Research Article

Effect of Fennel Seed Supplementation into Broiler Diet on Their Growth, Physiological, and Immunological Performance

Farid S. Nassar[1,2], Osama A. El-Sayed[3], Saidi Ouassaf[4], Ahmed O. Abbas[1,2*]

[1]Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia;[2]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza P.O. Box 12613, Egypt; [3]Poultry Breeding Department, Animal Production Research Institute, Dokki, Giza, Egypt; [4]Department of Finance, School of Business, King Faisal University, Al-Ahsa 31982, Saudi Arabia.

Abstract | The current research sought to determine the impact of dietary fennel seed (FS) inclusion on broiler chickens' productive performance, slaughter traits, biochemical components, cholesterol profile, stress indicators, and immune status. Four hundred male Cobb500 broiler chickens were grown on floor pens and fed a mash-based corn-soybean diet from one day to 21 days old. From 22-42 days of age, the birds were divided into four experimental groups (10 replicate pens × 10 chicks each) in which the birds were fed on a finisher-based diet supplemented with 0, 10, 20, and 30 g/kg FS powder (FS0, FS10, FS20, and FS30 groups, respectively). Data were analyzed using one-way ANOVA with a polynomial contrasts test to explore the linear and quadratic trends of the FS increasing levels in broiler diets. The overall amount of feed consumed was unaffected by the FS diets. Still, increasing dietary FS levels enhanced the broilers' final weights, gains, and feed-to-gain ratios ($p < 0.05$). As the dietary FS levels increased in broiler diets, weights for the carcass yield, breast, liver, and spleen increased substantially ($p < 0.05$). The plasma total protein and triiodothyronine hormone levels were remarkably ($p < 0.05$) enhanced by adding FS to broiler meals. At the same time, the concentrations of alanine transferase, aspartate transferase, and uric acid were significantly ($p < 0.05$) lowered. Additionally, the triglycerides were dramatically ($p < 0.05$) decreased while the high-density lipoprotein composed a higher proportion than the low-density-lipoprotein of the total cholesterols ($p < 0.05$). Additionally, as dietary FS levels increased, plasma corticosterone (CORT) and malondialdehyde (MDA) levels as stress markers in broilers reduced significantly ($p < 0.05$). On the other hand, adding FS to broiler diets considerably ($p < 0.05$) improved both the humoral and cellular immunity, as determined by the antibody (AB) titers and the phytohemagglutinin-wattle reaction (PHA-WR), respectively. It was observed that most of these measures showed a linear trend response to the rise in FS levels up to 30 g/kg of broiler diets. The FS30 group presented the lowest values of CORT and MDA by approximately 21% and 15%, respectively, and the maximum levels of AB titer and PHA-WR by 38% and 3-fold, respectively. These findings suggested that dietary supplementation with FS would be a useful nutritional strategy to enhance poultry production.

Keywords | Fennel seeds, Broiler production, Slaughter performance, Biochemical components, Cholesterol profile, Stress indicators, Immune status.

Received | September 29, 2023; **Accepted** | October 20, 2023; **Published** | January 15, 2024
***Correspondence** | Ahmed O Abbas, Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa 31982, Saudi Arabia; **Email:** aabbas@kfu.edu.sa
Citation | Nassar FS, El-Sayed OA, Ouassaf S, Abbas AO (2024). Effect of fennel seed supplementation into broiler diet on their growth, physiological, and immunological performance. Adv. Anim. Vet. Sci. 12(2): 239-248.
DOI | http://dx.doi.org/10.17582/journal.aavs/2024/12.2.239.248
ISSN (Online) | 2307-8316

Copyright: 2024 by the authors. Licensee ResearchersLinks Ltd, England, UK.
This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

INTRODUCTION

Natural products have received a lot of interest as nutritional tactics for enhancing the well-being and productivity of chickens in intensive production systems. According to previous studies (Shuaib et al., 2020; Alzarah et al., 2021; Khan et al., 2021; Al-Otaibi et al., 2022; Abbas et al., 2022), medicinal herbs could be introduced to both mammals and birds to promote the growth and health of these animals. These herbs may improve chickens' performance without resulting in any pathological abnormalities or changes to the blood profiles (Khan et al., 2019; Hafeez et al., 2021).

Fennel (*Foeniculum vulgare*) is a family of the most commonly medicinal aromatic flowering plants called Umbellifers or Apiaceae (Khan et al., 2019; Shojaiefar et al., 2022). According to Rather et al. (2016), it protects the liver, fights free radicals, prevents blood clots, reduces inflammation, and kills bacteria and fungi. Fennel seeds (FS) may reduce rat neuronal toxicity by maintaining antioxidative stress indicators and amyloid precursor protein isoforms (Koppula and Kumar, 2013; Bhatti et al., 2018). Additionally, the anti-inflammatory, analgesic, and antioxidative properties of the methanolic extract of FS protect nerve damage in a mouse model (Imran et al., 2019). Furthermore, fennel seed's phenolic components promote human health and growth, and their organic extracts exhibit antibacterial action against several diseases (Mehra et al., 2022).

According to previous literature, various levels of FS have been used to improve digestion, increase growth rate, and enhance egg features in chickens kept at regular temperatures (El-Deek et al., 2003; Al-Sagan et al., 2020; Khan et al., 2022). We know very little study on the impact of FS addition on the performance of chickens. Therefore, the current research aimed to ascertain the impact of dietary FS addition at different levels on the overall performance of broiler chickens, including their growth aspects, slaughter traits, biochemical components, cholesterol profile, stress indicators, and immunological status.

MATERIALS AND METHODS

Ethical Statement

This experiment followed the guidelines that King Faisal University's research ethical commission in Saudi Arabia suggested.

Fennel Seed (Fs) Analysis

Fresh FS were obtained from Majid Al Futtaim Carrefour & Society in the Saudi Arabia city of Riyadh (https://www.carrefourksa.com/mafsau/en/). FS were thoroughly cleaned, shade-dried for 2 wk at room temperature, and then reduced to powdery using a mill machine. The techniques provided by the Association of Official Analysis Chemists, AOAC, were used to conduct the proximate chemical analysis for the FS samples (AOAC, 2005). The antioxidant capacity (AOC), flavonoid contents (FC), and polyphenolic contents (PC) of the FS samples were assayed following the procedures outlined in prior research (Barakat et al., 2022). In conclusion, 10 mL of 70% methanol was used to extract 1 g of FS samples, and then the supernatant was taken out and adjusted to a final volume of 10 mL. A mixed solution of the FS methanolic extract (0.1 mL) and DPPH solution (2.9 mL) was measured spectrophotometrically at 517 nm and left in the dark for an hour to determine the AOC. A calibration curve was created by connecting the anti-radical capacity of DPPH to the antioxidant standard Trolox. Micromoles of Trolox-Equivalents (TE) per 100 g of FS were used to represent the AOC. An aliquot of the supernatant was mixed with Folin-Ciocalteu reagent (1:10) in equal volume and then incubated for 5 min to determine the PC. Na2CO3 (7.5%) was added to stop the reaction. The PC was determined as grams of Gallic acid equivalents (GAE) per 100 g of FS by comparing an optical density reading at 765 nm taken 60 minutes after incubation to a reference curve constructed from GA standard solution. A further equal volumes of the methanolic extract and 2% AlCl3 were mixed, and the results were observed for 60 minutes at 420 nm. For every 100 grams of FS, the FC was expressed as quercetin-equivalent (QE) grams. Table 1 provides an overview of all the data obtained from the FS analysis.

Experimental Design

An industrial corporation (Al Watania Poultry Co., Riyadh, Saudi Arabia) provided 400 one-day-old Cobb500™ male broiler chickens to support the current research. The chicks were raised on floor pens of 125 × 90 × 60 cm^3 measurements with 10 chicks each on a litter of wood shavings (5 cm deep). According to the Cobb-500 management recommendations ("Cobb500 Broiler Performance & Nutrition Supplement.," 2022), the chicks were raised in a conventional setting with a mashed corn-soya meal during the first 21 days of age. From day 22 to 42 of age, the chicks were divided according to the FS supplementation level into four treatment groups with ten replicate pens each. The birds in each group received a basal diet complemented with 0, 10, 20, or 30 g/kg FS powder (FS0, FS10, FS20, and FS30, respectively). All birds received unlimited access to food and water throughout the investigation. The diet was determined using the AOAC methodologies (AOAC, 2005), and the formulation and nutritional values have been presented in Table 2.

Table 1: The chemical analysis of fennel seeds (FS).

Item	Contents per 100 g FS
Proximate composition	
Moisture (g)	88.16
Crude protein (g)	2.14
Total lipids (g)	0.27
Carbohydrate (g)	8.02
Crude fiber (g)	2.82
Energy (MJ)	0.14
Minerals	
Calcium (mg)	46.53
Phosphorus (mg)	54.15
Sodium (mg)	49.21
Potassium (mg)	367.48
Iron (mg)	0.85
Zinc (mg)	0.24
Amino acids	
Isoleucine (mg)	0.66
Leucine (mg)	0.83
Tryptophan (mg)	0.61
Phenylalanine (mg)	0.47
Bioactive properties*	
Total polyphenols (g GAE)	7.204
Total flavonoids (g QE)	0.537
Antioxidant capacity (mmol TE)	0.922

* GAE, Gallic acid equivalent; QE, Quercetin equivalent; TE, Trolox equivalent.

Productive Traits

Broiler weights were determined at the initial (IBW) and final (FBW) days of the trial (22 and 42 days of age, respectively). The body weight gain was computed for each replication in the treatment group (BWG = FBW – IBW). The total feed intake for each replication in the treatment group was determined daily (TFI = feed introduced – remaining feed). Based on a calculation of the TFI per unit of BWG, the feed conversion ratio (FCR) was detected per replication.

Slaughter Traits And Internal Organs

Two birds per replication were individually weighed and killed at the end of the study (a total of 20 birds per treatment group). The birds' heads, necks, and feet were cut off after being heated in water at 54°C for 2 minutes. The hot carcass was promptly divided from the internal organs, intestines, and abdominal fat. The carcass yield was determined as a percentage of the live body weight. The weights of the breast, thigh, abdominal fat, intestines, and internal organs, including the spleen, liver, gizzard, and heart, were separately determined as percentages of the live body weight.

Table 2: Ingredients and nutritional values of the basal diet.

Ingredients	g/kg as fed
Soybean meal	300.0
Yellow corn	620.0
Vegetable oil	40.0
Dicalcium phosphate	15
Limestone	10
Salt (NaCl)	4.5
Premix*	5.5
Methionine	2.5
Lysine	1.5
Threonine	0.5
Tryptophan	0.5
Calculated nutrients	
Metabolizable energy (MJ/kg)	13.8
Crude protein (g/kg)	182.1
Calcium (g/kg)	8.3
None phytase phosphorus, NPP (g/kg)	4.7
Determined nutrients	
Crude protein (g/kg)	178.5
Crude fat (g/kg)	62.8
Crude fiber (g/kg)	32.8
Calcium (g/kg)	7.9
NPP (g/kg)	4.1

* Premix (contents per kg of the diet): 10 KIU vit A, 5 KIU vit D_3, 65 IU vit E, 3 mg vit K, 3 mg vit B_1, 9 mg vit B_2, 4 mg vit B_6, 0.02 mg vit B_{12}, 0.20 mg biotin, 20 mg niacin, 15 mg pantothenic acid, 2 mg folic acid, 500 mg choline chloride; 100 mg Mn, 100 mg Zn, 40 mg Fe, 15 mg Cu, 1 mg Iodine, and 0.35 mg Se.

Cholesterol Profile

Two birds per treatment group had blood drawn from the brachial vein at 42 days using heparinized tubes and syringes. The plasma was harvested by 10 min centrifugation at 2000 ×g at 4 °C. As previously described (Alaqil et al., 2020) and by the colorimetric methods of enzymatic diagnostic kits (ab65390, Abcam, Cambridge, MA, USA), the triglycerides (TG) and cholesterol (CH) profile, including total CH (TCH), high-density-lipoprotein CH (HDL-CH), and low-density-lipoprotein CH (LDL-CH), were quantified in the plasma samples. Briefly, 100 µL of the precipitation buffer (2X) and the plasma were pipetted together and then centrifuged twice for 10 minutes at 2000 ×g at room temperature. The supernatant was used to aspirate the HDL-CH fraction carefully, and 200 L of PBS was added to the residue to extract the LDL-CH fraction. Fifty µL of the sample or the standard solution was added to each microplate well before complementing with a

further fifty µL of the reaction reagent. The microplates were incubated for 60 minutes at 37 °C in the dark, and then the OD was measured at 570 nm on a reader device (ELx808TM, BioTek Instruments, Winooski, VT, USA). The CH profile was estimated using the formula A/V*D*100, where A is the CH standard value (g), V is the sample volume (µL), and D is either 1 or 2 depending on whether TCH or its fractions (HDL- and LDL-CH) are included.

Biochemical Components

At 42 d of age, plasma samples were obtained as mentioned before (2 samples per treatment group). The total protein (TP), uric acid (UA), alanine transferase (ALT), and aspartate transferase (AST) levels were measured using an automated scanning spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK) and available commercial colorimetric kits (Abcam, Waltham, MA, USA). The triiodothyronine (T_3) hormone was determined using chicken's ELISA kits (MBS269454, MyBioSource, San Diego, CA, USA) and processed by using a microplate reader (ELx808™, BioTek Instruments, Winooski, VT, USA).

Stress Indicators

corticosterone (CORT) and malondialdehyde (MDA) concentrations were determined in the collected plasma samples. ELISA kits (MBS701668, MyBioSource, San Diego, CA, USA) were used to measure the chicken CORT. The CORT accuracy tests were 0.5-20 ng/mL detection range, ≤ 8% intra-assay CV, and ≤ 10% inter-assay CV. The MDA was measured following the manufacturer's instructions for colorimetric assay kits (MBS9718963, MyBioSource Inc., San Diego, CA, USA). In brief, 300 µL of thiobarbituric acid (TBA) solution was added to 100 µL of plasma, which was incubated for 30 minutes at 95 °C then chilled for 10 minutes in an ice bath. After that, supernatants obtained by centrifugation for 10 minutes at 10000 ×g at 25 °C were divided into 200 µL aliquots and put into 96-well microplates for reading the absorbance at 532 nm.

Immune Status

The broiler humoral immune response was detected using the antibody (AB) titer against sheep red blood cells (SRBC) according to minor modified procedures of Bhatti et al. (2017). In brief, one milliliter of SRBC (5% in saline) was injected at 36 days of age into 2 birds per replication in each group. Blood sera were obtained on day 42 of age using 10 minutes of centrifugation at 400 ×g at 4°C. In a 96-well tray, ten serials of doubled dilutions were created using saline solution. Pipetting 2% SRBC into each well was followed by a 30-minute incubation period at 37 °C. The AB titer was then shown using the log2 of the reciprocal values of the most recent agglutinated dilution. To examine the cell-mediated immune response, two birds per replicate in each group at the end of the trial (42 days of age) were intradermally injected with a mitogenic phytohemagglutinin (PHA) (Thermo Fisher Scientific) in a designated location of the wattle. Once a swelling appeared in the predetermined region at least 24 hours following injection, the rise in wattle thickness was assessed to be a positive response to the immunological test (Al-Khalifa, 2016).

Statistical Analysis

The SPSS software program (version 22.0; IBM Corp., NY, USA, 2013) was used to analyze the data using one-way analysis of variance (ANOVA). A polynomial contrasts test was performed to investigate the linear and quadratic trends of the FS increasing levels in broiler diets on all features. The experimental unit for the data on productive performance was the pen (n = 10), while the experimental unit for the other parameters was the bird (n = 20). A p-value of less than 5% was used to perform statistical significance.

RESULTS

Productive Traits

Figure 1 displays the productive traits influenced by the FS addition in broiler diets. The TFI of the broilers was unaffected by the FS diets. A linear rise in the FBW and BWG and a linear reduction in the FCR were found by adding FS to the broiler diet ($p < 0.05$). The broiler performance traits increased to its highest level in the FS30 group.

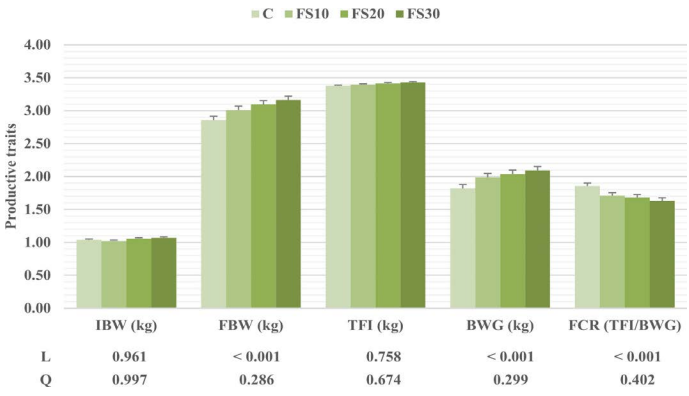

Figure 1: Effect of dietary supplementation with different levels of fennel seed (FS) on the productive traits of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables: IBW, initial body weight; FBW, final body weight; TFI, total feed intake; BWG, body weight gain; and FCR, feed conversion ratio. Bars express the means ± standard error of means (n = 10 replicate pens per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable.

Links Researchers

Slaughter Traits And Internal Organs

Figures 2 and 3 show the slaughter traits and internal organs influenced by the FS addition in broiler diets. As the dietary FS level increased, a linear increase ($p < 0.05$) in the carcass yield and intestinal weight was recorded. There was a linear and quadratic expansion ($p < 0.05$) in the breast yield when the dietary intake of FS increased, recording the greatest breast yield in the FS20 group. The thigh yield did not vary significantly between the FS groups. Broiler gizzard and heart indices did not vary across FS groups (p > 0.05). With increasing the FS levels in the broiler diet, a linear (p 0.05) rise in the liver index, as well as linear and quadratic rising trends (p < 0.05) in the abdominal fat and spleen indices, were observed.

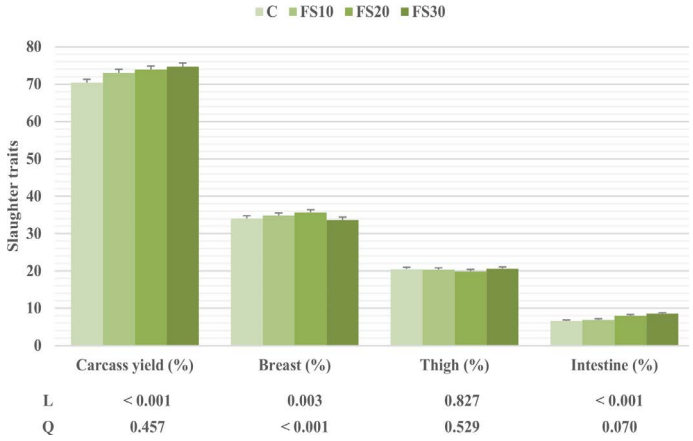

Figure 2: Effect of dietary supplementation with different levels of fennel seed (FS) on the slaughter traits of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables of slaughter traits are determined as a percentage of the live body weight. Bars express the means ± standard error of means ($n = 20$ birds per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable.

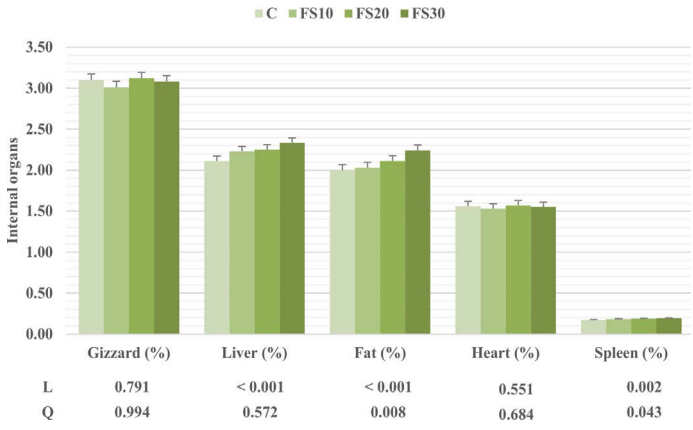

Figure 3: Effect of dietary supplementation with different levels of fennel seed (FS) on the internal organs of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables of internal organs are determined as a percentage of the live body weight. Bars express the means ± standard error of means ($n = 20$ birds per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable.

Plasma Cholesterols

Figure 4 displays the impact of FS addition in broiler diets on their plasma cholesterol profile. As the dietary FS level increased, TG and LDL-CH plasma concentrations were linearly ($p < 0.05$) reduced, and the TCH was linearly and quadratically declined. In contrast, the concentration of HDL-CH was linearly and quadratically ($p < 0.05$) increased. The lowest concentrations of TG, TCH, and LDL-CH and the highest HDL-CH were found in the FS30 group.

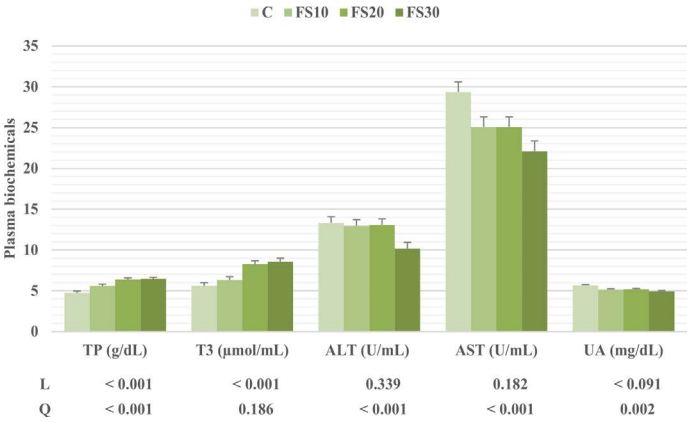

Figure 4: Effect of dietary supplementation with different levels of fennel seed (FS) on the plasma biochemicals of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables: TP, total protein; T3, triiodothyronine hormone; ALT, alanine aminotransferase enzyme; AST, aspartate aminotransferase enzyme; and UA, uric acid. Bars express the means ± standard error of means ($n = 20$ samples per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable.

Plasma Biochemicals

Figure 5 summarizes the plasma biochemical components of broilers as influenced by the FS treatment. Increasing the FS levels in broiler diets resulted in a linear and quadratic ($p < 0.05$) increase in the plasma TP. The plasma T_3 concentration increased linearly ($p < 0.05$) when increasing the FS level in the diets. The greatest T_3 concentration was found in the FS30 group. By raising the FS level in broiler diets, ALT, AST, and UA plasma concentrations were quadratically ($p < 0.05$) decreased, with the lowest values found in the FS30 group.

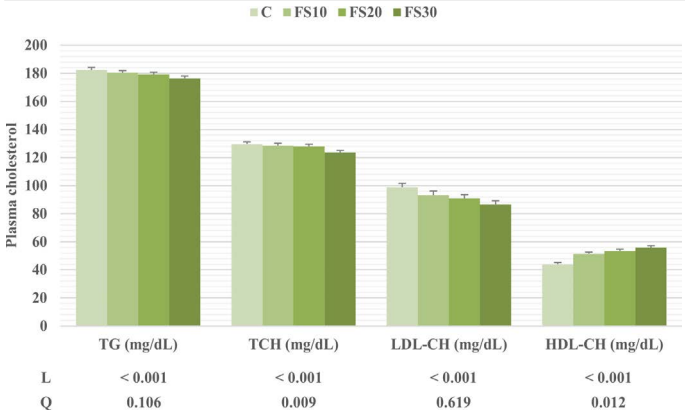

Figure 5: Effect of dietary supplementation with different levels of fennel seed (FS) on the plasma cholesterols of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables: TG, triglycerides; TCH, total cholesterol; LDL-CH, low-density lipoprotein cholesterol; and HDL-CH, high-density lipoprotein cholesterol. Bars express the means ± standard error of means (n = 20 samples per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable

Stress Indicators

Figure 6 illustrates broilers' stress indicators as influenced by the FS treatment. The plasma CORT and MDA concentrations were linearly ($p < 0.05$) decreased when the FS levels in the broiler diet increased. The FS30 group presented the lowest values of CORT and MDA by approximately 21% and 15%, respectively.

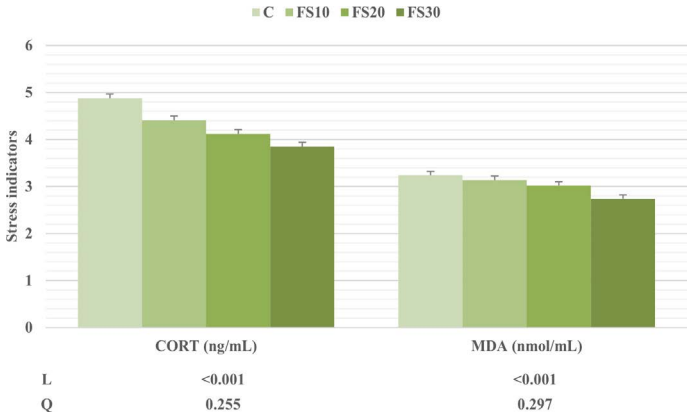

Figure 6: Effect of dietary supplementation with different levels of fennel seed (FS) on the stress indicators of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables: CORT, corticosterone hormone; and MDA, malonedialdehyde. Bars express the means ± standard error of means (n = 20 samples per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable.

Immune Status

Figure 7 presents the results of humoral and cellular immune status as influenced by FS supplementation in broiler diets. A linear AB titer increment and a linear and quadratic PHA-WR increment were observed with increasing the dietary levels of FS in the broiler diets ($p < 0.05$). The maximum AB titer and PHA-WR levels were obtained in the FS30 group, recording 38% and 3-fold values higher than in the control group.

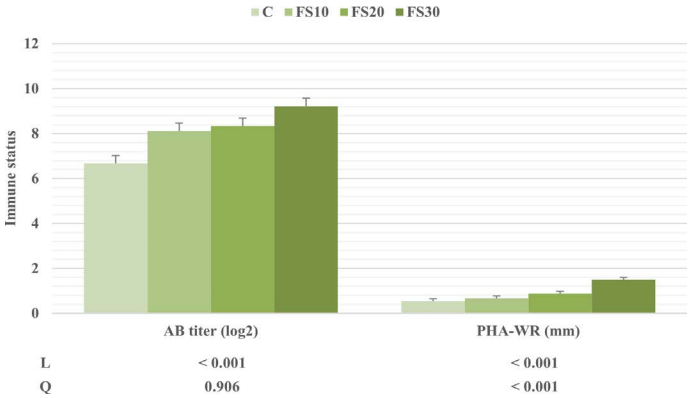

Figure 7: Effect of dietary supplementation with different levels of fennel seed (FS) on the immune status of broilers. Treatment groups: C, FS10, FS20, and FS30 indicate the supplementation of fennel seed at the rate of 0, 10, 20, and 30 g/kg of diet, respectively. Variables: AB titer, antibody titer against sheep red blood cells; and PHA-WR, phytohemagglutinin-wattle reaction. Bars express the means ± standard error of means (n = 20 samples per treatment group). *P*-value of the linear (L) and quadratic (Q) effects of FS levels are indicated for each variable.

DISCUSSION

The present study displayed that broiler production was enhanced as the FS level increased in the diets. Although FS supplementation did not affect the TFI of the broilers, the FBW, BWG, and FCR were linearly improved by FS supplementation. Per these findings, it was reported that adding FS to the diet had no impact on the feed consumption of male broilers (Safaei-Cherehh et al., 2020) or Japanese quails (Buğdayci et al., 2018). Other researchers found that FS supplementation increased the palatability and appetite of poultry for the diet, resulting in increased feed consumption (Ragab, 2007; Saki et al., 2014). The improvement in growth performance in the FS-treatment groups could be attributed to fennel's antibacterial and antifungal characteristics, which highly promote nutrient absorption (Elgayyar et al., 2001). It was also suggested that FS contains essential oils, volatile compounds, flavonoids, phenolics, and vitamins, which are required for broiler growth (El-Deek et al., 2003; Badgujar et al., 2014; Gharaghani et al., 2015).

Links Researchers

In addition, the linear improvement in FCR and BWG may contribute to the increase in the carcass yield and slaughter performance of broilers fed on increasing levels of FS in the current study. The present study also displayed a linear increase in the breast muscle, intestine, and liver weights of the broiler carcass as dietary FS increased, indicating the beneficial effects of FS on muscle formation, adipocyte production, protein digestion, and growth performance (Mohammadabadi et al., 2021). The increased spleen index in the FS groups could indicate the healthy immune system of the treated broilers (Smith and Hunt, 2003).

The current study shows a significant impact of FS on the cholesterol profile. In the broilers treated with increasing levels of FS, our data showed a significant drop in the plasma TG, TCH, and LDL-CH and an increase in the HDL-CH. Similar results were obtained in previous studies on broilers (Abul-Jabbar AL-Zuhairi et al., 2018; Safaei-Cherehh et al., 2020). In addition, dietary FS reduced blood cholesterol levels in hypercholesteremic mice (Oulmouden et al., 2014). These findings demonstrated that FS supplementation significantly impacts poultry's lipid metabolism (Abul-Jabbar AL-Zuhairi et al., 2018). The increase in unsaturated fatty acids in FS may be responsible for the decrease in TCH (Mahmud, 2014). It was shown that the hypolipidemic effect of fennel seeds may be due to the activity of 3-hydroxy-3-methyl-glutaryl coenzyme reductase, which mediates cholesterol degradation in the liver (Gharaghani et al., 2015). Moreover, it was suggested that the presence of polyphenols and flavonoids, as demonstrated in Table 1, may contribute to the hypolipidemic activity, lowering the LDL-CH and increasing the HDL-CH (Castro-Barquero et al., 2020; Sun et al., 2021). On the contrary, other investigators found that dietary FS oil did not affect blood cholesterol levels in other poultry species (Kaya et al., 2013; Belenli et al., 2015). These variations could be explained by the ration combinations, animal type, and dietary quantities of fennel seeds' active components.

The decreased ALT, AST, and UA in the FS-treated broilers could be explained by the ability of FS administration to maintain liver and renal functions (Ragab, 2007). In addition, several studies reported that FS administration has a hepatoprotective effect against oxidative stress and improves the levels of liver enzymes in rats (Barakat et al., 2022). Due to the hepatoprotective qualities of FS and the fact that a healthy liver controls the metabolism of proteins and amino acids, the higher plasma TP in FS-treated groups may also result from these properties (Charlton, 1996). Additionally, it was revealed that the hepatoprotective effects of medicinal fungi on rats increased plasma albumin, the main plasma protein produced by the liver and then transported throughout the body by blood circulation (Chiu and Hua, 2016). Moreover, plasma T_3 elevation by FS treatment in the current investigation can directly raise the broiler metabolism (Al-Ardi, 2020) and improve performance (Elnagar et al., 2010).

Due to their high metabolic rate, continuous selection for high growth rate, and intensive production system, commercial broilers are already very susceptible to unfavorable or stressful conditions (Loyau et al., 2013). In the present study, the main product of the hypothalamic-pituitary-adrenal (HPA) axis response to stress, CORT (Charmandari et al., 2005), and the main measurement for oxidative stress status, MDA (Pham-Huy et al., 2008), were assessed in the control and FS-treated birds as stress indicators. We found that MDA and CORT levels were linearly decreased as dietary FS levels increased, and this could be linked to the FS's polyphenols and flavonoids in the present study. These components, in turn, have a direct capacity to eliminate free radicals (Barakat et al., 2022). Previous studies have shown that the administration of phenolic compounds from fennel seed significantly lowered MDA levels and returned antioxidant enzyme levels to normal levels in broilers (Ghiasvand et al., 2021) and in rats (Samadi-Noshahr et al., 2021), especially in those animals suffered from stress. Moreover, lowering blood cholesterol levels in the same groups may be responsible for lowering the CORT levels in the FS-treated broilers (Pavlík et al., 2016).

The results evidenced a positive effect of FS supplementation in broiler diets on their humoral and cellular-mediated immune response, as indicated by the increase in the AB titer and PHA-WR. These results were supported by previous studies (Kazemi-Fard et al., 2013; Safaei-Cherehh et al., 2020; Fatima et al., 2022). The increased spleen weight of FS-treated broilers may explain the increase of broiler immunity in the same groups (Smith and Hunt, 2003). Additionally, it is believed that FS's bioactive components and antioxidant properties contribute to improving the immune response in birds by protecting cells from oxidative damage and enhancing cell function and proliferation (Ma et al., 2005). It is thought that thyroid hormones play a significant role in generating antibodies by supplying the energy needed to transform bone marrow cells into plasma cells (Shawky et al., 2020). This finding is consistent with our observation that a significant increase in the T_3 hormone of broilers in the same group accompanied the enhanced humoral immunity in the broilers fed on FS.

CONCLUSIONS

The study concluded that adding FS to broiler finisher diets positively impacted their productive performance and carcass yield. Some plasma measurements related to

growth, liver and kidney functions, and cholesterol profile were also enhanced by FS treatment. Furthermore, broilers treated with FS had lower stress markers and higher levels of humoral and cellular immunity. Most of these parameters expressed a linear trend in response to increased FS levels up to 30 g/kg of broiler diets. These results suggested that dietary supplementation with FS might be a useful nutritional strategy for poultry production.

ACKNOWLEDGMENTS

The facilities and resources used to conduct this work were provided by the Deanship of Scientific Research and Vice Presidency for Graduate Studies and Scientific Research at King Faisal University in Saudi Arabia, for which the authors are grateful.

NOVELTY STATEMENT

The current work examines the effect of fennel seed supplementation in broiler diets on their growth performance, carcass yield, biochemical components, cholesterol profile, stress indicators, and immune responses. The results of this study evidence positive effects of fennel seed on broiler performance and concluded that fennel seed supplementation could be employed as a promising nutritional strategy in poultry production.

FUNDING

The current study was funded by the Deanship of Scientific Research and Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Project no: GRANT5278).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

REFERENCES

Abbas A. O., A. A. Alaqil, G. M. K. Mehaisen, N. N. Kamel. (2022). Effect of Dietary Blue-Green Microalgae Inclusion as a Replacement to Soybean Meal on Laying Hens' Performance, Egg Quality, Plasma Metabolites, and Hematology. Animals. 12:2816 Available at https://www.mdpi.com/2076-2615/12/20/2816/htm (verified 30 October 2022).

Abul-Jabbar AL-Zuhairi Z., W. Sameer Abdullah, R. Kahat Majal. (2018). Effect the dietary supplementation of Cariander (*Coriandrum sativum L.*) and Fennel (*Foeniculum vulgares*) seed powder and their mixture in productional and physiological performance of broiler. Al-Qadisiyah J. Vet. Med. Sci. 17:27–28 Available at www.qu.edu.iq/journalvm.

Alaqil A. A., A. O. Abbas, H. S. El-Beltagi, H. K. Abd El-Atty, G. M. K. Mehaisen, E. S. Moustafa. (2020). Dietary supplementation of probiotic lactobacillus acidophilus modulates cholesterol levels, immune response, and productive performance of laying hens. Animals. 10:1–12 Available at https://pubmed.ncbi.nlm.nih.gov/32899938/ (verified 5 April 2021).

Al-Ardi M. H. (2020). The uses of gold nanoparticles and *Citrullus colocynthis L.* nanoparticles against *Giardia lamblia in vivo*. Clin. Epidemiol. Glob. Health. 8:1282–1286. Available at https://doi.org/10.1016/j.cegh.2020.04.028 (verified 20 December 2023).

Al-Khalifa H. (2016). Immunological techniques in avian studies. Worlds Poult Sci J 72:573–584 Available at https://www.tandfonline.com/doi/full/10.1017/S0043933916000532 (verified 24 August 2020).

Al-Otaibi M. I. M., H. A. M. Abdellatif, A. K. A. Al-Huwail, A. O. Abbas, G. M. K. Mehaisen, E. S. Moustafa. (2022). Hypocholesterolemic, Antioxidative, and Anti-Inflammatory Effects of Dietary *Spirulina platensisis* Supplementation on Laying Hens Exposed to Cyclic Heat Stress. Animals. 12:2759 Available at http://creativecommons.org/licenses/by/4.0/.

Al-Sagan A. A., S. Khalil, E. O. S. Hussein, Y. A. Attia. (2020). Effects of Fennel Seed Powder Supplementation on Growth Performance, Carcass Characteristics, Meat Quality, and Economic Efficiency of Broilers under Thermoneutral and Chronic Heat Stress Conditions. Animals. 2020., 10: 206 10:206 Available at https://www.mdpi.com/2076-2615/10/2/206/htm (verified 6 June 2023).

Alzarah M. I., A. A. Alaqil, A. O. Abbas, F. S. Nassar, G. M. K. Mehaisen, G. F. Gouda, H. K. Abd El-Atty, E. S. Moustafa. (2021a). Inclusion of *Citrullus colocynthis* seed extract into diets induced a hypolipidemic effect and improved layer performance. Agriculture. (Switzerland) 11:1–13. Available at https://doi.org/10.3390/agriculture11090808 (verified 20 December 2023).

Alzarah M. I., F. Althobiati, A. O. Abbas, G. M. K. Mehaisen, N. N. Kamel. (2021b). *Citrullus colocynthis* seeds: A potential natural immune modulator source for broiler reared under chronic heat stress. Animals. 11:1–13. Available at https://doi.org/10.3390/ani11071951 (verified 20 December 2023).

AOAC. (2005). Association of Official Analysis Chemists International. Official Methods of Analysis of AOAC International. 18th ed. Washington, D.C.

Badgujar S. B., V. V. Patel, A. H. Bandivdekar. (2014). *Foeniculum vulgare* Mill: A review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. Biomed Res Int 2014.

Barakat H., I. A. Alkabeer, T. Aljutaily, M. S. Almujaydil, R. M. Algheshairy, R. M. Alhomaid, A. S. Almutairi, A. Mohamed. (2022). Phenolics and Volatile Compounds of Fennel (*Foeniculum vulgare*) Seeds and Their Sprouts Prevent Oxidative DNA Damage and Ameliorates CCl4-Induced Hepatotoxicity and Oxidative Stress in Rats. Antioxidants. 11.

Belenli, D., D. Udum, S. Cengiz, U. Polat. (2015). Influence of Various Volatile Oils as a Dietary Supplement on Biochemical and Performance Parameters in Broilers. J. Biol.

Environ. Sci. 9:47–55 Available at https://www.researchgate.net/publication/311186498.

Bhatti S., S. A. Ali Shah, T. Ahmed, S. Zahid. (2018). Neuroprotective effects of *Foeniculum vulgare* seeds extract on lead-induced neurotoxicity in mice brain. https://doi.org/10.1080/01480545.2018.1459669 41:399–407 Available at https://www.tandfonline.com/doi/abs/10.1080/01480545.2018.1459669 (verified 6 June 2023).

Bhatti S. A., M. Z. Khan, M. K. Saleemi, M. Saqib, A. Khan, Z. Ul-Hassan. (2017). Protective role of bentonite against aflatoxin B1- and ochratoxin A-induced immunotoxicity in broilers. J. Immunotoxicol. 14:66–76.

Buğdayci K. E., F. K. Oğuz, M. N. Oğuz, E. Kuter. (2018). Effects of fennel seed supplementation of ration on performance, egg quality, serum cholesterol, and total phenol content of egg yolk of laying quails. Rev. Brasil. Zoot. 47.

Castro-Barquero S., A. Tresserra-Rimbau, F. Vitelli-Storelli, M. Doménech, J. Salas-Salvadó, V. Martín-Sánchez, M. Rubín-García, P. Buil-Cosiales, D. Corella, M. Fitó, D. Romaguera, J. Vioque, Á. M. Alonso-Gómez, J. Wärnberg, J. A. Martínez, L. Serra-Majem, F. J. Tinahones, J. Lapetra, X. Pintó, J. A. Tur, A. Garcia-Rios, L. García-Molina, M. Delgado-Rodriguez, P. Matía-Martín, L. Daimiel, J. Vidal, C. Vázquez, M. Cofán, A. Romanos-Nanclares, N. Becerra-Tomas, R. Barragan, O. Castañer, J. Konieczna, S. González-Palacios, C. Sorto-Sánchez, J. Pérez-López, M. A. Zulet, I. Bautista-Castaño, R. Casas, A. M. Gómez-Perez, J. M. Santos-Lozano, M. Á. Rodríguez-Sanchez, A. Julibert, N. Martín-Calvo, P. Hernández-Alonso, J. V. Sorlí, A. Sanllorente, A. M. Galmés-Panadés, E. Cases-Pérez, L. Goicolea-Güemez, M. Ruiz-Canela, N. Babio, Á. Hernáez, R. M. Lamuela-Raventós, R. Estruch. (2020). Dietary Polyphenol Intake is Associated with HDL-Cholesterol and A Better Profile of other Components of the Metabolic Syndrome: A PREDIMED-Plus Sub-Study. Nutrients 12:689 Available at https://doi.org/10.3390/nu12030689 (verified 9 June 2023).

Charlton M. R. (1996). Protein metabolism and liver disease. Baillieres Clin. Endocrinol. Metab. 10:617–635.

Charmandari E., C. Tsigos, G. Chrousos. (2005). Endocrinology of the stress response. Annu Rev Physiol 67:259–284 Available at http://www.annualreviews.org/doi/10.1146/annurev.physiol.67.040403.120816 (verified 6 April 2021).

Chiu H. W., K. F. Hua. (2016). Hepatoprotective effect of wheat-based solid-state fermented antrodia cinnamomea in carbon tetrachloride-induced liver injury in rat. PLoS One. 11:1–17.

Cobb500 Broiler Performance & Nutrition Supplement. (2022). Available at https://www.cobb-vantress.com/en_US/products/cobb500/ (verified 7 June 2022).

El-Deek A. A., Y. A. Attia, M. M. Hannfy. (2003). Effect of anise (*Pimpinella anisum*), ginger (*Zingiber officinale roscoe*) and fennel (*Foeniculum vulgare*) and their mixture on performance of broilers. Archiv. fur Geflugelkunde. 67:92–96.

Elgayyar M., F. A. Draughon, D. A. Golden, J. R. Mount. (2001). Antimicrobial Activity of Essential Oils from Plants against Selected Pathogenic and Saprophytic Microorganisms. J. Food Prot. 64:1019–1024.

Elnagar S. A., S. E. Scheideler, M. M. Beck. (2010). Reproductive hormones, hepatic deiodinase messenger ribonucleic acid, and vasoactive intestinal polypeptide-immunoreactive cells in hypothalamus in the heat stress-induced or chemically induced hypothyroid laying hen. Poult. Sci. 89:2001–2009.

Fatima A., N. Chand, S. Naz, M. Saeed, N. U. Khan, R. U. Khan. (2022). Coping heat stress by crushed fennel (*Foeniculum vulgare*) seeds in broilers: Growth, redox balance and humoral immune response. Livest. Sci. 265.

Gharaghani H., F. Shariatmadari, M. A. Torshizi. (2015). Effect of Fennel (*Foeniculum vulgare* Mill.) Used as a Feed Additive on The Egg Quality of Laying Hens Under Heat Stress. Brazilian J. Poult. Sci. 17:199–207 Available at https://www.scielo.br/j/rbca/a/8pBT9XQCxHM5Gh7nGNYn9Cn/?format=html&lang=en (verified 6 June 2023).

Ghiasvand A. R., A. Khatibjoo, Y. Mohammadi, M. Akbari Gharaei, H. Shirzadi. (2021). Effect of fennel essential oil on performance, serum biochemistry, immunity, ileum morphology and microbial population, and meat quality of broiler chickens fed corn or wheat-based diet. Br. Poult. Sci. 62:562–572 Available at https://www.tandfonline.com/doi/abs/10.1080/00071668.2021.1883551 (verified 9 June 2023).

Hafeez A., W. Akram, A. Sultan, Y. Konca, T. Ayasan, S. Naz, W. Shahzada, R. U. Khan. (2021). Effect of dietary inclusion of taurine on performance, carcass characteristics and muscle micro-measurements in broilers under cyclic heat stress. https://doi.org/10.1080/1828051X.2021.1921627 20:872–877 Available at https://www.tandfonline.com/doi/abs/10.1080/1828051X.2021.1921627 (verified 5 June 2023).

Imran A., L. Xiao, W. Ahmad, H. Anwar, A. Rasul, M. Imran, N. Aziz, A. Razzaq, M. U. Arshad, A. Shabbir, J. L. Gonzalez de Aguilar, T. Sun, G. Hussain. (2019). *Foeniculum vulgare* (Fennel) promotes functional recovery and ameliorates oxidative stress following a lesion to the sciatic nerve in mouse model. J. Food Biochem. 43:e12983 Available at https://onlinelibrary.wiley.com/doi/full/10.1111/jfbc.12983 (verified 6 June 2023).

Kaya A., H. Kaya, M. Macİt, Ş. Çelebİ, N. Esenbuğa, M. A. Yörük, M. Karaoğlu. (2013). Effects of dietary inclusion of plant extract mixture and copper into layer diets on egg yield and quality, yolk cholesterol and fatty acid composition. Kafkas Univ. Vet. Fak. Derg. 19:673–679.

Kazemi-Fard M., H. Kermanshahi, M. Rezaei, A. Golian. (2013). Effect of Different Levels of Fennel Extract and Vitamin D 3 on Performance, Hatchability and Immunity in Post Molted Broiler Breeders. Iran J. Appl. Anim. Sci. 3:733–745 Available at www.ijas.ir.

Khan R. U., A. Fatima, S. Naz, M. Ragni, S. Tarricone, V. Tufarelli.(2022). Perspective, Opportunities and Challenges in Using Fennel (*Foeniculum vulgare*) in Poultry Health and Production as an Eco-Friendly Alternative to Antibiotics: A Review. Antibiotics. 11.

Khan R. U., S. Naz, Z. Nikousefat, V. Tufarelli, M. Javdani, M. S. Qureshi, V. Laudadio. (2019). Potential applications of ginger (*Zingiber officinale*) in poultry diets. https://doi.org/10.1017/S004393391200030X 68:245–252 Available at https://www.tandfonline.com/doi/abs/10.1017/S004393391200030X (verified 5 June 2023).

Khan A., M. Tahir, I. Alhidary, M. Abdelrahman, A. A. Swelum, R. U. Khan. (2021). Role of dietary *Moringa oleifera* leaf extract on productive parameters, humoral immunity and lipid peroxidation in broiler chicks. https://doi.org/10.1080/10495398.2021.1899936 33:1353–1358 Available at https://www.tandfonline.com/doi/abs/10.1080/10495398.2021.1899936 (verified 5 June 2023).

Koppula S., H. Kumar. (2013). *Foeniculum vulgare* Mill (Umbelliferae) Attenuates Stress and Improves Memory in

Wister Rats. Trop. J. Pharmaceut. Res. 12:553–558 Available at https://www.ajol.info/index.php/tjpr/article/view/93274 (verified 6 June 2023).

Loyau T., C. Berri, L. Bedrani, S. Métayer-Coustard, C. Praud, M. J. Duclos, S. Tesseraud, N. Rideau, N. Everaert, S. Yahav, S. Mignon-Grasteau, A. Collin. (2013). Thermal manipulation of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. J. Anim. Sci. 91:3674–3685 Available at https://pubmed.ncbi.nlm.nih.gov/23736053/ (verified 9 June 2023).

Ma D., A. Shan, Z. Chen, J. Du, K. Song, J. Li, Q. Xu. (2005). Effect of *Ligustrum lucidum* and *Schisandra chinensis* on the egg production, antioxidant status and immunity of laying hens during heat stress. Arch. Anim. Nutr. 59:439–447 Available at https://pubmed.ncbi.nlm.nih.gov/16429829/ (verified 9 June 2023).

Mahmud H. A. (2014). Response Of Growing Japanese Quail To Different Levels Of Fennel Seeds Meal. Egyptian Poult. Sci. J. 34:795–807 Available at https://epsj.journals.ekb.eg/article_5362.html (verified 6 June 2023).

Mehra N., G. Tamta, V. Nand, J. P. Singh. (2022). Nutritional profiling, antibacterial potential, and cluster analysis in *Foeniculum vulgare* seeds against human pathogenic bacteria. J. Food Process Preserv. 46:e16763 Available at https://onlinelibrary.wiley.com/doi/full/10.1111/jfpp.16763 (verified 6 June 2023).

Mohammadabadi M., S. H. Masoudzadeh, A. Khezri, O. Kalashnyk, R. V. Stavetska, N. I. Klopenko, V. P. Oleshko, S. V. Tkachenko. (2021). Fennel (*Foeniculum vulgare*) seed powder increases Delta-Like Non-Canonical Notch Ligand 1 gene expression in testis, liver, and humeral muscle tissues of growing lambs. Heliyon 7 Available at https://doi.org/10.1016/j.heliyon.2021.e08542 (verified 6 June 2023).

Oulmouden F., N. Ghalim, M. E. Morhit, H. Benomar, E. M. Daoudi, S. Amrani, L. Pasteur, H. Rayad. (2014). Hypolipidemic and Anti -Atherogenic effect of methanol extract of Fennel (*Foeniculum vulgare*) in hypercholesterolemic mice.

Pavlík A., P. Sláma, L. Mazalová, E. Kabourková. (2016). Total cholesterol and corticosterone concentration relationship in blood plasma of laying hens. J. Microbiol. Biotechnol. Food Sci. 05:17–19.

Pham-Huy L. A., H. He, C. Pham-Huy. (2008). Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci. 4:89–96 Available at www.ijbs.org (verified 1 April 2021).

Ragab M. S. (2007). Effects Of Using Fennel Seeds In Growing Japanese Quail Diets Varying In Their Protein Content With Or Without Enzyme Supplementation. Fayoum J. Agricult. Res. Develop. 21:113–136 Available at https://fjard.journals.ekb.eg/article_197555.html (verified 6 June 2023).

Rather M. A., B. A. Dar, S. N. Sofi, B. A. Bhat, M. A. Qurishi. (2016). *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. Arabian J. Chem. 9:S1574–S1583.

Safaei-Cherehh A., B. Rasouli, P. A. Alaba, A. Seidavi, S. R. Hernández, A. Z. M. Salem. (2020). Effect of dietary *Foeniculum vulgare* Mill. extract on growth performance, blood metabolites, immunity and ileal microflora in male broilers. Agroforest. Syst. 94:1269–1278 Available at https://link.springer.com/article/10.1007/s10457-018-0326-3 (verified 6 June 2023).

Saki A. A., M. Kalantar, E. Rahmatnejad, F. Mirzaaghatabar. (2014). Health Characteristics and Performance of Broiler Chicks in Response to *Trigonella foenum graecum* and *Foeniculum vulgare*. Iran J. Appl. Anim. Sci. 4:387–391 Available at https://ijas.rasht.iau.ir/article_513702.html (verified 6 June 2023).

Samadi-Noshahr Z., M. A. R. Hadjzadeh, R. Moradi-Marjaneh, A. Khajavi-Rad. (2021). The hepatoprotective effects of fennel seeds extract and trans-Anethole in streptozotocin-induced liver injury in rats. Food Sci. Nutr. 9:1121–1131 Available at https://onlinelibrary.wiley.com/doi/full/10.1002/fsn3.2090 (verified 9 June 2023).

Shawky S. M., S. I. Fathalla, I. S. Zahran, K. M. Gaafar, M. K. Hussein, I. S. Abu-Alya. (2020). Immunological Stimulant Effect Of Linseed Oil And Fennel Oil Supplemented Diet On Broilers. Adv. Anim. Vet. Sci. 8:771–776.

Shojaiefar S., M. R. Sabzalian, A. Mirlohi, M. H. Mirjalili. (2022). Seed yield stability with modified essential oil content and composition in self-compatible progenies of bitter fennel (*Foeniculum vulgare* Mill.). Ind. Crops Prod. 182:114821.

Shuaib M., N. Ullah, A. Hafeez, I. A. Alhidary, M. M. Abdelrahman, R. U. Khan. (2020). Effect of dietary supplementation of wildCumin (*Bunium persicum*) seeds on performance, nutrient digestibility and circulating metabolites in broiler chicks during the finisher phase. https://doi.org/10.1080/10495398.2020.1844222 33:871–875 Available at https://www.tandfonline.com/doi/abs/10.1080/10495398.2020.1844222 (verified 5 June 2023).

Smith K. G., J. L. Hunt. (2003). On the use of spleen mass as a measure of avian immune system strength. Oecologia. 2003 138:1 138:28–31 Available at https://link.springer.com/article/10.1007/s00442-003-1409-y (verified 19 November 2022).

Sun P., L. Zhao, N. Zhang, J. Zhou, L. Zhang, W. Wu, B. Ji, F. Zhou. (2021). Bioactivity of Dietary Polyphenols: The Role in LDL-C Lowering. Foods. 10 Available at https://doi.org/10.3390%2Ffoods10112666 (verified 9 June 2023).