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Oxidative stress and effects of dill (*Anethum graveolens* dhi) powder on the performance and health status of broilers

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Abstract

Effects of dill powder (*Anethum graveolens* dhi) on performance and biochemical traits, immune response, blood cells and microbial population of cecum broilers after stress induced with dexamethasone were investigated. Two hundred and forty broilers were arrayed in completely randomized design with six experimental groups, five replications and eight broilers per replication. The experimental groups consisted of G1: control diet without additives, G2: control diet supplemented with 1.5% dill powder, G3: control diet supplemented with 1.5% dill powder under dexamethasone stress, G4: control diet supplemented with 3% dill powder, G5: control diet supplemented with 3% dill powder under dexamethasone stress, and G6: control diet under dexamethasone stress. After 31, 33, 38 and, 40 days, 2 mg/kg bodyweight (BW) dexamethasone was injected in right chest muscle in groups 3, 5, 6, and samples were taken after 35 and 42 days. Use of 3% dill powder reduced cecum microbial population significantly. Under dexamethasone oxidative stress conditions dill powder improved biochemical traits and the number of white blood cells significantly. Without stress induced by dexamethasone injection, use of 3% dill powder improved internal organs, increased the length of the digestive tract, improved weight gain, reduced FCR and improved antibody titers to Newcastle disease (ND) and avian influenza (AI) viruses. All levels of dill powder prevented negative impacts of dexamethasone on functional and biochemical traits. Thus, 3% dill powder has beneficial effects on performance, immune response, blood cell and microbial population, and could be used as a growth promoter and to prevent oxidative stress.

Keywords: avian influenza, biochemical traits, broiler, microbial, oxidative stress
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Introduction

Birds are sensitive to stress. Stress reduces feed consumption, growth, and the ability to digest amino acids, alters amino acid requirements, and changes carcass characteristics, causing economic losses. These negative effects are accompanied by deterioration in meat quality, animal welfare, and immune functions (Feizi *et al.*, 2012; Kang *et al.*, 2020).

The use of medicinal herbs as additives to poultry feed and even for the treatment of diseases is considered low cost and non-hazardous, has not side effects, or complications for the environment (Khosravi Nia *et al*., 2009; Sevim & Ayasan, 2020). Dill (*Anethum graveolens dhi*), which belongs to the *Apiaceae* family, is native to Palestine and has been exported widely. In Iran, dill is an annual plant that is cultivated naturally in Tabriz, Khorasan, and Tafresh. Its roots and seeds were used in traditional medicine to treat gastrointestinal, lipid, spasmodic and microbial problems (Kamkar, 2009). The active ingredients include two major compounds, namely de-carvone and limonene. These ingredients have antioxidant effects, stabilize liver cell membrane and reduce the release of enzymes to the blood (Bahadori *et al.*, 2013; Chahal *et al.*, 2017). In addition, dill has been reported to have anti-cancer, anti-diabetic and antioxidant effects (Bahadori *et al.*, 2013). It is a rich source of essential oils, calcium, potassium, magnesium, phosphorus, sodium, vitamin A and niacin. The fruits of this plant contain 1 - 4% essential oil, with the main components of carvone (30 - 60%), limonene (36%), and α-phellandrene (20.61%), and other compounds such as pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, and isomyristicin.

Glucocorticoids such as dexamethasone play an important role in counteracting stressful conditions. In high concentrations, they have a catabolic effect on skeletal muscle, inhibiting protein synthesis, and

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increasing degradation of muscle fibres through proteolysis (Goldberg *et al*., 1980; Smith *et al.*, 1990). Glucocorticoids also weaken the immune system (Strehl *et al.*, 2019), reduce consumption of food in the short term by stimulating insulin secretion from the pancreas (Strack *et al.*, 1995), and inhibit production of thyroid-releasing hormone in the hypothalamus (Haugen, 2009). These effects can lead to reduced growth and muscle weight (Hosseini Siyar & Farahvar, 2017). In broilers, the adverse effects of dexamethasone can be mitigated using corticosteroids (Hosseini Siyar & Farahvar, 2017).

The use of medicinal plants, including green tea and *Eriobotrya Japonica,* has been shown to reduce the negative impacts of stress in broilers (Azizipori *et al*., 2021) and other species (Noh *et al*., 2015). Preliminary evidence suggested multi-faceted benefits of supplementing broiler diets with dill (Hammod *et al*., 2020). Thus, the purpose of this study was to investigate the effects of dill powder on performance, visceral, and biochemical traits, blood cells, immune response and microbial population in the cecum of broilers after induction of stress using dexamethasone.

Materials and Methods

Experiments were carried out based on procedures and guidelines approved by the Animal Care Committee of the Iranian Council of Animal Care 1995 (No. IACUC95). The animal experiment was carried out in accordance with the procedures and guidelines documented by Xu *et al*. (2003).

In this experiment, 240 male Ross-308 broilers (initial weight 42 ± 2 g) were used in a completely randomized design with six experimental groups, five replications and eight broilers per replication. The base diet was formulated with WUFFDA software (Pesti *et al.*, 2014) based on the requirements of Ross-308 broilers (Table 1). The experimental groups included G1: control diet without any additives, G2: control diet with 1.5% dill powder, G3: control diet with 1.5% dill powder under dexamethasone stress, G4: control diet with 3% dill powder, G5: control diet with 3% dill powder under dexamethasone stress, and G6: control diet under dexamethasone stress. At days 31, 33, 38, and 40, 2 mg/kg BW of dexamethasone was injected in the right chest muscle of the birds in G3, G5, and G6, and samples were collected at days 35 and 42. Dexacoid®, which is produced by Nasr Fariman Co. (Iran), was used as the source of dexamethasone. Each ml of drug contained 4 mg of dexamethasone sodium phosphate. The dill used in this study was freshly prepared from the summer crop grown in Malayer County, Hamadan Province, Iran. After removing the excess parts of the plant, it was dried in shade with free air flow and ground in the laboratory of Malayer University. The dill powder that was obtained was stored at ambient temperature in the laboratory until it was used. Its chemical composition on a dry matter basis was 0.246% phenolic acid, 5.5% ash, 86% dry matter, 0.3% phosphorus, 2.8% calcium, 3% crude fat, and 34% crude protein.

The performance of the broilers was characterized by their BW, feed consumption (FC) and feed conversion ratio (FCR). For this purpose, the amount of feed consumed by the broilers in each pen was recorded daily. Bodyweight was recorded weekly, and broilers were not fed for three hours before weighing to empty the contents of gastrointestinal tract. The FCR ratio was measured at the end of each week and at the end of the period. The weights were measured with a digital scale with an accuracy of 0.01 g.

Two chicks from each pen were selected at six weeks old, and individual blood samples were collected from the brachial vein into non-heparinized tubes. The tubes were centrifuged at 3000 x gravity for 10 minutes and the isolated serum was stored at −20 °C for further analysis. The serum samples were analysed for liver enzymes, namely alanine aminotransferase (ALT), a aminotransferase (AST), and alkaline phosphatase (ALP) and for the biochemical parameters phosphorus, calcium, triglycerides, and cholesterol, as well as blood proteins, total protein, globulin and albumin using commercial kits (Boehringer Mannheim, Hitachi 704 automatic analyser, Japan). Antibody titers against Newcastle disease (ND) and avian influenza (AI) viruses were done with the hemagglutination inhibition test technique. Differential blood cells counts were performed using a Neuobauer® hemacytometer slide (Campbell, 1997; Zhang *et al*., 2009; Hedayati *et al*., 2018).

After slaughter, 1 g of the contents of the cecum was collected with sterile forceps and transferred to a sterile sample container. All samples were transferred to the laboratory at 4 °C under completely sterile conditions, and the microbial population was evaluated in a sterile phosphate buffer solution at pH 7.2 by serial dilution to seven concentrations. The authors used 1 ml of each sample on the culture medium, namely salmonella shigella agar, MacConkey agar, and eosin methylene blue culture media, which had previously been prepared for growing coliforms, Salmonella, and Escherichia coli bacteria. The culture was done under the hood and in the presence of flame. Finally, the samples were incubated at 37 °C for 24 hours (Akbari *et al*., 2004; Hedayati *et al*., 2018).

The data were analysed as a 2 x 3 factorial design with version 9.2 of SAS (SAS Institute Inc., Cary, North Carolina, USA). Comparison of treatment means was performed with Duncan's multiple range test (1995) and differences among treatments were considered significant at $P \leq 0.05$.

Table 1 Diets prepared for broiler chickens in a study of mitigation of stress response by supplementation with dill powder

Ingredients, %	Days 1 to 21	Days 22 to 42
Corn	54.32	62.4
Soybean meal (44% crude protein	39.08	31.80
Oil	2.16	1.80
Shell	0.09	0.82
Dicalcium phosphate	2.05	1.84
Sodium chloride	0.37	0.31
Vitamin and mineral supplement[1]	0.50	0.50
L-Lysine hydrochloride 78%	0.07	0.23
DL-Methionine 99%	0.20	0.27
L-Threonine	0.09	0.07
Lysine	0.26	0.17
Analysed composition, % unless otherwise stated		
Metabolizable energy, MJ/kg	2900	3000
Crude protein	22.16	19.2
Calcium	1.00	0.90
Available phosphorous	0.50	0.45
Threonine	0.79	0.71
Lysine	1.15	0.96
Methionine + cysteine	0.83	0.78
Methionine	0.50	0.48
Dietary cation anion balance, mq/kg	232	204

[1]Vitamin A: 15000 IU, vitamin D_3: 1500 IU, vitamin E_2: 20 mg, vitamin K_3: 0.7 mg, vitamin B12: 0.02 mg, niacin: 22.5 mg, thiamine: 5 mg, folic acid: 0.7 mg, pyridoxine: 1.6 mg, riboflavin: 5 mg, pantothenic acid: 25 mg, choline chloride: 175 mg, manganese: 60 mg, zinc: 45 mg, iodine: 1.25 mg, selenium: 0.4 mg, copper: 10 mg, iron: 72 mg per kg diet

Results and Discussion

The effects of supplementation with dill and induced stress were found to interact significantly, affecting FW, FC, and FCR at 21 and at 42 days (Table 2). When there was no induced stress, the weights at 21 and 42 days increased as the level of supplemental dill increased. However, under the stressed condition, BW at 21 days was reduced in G3 compared with G6, with birds in G5 being heavier than those in G6 and G3. Without stress, feed consumption to 21 days was increased from G1 to G2 to G4, with the increase from G1 to G2 being smaller than the increase from G2 to G4. However, under induced stress, the increases in feed consumption to 211 days were smaller than without stress and approximately equal between G6 and G3 and G3 and G5. From 22 to 42 days, FC decreased progressively with increasing levels of dill supplementation when the broilers were not subjected to stress. However, with induced stress, FC increased from G6 to G3 and then decreased from G3 to G5. The net effect of these changes in FW and FC on FCR were for FCR to decrease markedly with the supplementation of dill relative to G1, with the difference between G2 and G4 being relatively small in both periods. Conversely, under the imposed stress condition, FCR for days 1 to 21 showed relatively small differences in G6, G3 and G5. However, under the imposed stress G5 produced markedly better FCR than G6 and G3.

Various forms of stress activate the hypothalamic-pituitary adrenal axis directly or indirectly, leading to the release of corticosterone into the blood. In broilers, a mimicked stressful condition induced by corticosterone injections led to reduced FCR (Mehaisen *et al*., 2017). Other studies mimicked stress conditions by adding corticosterone to the diet or drinking water or by intramuscular injection (Fu *et al*., 2014; Zulkifli *et al*., 2014). Bahadori *et al*. (2013) observed that various levels of dill supplementation increased both FC and weight gain. However, these effects did not translate to improved FCR. The mechanism that explained reduced weight of broilers in response to glucocorticoids is its proteolytic effect on skeletal muscle

cells. Glucocorticoids increase the production of free radicals, inducing atrophy of the muscle fibres (Furukawa *et al*., 2016). Using 0.5% of dill powder supplement increased the total weight of digestive tract organs, and improved FCR. Also, supplementation of diets for rabbits with 0.5% dill powder increased final weight significantly and the percentage of total viscera, and improved FCR, perhaps in response to the aromatic compounds in dill powder stimulating appetite (Ibrahim, 2005).

Table 2 Effects of dill powder and dexamethasone on functional characteristics of broilers

Experimental groups			Days 1 to 21			Days 1 to 42		
	Stress	Dill, %	FCR	FC	FW	FCR	FC	FW
G1	No	0.0	1.10[a]	718.4[e]	527.0[e]	1.91[a]	3294.9[a]	1843.5[d]
G2	No	1.5	0.98[c]	778.7[b]	580.0[c]	1.64[c]	3283.0[b]	1890.5[c]
G3	Yes	1.5	0.96[e]	739.6[d]	551.7[d]	1.82[b]	3286.3[b]	1886.2[c]
G4	No	3.0	0.98[c]	786.5[a]	614.8[a]	1.60[d]	3264.3[d]	1945.2[a]
G5	Yes	3.0	0.97[d]	766.1[c]	615.3[a]	1.66[c]	3277.8[c]	1911.4[b]
G6	Yes	0.0	0.99[b]	707.9[f]	592.9[b]	1.88[a]	3251.0[e]	1831.9[e]
Standard error								
	Stress		0.013	3.8	9.1	0.018	4.1	5.2
	Dill		0.007	2.2	5.2	0.010	2.8	3.1
	Stress x dill		0.009	2.7	6.4	0.013	3.2	3.7
P-Value								
	Stress		0.002	<0.001	0.124	0.001	0.012	0.003
	Dill		<0.004	<0.001	<0.001	<0.001	0.007	<0.001
	Stress x dill		0.006	0.011	0.001	<0.001	<0.001	0.042

FCR: feed conversion ratio, FC: feed consumption, FW: final weight
[a,b,c,d,e] Within a column, means with a common superscript were not different with probability P <0.05

The effects of supplementation with dill and induced stress interacted significantly to affect carcass traits that were recorded when the broilers were 35 and 42 days old (Tables 3 and 4). At 35 days, carcass weight was lower in G1 and G6 (P <0.05). In the stressed groups, G5 increased carcass weight (P <0.01). At 42 days, G4 and G5 increased carcass weight, and the length and width of chest and thighs relative to G1 and G6 (P <0.01). Bahadori *et al*. (2013) reported that percentage of thigh in groups supplemented with dill powder was higher than that of control (P <0.05) and the weight of the supplemented group was lower than that of control (P <0.05). Glucocorticoids released in response to stress trigger proteolytic mechanisms in muscles, especially skeletal muscles, and cause muscle breakdown (Furukawa *et al*., 2016). The active ingredients of some medicinal herbs affect the dexamethasone signalling pathway and prevent induced proteolysis. For example, antioxidant compounds in the essential oil from *Eriobotrya japonica* prevented atrophy induced in muscle fibres by dexamethasone (Noh *et al*., 2015). The weights of carcass and carcass components were affected significantly by the gradual effect of diet with dill seed (Rafiei-Tari, 2015). Adding essential oil from *Coriandrum sativum* to the diet of broilers was shown to improve their performance, growth, and carcass traits (Ghazanfari & Mohammadi, 2015).

Table 3 Effects of dill powder and dexamethasone on length, width and weight of carcasses, thighs and breast of 35-day-old broiler chickens

Experimental groups			Leg width, cm	Leg length, cm	Breast width, cm	Breast length, cm	Breast weight, %	Leg weight, %	Carcass weight, %
	Stress	Dill, %							
G1	No	0.0	4.1[b]	19.7[b]	3.4[c]	14.6[b]	33.16[c]	29.00[e]	65.62[d]
G2	No	1.5	4.2[b]	20.4[c]	4.2[a]	15.0[c]	33.41[b]	29.37[c]	69.23[b]
G3	Yes	1.5	4.2[b]	20.5[c]	3.9[c]	15.2[c]	33.63[c]	30.29[b]	70.04[a]
G4	No	3.0	4.4[c]	20.5[c]	3.8[c]	15.7[a]	33.19[c]	30.19[b]	69.96[b]
G5	Yes	3.0	4.9[a]	20.8[a]	4.5[a]	15.6[a]	34.51[a]	30.96[a]	69.96[b]
G6	Yes	0.0	4.0[b]	19.0[d]	3.3[c]	14.1[d]	32.28[e]	28.84[d]	65.80[c]
Standard error									
	Stress		0.07	0.14	0.26	0.13	0.23	0.07	0.14
	Dill		0.04	0.08	0.15	0.08	0.13	0.04	0.12
	Stress x dill		0.05	0.10	0.18	0.09	0.16	0.05	0.10
P-Value									
	Stress		0.010	0.258	0.126	0.088	0.135	<0.001	0.002
	Dill		<0.001	<0.001	0.009	<0.001	<0.001	<0.001	<0.001
	Stress x dill		0.001	0.001	0.062	0.008	<0.001	<0.001	0.445

[a,b,c,d,e] Within a column, means with a common superscript were not different with probability *P* <0.05

Table 4 Effects of dill powder and dexamethasone on length, width and weight of carcasses, thighs, and breasts of 42-day-old broiler chickens

Experimental groups			Leg width, cm	Leg length, cm	Breast width, cm	Breast length, cm	Breast weight, %	Leg weight, %	Carcass weight, %
	Stress	Dill, %							
G1	No	0.0	4.4[c]	20.4[b]	3.8[d]	14.6[c]	33.16[c]	22.6[c]	64.7[b]
G2	No	1.5	4.8[b]	20.9[a]	4.4[b]	15.0[b]	33.41[b]	22.4[d]	62.1[c]
G3	Yes	1.5	4.7[b]	21.2[a]	4.1[c]	15.2[b]	33.63[c]	23.8[b]	60.7[d]
G4	No	3.0	4.9[a]	21.4[a]	4.7[a]	15.7[a]	33.19[c]	24.3[a]	67.6[a]
G5	Yes	3.0	4.7[b]	20.2[b]	4.1[c]	15.6[a]	34.51[a]	21.5[e]	62.9[c]
G6	Yes	0.0	4.1[b]	19.0[c]	3.7[d]	14.1[d]	32.28[e]	21.0[f]	64.3[b]
Standard error									
	Stress		0.04	0.21	0.06	0.13	0.23	0.14	0.57
	Dill		0.02	0.12	0.04	0.08	0.13	0.08	0.45
	Stress x dill		0.03	0.15	0.05	0.09	0.16	0.09	0.48
P-Value									
	Stress		<0.001	0.001	<0.001	0.088	0.135	<0.001	<0.001
	Dill		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Stress x dill		0.049	0.004	0.001	0.008	<0.001	<0.001	0.001

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

Table 5 Effects of dill powder and dexamethasone on lymphatic organs of broiler chickens

Experimental groups			35 days old			42 days old		
	Stress	Dill, %	Spleen, %	Thymus, %	Bursa, %	Spleen, %	Thymus, %	Bursa, %
G1	No	0.0	0.14[b]	0.48[c]	0.41[b]	0.31[b]	0.36[d]	0.42[b]
G2	No	1.5	0.15[b]	0.62[b]	0.53[d]	0.34[a]	0.43[c]	0.48[a]
G3	Yes	1.5	0.15[b]	0.39[e]	0.59[c]	0.33[a]	0.36[d]	0.48[a]
G4	No	3.0	0.26[a]	0.70[a]	0.65[a]	0.35[a]	0.54[a]	0.49[a]
G5	Yes	3.0	0.15[b]	0.43[d]	0.63[a]	0.35[a]	0.49[b]	0.48[a]
G6	Yes	0.0	0.10[c]	0.35[f]	0.31[e]	0.28[c]	0.36[d]	0.39[c]
Standard error								
	Stress		0.020	0.006	0.014	0.004	0.010	0.004
	Dill		0.001	0.003	0.008	0.002	0.008	0.002
	Stress x dill		0.014	0.004	0.010	0.003	0.009	0.003
P-value								
	Stress		0.002	<0.001	0.035	0.002	<0.001	0.003
	Dill		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Stress x dill		0.014	<0.001	<0.001	0.033	0.013	0.015

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

The interaction of induced stress and supplemental dill (*P* <0.01) affected the percentage weight of the internal organs (Tables 6 and 7). The highest relative weight of internal organs on day 35 was related to G4, which showed a significant increase compared with other groups (*P* <0.01). Injection of dexamethasone produced significant increase in heart weight at day 42 compared with the corresponding unstressed groups that received the same amount of dill powder (*P* <0.01). This increase in weight of the heart may be because of free radicals created by dexamethasone, which increase signalling and coagulation and transcription factors in cardiac muscle cells, causing hypertrophy and increasing heart weight (Sevim & Ayasan, 2020). The effect of dexamethasone with dill showed that using dill powder prevented atrophy induced by dexamethasone in digestive organs. Glucocorticoids have an anabolic effect on the liver, and the lipid layer increased (Mohammadi, 2020) in broilers that received corticosteroids.

The interaction effects of supplementation with dill powder and dexamethasone affected small intestine length in the broilers significantly (Table 8). At 35 days, the greatest intestinal length was observed in G5, which was higher than other groups (*P* <0.01). At 42 days, G6 had the greatest relative length of small intestine (*P* <0.01). The use of 0.5% dill powder supplementation has been shown to increase final weight and total weight of digestive organs and improved FCR (Ibrahim, 2005). The results of various levels of *Coriandrum sativum* essential oil in broilers shows that adding it to the diet of broilers improved growth and carcass traits (Alagawany *et al.,* 2019). But the effect of adding *Ferula gummosa* root to diet was not significant on performance and microbiology of intestine and apparent digestibility of nutrients in broilers on the length of gastrointestinal tract (*P* <0.05) (Abdollahi Zaveh *et al*., 2013).

The interaction effects of the levels of dill powder with dexamethasone-induced stress affected the serum biochemical traits of the broilers significantly at 35 and 42 days old (Table 9). When the birds were 42 days old, serum calcium was increased significantly with G5 compared with the other groups (*P* <0.01). The lowest levels of serum cholesterol and triglycerides were observed in G2 (*P* <0.01). Generally, dexamethasone injection increased serum triglycerides and cholesterol compared with the unstressed groups (*P* <0.01). The mechanism by which stress increases blood lipids is not well established, but it has been suggested that stress encourages the body to produce metabolic fuels, with the liver producing and secreting low-density lipoproteins, leading to an increase in serum cholesterol (Calderon *et al*., 1998). Rafiei-Tari (2015) observed that quail fed dill powder had non-significantly reduced levels of cholesterol and low-density lipoproteins compared with the control group.

Table 6 Effects level of dill powder and dexamethasone on the visceral organs of 35-day-old broiler chickens

Experimental groups			Heart	Liver	Proventriculus	Crop	Gizzard	Pancreas
	Stress	Dill, %						
G1	No	0.0	1.0[c]	3.1[c]	0.98[b]	1.24[b]	4.8[b]	0.51[e]
G2	No	1.5	1.1[b]	3.8[b]	1.02[b]	1.22[b]	5.4[d]	0.67[b]
G3	Yes	1.5	1.0[c]	3.0[c]	1.19[a]	1.33[b]	5.5[d]	0.62[c]
G4	No	3.0	1.3[a]	4.5[a]	1.26[a]	1.30[b]	6.2[c]	0.70[a]
G5	Yes	3.0	1.1[b]	4.8[a]	1.25[a]	1.47[a]	6.5[a]	0.55[d]
G6	Yes	0.0	1.1[c]	3.0[c]	0.95[b]	0.84[c]	3.9[e]	0.41[f]
Standard error								
Stress			0.018	0.11	0.04	0.06	0.10	0.013
Dill			0.013	0.07	0.02	0.03	0.06	0.007
Stress x dill			0.016	0.09	0.03	0.04	0.07	0.009
P-value								
Stress			<0.001	0.013	0.186	0.428	0.018	<0.001
Dill			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Stress x dill			<0.001	0.001	0.028	<0.001	<0.001	0.001

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability $P < 0.05$

Table 7 Effects of dill powder and dexamethasone on visceral organs of 42-day-old broiler chickens

Experimental groups			Heart	Liver	Proventriculus	Crop	Gizzard	Pancreas
	Stress	Dill, %						
G1	No	0.0	1.06[b]	3.01[a]	1.03[b]	1.23[b]	5.7[b]	0.56[b]
G2	No	1.5	0.82[c]	3.5[a]	1.19[a]	1.39[a]	6.6[b]	0.63[a]
G3	Yes	1.5	1.00[b]	3.2[b]	1.10[c]	1.19[b]	6.5[c]	0.60[c]
G4	No	3.0	1.00[b]	3.3[b]	1.13[d]	1.39[a]	6.9[a]	0.64[a]
G5	Yes	3.0	0.91[d]	3.1[c]	1.10[c]	1.23[b]	6.8[a]	0.64[a]
G6	Yes	0.0	1.13[a]	3.1[c]	0.96[e]	1.11[c]	6.7[b]	0.51[b]
Standard error								
Stress			0.015	0.02	0.016	0.016	0.08	0.008
Dill			0.008	0.01	0.009	0.009	0.05	0.006
Stress x dill			0.010	0.02	0.011	0.011	0.06	0.006
P-value								
Stress			0.001	<0.001	<0.001	<0.001	0.002	0.001
Dill			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Stress x dill			<0.001	<0.001	0.002	0.087	0.001	0.053

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability $P < 0.05$

Table 8 Effects of dill powder and dexamethasone on length of duodenum, jejunum and ileum of the small intestine of broiler chickens

Experimental groups			35 days old			42 days old		
	Stress	Dill, %	Duodenum, cm	Jejunum, cm	Ileum, cm	Duodenum, cm	Jejunum, cm	Ileum, cm
G1	No	0.0	18.8^{d}	42.83^{c}	40.5^{b}	18.1^{c}	42.0^{d}	38.0^{d}
G2	No	1.5	19.0^{c}	42.93^{a}	37.1^{d}	18.5^{b}	42.6^{c}	38.4^{b}
G3	Yes	1.5	19.5^{b}	42.96^{a}	38.6^{c}	18.4^{b}	42.3^{b}	38.2^{c}
G4	No	3.0	19.2^{b}	42.73^{c}	38.3^{c}	19.0^{a}	42.8^{a}	38.6^{a}
G5	Yes	3.0	19.9^{a}	43.06^{a}	41.6^{a}	18.5^{b}	42.3^{b}	38.2^{c}
G6	Yes	0.0	18.5^{d}	42.19^{d}	38.0^{c}	18.1^{c}	41.8^{e}	37.9^{d}
Standard error								
	Stress		0.07	0.06	0.38	0.04	0.05	0.04
	Dill		0.04	0.03	0.22	0.03	0.04	0.03
	Stress x dill		0.05	0.04	0.27	0.03	0.04	0.03
P-value								
	Stress		0.001	0.026	0.009	<0.001	<0.001	<0.001
	Dill		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Stress x dill		<0.001	<0.001	<0.001	<0.001	0.033	0.002

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

Table 9 Effects of dill powder and dexamethasone on serum constituents of broiler chickens (mg/dl)

Experimental groups			35 days old				42 days old			
	Stress	Dill, %	CHOL, mg/dl	TG, mg/dl	P, mg/dl	Ca, mg/dl	CHOL, mg/dl	TG, mg/dl	P, mg/dl	Ca, mg/dl
G1	No	0.0	158^{b}	122^{d}	9.44^{c}	10.1^{c}	141^{b}	84^{d}	9.5^{d}	11.5^{d}
G2	No	1.5	143^{c}	120^{e}	10.10^{b}	11.5^{a}	133^{c}	78.3^{f}	9.9^{c}	13^{b}
G3	Yes	1.5	169^{a}	139^{c}	9.63^{d}	11^{b}	155.4^{a}	87^{c}	9.6^{d}	12.3^{c}
G4	No	3.0	147^{c}	112^{f}	10.42^{a}	11.4^{a}	130^{d}	79.6^{e}	10.8^{a}	13.3^{b}
G5	Yes	3.0	159^{b}	144^{b}	9.33^{c}	11.5^{a}	156.4^{a}	88.3^{b}	9.6^{d}	13.7^{a}
G6	Yes	0.0	176^{a}	153^{a}	9.69^{d}	11.4^{a}	158^{a}	89.6^{a}	10.5^{b}	12.6^{c}
Standard error										
	Stress		3.3	2.1	0.12	0.13	0.13	0.43	0.094	0.13
	Dill		1.9	1.2	0.07	0.07	0.08	0.24	0.073	0.08
	Stress x dill		2.3	1.4	0.09	0.09	0.09	0.30	0.079	0.09
P-value										
	Stress		<0.001	<0.001	<0.001	0.010	0.028	<0.001	0.024	0.028
	Dill		<0.001	<0.001	0.009	<0.001	<0.001	<0.001	0.001	<0.001
	Stress x dill		<0.001	0.003	<0.001	<0.001	<0.001	0.007	<0.001	<0.001

CHOL: cholesterol, TG: triglyceride, P: phosphorus, Ca: calcium
[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

The level of dill powder supplementation interacted (P <0.05) with the injection of dexamethasone to affect liver enzyme levels in the broilers at 35 and 42 days (Table 10). At 35 days, ALP was increased in G5 (P <0.01). The lowest level of AST was observed in the G1 (P <0.01). On day 42, the levels of liver enzymes were lowest in the stressed groups (P <0.01). The highest levels of the hepatic enzymes were observed in G4 (P <0.01). Measuring plasma levels of ALP, ALT and AST is an index for the diagnosis of membrane damage in liver cells and tissues because of medicines or certain diseases (Mohammadi, 2020). The aqueous extract of dill has been shown to reduce plasma levels of lactate dehydrogenase (Fathi & Heidari, 2016). Ingestion of cortisol acetate increased the activity of ALT significantly (Kang *et al.*, 2020). Dexamethasone is a stronger glucocorticoid than cortisol, which increases the activity of ALT in liver and plasma tissue and its effect is dose dependent (Jackson *et al*., 2008). Thus, it was observed in this study, there was a possibility of liver tissue damage because of the injection of dexamethasone. Feeding dill powder could mitigate this effect.

Table 10 Effects of dill powder and dexamethasone on enzyme levels in liver of broiler chickens

Experimental groups			35 days old			42 days old		
	Stress	Dill, %	ALT, mg/dl	AST, mg/dl	ALP, mg/dl	ALT, mg/dl	AST, mg/dl	ALP, mg/dl
G1	No	0.0	2.9^{c}	243^{d}	4148^{c}	3.1^{b}	285^{b}	3187^{c}
G2	No	1.5	3.8^{b}	264^{b}	4037^{d}	3.2^{b}	290^{a}	3223^{b}
G3	Yes	1.5	4.0^{a}	250^{c}	4183^{b}	3.0^{c}	288^{b}	3153^{d}
G4	No	3.0	2.5^{d}	273^{a}	3038^{d}	3.5^{a}	294^{a}	3262^{a}
G5	Yes	3.0	3.7^{b}	262^{b}	4261^{a}	3.1^{b}	286^{b}	3222^{b}
G6	Yes	0.0	2.2^{e}	244^{d}	4020^{e}	3.1^{b}	283^{b}	3115^{e}
Standard error								
Stress			0.036	1.6	5.9	0.06	1.3	8.1
Dill			0.21	1.0	3.4	0.03	1.0	4.7
Stress x dill			0.26	1.1	4.1	0.04	1.1	5.7
P-value								
Stress			<0.001	<0.001	<0.001	0.003	0.001	<0.001
Dill			<0.001	<0.001	<0.001	0.006	<0.001	<0.001
Stress x dill			<0.001	0.003	<0.001	0.010	0.055	0.030

ALT: alanine aminotransferase. AST: aspartate aminotransferase, ALP: alkaline phosphatase
[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability P <0.05

The highest level (P <0.01) of serum total protein was seen in G6 (Table 11). Generally the imposition of stress increased serum protein levels (P <0.01). It was shown that an increase in total protein in broilers treated with corticosteroids was associated with a reduction in BW and weights of the immune system organs (Kang *et al.*, 2020). Glucocorticoids and their synthetic compounds, such as dexamethasone, inhibit skeletal muscle growth and increase protein catabolism in broilers (Kang *et al*., 2020). In addition, an increase in total protein is because of increased gluconeogenesis of degraded proteins that are activated by glucocorticoids (Kang *et al.*, 2020). The use of 2% *Coriandrum sativum* seeds increased total serum protein significantly (Asadi Firoozabadi & Taherpour, 2014). Also, concentration of albumin in groups with 2% and 3% *Coriandrum sativum* seeds was about 7.32% and 6.11% more (P <0.05) (Farah, 2011).

The levels of monocytes and basophils were not affected by the interaction of induced stress with the level of dill supplementation (P >0.05). However, the levels of eosinophils, lymphocytes and heterophils, as well as the haemoglobin content and haematocrit were affected by this interaction (Tables 12 and 13). The highest levels of lymphocytes were observed in G2 at day 35 and in G4 at day 42. The percentage of heterophils was greatest in G6 (P <0.01). On day 42, the haematocrit and haemoglobin contents were lowest in G4 and highest in G3 (P < 0.05). Ghazanfari & Mohammadi (2015) found no significant effects on the complete blood count attributable to the level of *Coriandrum sativum* essential oil in the diet of broilers (P <0.05). However, the immune system function may be improved through the use of medicinal herbs (Lavinia *et al*., 2009; Mousa-Ali, 2016; Mohammadi, 2020).

Table 11 Effects of dill powder and dexamethasone on serum proteins of broiler chickens

Experimental groups			35 days old			42 days old		
	Stress	Dill, %	Total protein, mg/dl	Albumin, mg/dl	Globulin, mg.dl	Total protein, mg/dl	Albumin, mg/dl	Globulin, mg/dl
G1	No	0.0	3.5[b]	1.74[c]	1.78[d]	4.12[c]	1.6 [b]	2.5 [b]
G2	No	1.5	3.2[b]	1.64[d]	1.63[e]	4.00[c]	1.5[b]	2.5[b]
G3	Yes	1.5	4.3[a]	2.00[a]	2.34[b]	4.57[b]	2.2[a]	2.4[b]
G4	No	3.0	3.0[b]	1.28[e]	1.63[e]	3.93[c]	1.5[b]	2.4[b]
G5	Yes	3.0	4.1[a]	1.82[b]	2.25[c]	4.45[b]	1.9[a]	2.5[b]
G6	Yes	0.0	4.2[a]	1.69[d]	2.54[a]	4.80[a]	2.0[a]	2.8[a]
Standard error								
Stress			0.09	0.054	0.039	0.031	0.02	0.03
Dill			0.07	0.031	0.029	0.017	0.01	0.02
Stress x dill			0.08	0.038	0.032	0.021	0.01	0.02
P-value								
Stress			<0.001	<0.001	<0.001	<0.001	<0.001	0.003
Dill			<0.001	<0.001	<0.001	0.015	0.063	<0.001
Stress x dill			0.002	<0.001	0.033	0.845	0.012	<0.001

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

Table 12 Effects of dill powder and dexamethasone on blood cell percentages of 35-day-old broiler chickens

Experimental groups			Eosinophil	Monocyte	Lymphocyte	Hetrophil	Basophil	Hemoglobin	Hematocrit
	Stress	Dill, %							
G1	No	0.0	2.00[b]	1.39	61.00[c]	35.06[b]	0.55	11.15[b]	33.6[b]
G2	No	1.5	2.00[b]	1.39	64.30[a]	31.98[f]	0.33	11.10[b]	33.0[b]
G3	Yes	1.5	2.00[b]	2.00	62.00[b]	34.00[e]	0.00	10.80[c]	32.3[c]
G4	No	3.0	2.00[b]	2.00	61.30[c]	34.37[c]	0.33	11.20[b]	32.0[c]
G5	Yes	3.0	3.00[a]	1.72	61.00[c]	33.62[d]	0.66	11.70[a]	34.6[a]
G6	Yes	0.0	2.00[b]	1.39	59.00[d]	37.28[a]	0.33	10.20[d]	33.3[b]
Standard error									
	Stress		0.13	0.32	0.56	0.32	0.27	0.13	0.24
	Dill		0.07	0.21	0.32	0.21	0.15	0.10	0.14
	Stress x dill		0.09	0.24	0.39	0.24	0.19	0.11	0.17
P-value									
	Stress		0.002	0.386	0.022	0.045	0.622	0.015	0.018
	Dill		0.011	0.468	<0.001	<0.001	0.391	0.021	0.014
	Stress x dill		0.011	0.468	0.042	0.001	0.391	<0.001	<0.001

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

Table 13 Effects of dill powder and dexamethasone on blood cell percentages in 42-day-old broiler chickens

Experimental groups			Eosinophil	Monocyte	Lymphocyte	Hetrophil	Basophil	Haemoglobin	Haematocrit
	Stress	Dill, %							
G1	No	0.0	3.00[a]	1.30	61.60[d]	33.77[b]	0.33	11.4[c]	34.3[b]
G2	No	1.5	2.00[b]	1.30	64.00[b]	32.04[c]	0.66	11.6[b]	35.3[a]
G3	Yes	1.5	2.00[b]	2.00	61.30[d]	34.37[a]	0.33	11.7[a]	36.0[a]
G4	No	3.0	3.00[a]	1.30	65.30[a]	30.07[d]	0.33	10.2[d]	32.3[c]
G5	Yes	3.0	2.00[b]	2.00	61.60[d]	33.74[b]	0.66	11.2[c]	33.6[b]
G6	Yes	0.0	1.40[c]	1.00	62.60[c]	34.00[a]	1.00	11.3[c]	34.0[b]
Standard error									
Stress			0.15	0.23	0.30	0.19	0.30	0.10	0.27
Dill			0.11	0.13	0.17	0.11	0.17	0.05	0.15
Stress x dill			0.12	0.16	0.21	0.14	0.21	0.07	0.19
P-value									
Stress			<0.001	0.111	<0.001	<0.001	0.390	0.001	0.030
Dill			0.010	0.091	0.004	<0.001	0.822	<0.001	<0.001
Stress x dill			<0.001	0.091	<0.001	0.010	0.287	0.001	0.033

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

On days 35 and 42, G4 and G5 had significantly reduced levels of cecal Salmonella, *Escherichia coli* and coliform (Table 14). On day 42, G3 also showed a reduced level of bacterial colonization in the cecum (*P* <0.01). Essential oils induce the death of pathogenic bacteria because they are hydrophobic. When they reach the bacterial cell wall they damage its structure, allowing cellular contents to escape into the intercellular space, leading to cell death (Mousa-Ali, 2016; Mohammadi, 2020). Because the microbial population in the gastrointestinal tract is reduced, the rate of degradation of proteins and amino acids is also reduced and more of the nutrients are absorbed (Mohammadi *et al*., 2021). The increased availability of amino acids improves the carcass percentage, reducing the conversion of protein to lipid and lowering the amount of lipid accumulation. In the current study, the use of dill powder as an antibacterial growth promoter improved the health of the broiler digestive tract under the stressful conditions caused by dexamethasone, leading to increased growth and enhanced feed conversion.

Unstressed broilers produced greater antibody titers in response to challenges with Newcastle disease and avian influenza virus than stressed broilers (*P* <0.01) (Table 15). Flavonoid compounds that are present in dill are potent antioxidants that improve the immune system of the birds (Dehpour *et al*., 2009; Kavoosi & Rowshan, 2013). Ghalamkari *et al*. (2011) showed that Satureja powder had no effect on Newcastle virus antibody titer (*P* <0.05). However, Newcastle and influenza titers increased when birds were provided with garlic and cinnamon powders simultaneously (*P* <0.05) (Mohammadi *et al*., 2021).

Table 14 Effects of dill powder and dexamethasone on bacterial population in cecum of broiler chickens (log_{10})

Experimental groups			35 days old			42 days old		
	Stress	Dill, %	E.coli, log_{10}	Coliform, log_{10}	Salmonella, log_{10}	E.coli, log_{10}	Coliform, log_{10}	Salmonella, log_{10}
G1	No	0.0	10.58^{a}	10.27^{a}	10.54^{b}	10.90^{a}	10.56^{b}	10.84^{a}
G2	No	1.5	10.66^{a}	10.51^{a}	10.48^{b}	10.74^{b}	10.74^{a}	10.45^{b}
G3	Yes	1.5	10.34^{a}	10.41^{a}	10.69^{b}	10.37^{c}	10.31^{d}	10.81^{a}
G4	No	3.0	9.80^{b}	8.97^{c}	9.04^{d}	10.48^{c}	10.36^{d}	10.16^{c}
G5	Yes	3.0	9.60^{b}	9.95^{b}	10.14^{c}	10.63^{b}	10.57^{c}	10.49^{b}
G6	Yes	0.0	10.32^{a}	10.2^{a}	10.34^{b}	10.79^{b}	10.76^{a}	10.79^{a}
Standard error								
	Stress		0.191	0.079	0.114	0.055	0.053	0.047
	Dill		0.110	0.048	0.065	0.043	0.033	0.027
	Stress x dill		0.135	0.058	0.080	0.046	0.039	0.033
P-value								
	Stress		0.125	0.001	0.002	0.008	0.875	<0.001
	Dill		0.002	<0.001	<0.001	<0.001	0.008	<0.001
	Stress x dill		0.952	<0.001	<0.001	<0.001	<0.001	0.002

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

Table 15 Effects of level of dill powder and dexamethasone on antibody titers against Newcastle disease and avian influenza in broiler chickens

Experimental groups		35 days old		42 days old	
Stress	Dill, %	Avian influenza. log_2	Newcastle disease, log_2	Avian Influenza, log_2	Newcastle disease, log_2
No	0.0	6.00^{a}	2.33^{a}	6.00^{a}	3.00^{b}
No	1.5	5.00^{b}	1.66^{b}	5.00^{b}	4.00^{a}
Yes	1.5	5.00^{b}	2.00^{b}	4.00^{c}	2.00^{c}
No	3.0	5.33^{b}	1.66^{b}	6.00^{a}	4.66^{a}
Yes	3.0	5.33^{b}	2.33^{a}	5.00^{b}	2.00^{c}
Yes	0.0	5.66^{b}	1.33^{b}	3.00^{d}	1.00^{d}
Standard error					
	Stress	0.19	0.07	0.57	0.27
	Dill	0.11	0.04	0.33	0.15
	Stress x dill	0.13	0.05	0.40	0.19
P-value					
	Stress	0.777	1.000	<0.001	<0.001
	Dill	0.245	0.088	<0.001	0.001
	Stress x dill	0.920	0.113	<0.001	0.397

[a,b,c,d,e,f] Within a column, means with a common superscript were not different with probability *P* <0.05

Conclusions

Adding dill powder to the diet of broiler chickens mitigated the damaging effects of dexamethasone-induced stress. Therefore, dietary supplementation with dill powder could be recommended to reduce the

effects of stress in poultry.

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Authors' Contributions

AJ and MH (https://orcid.org/0000-0002-1649-280X) established the experimental shed, arranged materials, and performed the methodology. AJ and MH contributed to sample collection, and captured data. MM (https://orcid.org/0000-0002-8067-4739) and SKH (https://orcid.org/0000-0001-8383-9546) analysed the data, and MH wrote the manuscript.

Conflict of Interest Declaration

Authors have no conflict of interests to declare.

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