 mg; niacin, 22 mg; pyridoxine, 4 mg; biotin, 0.03 mg; folic acid, 1 mg; Ca-pantotenate, 40 mg; choline chloride, 840 mg; ethoxyquin, 0.125 mg.

[2] Supplied per kilogram of diet: Zn, 65 mg; Mn, 75 mg; Cu, 6 mg; Se, 0.2 mg; I, 1 mg; Fe, 75 mg.

Nipples drinkers were installed in each cage to provide free access to drinking water. The hens were exposed to a 16L:8D photoperiod program and temperature was set at 21°C throughout the experiment. Body weights were measured at the beginning and end of the 9-week experimental period. Experimental diets were: 1) positive control (PC) diet (basal diet); 2) negative control (NC) diet (basal diet supplemented with 30 mL $CCl_4$/kg diet); 3) NC diet plus 50 mg fennel extract/kg diet (50F); 4) NC diet plus 100 mg fennel extract/kg diet (100F). The experimental protocols were reviewed and approved by the Animal Care

Committee of the Ferdowsi University of Mashhad, Mashhad, Iran.

**Sample collection and measurements**

Egg production and the number of cracked eggs were recorded daily and expressed as a weekly basis. The difference between feed offered and feed remaining during each week was used to measure feed intake. Blood samples were taken from the wing veins of two birds per cage (24 birds/treatment) at the end of the experiment (44 weeks of age) in 10 mL glass tubes and centrifuged at 2,000 × g for 15 min at 4°C to separate serum samples (Hadavi *et al*., 2015). The following were measured in serum: concentrations of Ca and P; lipids including cholesterol (CHL), triglyceride (TG), low density lipoprotein-cholesterol (LDL-c), and high density lipoprotein-cholesterol(HDL-c); and enzymes of hepatic origin including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). The lipid particles and enzymes were measured using an autoanalyzer (Selectra Evital scientific, Dieren, Netherlands).

At the end of the experiment, one laying hen/cage (12 birds/treatment) was killed by cervical dislocation to determine bone mineralization and strength. Left tibia of the hen was separated and cleaned from adherent tissues. The biomechanical strength of left tibia was determined using Instron Universal Testing Machine (Model H5KS, Tinius Olsen Company, PA, USA). The distance between two steel bars was 5 cm, and a 10 mm diameter cross head breaking probe (50 kg) approached the bone at 5 mm $min^{-1}$ until the bone was broken. Using the software (Q Mat), ultimate shear force, maximal deflection before fracture, shear fracture energy, and stiffness (tangent to the angle α) were calculated. Sheared tibia pieces were collected, dried over night at 105°C and burned at 600°C for 16 h for mineral (Ca and P) content determination based on AOAC (2005). The concentration of Ca was determined by atomic absorption spectrophotometry (Varian SpectrA 50B Atomic Absorption Spectrometer: Varian Ltd, CA, USA) according to AOAC procedures (method 927.02; AOAC, 2005), and total P was measured calorimetrically using the molybdovanadate method (method 965.17; AOAC, 2005).

**Statistical analysis**

All data were analyzed in a completely randomized design using the general linear models of SAS (2007). Differences among means of treatments were assessed using Tukey's multiple range test and were considered to be significant if $P < 0.05$.

**Results and Discussion**

**Egg production and feed intake**

The effects of fennel extract on egg production (EP), cracked egg, and feed intake (FI) of laying hens administered $CCl_4$ are presented in Tables 2, 3, and 4, respectively. Supplementation of fennel extract significantly improved ($P < 0.05$) performance attributes compared to NC but to levels below PC. Applegate *et al*. (2009) showed that toxic materials can destroy the structure of epithelial cells in the intestine, which may lead to a reduction in egg production of laying hens. These results are consistent with findings of Kazemi-Fard *et al*. (2013), who demonstrated that dietary fennel extract at 50 mg/kg or 100 mg/kg increased egg production in broiler breeders.

Fennel extract has phytoestrogenic compounds like anethole which supports egg production (Sachdev *et al*., 2011). Improvements in egg production may be due to the presence of unsaturated fatty acids (especially linolenic acid in fennel extract) which are essential for egg production (Sachdev *et al*., 2011). It is hypothesized that the aromatic characteristics of various herbal extracts and essential oils may increase the palatability of feedstuffs, resulting in higher feed intake in poultry (Williams and Losa, 2001). Gharaghani *et al*. (2015) demonstrated that fennel added to layers diet may scavenge oxidative adducts from reproductive organs, alleviating the negative effects of heat stress on eggshell calcification and subsequently decrease the number of broken eggs. We show that $CCl_4$ toxicity negatively affects egg production and feed intake but this was ameliorated by fennel extract.

**Table 2.** Effects of fennel extract on egg production in Hy-Line W-36 laying hens administered $CCl_4$ from 36 to 44 weeks of age

| Treatments[1] | Egg production (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | wk 37 | wk 38 | wk 39 | wk 40 | wk 41 | wk 42 | wk 43 | wk 44 | wk 37-44 |
| PC | 84.36$^{a}$ | 83.08$^{a}$ | 81.23$^{a}$ | 79.86$^{a}$ | 79.45$^{a}$ | 78.21$^{a}$ | 74.45$^{a}$ | 76.64$^{a}$ | 80.52$^{a}$ |
| NC | 68.25 $^{c}$ | 63.93$^{c}$ | 60.45$^{c}$ | 63.49$^{b}$ | 60.49$^{c}$ | 62.78$^{c}$ | 56.19$^{c}$ | 53.42$^{c}$ | 61.83$^{c}$ |
| NC+50F | 74.38$^{bc}$ | 71.66$^{bc}$ | 71.65$^{b}$ | 68.14$^{ab}$ | 68.78$^{bc}$ | 69.78$^{bc}$ | 59.78$^{c}$ | 66.43$^{b}$ | 68.54$^{b}$ |
| NC+100F | 77.43$^{b}$ | 71.58$^{b}$ | 72.75$^{b}$ | 72.62$^{ab}$ | 72.64$^{b}$ | 70.95$^{b}$ | 61.45$^{b}$ | 64.21$^{b}$ | 69.75$^{b}$ |
| *SEM*[2] | 1.460 | 1.821 | 1.366 | 2.330 | 1.793 | 1.684 | 2.380 | 1.579 | 0.773 |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[1]PC: positive control diet (basal diet only), NC: negative control diet (basal diet supplemented with 30 mL $CCl_4$/kg diet to induce chronic damage and oxidation in the liver), 50F: 50 mg fennel extract/kg diet, 100F: 100 mg fennel extract/kg of diet.
[2]*SEM*: standard error of means.
[a-c]Means within the same column with uncommon superscript differ significantly ($P < 0.05$)

**Table 3**. Effect of fennel extract on cracked egg of Hy-Line W-36 laying hens administered $CCl_4$from 36 to 44 weeks of age

| Treatments[1] | Cracked eggs (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | wk 37 | wk 38 | wk 39 | wk 40 | wk 41 | wk 42 | wk 43 | wk 44 | wk 37-44 |
| PC | 0.06$^{c}$ | 0.89$^{c}$ | 0.76$^{c}$ | 0.42$^{c}$ | 0.74$^{c}$ | 0.51$^{c}$ | 0.52$^{c}$ | 1.45$^{c}$ | 0.56$^{c}$ |
| NC | 10.45$^{a}$ | 12.75$^{a}$ | 10.93$^{a}$ | 11.89$^{a}$ | 10.84$^{a}$ | 11.62$^{a}$ | 12.34$^{a}$ | 15.01$^{a}$ | 9.87$^{a}$ |
| NC+50F | 6.86$^{ab}$ | 9.35$^{ab}$ | 6.41$^{ab}$ | 9.08$^{ab}$ | 5.64$^{ab}$ | 8.02$^{ab}$ | 9.15$^{ab}$ | 9.15$^{b}$ | 7.12$^{b}$ |
| NC+100F | 3.67$^{b}$ | 7.42$^{b}$ | 4.31$^{b}$ | 6.45$^{b}$ | 3.91$^{b}$ | 5.73$^{b}$ | 8.41$^{b}$ | 9.04$^{b}$ | 5.98$^{b}$ |
| *SEM*[2] | 1.024 | 1.095 | 1.069 | 0.997 | 1.264 | 1.078 | 1.580 | 0.897 | 0.452 |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[1]PC: positive control diet (basal diet only), NC: negative control diet (basal diet supplemented with 30 ml $CCl_4$/kg diet to induce chronic damage and oxidation in the liver), 50F: 50 mg fennel extract/kg diet, 100F: 100 mg fennel extract/kg of diet.
[2]*SEM*: standard error of means.
[a-c]Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

**Table 4**. Effect of fennel extract on feed intake of Hy-Line W-36 laying hens administered $CCl_4$ from 36 to 44 weeks of age

| Treatments[1] | Feed intake (g/hen/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | wk 37 | wk 38 | wk 39 | wk 40 | wk 41 | wk 42 | wk 43 | wk 44 | wk 37-44 |
| PC | 101.46$^{a}$ | 105.21$^{a}$ | 106.11$^{a}$ | 108.12$^{a}$ | 107.72$^{a}$ | 115.51$^{a}$ | 110.11$^{a}$ | 113.84$^{a}$ | 108.36$^{a}$ |
| NC | 77.86$^{c}$ | 75.23$^{c}$ | 75.31$^{c}$ | 81.47$^{b}$ | 78.81$^{c}$ | 82.42$^{c}$ | 79.42$^{b}$ | 76.97$^{b}$ | 79.02$^{c}$ |
| NC+50F | 85.12$^{b}$ | 87.42$^{bc}$ | 86.47$^{bc}$ | 90.41$^{b}$ | 90.45$^{bc}$ | 90.53$^{c}$ | 89.83$^{ab}$ | 92.68$^{ab}$ | 89.24$^{b}$ |
| NC+100F | 84.62$^{b}$ | 89.41$^{b}$ | 89.96$^{b}$ | 92.64$^{ab}$ | 93.37$^{b}$ | 96.40$^{b}$ | 92.47$^{ab}$ | 95.94$^{ab}$ | 90.43$^{b}$ |
| *SEM*[2] | 2.058 | 3.012 | 2.826 | 3.944 | 3.180 | 3.981 | 4.382 | 4.999 | 1.579 |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[1]PC: positive control diet (basal diet only), NC: negative control diet (basal diet supplemented with 30 ml $CCl_4$/kg diet to induce chronic damage and oxidation in the liver), 50F: 50 mg fennel extract/kg diet, 100F: 100 mg fennel extract/kg of diet.
[2]*SEM*: standard error of means.
[a-c] Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

**Serum parameters**

Birds that received 100F showed an improvement in serum parameters such as higher ($P < 0.05$) concentrations of Ca and P, lower ($P < 0.05$) levels of TG, CHL, and LDL-c; and higher ($P < 0.05$) concentration of ALT compared to NC group (Table 5). The mechanisms underlying the changes in Ca and P concentrations in serum after fennel treatment are poorly understood, but maybe related to the elevated levels of bone mineralization. Our results are in agreement with Tollba and Hassan (2003) who reported that adding fennel grounds to broiler diets under stressful conditions reduced plasma CHL. This hypercholesterolemia may be related to the mode of action of fennel in bird metabolism. Fennel competes with CHL at binding sites or interferes with the CHL biosynthesis in the liver

by increasing conversion of hepatic CHL to bile salts due to loss of complexes of these substances in the feces (Tollba and Hassan, 2003). Serum lipids were elevated in $CCl_4$-treated Japanese quails compared to those fed a control diet (Samadi *et al.*, 2015). A lipid metabolism disorder caused by hepatic injury and oxidant production in $CCl_4$-treated birds could be the main factor driving this increased level of serum lipids, while a high dose of fennel extract (i.e. 100F) could scavenge oxidants in injured liver, improve liver health, and reduce lipid leakage to the serum (Tollba and Hassan, 2003).

**Table 5.** Effect of fennel extract on serum minerals, lipids, and hepatic enzymes of Hy-Line W-36 laying hens administered $CCl_4$ at 44 weeks of age

| Treatments[1] | Serum minerals (mg/dL) | | Serum lipids (mg/dL) | | | | Serum hepatic enzymes (IU/L) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ca[2] | P | TG | CHL | HDL | LDL | ALT | AST | ALP |
| PC | 49.76[a] | 8.26[a] | 72.13[b] | 140.46[b] | 114.28 | 23.43[b] | 1.84[d] | 124.22[c] | 2035.4[c] |
| NC | 35.83[c] | 4.36[b] | 89.42[a] | 165.27[a] | 112.96 | 44.89[a] | 5.96[a] | 172.13[a] | 3471.5[a] |
| NC+50F | 44.75[b] | 6.72[ab] | 74.74[b] | 137.29[b] | 115.57 | 19.07[b] | 3.91[b] | 164.89[ab] | 3143.1[ab] |
| NC+100F | 46.94[ab] | 5.99[ab] | 68.89[b] | 138.48[b] | 113.47 | 21.84[b] | 3.32[c] | 151.93[b] | 2865.9[b] |
| *SEM*[3] | 1.462 | 0.465 | 3.080 | 2.843 | 2.537 | 1.865 | 0.259 | 3.042 | 84.116 |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[1]PC: positive control diet (basal diet only), NC: negative control diet (basal diet supplemented with 30 ml $CCl_4$/kg diet to induce chronic damage and oxidation in the liver), 50F: 50 mg fennel extract/kg diet, 100F: 100 mg fennel extract per kg of diet.
[2]Ca: calcium; P: phosphorous; TG: triglyceride; CHL: cholesterol; LDL: low density lipoprotein; HDL: high density lipoprotein; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase.
[3] *SEM*: standard error of means.
[a-d] Means within the same column with uncommon superscript differ significantly ($P < 0.05$).

In the present study, $CCl_4$ toxicity increased ($P < 0.05$) the serum concentration of hepatic enzymes in laying hens, reflecting liver damage. These findings are in agreement with those of Samadi *et al.* (2015) who reported that Japanese quail intraperitoneally treated with $CCl_4$ showed a significant increase in the serum concentrations of the hepatic enzymes AST, ALT and ALP. $CCl_4$ induces liver damage and disrupts hepatic metabolism, resulting in increased serum concentrations of hepatic enzymes (Mandrekar and Szabo, 2009). Ali *et al.* (2010) reported that $CCl_4$ induced hepatotoxicity in mice manifested biochemically by significant elevation of activities of liver enzymes such as ALT and AST.

**Table 6**. Effect of fennel extract on bone mineralization and strength characteristics of $CCl_4$ intoxicated Hy-Line W-36 laying hens at 44 weeks of age

| Treatments[1] | Tibia mineralization | | Tibia strength characteristics | | | |
|---|---|---|---|---|---|---|
| | Ca[2] (%) | P (%) | Shear force (N) | Fracture deflection (mm) | Fracture energy (N*mm) | Stiffness (N/mm) |
| PC | 38.27[a] | 23.18[a] | 140.83[a] | 0.524 | 30.12 | 240.23[a] |
| NC | 29.94[c] | 19.02[c] | 74.15[c] | 0.516 | 30.06 | 165.02[c] |
| NC+50F | 32.99[bc] | 20.14[bc] | 95.99[bc] | 0.503 | 30.62 | 195.00[bc] |
| NC+100F | 34.03[b] | 20.98[b] | 116.12[b] | 0.528 | 31.03 | 198.13[b] |
| *SEM*[3] | 0.804 | 0.573 | 5.275 | 0.032 | 1.512 | 7.535 |
| *P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

[1]PC: positive control diet (basal diet only), NC: negative control diet (basal diet supplemented with 30 ml $CCl_4$/kg diet to induce chronic damage and oxidation in the liver), 50F: 50 mg fennel extract/kg diet, 100F: 100 mg fennel extract per kg of diet.
[2]Ca: calcium; P: phosphorous.
[3] *SEM*: standard error of means.
[a-c] Means within the same column with uncommon superscript differ significantly ($P< 0.05$).

**Bone mineralization and strength characteristics**

The effects of experimental diets on bone mineralization and strength characteristics are presented in Table 6. Neither doses of fennel extract (50 mg/kg diet and 100 mg/kg diet) improved bone parameters compared to PC group. Nonetheless, birds that received NC

supplemented with 100F showed enhanced ($P < 0.05$) Ca retention, tibia shear force, and stiffness compared to NC birds.

In regard to effects of fennel extract on bone parameters, the present results are in agreement with those of Tahmasbi *et al*. (2012) who reported that administration of an alcoholic fennel extract improved bone calcification of laying hens in the late phase of production. Anethole (the major component of the fennel extract) has estrogenic effects (as a phytoestrol). Estrogen (as a sterol) is a powerful hormonal modulator of Ca metabolism and increases Ca uptake in the gut by activating 1-α-hydroxylase in the kidney (Tanaka *et al*., 1978). In birds, as in mammals, estrogen has a major influence on osteogenesis (Wilson and Thorp, 1998). Stiffness is also a vital factor of efficient locomotion of laying hens kept in cages. Kim *et al*. (2011) showed that stiffer bones could help improve leg movements and prevent fatigue in laying hen.

**Conclusion**

We conclude that adding $CCl_4$ negatively influenced laying hens' production, serum parameters, and bone parameters, but adding fennel extract at 100 mg/kg diet could improve these parameters in laying hens administered $CCl_4$. However, these levels were lower compared to those in the positive control.

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