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EFFECT OF MEDICINAL PLANTS AND VITAMIN E ON PRODUCTIVE PERFORMANCE, SOME PHYSIOLOGICAL, IMMUNOLOGICAL PARAMETERS AND ANTIOXIDANT STATUS OF BROILER UNDER COLD STRESS

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ABSTRACT

This study was conducted to clarify the effect of adding different levels of medicinal plants and vitamin E supplements on productive performance, serum physiological, immunological parameters, and antioxidant status of broiler under cold stress 15 ˚C±2 for 35 days. A total of 600 Ross one-day-old broiler chicks were distributed randomly into eight treatments with five replicates for each (15 birds/replicate) as follows: T1= standard ration (negative control); T2= 50 mg vitamin E /kg ration (positive control); T3= 5g Dill powder /kg ration; T4= 10g Dill powder/kg ration; T5= 5g Adiantum powder /kg ration; T6= 10g Adiantum powder /kg ration; T7=5g Crataegus powder /kg ration and T8=10g Crataegus powder /kg ration. Results showed that adding medicinal plants had a significant effect ($p<0.05$) on live body weight, weight gain, feed conversion ratio, mortality percentage, and production index, as well as a significant effect on serum protein, lipid profile, T3, T4, corticosterone hormone, significantly lower malondialdehyde (MDA) stimulator oxidative enzymes and higher the concentration of antioxidant enzymes total antioxidant capacity (TAC) in all additive treatments compared with control groups, all helped to reduce oxidative stress as well as boost antibody titers of ND and IBD when compared with negative and positive control.

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INTRODUCTION

Animals face a variety of environmental stressors every day. Cold stress occurs when the surrounding temperature decreases below 18 °C. During winter months, the overall ambient temperature ranges from -10 to +5 °C in many regions of the world. Because birds are homoeothermic animals, they can only thrive in a limited range of thermoneutral environments. The birds are homoeothermic that can live comfortably only in a relatively narrow zone of thermoneutrality as well as sensitive to stress. There are several stressors, such as cold stress, heat stress, high stocking density, and, diseases that can affect birds and cause several deleterious changes. Stress decreases feed consumption growth, and changes carcass characteristics and causing economic losses. These adverse effects are accompanied by a decline in meat quality, animal welfare and immunological function (Kang *et al*., 2020). Animal welfare and health will be negatively impacted by cold stress, resulting in decreased production performance, immune imbalance and decreased antioxidant capacity.

Consequently, the development of ways to establish adaptability to low temperatures is a main task in animal production. Poultry is able to create cold acclimation through endocrine, metabolic, autonomic regulatory and behavioral systems when they are repeatedly exposed to cold temperatures (Li *et al*., 2017 ; Liu *et al*., 2020). Besides, the use of medicinal herbs as supplements for chicken feed and even disease treatment is deemed low-cost and non-hazardous with no side effects or environmental consequences (Sevim and Ayaşan, 2020). In addition, herbs and herbal products are used in poultry diets as a replacement for synthetic products in order to promote the effective use of feed nutrients, which may ultimately result in more rapid body weight gain, higher production rates, and improved feed efficiency. Furthermore, active components of herbs may improve digestion and stimulate immune function in broilers (Ghazalah and Ali, 2008). Herbal extracts are the most common natural feed additives used for improving poultry and animal production (Alagawany *et al* ., 2021; Abou-Kassem *et al*., 2022 ; and Arif *et al*., 2022). Numerous phytogenic poultry feed additives for instance aromatic herbs or fragrance oils, have also been researched in recent decades (Abd Elkader *et al*., 2021 ; Reda *et al*., 2021).

Because of rising concerns about food contamination and the side effects of antibiotic overuse, more people are turning to medicinal plants as natural treatment options. Compared to antibiotics, herbs are less expensive, more widely available, and leave no hazardous residue in animal products. Improved performance, increased immune cell proliferation, relief from the intestinal challenge, decreased oxidative stress, reduced mortality and elevated antibody titers are just some of the many biological activities performed by phytochemicals (such as flavonoids, phenols, saponins, alkaloids, etc.) in animals (Oluwafemi *et al*., 2020). Therefore, to deal with the problems caused by cold stress on poultry the use of medical plants as natural antioxidant and vitamin E as an artificial antioxidant has been considered (Ameen., *et al*., 2023).

As a result, this study aimed to investigate the effects of different levels of some medical plants and vitamin E on some physiological parameters, immunity and antioxidant status of broiler chicks under cold stress. Consequently, these medical plants were added to the diets to recognize the attitude of broiler chickens with this condition.

MATERIALS AND METHODS

Ethical approve

The experiment was approved by the Animal Ethics Committee of the College of Agricultural Engineering Sciences, animal production department (Approval No: AEC 1922022).

Experimental animals

The present study was carried out in January 2022, in Akre commercial poultry project, Kurdistan Region-Iraq. The experiment was performed on day-old of 600 Ross chicks, randomly distributed into 8 treatments with 5 replicates and each replicate consisted of 15 birds reared for 35 days under cold stress. The chicks were

brought from a commercial hatchery (Eiteemad Hatchery) in Bardarash region and kept in cages (1.5m x 1m x 1m) width, length and high respectively with separate doors for each cage. The ration was formulated to meet the nutrient requirements of chicks according to (NRC, 1994).

The birds were fed with basal diet without any plant supplement in T1 (negative control), 50 mg/kg diet vitamin E in T2 (positive control), 5 g/kg diet Dill in T3, 10 g/kg diet Dill in T4, 5 g/kg diet Adiantum in T5, 10 g/kg diet Adiantum in T6, 5 g/kg diet Crataegus in T7 and 10 g/kg diet Crataegus in T8 added to standard diet as powder. Forty litter cages had one feeder and one drinker in each replicate; feed and drinking water were given *ad libitum* and three phases of feeding were adopted as shown in Table (1). The house was controlled in temperature and humidity by a thermostat and all treatments have had the same conditions. From 14 d until 35 d of age, temperature-simulated cyclic cold stress (CS): was maintained at 15 ± 2°C for 6 h from 10: am. to 4: pm. and then was slowly reduced until reaching the comfort temperature (21–24°C) at 35 days of age. Lighting was provided 23 hours per day with 1hour of darkness, apart from d 1 to 7 when 23 hours of lighting were provided for according to the Rose 308 broiler management handbook, 2018. During 35 days of the experiment data of productive performance was collected: live body weight LBW(g), Body weight gain BWG (g), Feed intake FI (g/bird), Feed conversion ratio FCR (g feed/ g weight), Mortality% and production index (PI) had been recorded weekly. On the last day (35days) of the experiment, two birds male and female from each replicates of all treatments were randomly chosen and weighed, then four ml of blood from the wing vein (brachial vein) collected in a tube without anticoagulants to obtain serum.

The collected blood samples were centrifuged at 3000 cycle /minute for 20 minutes and putting a sticker with markings on the chicken legs to differentiate between male and female after slaughter. Then serum biochemical tests were taken: glucose (mg/dL), cholesterol (mg/dL) by Cobas Integrata400 plus and triglyceride (mg/dL), total protein, albumin and globulin as well as thyroxine T4 (ng/mL) and triiodothyronine T3 (ng/mL) by Cobas e411. Furthermore, corticosterone hormone concentration in plasma was determined using ELISA according to the instructions of the kit included in the Buyer's Guide for Life Science Boi-compare. Moreover, immunity tests against Newcastle Disease ND antibody titers and Infectious Bronchitis Virus IBV antibody titers. Besides, antioxidant status (TAC) and (MDA) were determined by ELISA according to the instructions of the kit included in the Enzo Life Science.

Statistical analysis

Data were subjected to one- way analysis of variance in completely randomized design (C.R.D.) by using SPSS program (2015). Then by using Duncan's multiple range test at probability ($P<0.05$) means were checked for statistical differences in different groups.

Table (1): Broiler (Ross 308) diet Ingredients and chemical compositions.

Ingredients	Starter (1-14d)	Grower (15-21d)	Finisher (22- 35d)
Wheat	65	66	69
Soya bean meal	27	25	23
Sun flower oil	3.0	3.5	3.8
Limestone	1.2	1.2	1.0
Premix	3.6	3.7	2
Antitoxin	0.2	0.6	1.2
Total	100	100	100
CalculatedNutrient Composition	Starter	Grower	Finisher
Metabolic energy kcal/kg	2900	3000	3100
Crud protein %	23.35	22.50	21.88
Fiber %	2.31	2.49	2.59
Ash%	5.48	5.66	5.88
Fat %	3.89	4.04	4.12

*Each 1 Kg contains: Vitamin A 400000 IE, vitamin B6 40mg, Vitamin D3 80000 IE, Cholin chloride 1000 mg, vitamin E (acetate) 1000 IE, Cholecalciferol 2.000 mg, Vitamin K3 60 mg, Fe: Fe (II) sulf. 2000 mg, Vitamin B130 mg, Cu: CuSO4.5H2O 200 mg, vitamin B2 200 mg, Zn: ZnOxyde 1600 mg, Pantothenic acid 300 mg, Mn: Mn (II) oxide 2400 mg, Niacin 1200mg, Co: Co (II) carbon 6 mg, Biotin 2000 mcg, I: Ca – Iodate 30 mg, Vitamin B12 600 mcg, Se: Na – selenite 4 mg and Folic acid 20 mg.

Quantitative detection of the most important active compounds presents in (Dill, Adiantum and Crataegus) leaf extract powder

Aquantitative detection of the most important active substances found in **(Dill, Adiantum and Crataegus**) leaf powder was carried out in the Ministry of Science and Technology laboratories in Baghdad/ Iraq, according to (Tofighi *et al*.,2016). The active compounds of the three plants were analyzed by Chromatography HPLC (SYKAMN HPLC CHROMATOGRAPHIC SYSTEM) and the results as follows in tables (2), (3) and (4).

Table (2): Active compounds of Dill extract powder.

No	**Total content of active compounds**	Concentration
1	Total phenolic content (mg / 100 gm)	451.25
2	Total flavonoid content (mg / 100gm)	298.58
3	Total alkaloid content %	33.21
4	Total glycoside content %	16.58
5	Total saponins content %	6.25
6	Total carotene content %	3.65
No	**Active compound**	Concentration
1	Quercetin (mg / kg)	48.5
2	Carvone (mg / kg)	135.59
3	Limonene (mg / kg)	174.5
4	Thymol (mg / kg)	39.15

Table (3): Active compounds of Adiantum extract powder.

No	**Total content of active compounds**	Concentration
1	Total phenolic content (mg / 100 gm)	312.0
2	Total flavonoid content (mg / 100gm)	244.5
3	Total alkaloid content %	18.9
4	Total glycoside content %	22.5
5	Total saponins content %	4.5
6	Total carotene content %	12.0
No	**Active compound**	Concentration
1	Quercetin (mg / kg)	45.8
2	Rutin (mg / kg)	15.3
3	Luteolin (mg / kg)	20.6
4	Kaempferol (mg / kg)	29.5

Table (4): Active compounds of Crataegus extract powder.

No	**Total content of active compounds**	Concentration
1	Total phenolic content (mg / 100 gm)	264.1
2	Total flavonoid content (mg / 100gm)	188.5
3	Total alkaloid content %	15.9
4	Total glycoside content %	6.9
5	Total saponins content %	3.5
6	Total carotene content %	6.2
No	**Active compound**	Concentration
1	Quercetin (mg / kg)	41.2
2	Kaempferol (mg / kg)	16.8
3	Thymol (mg / kg)	112.2
4	Limonene (mg / kg)	93.5

RESULTS AND DISCUSSION

Tables (5 and 6) showed that the inclusion of different levels of Dill, Adiantum, Crataegus, vitamin E on live body weight and body weight gain were found significant differences at 14-35 days, these findings are supported by the findings of Tayeb *et al*. (2019) who found a significant effect of medicinal plants on live body weight and weight gain in a group containing 3 g/kg of Adiantum for 42 days. As it is known cold stress significantly reduces the growth performance of broiler chickens (Zhou *et al*., 2021) and has a negative effect on production performance (Gong *et al*., 2023). It is evident in this experiment when adding medicinal plants, especially Adiantum had a positive effect on growth performance.

The significant improvement of the final live body and weight gain of broiler treatment fed Adiantum with 10 g/kg feed might be due to the fact that Adiantum had six flavonoid compounds: kaempferol, luteolin, quercetin and rutin were identified (AL-Khesraji *et al*., 2017) and these compounds may be the reason for appetite and thus increased feed consumption which led to take excess energy from the diet and spend excess energy for growth which let to improvement in body weight gain.

Table (5): Effect of adding medicinal plants and vitamin E on live body weight (g).

Days / Treatment	Live Body weight (g)				
	7 days	14 days	21 days	28 days	35 days
T1	141.74a ±0.44	304.61e ±1.18	546.31f ±0.89	877.79f ±4.55	1419.64d ±6.12
T2	142.09a ±0.16	305.46de ±1.41	575.99e ±1.56	936.17e ±1.81	1397.69d ±16.38
T3	142.19a ±0.58	308.09de ±1.84	586.41d ±0.64	933.77e ±1.35	1480.97c ±31.83
T4	139.15a ±1.51	313.12cd ±2.54	590.82c ±1.09	960.34d ±2.04	1538.98b ±18.51
T5	143.43a ±2.06	313.12cd ±2.42	593.55c ±0.90	959.84d ±2.68	1553.47b ±10.31
T6	140.43a ±0.73	341.23a ±1.40	617.36a ±0.74	1059.46a ±1.02	1650.22a ±4.16
T7	136.98a ±3.83	317.81bc ±5.45	607.33b ±1.14	987.93c ±1.91	1547.62b ±14.67
T8	139.60a ±1.91	323.89b ±2.38	609.56b ±2.46	1002.15b ±11.76	1556.96b ±15.50
Sig.	N.S	0.05	0.05	0.05	0.05

*a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S=non- significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (6): Effect of adding medicinal plants and vitamin E on body weight gain (g).

Days / Treat.	Body weight gain (g)					
	7 days	14 days	21 days	28 days	35 days	1-35 days
T1	97.74a ±0.44	162.86e ±1.42	241.69e ±1.30	331.48f ±4.93	541.84a ±3.07	1375.64d ±6.12
T2	89.09a ±0.16	163.36e ±1.9	270.53d ±2.49	360.18de ±3.17	461.52b ±17.19	1353.69d ±16.38
T3	98.19a ±0.58	165.90e ±1.90	278.32bcd ±1.32	347.35e ±0.96	547.20a ±30.60	1436.97c ±31.83
T4	95.15a ±1.51	174.53cd ±1.87	277.14cd ±2.30	369.52cd ±2.60	578.63a ±16.84	1494.98b ±18.51
T5	99.43a ±2.06	169.65de ±3.36	280.42bc ±1.64	366.29cd ±2.62	593.62a ±9.29	1509.47b ±10.31
T6	96.43a ±0.73	200.80a ±2.03	276.12cd ±1.31	442.09a ±0.54	590.76a ±3.69	1606.22a ±4.16
T7	92.98a ±3.83	180.82bc ±2.60	289.52a ±5.15	380.59bc ±2.20	559.68a ±16.42	1503.62b ±14.67
T8	95.60a ±1.91	184.29b ±2.79	285.66ab ±3.35	392.59b ±13.12	554.51a ±22.39	1512.96b ±15.50
Sig.	N.S	0.05	0.05	0.05	0.05	0.05

*a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non- significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Moreover, the active compounds in Adiantum as shown in Table 3 such as luteolin and quercetin may be increase appetizing and increase feed consumption as well as rise in gut enzymes and HCL activities and bile in the small intestine and increase of nutrient absorption and finally increase live body weight.

Tables (7 and 8) showed that total feed intake (FI) and feed conversion ratio under cold stress showed no significant differences (P<0.05) on both of them at 7 days. However, when feeding medicinal plants and vitamin E at 14 days and exposed to cold stress significant differences (P<0.05) were found in feed intake at14-35days and feed conversion ratio at 21-35 days compared with T1and T2. The current results agreed with Tayeb *et al*., (2019) who reported that the groups (Adiantum 3 g/kg) have a considerably better feed conversion ratio than the control group and improved feed intake. The improvement of feed intake and feed conversion ratio may be due to the phenolics and terpenoids (2.73 %) (Al-Snafi, 2015) of leaves and stem of Adiantum, which activates appetite mechanisms in poultry and contributes to balancing the gastrointestinal microflora system (Goliomytis *et al*., 2014) thus improved feed conversion ratio. Additionally, Adiantum contains fiber as (67.23 %). Fiber can avoid digestion and absorption in the small intestine which makes it able to affect the way other nutrients are absorbed and metabolized in the gastrointestinal tract. When birds are exposed to cold stress, they gather around each other, which leads to cannibalism, while the presence of fibers in medicinal plants reduces it and then improving the feed conversion ratio (Ibrahim *et al*., 2011).

Table (7): Effect of adding medicinal plants and vitamin E on feed intake (g).

Days / Treat.	7 days	14 days	21 days	28 days	35 days	1-35 days
T1	103.11a ±1.03	190.84d ±1.65	354.01 bc ±1.78	536.22d ±9.78	956.40a ±6.58	2140.60bc ±12.55
T2	103.44a ±0.86	191.35d ±2.01	381.21a ±4.88	573.92b ±5.60	809.16b ±31.41	2059.09c ±28.81
T3	101.95a ±1.23	195.52d ±2.95	381.96a ±2.03	547.41cd ±0.77	950.18a ±53.60	2177.04ab ±54.21
T4	99.99a ±1.88	205.35bc ±2.84	379.00a ±15.22	566.09bc ±4.48	958.36a ±27.53	2208.81ab ±26.55
T5	103.07a ±1.12	199.78cd ±4.95	372.75ab ±3.41	554.17bcd ±4.30	998.96a ±15.52	2228.74ab ±17.32
T6	101.63a ±1.77	228.96a ±2.05	340.68c ±2.64	626.83a ±2.61	966.91a ±14.83	2265.04a ±17.25
T7	97.19a ±3.93	209.44b ±3.46	371.32ab ±6.88	560.05bcd ±2.69	937.95a ±26.07	2175.97ab ±24.86
T8	102.65a ±2.87	214.14b ±3.13	361.19ab ±3.85	570.56bc ±18.79	946.34b ±38.19	2194.90ab ±26.40
Sig.	N.S	0.05	0.05	0.05	0.05	0.05

*a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (8): Effect of adding medicinal plants and vitamin E on feed conversion ratio (g feed/g weight gain).

Days / Treat.	Feed conversion ration					
	7 days	14 days	21 days	28 days	35 days	1-35 days
T1	1.06a ±0.01	1.17a ±0.01	1.46a ±0.03a	1.62a ±0.01a	1.77a ±0.02a	1.56a ±0.02a
T2	1.05a ±0.01	1.17a ±0.01	1.41b ±0.01b	1.59b ±0.03b	1.75ab ±0.04ab	1.52b ±0.03b
T3	1.04a ±0.01	1.18a ±0.01	1.37bc ±0.01bc	1.58c ±0.03c	1.74b ±0.01b	1.51b ±0.01b
T4	1.05a ±0.01	1.18a ±0.01	1.37bc ±0.05bc	1.53d ±0.01d	1.66e ±0.01e	1.48c ±0.01c
T5	1.04a ±0.01	1.18a ±0.03	1.33cd ±0.01cd	1.51e ±0.01e	1.68d ±0.01d	1.48c ±0.01c
T6	1.05a ±0.16	1.14a ±0.02	1.23e ±0.01e	1.42h ±0.01h	1.64f ±0.02f	1.41e ±0.01e
T7	1.05a ±0.01	1.16a ±0.01	1.28de ±0.01de	1.47f ±0.03f	1.68de ±0.04de	1.45d ±0.02d
T8	1.07a ±0.015	1.16a ±0.010	1.26e ±0.04e	1.45g ±0.04g	1.71c ±0.01c	1.45d ±0.01d
Sig.	N.S	N.S	0.05	0.05	0.05	0.05

* a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (9) presented that the mortality percentages were significantly (P<0.05) lower in the treatments of medicinal plants and vitamin E in T6, T8, T3, T4, T7 and T2, T5 compared with T1, the higher mortality percentages were recorded in T1 (24.00) without supplemented medicinal plants compared with other treatments and the lower mortality percentages were in T6 and T8 (2.67). The best value of PI was significantly (p<0.05) higher in T6, T7, T7, T8, T4, T5, T3 and T2 respectively compared with the negative control (T1). Production index achieved high values in all treatments of medicinal plants and vitamin E as compared with control without supplementation T1. The current results disagreed with Alkado *et al*., (2022) who found medicinal plant as Chamomile was non- significant effect on the percentage of mortality % under heat stress. The higher values of production index due to lower mortality percentages and body weight gain which led to higher income production index (PI). In addition, the flavonoids and quercetin found in Adiantum have shown antioxidant effects that benefit laying chickens, especially in stressful situations. They enhance the chickens' defense systems, while also reducing lipid peroxidation levels. This could potentially support the chickens' health and productivity, particularly when they face stress (Iskender *et al*., 2016).

Table (9): Effect of adding medicinal plants and vitamin E on mortality and production index at 35 days.

Traits / Treatment	Mortality rate	Production index
T1	24.00a ±13.09a	198.24c ±34.18c
T2	5.33b ±3.26b	248.44bc ±8.31bc
T3	4.00b ±1.63b	268.39bc ±9.11bc
T4	4.00b ±1.63b	285.74abc ±6.55abc
T5	5.33b ±1.33b	284.52bc ±3.21abc
T6	2.67b ±1.63b	325.49a ±5.81a
T7	4.00b ±1.63b	293.44ab ±6.90ab
T8	2.67b ±2.10b	286.12abc ±6.10abc
Sig.	0.05	0.05

*a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adaintum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (10) focused on effect of adding medicinal plants and vitamin E on blood serum thyroid hormones under cold stress conditions. The hormones data revealed that there were significant differences (P<0.05) between treatments addition compared with T1 on triiodothyronine hormone of both male and female of broiler, concerning related with thyroxine hormone no significant differences were found in male broiler. However, significant differences were found in female broiler compared with T1 except T5 found no significant differences compared with T1. The higher concentration of Thyroxine hormone of female broiler was in T6 (supplement Adiantum 10 mg/kg feed) because the supplementation of Adiantum was stimulate oropharyngeal receptors which are directly linked to the appetite center located in the hypothalamus and may be induce the secretion of thyroxin and triiodothyronine from thyroid gland that enhance protein, carbohydrate and fat metabolism (Scans, 2015). Concerning stress hormone (corticosterone) significant differences (P<0.05) were found in treatments addition in male and female broiler compared with T1. Generally, corticosterone hormone concentration increase during cold stress and decrease with diet supplemented with medicinal plants and vitamin E. The reason for significant differences due to medicinal plants stimulate the release of (T3, T4) hormones and lower corticosterone levels in chicken blood and may operate as an anti-stressor to modulate thyroid action (Mokhtari *et al*., 2018). The current findings were similar to those reported by Tayeb *et al*. (2019) who discovered that varies amounts of thyme, rosemary, adiantum and their combination significantly affect hormonal response.

Table (10): Effect of adding medicinal plants and vitamin E on thyroid hormones (T3 and T4) and corticosterone in male and female broiler at 35 days.

Traits / Treatment	T3 (ng/ml)		T4(ng/ml)		Corticosterone (ng/ml)	
	Male	Female	Male	Female	Male	Female
T1	1.32b ±0.06	1.27b ±0.03	3.29a ±0.15	3.21b ±0.11	19.38a ±0.03	19.32a ±0.08
T2	1.44a ±0.02	1.46a ±0.02	3.34a ±0.07	3.24ab ±0.09	17.71b ±0.50	17.64b ±0.51
T3	1.45a ±0.02	1.43a ±0.03	3.31a ±0.02	3.27ab ±0.02	17.38b ±0.13	17.24b ±0.07
T4	1.43a ±0.01	1.44a ±0.01	3.36a ±0.03	3.34ab ±0.05	16.34c ±0.20	16.11c ±0.21
T5	1.44a ±0.01	1.43a ±0.02	3.36a ±0.05	3.19b ±0.05	14.10de ±0.53	14.04d ±0.52
T6	1.45a ±0.12	1.47a ±0.02	3.41a ±0.02	3.57a ±0.19	13.26e ±0.05	13.15e ±0.05
T7	1.44a ±0.01	1.42a ±0.18	3.34a ±0.02	3.24ab ±0.06	14.35d ±0.30	14.13d ±0.18
T8	1.45a ±0.01	1.44a ±0.02	3.35a ±0.01	3.36ab ±0.13	14.91d ±0.29	14.76d ±0.22
Sig.	0.05	0.05	N.S	0.05	0.05	0.05

*a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (11) revealed a significantly (P<0.05) higher value of albumin concentration in blood serum in all treatments of supplementation in the treatments T4, T8, T5, T7 and T6 of male broiler serum compared with the control T1, while T2, T3 no significant effect on T1. Likely, recorded significant increase in all treatments with supplemented herbal compared with T1 under cold stress of female broiler. In addition, serum globulin recorded a significant difference between all supplemented treatments compared with T1 of male and female broiler. Additionally, we found significant differences (P<0.05) on total protein values compared with T1 and T2.

The current results agreed with finding of Ahmadipour *et al*., (2017) chickens that received Crataegus extract in the drinking water at a level of 0.1 and 0.2 ml/L had a higher concentration of total protein, albumin and globulin than the control group (p<0.05). Additionally, Kamel and Hamed, (2021) discovered that feeding broiler chicks on different levels of Adiantum significantly increased (P<0.05) albumin and total protein compared to the control group. Conversely, Tayeb *et al*., (2019) noticed medicinal plant treatments had no significant effect on the total protein, albumin and globulin compared to control group. The improvement of biochemical parameters may be attributable to the biological action of medicinal plants which boost the immune response (El-Ghamry, 2004).

Table (11): Effect of adding medicinal plants and vitamin E on albumin, globulin, total protein in male and female broiler at 35 days.

Traits / Treat.	Albumin (g/dL)		Globulin(g/dL)		Total protein(g/dL)	
	Male	Female	Male	Female	Male	Female
T1	1.38c ±0.02	1.25c ±0.15	1.42b ±0.05	1.55b ±0.17	2.80c ±0.03	2.80d ±0.03
T2	1.44c ±0.01	1.32bc ±0.12	2.25a ±0.04	2.33a ±0.14	3.70ab ±0.04	3.65b ±0.04
T3	1.45c ±0.01	1.42abc ±0.01	2.07a ±0.13	2.07a ±0.13	3.52b ±0.13	3.49c ±0.13
T4	1.65a ±0.01	1.61a ±0.02	2.18a ±0.01	2.20a ±0.03	3.83a ±0.02	3.81a ±0.01
T5	1.60ab ±0.02	1.60a ±0.03	2.08a ±0.16	2.24a ±0.04	3.68ab ±0.14	3.84a ±0.02
T6	1.43c ±0.01	1.49ab ±0.04	2.29a ±0.16	2.33a ±0.04	3.73ab ±0.16	3.83a ±0.02
T7	1.55b ±0.05	1.51ab ±0.06	2.28a ±0.02	2.32a ±0.05	3.83a ±0.05	3.83a ±0.02
T8	1.63a ±0.02	1.62a ±0.03	2.15a ±0.05	2.16a ±0.05	3.78ab ±0.03	3.78ab ±0.03
Sig.	0.05	0.05	0.05	0.05	0.05	0.05

*a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (12) observes that total cholesterol concentration revealed significant differences (P<0.05) between herbal treatments compared with T1 and T2, the highest value in T1 male broiler followed by T7, T2, T3, T4, T7, T8, T5 and T6. Additionally, there were significant differences in T4, T5, T6, T7 and T8 with other treatments T1and T2 in female broiler, while T3 did not significantly differ with T1 and T2. Dietary supplementation of medicinal herbs decreased serum triglyceride levels in male and female birds reared under cold stress. Serum triglyceride in all herbal supplemented groups particularly T6 of both male and female were significantly different compared of T1 which had the highest triglyceride level in male and female broiler.

The current findings agreed with Tayeb *et al*., (2019) who declared medicinal plants was significantly affected on the cholesterol level by treated groups compared to control group as well as Abdul-Majeed *et al*., (2023) who found medicinal plants was a significant elevation in cholesterol and triglyceride compared with other groups. On the other hand, the present results are disagreed with finding of Torki *et al*., (2015) no significant effect of the experimental diets on the serum content of cholesterol and albumin of hens. Cold stress alters the concentration of serum cholesterol components such as high-density lipoprotein cholesterol and LDL, suggesting that it can accelerate the breakdown of fat and give the body energy while the biochemical parameters were improved when we supplemented with medicinal plants and this improvement may be attributable to the biological action of these herbs especially Adiantum which boost the immune response (El-Ghamry, 2004). In the

current results the low concentration of cholesterol in the blood plasma in the Adiantum treatments of both male and female when compared to the control T1and T2 may be related to the high percentages of fiber (67.23%) which leads to reduced absorption of diet cholesterol in the gastrointestinal tract (GI) (Al Snafi, 2015). The decrease in cholesterol levels in T6 containing Adiantum powder may be attributable to the hypocholesterolemic qualities attributed to the defatted section of the leaves containing rutin (Al-Snafi, 2015). Rutin exerted anticholesterolemic effects, lowering total cholesterol and triglycerides.

Table (12): Effect of adding medicinal plants and vitamin E on triglycerides and cholesterol in male and female broiler at 35 days.

Traits / Treatment	Triglycerides (mg/dL)		Cholesterol (mg/dL)	
	Male	Female	Male	Female
T1	85.50a ±0.85	84.98a ±0.51	135.61a ±0.21	134.58a ±0.47
T2	82.40a ±0.99	80.95a ±0.83	134.25a ±0.58	132.45a ±0.52
T3	81.92a ±0.99	81.11a ±0.44	128.52bc ±2.44	124.10b ±3.80
T4	72.95b ±1.54	69.81b ±1.31	127.02bc ±1.37	122.81b ±0.99
T5	62.04d ±1.73	61.19d ±1.19	126.28c ±0.71	125.48b ±2.14
T6	56.54e ±0.36	54.09e ±0.49	125.98bc ±1.11	124.16b ±0.87
T7	66.29cd ±0.99	64.74cd ±0.39	129.66b ±0.93	127.40b ±0.96
T8	68.44bc ±3.46	66.77bc ±3.15	127.46bc ±0.84	125.25b ±0.91
Sig.	0.05	0.05	0.05	0.05

* a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (13) indicates the influence of medicinal plants and vitamin E on broilers immune responses against newcastle disease virus (NDV) and infectious bursal disease (IBD). The results revealed that antibody titers against NDV and IBD were significantly increased (P<0.05) in treatments provided with selected herbals, which led to a close result of T2, T3, T4, T5, T6, T7, T8 compared with TI which was recorded as the lowest value. Additionally, there were significant differences in both male and female broiler immune response T6 (supplemented with 10 mg Adiantum /kg feed) recorded the best value of antibody titers against NDV and IBD in male and female broiler. The findings agree with the result supported by Farhadi *et al*. (2020) who observed that broilers which were given medicinal plants had significantly (P≤0.05) higher humoral immunity against ND and IBD disease. According to our findings the use of medicinal plants particularly Adiantum, had a good impact on antibody titers. The effects of using Adiantum supplements to increase antibodies against ND and IBD may be attributed to the stimulation of the growth of

immunological organs like the thymus and spleen as well as increased antibody production against ND and IBD. Additionally, polysaccharides derived from the medicinal plants dramatically enhanced antibody titers in vaccinated chickens (Qiu and Cui, 2008).

Table (13): Effect of adding medicinal plants on broiler immunity response against Newcastle disease virus (NDV) and infectious bursal disease (IBD) in male and female broiler at 35 days.

Traits / Treatment	Newcastle titer		Infectious bursal titer	
	Male	Female	Male	Female
T1	2450.52d ±5.66	2431.68e ±22.00	1276.44d ±12.00	1269.71c ±18.06
T2	3357.60c ±19.26	3306.69d ±19.38	1342.89cd ±47.70	1631.10bc ±42.15
T3	3415.92bc ±24.48	3453.09bc ±13.10	1457.24b ±12.87	1441.09ab ±17.84
T4	3487.54ab ±46.11	3532.40ab ±41.93	1382.76bc ±24.65	1340.36bc ±46.81
T5	3581.72a ±43.26	3559.11a ±55.11	1444.67b ±10.75	1432.78ab ±19.06
T6	3587.52a ±60.78	3569.51a ±19.20	1569.14a ±14.21	1528.05a ±13.86
T7	3459.12bc ±11.94	3403.06c ±18.76	1461.18b ±53.22	1399.20b ±48.95
T8	3498.69ab ±27.89	3497.69ab ±27.41	1460.74b ±7.70	1424.50b ±25.92
Sig.	0.05	0.05	0.05	0.05

* a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

Table (14) showed the results of total antioxidant capacity (TAC) were significantly (P<0.05) higher concentration in the serum of male broiler in all treatments of Dill, Adiantum, Crataegus and then vitamin E T6, T5, T8, T3, T7, T4 and T2 compared with negative control T1as well as were significantly higher concentration in serum of female broiler in all treatments T6, T5, T7, T8, T3, T4 and T2 compared with negative control T1.

However, malondialdehyde (MDA) had a significantly (P<0.05) lower concentration in serum of male broiler in all treatments T7, T8, T5, T6, T3, T5 and T4 compared with control T1. Besides, significantly lower concentration in serum of female broiler in all treatments T7, T8, T5, T6, T3, T5 and T4 compared with control T1. The current result was in agreement of Ahmadipour *et al*., (2019) employed hydroalcoholic extract of Adiantum at 100, 200 and 400 mg/kg/day in adult male mice, significantly increased serum TAC in chronic stress-exposed mice decreased MDA serum.

Cold stress leads to oxidative stress when the anti-oxidation is out of balance while medicinal plants supplements may improve total antioxidant capacity (TAC) and reduce levels of malondialdehyde (MDA) in the blood stream due to the

antioxidant activity of medicinal plants and antioxidants can reduce oxidative stress caused by reactive oxygen species (ROS) and unstable chemicals created by the body in response to environmental stress (Arain *et al*., 2018). Many active components of herbs and spices can prevent lipid peroxidation by reducing free radicals. Besides, medicinal plants contain compounds like phenolic compounds found in herbs and spices such as (flavonoids, tannins, phenolic acids, and phenolic terpenes) which possess antioxidant properties and play a protective role against oxidative reactions which supports the animal's health (Abdul-Majeed *et al*.,2022). These components are effective in preventing lipid peroxidation by neutralizing harmful free radicles and integrating these antioxidants in diets help conflict the damaging effects of oxidative stress caused by various environmental factors including cold stress (Bellucci *et al*., 2022).

Table (14): Effect of adding medicinal plants on MDA and TAC in male and female broiler at 35 days.

Traits / Treatment	MDA (mmol/ml)		TAC (mmol/ml)	
	Male	Female	Male	Female
T1	2.84a ±0.01	2.81a ±0.01	1.51g 0.01	1.52f ±0.02
T2	2.52b ±0.01	2.52b ±0.02	3.14f ±0.02	3.14e ±0.02
T3	2.41d ±0.01	2.41c ±0.01	3.72c ±0.01	3.56cd ±0.14
T4	2.46c ±0.01	2.46c ±0.01	3.41a ±0.01	3.39d ±0.02
T5	2.31f ±0.01	2.31d ±0.01	4.14e ±0.02	4.09b ±0.02
T6	2.34e ±0.01	2.34d ±0.01	4.63a ±0.01	4.56a ±0.02
T7	2.06g ±0.01	2.06f ±0.02	3.64d ±0.01	3.62c ±0.01
T8	2.26h ±0.02	2.26e ±0.03	3.73c ±0.02	3.43cd ±0.13
Sig.	0.05	0.05	0.05	0.05

* a,b,c Means with different superscripts in the same column differ significantly (P<0.05) and N. S= non-significant. T1= standard diet, T2= 50 mg vitamin E, T3= 5 g Dill, T4= 10 g Dill, T5= 5 g Adiantum, T6= 10 g Adiantum, T7= 5 g Crataegus, T8= 10 g Crataegus.

CONCLUSIONS

In general, the medicinal plants powder supplements in broiler's diet have a beneficial effect on most productive performance. Improved disease resistance in the birds by decreasing mortality rate, increasing antibody titer against disease, sustaining the antioxidant statues (activate TAC) and reducing (MDA) these changes enable the rearing of healthy broiler and more immunity chicks. Adiantum supplements also affect and enhance physiological traits under cold stress.

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CONFLICT OF INTEREST

The authors certify that they have no affiliations with any organization entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

تأثير النباتات الطبية وفيتامين E في الاداء الانتاجي وبعض المعايير الفسلجية والمناعية وحالة مضادات الاكسدة لفروج اللحم تحت اجهاد البرودة

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الخلاصة

أجريت هذه الدراسة لتوضيح تأثير إضافة مستويات مختلفة من النباتات الطبية و فيتامين E في الأداء الإنتاجي وبعض المعايير الفسلجية والمناعية وحالة مضادات الأكسدة لفروج اللحم تحت اجهاد البرودة 15 درجة مئوية ± 2 لمدة 35 يوماً. تم توزيع 600 فروج اللحم Ross بعمر يوم واحد عشوائياً على ثماني معاملات بواقع خمس مكررات لكل منها (15 طائر/مكرر) على النحو التالي: T1= عليقة قياسية (سيطرة سالبة) , T2=50 ملغ من فيتامين E / كغم علف (سيطرة موجبة), T3= 5 غرام من مسحوق الشبت / كغم علف, T4= 10 غرام من مسحوق الشبت/كغم علف, T5= 5 غرام من مسحوق كزبرة البئر / كغم علف, T6=10غرام مسحوق كزبرة البئر / كغم علف, T7= 5 غرام مسحوق اوراق الزعرور / كغم , T8= 10 غرام مسحوق اوراق الزعرور / كغم علف. أظهرت النتائج أن إضافة النباتات الطبية كان له تأثير معنوي ($P<0.05$) على وزن الجسم الحي، الزيادة الوزنية، كفاءة التحويل الغذائي، نسبة الهلاكات والدليل الإنتاجي بالإضافة إلى التأثير معنوي ($P<0.05$) على تقليل تراكيز الانزيمات المحفزة للاكسدة ثنائي الملون الديهايد (MDA) وارتفعت تركيز الانزيمات المضادة للاكسدة ((TAC في جميع معاملات الاضافة مقارنة مع مجموعة السيطرة ، كلها ساعدت في تقليل الإجهاد التأكسدي بالإضافة إلى زيادة الأجسام المضادة لـ ND وIBD بالمقارنة مع السيطرة السالبة والموجبة.

الكلمات المفتاحية: اداء الفروج, زعرور , شبت , كزبرة البئر , مناخ بارد , مضادات اكسدة.

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