EFFECT OF EUCALYPTUS LEAVES AND ITS SUPPLEMENTATION WITH DIET ON BROILER PERFORMANCE, MICROBIAL AND PHYSIOLOGICAL STATUES TO ALLEVIATE COLD STRESS

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ABSTRACT

The aim of this study was to investigate the effects of *Eucalyptus Camaldulensis* leaves powder (EUP) and oil (EUO) to alleviate cold stress and their effects on broiler performance, duodenum morphology and enzyme secretion, microbial counts, oxidative index expression levels of heat shock protein, vitamin E and TBARS (thiobarbituric acid-reactive substances) and meat sensory evaluation. Six hundred thirty-one day-old unsexed chicks were randomly divided into 7 groups: control (basal diet), EuP (0.5, 1, & 2) %, EuO (0.05, 0.1 & 0.2) %, the chicks kept under the temperature of 17°C afternoon and 15°C at night for under cold stress condition.The results of dietary addition with Eucalyptus powder-EuP (1.0) % or (EuO) oil (0.05, 0.1 and 0.2) % were significantly ($p \leq 0.05$) higher in body weight (BW), body weight gain (BWG), economic profit (EP), small intestine relative length, duodenum (more number of goblet cells, villus height, crypt depth, improved the secretion of amylase, lipase, trypsin and chymotrypsin, total count of *Lactobacillus*), blood vitamin E, the thigh and breast meat sensory evaluation (juiciness and flavor). While, decreased broiler mortality, duodenum: (pH acidity. *E. Coli, Coliforms perfringens* and *Salmonella typhimurium*) blood TBARS concentration, Heat shock protein (Hsp: 40, 70 and 90), less hardness and TBARS in the thigh and breast of broiler meat compared with the control. In the most characteristics the age 35 d superiority 42 d.

Keywords: Eucalyptus leaves, oil, broiler, body performance, microbial, physiology.

تأثير إضافة أوراق الكالبتوس وزيتها في علف فروج اللحم على الأداء الإنتاجي والحالة الميكروبية والفسيولوجية للتخفيف من الإجهاد البارد

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المستخلص

الهدف من الدراسة معرفة تأثير مسحوق أوراق الكالبتوس (EUP) وزيتها (EUO) للتخفيف من الإجهاد البارد وتأثيراتها على أداء الفروج، تشريح والأنزيمات الفارزة من الاثني عشر والأعداد الميكروبية، مؤشر الأكسدة (بروتينات الصدمة الحرارية، فيتامين E، TBARS) و مستوى والتقييم الحسي للحم. تم توزيع ستمائة و ثلاثون فرخة فروج اللحم بعمر يوم واحد إلى 7 مجموعات: السيطرة (العليقة القياسية) مسحوق اوراق الكالبتوس (0.5 ، 1 و 2)٪ وزيت الكالبتوس (0.05 ، 0.1 و 0.2)٪ ، ربيت الأفراخ تحت درجة حرارة 17 درجة مئوية بعد الظهر و 15 درجة مئوية ليلا تحت ظروف الإجهاد البارد. كانت نتائج إضافة العلف بمسحوق الأوكالبتوس (1.0)٪ أو زيتها (0.05 ،0.1 و 0.2)٪ عالي معنوياً ($p \leq 0.05$) في وزن الجسم، الزيادة الوزنية، الربح الاقتصادي، الطول النسبي الأمعاء الدقيقة، زيادة أعداد الخلايا الكأسية و الزغابات وعمق الخبايا و تحسين إفراز أنزيمات (الأميليز ، اللايبيز ، التريبسين و الكايموتربسين) و العدد الكلي للبكتريا اللبنية في الاثني عشر، فيتامين E في الدم ، التقييم الحسي للحم الفخذ والصدر (العصيرية والنكهة). في حين انخفض معدل الهلاكات، حموضة الأس الهيدروجيني، أعداد الأي كولاي الكوليفورم بيرفيرينجرز والسالمونيلا تايفيريم في الاثني عشري، تركيز TBARS و بروتينات الصدمة الحرارية Hsp (40 ، 70 و90) في الدم، تقليل من صلابة لحم الفخذ والصدر وتركيز TBARS في اللحم، مقارنة مع مجموعة السيطرة. معظم الصفات ارتفعت بعمر 35 يوم مقارنة بعمر 42 يوم.

كلمات مفتاحية: انزيمات، مؤشر الاكسدة، التقييم الحسي، الاجهاد.

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INTRODUCTION

Poultry production is one of the fastest ways to full up the shortage of animal protein provide and consumption in Iraq. Herbs and herbal products are incorporated in poultry diets to replace synthetic products in order to stimulate and promote the effective use of feed nutrients which may subsequently result in more rapid body weight gain, higher production rates and improved feed efficiency, moreover, active components of herbs may improve digestion and stimulate the immune function in broilers (11). In the last few decades, there has been an exponential growth in the field of herbal medicine, they are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, bio-pesticides and food additives (1). Eucalyptus Cama-ldulensis is an important ethnomedicinal plant belonging to the family of myrtaceae (26), it is used for food and eucalyptol (1,8-cineole) is listed as a synthetic flavoring agent (3), color, aroma and preservation of food or beverages (5). The pharmacological studies revealed that Eucalypts possessed gastrointestinally, anti-inflammatory, antioxidant, antimicrobial (2). Medicinal eucalyptus oil is widely used for the relief of cold and influenza symptoms. It is a unique natural product having antiseptic properties and the power to clear the nasal passages and bronchial tubes making it easier to breathe (13). Eucalyptus oil has antibacterial activities against microorganisms salmonella type, Streptococcus A, Staphylococcus aureus (16). The use of EOs in enhancing productivity may give promising effects as growth and health promoter (20). The supplementation of 0.1 and 0.2 % Eucalyptus Camaldulensis leaves powder in broiler chicks were increased body weight and gain, immune statue, improved FCR and decreased the mortality percentage (25). This study aimed to know the effect of eucalyptus leaves and its oil in different levels on body performance, measuring duodenum (morphology, bacteria and enzymes) content, plasma antioxidant TBARS, vit. E and heat shock protein (Hsp) levels to alleviated cold stress during winter season.

MATERIALS AND METHODS

Experimental design

This study was conducted at Poultry farm/Dept. of Animal resources/College of Agriculture/University of Salahaddin-Erbil/Iraq. 630 one day old broiler chicks (Ross-308) birds were housed in floor pens of identical size (2 × 2 m) and brooded at 30, 28 and 25°C during the 1st, 2nd and 3rd weeks respectively. Cold stress was applied during 4th, 5th and 6th week for the remaining 17°C afternoon and 15°C at night during winter months (2/January to 12/February). The birds were distributed randomly in seven groups and three replicates each as follows: 1) Control: 0.0% (basal diet), three levels of eucalyptus leaves powder (EuP) added with basal diet (0.5, 1.0 and 2.0) %, groups three levels of eucalyptus leaves oil (EuO) added in basal diet (0.05, 0.1 and 0.20) %. The birds were provided feed and water *ad libitum*, the feed was prepared by Kosar company that contains 3000, 3100, 3175 kcal/kg metabolizable energy, 23, 21, 20 %) crude protein (starter (1-11d), grower (12-25d), finisher (26-42d) diet respectively. The birds being received with one hour of darkness following a period of 23 h light at the first week and 22h in (2-6) weeks.

Body performance determination

On days 35 and 42 of age, five birds, respect-tively from each replicate, were randomly selected, weighed, the feed intake, feed conversion ratio, body weight gain, mortality and economic profit were calculated.

Morphology of small intestine measurement

At age 35 and 42 days birds were killed by cervical dislocation. Subsequen-tly, the abdominal cavity was opened, and the small intestine were weighed. The contents of the duodenum was collected in plastic bottles to measure pH. Intestinal duodenum samples were dehydrated, cleared, and embedded in paraffin. Serial sections were cut at 5 µm and placed on glass slides. For all assays, sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Sections were examined by light microscopy (35) for determining number of goblet cells, villus height, crypt depth and villus to crypt (V/C) ratio.

Duodenum enzymes determinations

After thawing the frozen intestine in 4°C water for enzyme activities determina-tion. Duodenum mucosa was homogenized in cold water (100 mg/ml) and centrifuged for 5 rain at 1000g and 4°C. The amylase was measured with soluble starch as substrate (8). Trypsin was measured using Na-p-Toluolsulfonyl-L-arginine methyl ester hydrochloride as a substrate; chymotrypsin was measured using N-Benzoyl-L-tyrosine ethyl ester as a substrate according to the procedures description (39). Lipase was determined using Randox reagent kit. All digestive enzymes activities were expressed as units per milliliter (U/mL).

Duodenum microbial determination

Duodenum contents were cautiously kept in sterile petri dishes at -20°C until analyses in the laboratory. One gram of each homogenized sample was collected and transferred into 10 ml sterile saline solution for dilution. The next step, each sample was spread on selective agar plates as follows. MRS agar medium was used for *lactobacillus* bacteria and the Nutrient and McConkey agars were utilized for *E. Coli*, *Coliforms perfringens* (14) and *Salmonella typhimurium* was cultured in semisolid Salmonella Agar and incubated at 37°C for 24 h the colonies were determined.

Blood antioxidant and heat shock protein determinations

Blood samples were randomly collected from the wing vein of 5 broilers in tubes without anticoagulant from each group at age 35 and 42 days for each treatment to determined: TBARS level was determined using the thiobarbituric acid method. Results were expressed as mmol of malondialdehyde (MDA) equivalents/ mg protein of blood. Vitamin E level: α-tocopherol extraction was determined by high performance liquid chromatographic (HPLC). Determination of Hsp concentrations using kit produced by (Cusabio Biotech Co.,) the steps of determination explained in the instruction of the kit of chicken Hsp 40, Hsp 70 and Hsp 90 in various matrices and diluted with the sample diluent to produce samples with values within the dynamic range of the assay. The OD concentrations, determined by ELISA.

Sensory and TBARS analysis of meat

The **first set**: three samples of the thigh and breast meat from each treatment were chilled in (4 °C) and frozen at (-26°C) for 7 days before sensory analyses, the samples of meat were prepared as based in the method of (17), then served to the panelists. Hedonic scale (1 to 9) was used for hardness, juiciness and flavor analysis. The **second set**: samples of the thigh and breast muscle were determined TBARS, it was expressed as mmol of malondi-aldehyde (MDA) equivalents/mg protein of fresh meat. The concentration of MDA in the breast muscle was determined at the 35^{th} and 42^{nd} days after storing at 4°C by using the test kit purchased from (Nanjing, china) following the instructions of the test kit.

Eucalyptus Camaldulensis leaves and oil analysis

100 g of dried eucalyptus Camaldulensis leaves were analysed in a private laboratory. The chemical composition of Eucalyptus oil was analysed by gas chromatography coupled with mass spectrometry (GC-MS).

Table 1. Nutrient analysis of dried

	Nutrient Value	%
Ingredients	Water	51.81
	Fiber	17.38
	Protein	7.82
	Carbohydrates	5.86
	Fat	8.15
	Minerals	2.15
	Tannins	6.83
Volatile oils	1, 8 Cineole	35.17
	β-pinene	17.33
	Oxy-sesquiterpenes	16.38
	Cryptone	5.13
	α-pinene	4.68
	4- Terpineol	4.22
	Myrtenal	2.46
	α- Terpinolene	1.96
	α- Terpineol	1.79
	Trans-Pinocarveol	1.68
	α- Thujene	1.33
	Bicyclogirmacrene	0.84
	Myrcene	0.65
	Terpinene	0.53
	Other compounds	5.85

Eucalyptus and its oil

Statistical analysis: All data were analyzed using CRD (Completely Randomized Design) by SAS institute program (30). Duncan's

multiple range tests were used to compare differences among the treatments.

RESULTS AND DISCUSSION

Table 2. evaluated the broiler dietary addition with (EuP) Eucalyptus powder (0.5, 1.0 and 2.0) % or its (EuO) oil (0.05, 0.1 and 0.2) % on body performance. The results were significantly ($p \leq 0.05$) higher in body weight (BW), body weight gain (BWG) and economic profit (EP) in the groups 1.0 % EuP and all levels of EuO respectively compared with the control and other groups (0.5 and 2.0) % EuP at ages 35 and 42 days, however 0.2 % EuP was more lower BW compared with all groups besides with the control group. Also noticed dramatic improvement in the feed conversion ratio (FCR) in all the groups of EuO and 0.1% EuP, also in mortality percentage in all the groups of EuO and in both groups of EuP (0.5 and0.1%) at ages 35 and 42. While feed intake (FI) wasn't show any differences among all groups of EuP and EuO with the control at different ages. With regard to the age effect, the birds records more improvement in the FI, FCR and mortality percentage at age 35 days compared with 42 d.

Table 2. Effect of Eucalyptus Leaves and oil on broiler body performance and economic profit ($) to alleviate cold stress.

Traits	Age		Eucalyptus powder %			Eucalyptus oil %			SE	Effect of
	d	0.0 %	0.5	1.0	2.0	0.05	0.10	0.20		age
IBW g	1	———	———	———	43.20	———	———	———		
FI	35	4067.2 ^{a}	4091.5 ^{a}	4077.1 ^{a}	3753.5 ^{a}	3957.8 ^{a}	3920.4 ^{a}	3903.5 ^{a}	173	3967 ^{b}
g	42	5532.3 ^{a}	5332.6 ^{a}	5116.5 ^{a}	5246.0 ^{a}	5346.5 ^{a}	5220.0 ^{a}	5201.7 ^{a}	215	5285 ^{a}
BW	35	2120.4 ^{bc}	2193.2 ^{bc}	2230.5 ^{b}	2005.3 ^{c}	2355.0^{ab}	2412.0 ^{a}	2498.6 ^{a}	122	2259 ^{b}
g	42	2735.3 ^{c}	2820.6 ^{bc}	2859.1 ^{b}	2533.0 ^{d}	3055.3^{ab}	3110.2 ^{ab}	3205.3 ^{a}	149	2902 ^{a}
BWG	35	2077.2 ^{c}	2150.0 ^{bc}	2187.3 ^{b}	1962.1 ^{c}	2311.8^{ab}	2368.8 ^{ab}	2455.4 ^{a}	103	2216 ^{b}
g	42	2692.1 ^{bc}	2777.4 ^{bc}	2815.9 ^{b}	2489.8 ^{c}	3012.1^{ab}	3067.0 ^{ab}	3162.1 ^{a}	126	2859 ^{a}
FCR	35	1.958 ^{a}	1.903 ^{a}	1.864 ^{b}	1.912 ^{a}	1.712 ^{bc}	1.655 ^{bc}	1.590 ^{c}	0.11	1.78 ^{a}
	42	2.055 ^{ab}	1.920 ^{b}	1.817 ^{c}	2.107 ^{a}	1.775 ^{c}	1.702 ^{cd}	1.645 ^{d}	0.18	1.86 ^{a}
Mortal-	35	2.67 ^{a}	1.55 ^{b}	1.67 ^{b}	2.00 ^{a}	1.00 ^{b}	1.00 ^{b}	0.75 ^{b}	0.09	1.52 ^{b}
ity %	42	4.75 ^{ab}	3.90 ^{b}	3.50 ^{b}	6.50 ^{a}	2.75 ^{c}	2.50 ^{c}	1.75 ^{c}	0.10	3.66 ^{a}
EP	35	0.618 ^{bc}	0.634 ^{bc}	0.726 ^{b}	0.564 ^{c}	0.777 ^{b}	0.813 ^{ab}	0.901 ^{a}	0.12	0.726 ^{a}
$/kg	42	0.548 ^{c}	0.557 ^{c}	0.651 ^{b}	0.533 ^{c}	0.700 ^{ab}	0.717 ^{ab}	0.783 ^{a}	0.17	0.647 ^{b}

IBW: initial body weight, FI: feed intake, BW: body weight, BWG: body weight gain, FCR: feed conversion ratio, EP: economic profit. The same superscripts within rows means non-significant, $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$).

Table 3. the small intestine relative length to body weight (cm/g BW) was significantly ($p \leq 0.05$) higher in the groups of EuO (0.05, 0.1 and 0.2) % compared with the other groups of the study at ages 35 and 42 days. Also the duodenum morphology was significantly ($p \leq 0.05$) more number of goblet cells in the groups of EuP (0.5, 1.0) % and all the groups of EuO, so villus was more height and villus height to crypt depth (V/C) ratio were better in all the groups of EuP and EuO at the both ages, as well as crypt was more depth in the groups of EuP (0.5, 1.0) % and all the groups of EuO at age 35 d, also at age 42 d increased in all the groups of EuO. While the effect of age wasn't shown any difference among all the characteristics above between the two ages 35 and 42 days.

Table 3. Effect of Eucalyptus Leaves and oil on small intestine length and duodenum morphology changes to alleviate cold stress

Traits	Age		Eucalyptus powder %			Eucalyptus oil %			SE	Effect of
	d	0.0 %	0.5	1.0	2.0	0.05	0.10	0.20		age
Small intestine R.	35	12.01^{b}	12.55 ^{b}	12.98 ^{ab}	12.28 ^{b}	13.93 ^{a}	14.10 ^{a}	14.39 ^{a}	0.77	13.18 ^{a}
length (cm)/g	42	11.87 ^{b}	12.08 ^{b}	12.66 ^{ab}	11.95 ^{b}	13.80 ^{a}	13.85 ^{a}	14.08 ^{a}	0.95	12.90 ^{a}
Number of goblet	35	8.43 ^{c}	11.25 ^{b}	12.68 ^{ab}	9.32 ^{c}	12.90 ^{ab}	14.75 ^{a}	15.11 ^{a}	1.13	12.06 ^{a}
cells[1]	42	8.05 ^{c}	11.76 ^{b}	12.10 ^{b}	8.93 ^{bc}	12.39 ^{ab}	14.50 ^{a}	14.78 ^{a}	1.37	11.79 ^{a}
Villus height (µm)	35	852 ^{d}	1089 ^{c}	1175 ^{bc}	1130 ^{bc}	1291 ^{b}	1405 ^{ab}	1533 ^{a}	130	1211 ^{a}
	42	910 ^{d}	1127 ^{c}	1210 ^{bc}	1100 ^{c}	1327 ^{b}	1462 ^{ab}	1582 ^{a}	217	1245 ^{a}
Crypt width (µm)	35	88.0 ^{c}	93.9 ^{b}	97.3 ^{b}	89.0 ^{bc}	110.6 ^{ab}	112.4 ^{a}	114.8 ^{a}	4.3	100.9 ^{a}
	42	93.3 ^{cd}	95.0 ^{c}	96.8 ^{c}	87.8 ^{d}	109.1 ^{b}	114.1 ^{ab}	119.0 ^{a}	5.8	102.3 ^{a}
V/C	35	9.68 ^{c}	11.60 ^{b}	12.08 ^{ab}	12.70 ^{ab}	11.67 ^{b}	12.50 ^{ab}	13.35 ^{a}	0.83	11.94 ^{a}
	42	9.75 ^{c}	11.64 ^{b}	12.50 ^{ab}	12.53 ^{ab}	11.63 ^{b}	13.65 ^{a}	13.29 ^{a}	0.90	12.14 ^{a}

[1]number of goblet cells per mm of villus length. The same superscripts within rows means non-significant, $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$).

Table 4. explain the effect of effect of different levels of eucalyptus leaves powder or oil on duodenum enzymes concentration, the duodenum pH in the groups (1 , 2) % of EuP and all groups of EuO were significantly (p≤0.05) less acidity compared with the control and 0.5 % EuP at age 35 d, also the two levels of EuO (0.1, 0.2) % were less acidity at age 42 d. About the duodenum enzymes secretion, amylase was significantly (p≤0.05) higher in the groups of EuP (1.0 & 2.0) % and all the groups of EuO at ages 35 and 42 d. So lipase and trypsin at both ages and chymotrypsin at age 42 were significantly (p≤0.05) raised in all the groups of EuO and 1.0 % EuP, as well as chymotrypsin at age 35 d increased in all the groups of EuO and EuP in (0.5 & 1.0) %. Furthermore in the effect of age, pH at age 35 d was significantly (p≤0.05) less than the age 42, while there weren't any significant differences between the ages 35 and 42 d in the enzymes (amylase, lipase, trypsin and chymotrypsin). Table 5. depicts all the groups of eucalyptus leaves powder and oil were significantly (p≤0.05) turn up the total count of *Lactobacillus* at ages 35 and 42 d. however the added different levels of EuP and EuO were significantly (p≤0.05) turn down the harmful bacteria (*E. Coli, Coliforms perfringens*) nor in 2 % and *Salmonella typhimurium*) levels in all the groups of EuP & EuO of broilers duodenum at ages 35 and 42 days. The effect EuP and EuO on age had significantly (p≤0.05) increased *Lactobacillus* counts at age 35 d. But *E. Coli* and *Coliforms perfringens were* significantly (p≤0.05) decreased at age 35 d compared with the 42 d. While, the both ages had no shown any differences in *Salmonella typhimurium.*

Table 4. Effect of Eucalyptus Leaves and oil on duodenum digestive enzymes concentrations (ppm) to alleviate cold stress

Traits	Age	0.0	Eucalyptus powder %			Eucalyptus oil %				Effect
	d	%	0.5	1.0	2.0	0.05	0.10	0.20	SE	of age
pH	35	4.70 ^{c}	5.04 ^{bc}	5.28 ^{b}	5.73 ^{ab}	5.58 ^{b}	5.90 ^{ab}	6.09 ^{a}	0.371	5.47 ^{b}
	42	5.44 ^{b}	5.75 ^{b}	5.92 ^{ab}	6.39 ^{ab}	6.29 ^{ab}	6.36 ^{a}	6.48 ^{a}	0.293	6.09 ^{a}
Amylase	35	2.16 ^{c}	2.35 ^{bc}	2.87 ^{b}	2.21 ^{ab}	2.98 ^{b}	3.55 ^{ab}	4.19 ^{a}	0.103	2.90 ^{a}
	42	2.11 ^{c}	2.22 ^{bc}	2.60 ^{b}	2.24 ^{ab}	2.81 ^{b}	3.20 ^{ab}	3.87 ^{a}	0.126	2.72 ^{a}
Lipase	35	9.83 ^{c}	10.36 ^{bc}	11.25 ^{b}	10.66 ^{bc}	11.48 ^{b}	12.19 ^{ab}	12.89 ^{a}	0.409	11.24 ^{a}
	42	9.89 ^{c}	10.02 ^{bc}	11.00 ^{b}	10.43 ^{bc}	11.15 ^{b}	11.77 ^{ab}	12.65 ^{a}	0.355	10.99 ^{a}
Trypsin	35	24.47 ^{c}	25.67 ^{bc}	26.96 ^{b}	25.01 ^{bc}	27.55 ^{b}	28.08 ^{ab}	29.65 ^{a}	1.177	26.80 ^{a}
	42	24.60 ^{c}	25.30 ^{bc}	26.55 ^{b}	24.89 ^{bc}	27.08 ^{b}	27.44 ^{ab}	29.13 ^{a}	0.975	26.43 ^{a}
Chymotr	35	13.77 ^{d}	15.91 ^{c}	16.86 ^{bc}	14.46 ^{cd}	17.04 ^{b}	19.00 ^{ab}	20.17 ^{a}	0.822	16.74 ^{a}
ypsin	42	13.60 ^{c}	15.70 ^{c}	16.59 ^{bc}	14.41 ^{cd}	17.22 ^{b}	18.75 ^{ab}	19.51 ^{a}	0.903	16.54 ^{a}

The same superscripts within rows means non-significant, $^{a-c}$ Means within rows with different superscripts differ significantly at (P≤ 0.05).

Table 5. Effect of eucalyptus leaves powder or oil on bacterial counts (cfu×log 10^{-3}) to alleviate cold stress

Traits	Age		Eucalyptus powder %			Eucalyptus oil %				Effect
	d	0.0 %	0.5	1.0	2.0	0.05	0.10	0.20	SE	of age
Lactobacillus	35	5.84 ^{c}	6.73 ^{b}	7.32 ^{ab}	6.25 ^{b}	7.52 ^{ab}	7.95 ^{ab}	8.90 ^{a}	0.58	7.22 ^{a}
	42	6.05 ^{c}	6.58 ^{b}	6.93 ^{ab}	6.29 ^{bc}	6.97 ^{ab}	7.22 ^{ab}	7.85 ^{a}	0.77	6.84 ^{b}
E. Coli	35	10.35 ^{a}	6.28 ^{b}	4.45 ^{c}	5.31 ^{bc}	4.11 ^{c}	3.05 ^{cd}	2.13 ^{d}	0.19	5.10 ^{b}
	42	12.06 ^{a}	7.09 ^{b}	4.93 ^{c}	5.44 ^{bc}	4.39 ^{c}	3.29 ^{cd}	3.06 ^{d}	0.25	5.75 ^{a}
Coliforms	35	4.56 ^{a}	3.11 ^{b}	2.81 ^{bc}	3.89 ^{ab}	2.72 ^{bc}	2.38 ^{c}	2.09 ^{c}	0.13	3.07 ^{b}
perfringens	42	5.07 ^{a}	3.29 ^{b}	3.07 ^{bc}	3.85 ^{ab}	2.90 ^{bc}	2.59 ^{c}	2.37 ^{c}	0.17	3.31 ^{a}
Salmonella	35	3.91 ^{a}	2.67 ^{b}	2.19 ^{bc}	2.97 ^{b}	2.13 ^{bc}	1.89 ^{bc}	1.32 ^{c}	0.11	2.44 ^{a}
typh.	42	4.22 ^{a}	2.83 ^{b}	2.33 ^{b}	2.86 ^{b}	2.29 ^{b}	2.03 ^{bc}	1.41 ^{c}	0.18	2.57 ^{a}

The same superscripts within rows means non-significant, $^{a-c}$ Means within rows with different superscripts differ significantly at (P≤ 0.05).

Table 6. Confirmed the effect of eucalyptus leaves powder and its oil on some blood antioxidant profile and heat shock protein (Hsp) summation density to alleviate cold stress. The results shows significantly (p≤0.05) higher in vitamin E concentration in blood plasma of broiler intake diet added EuP (0.5 and 1.0) % and all the EuO groups at age 35 d, so at age 42 d increased in 1.0 % EuP and all the groups of EuO compared with control. However, TBARS was significantly (p≤0.05) lower in the group 1.0 % of EuP and all the

groups of EuO, so heat shock protein summation density (Hsp 40) had lower in all the groups of EuP and EuO at age 35 d, Hsp 40 at age 42 d, Hsp 70 & 90) were lower in the groups (1 & 2) % of EuP and all the groups of EuO at ages 35 and 42 d. According to the age effect, Vit. E was increased at age 35 d compared with the age 42 d, but TBARS and Hsp 70 were decreased at the age 35 d, while had not seen any differences between the ages 35 & 42 days in the Hsp 40 and 90. Table 7. shows the sensory evaluation and TBARS level was detected in the thigh and breast meat of the broiler throughout the eucalyptus leaves powder and oil additives. From the results had noticed the thigh meat hardness in both ages and TBARS at 35 d significantly ($p \leq 0.05$) lower in EuP (1 and 2) % and all groups of EuO, also recorded lower hardness and TBARS in breast meat in all the groups of EuP and EuO at age 35 d, and in EuP (1 and 2) % and all groups of EuO at age 42 d. While the thigh meat was significantly ($p \leq 0.05$) more juicy in the EuP (1 and 2) % and all groups of EuO at both ages, so the thigh meat flavor and breast meat juiciness and flavor were significantly ($p \leq 0.05$) improved in all the groups of EuP and EuO at both ages. The effect of age was significantly ($p \leq 0.05$) improved the hardness, TBARS and thigh and breast meat were more juicy at age 35 d compared with 42 d. however the thigh and breast meat flavor had not record any significant differences between the both ages.

Table 6. Effect of Eucalyptus Leaves and oil on some blood antioxidant profile and heat shock protein (Hsp) summation density to alleviate cold stress

Traits		Age d	0.0 %	Eucalyptus powder %			Eucalyptus oil %			SE	Effect of age
				0.5	1.0	2.0	0.05	0.10	0.20		
Antioxid ant-profile	TBARS	35	3.46 ^{a}	3.29 ^{ab}	2.88 ^{b}	3.53 ^{a}	3.02 ^{b}	2.84 ^{bc}	2.69 ^{c}	0.11	3.10 ^{b}
		42	3.85 ^{a}	3.51 ^{ab}	3.23 ^{b}	3.98 ^{a}	3.25 ^{b}	2.95 ^{bc}	2.82 ^{c}	0.18	3.37 ^{a}
	Vit. E	35	5.28 ^{c}	5.77 ^{b}	6.18 ^{b}	5.06 ^{c}	6.90 ^{b}	8.45 ^{ab}	10.23 ^{a}	0.52	6.84 ^{a}
		42	4.45 ^{c}	5.53 ^{bc}	5.89 ^{b}	4.83 ^{c}	6.04 ^{b}	7.11 ^{ab}	8.69 ^{a}	0.47	6.08 ^{b}
Hsp summ-ation density	Hsp 40	35	1815 ^{a}	1693 ^{b}	1544 ^{b}	1410 ^{bc}	1507 ^{b}	1215 ^{bc}	1033 ^{c}	133	1459 ^{a}
		42	1765 ^{a}	1722 ^{a}	1619 ^{b}	1397 ^{bc}	1598 ^{b}	1300 ^{bc}	1105 ^{c}	179	1500 ^{a}
	Hsp 70	35	3104 ^{a}	2993 ^{ab}	2263 ^{b}	1979 ^{c}	2200 ^{bc}	1680 ^{cd}	1496 ^{d}	175	2245 ^{b}
		42	3475 ^{a}	3255 ^{ab}	2740 ^{b}	2379 ^{c}	2483 ^{bc}	1685 ^{d}	1207 ^{e}	201	2460 ^{a}
	Hsp 90	35	2725 ^{a}	2678 ^{ab}	2190 ^{b}	1850 ^{bc}	1848 ^{bc}	1495 ^{c}	1318 ^{d}	152	2014 ^{a}
		42	2910 ^{a}	2777 ^{ab}	2346 ^{b}	2004 ^{bc}	1633 ^{c}	1467 ^{cd}	1402 ^{d}	169	2077 ^{a}

The same superscripts within rows means non-significant, $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$).

Table 7. Effect of eucalyptus leaves powder or oil on sensory evaluation and TBARS (mg malonaldehyde/100g tissue) concentration in thigh and breast meat of broiler

Traits		Age d	0.0 %	Eucalyptus powder %			Eucalyptus oil %			SE	Effect of age
				0.5	1.0	2.0	0.05	0.10	0.20		
Thigh meat	Hardness	35	4.32 ^{c}	4.22 ^{bc}	3.67 ^{b}	3.25 ^{ab}	3.29 ^{ab}	3.08 ^{ab}	2.57 ^{a}	0.23	3.50 ^{b}
		42	4.70 ^{c}	4.43 ^{bc}	4.03 ^{b}	3.70 ^{ab}	3.51 ^{ab}	3.43 ^{ab}	3.29 ^{a}	0.27	3.91 ^{a}
	juiciness	35	5.32 ^{c}	5.49 ^{bc}	5.65 ^{b}	5.60 ^{b}	5.76 ^{b}	5.90 ^{ab}	6.42 ^{a}	0.30	5.73 ^{a}
		42	5.09 ^{c}	5.12 ^{bc}	5.33 ^{b}	5.42 ^{ab}	5.33 ^{b}	5.52 ^{ab}	5.98 ^{a}	0.33	5.38 ^{b}
	Flavor	35	4.55 ^{c}	5.32 ^{b}	5.63 ^{b}	5.85 ^{b}	6.10 ^{ab}	6.28 ^{ab}	6.63 ^{a}	0.31	5.70 ^{a}
		42	4.48 ^{c}	5.39 ^{b}	5.72 ^{b}	6.05 ^{ab}	6.19 ^{ab}	6.31 ^{ab}	6.70 ^{a}	0.40	5.77 ^{a}
	TBARS	35	1.32 ^{a}	1.04 ^{b}	0.85 ^{bc}	1.18 ^{ab}	0.71 ^{bc}	0.57 ^{bc}	0.41 ^{c}	0.08	0.86 ^{b}
		42	1.59 ^{a}	1.33 ^{ab}	1.19 ^{b}	1.29 ^{ab}	0.93 ^{b}	0.84 ^{ac}	0.69 ^{c}	0.11	1.12 ^{a}
Breast meat	Hardness	35	5.38 ^{c}	4.80 ^{b}	4.31 ^{b}	4.01 ^{ab}	4.15 ^{ab}	3.93 ^{ab}	3.72 ^{a}	0.21	4.32 ^{b}
		42	5.71 ^{c}	5.09 ^{bc}	4.73 ^{b}	4.58 ^{ab}	4.40 ^{ab}	4.19 ^{ab}	4.05 ^{a}	0.30	4.72 ^{a}
	juiciness	35	3.81 ^{c}	4.12 ^{b}	4.51 ^{b}	4.19 ^{b}	4.57 ^{b}	5.09 ^{ab}	5.59 ^{a}	0.33	4.55 ^{a}
		42	3.45 ^{c}	3.88 ^{b}	4.29 ^{ab}	3.85 ^{b}	4.03 ^{b}	4.62 ^{ab}	4.80 ^{a}	0.37	4.13 ^{b}
	Flavor	35	3.86 ^{c}	4.69 ^{b}	5.02 ^{ab}	5.49 ^{ab}	5.30 ^{ab}	5.66 ^{ab}	5.80 ^{a}	0.29	5.12 ^{a}
		42	3.95 ^{c}	4.82 ^{b}	5.29 ^{ab}	5.68 ^{ab}	5.49 ^{ab}	5.79 ^{ab}	5.87 ^{a}	0.35	5.27 ^{a}
	TBARS	35	0.98 ^{d}	0.73 ^{c}	0.44 ^{b}	0.36 ^{ab}	0.43 ^{b}	0.30 ^{ab}	0.23 ^{a}	0.08	0.49 ^{b}
		42	1.28 ^{c}	1.09 ^{bc}	0.75 ^{b}	0.82 ^{b}	0.67 ^{b}	0.55 ^{ab}	0.42 ^{a}	0.11	0.80 ^{a}

The same superscripts within rows means non-significant, $^{a-c}$ Means within rows with different superscripts differ significantly at ($P \leq 0.05$). TBARS: thiobarbituric acid-reactive substances

The uses of eucalyptus leaves increased appetite and digestion stimulants and enhancing the physiological responses, help to sustain good health and welfare of the animals and improve their performance (27). In the present research the laboratory analysis of

Eucalyptus Camaldulensis leaves powered contains valuable nutrients (protein 7.82, carbohydrate 5.86, fat 8.15, minerals 2.15) % and important volatile oils (Cineole 35.17, β-pinene 17.33, Oxysesquiterpenes 16.38, Cryptone 5.13, α-pinene 4.68, 4-Terpineol 4.22, Myrtenal 2.46, etc.) % as shows in (Table 1). Thus, it's a rich source of secondary compounds with a variety of biological activities. The EO of eucalyptus contains 1,8-cineole as its major constituent (18). The eucalyptus oil in the leaves is grouped as medicinal, industrial, aromatic and flavoring, depending on their chemical composition (28), also the leaves contain polyunsaturated fatty acids PUFA (58.41%) including omega-3 and omega-6 due to their generalized beneficial health effects, also its rich source of vitamin E (1558.27 µg/g of dry weight) and Vitamin C (339.16 mg/g dry weight) are facilitating resistance to the oxidative stresses (12). In addition the leaves contain minerals like K, P, Ca, Mg, Fe, Zn, Na, Mn and Cu, may help a good balance of nutrients (21). The natural senescence process in eucalyptus leaves may also include a regulated reduction in chlorophyll content as well as an increase in non-green pigments, especially anthocyanin, the presence of anthocyanin in stressed or damaged mature foliage through the additional effects of cold-induced photo inhibition (34). The improvement of feed conversion is due to the active materials and the valuable nutrients that found in eucalyptus leaves causing greater efficiency utilized of the assumption of powder and EO of some herbs like eucalyptus might improve the palatability of feed due to their aromatic (1, 8-cineole) characteristics in eucalyptus could promote feed consumption when added to diets of poultry (38). There are remarkable *in vitro* and *in vivo* proofs that show that either EO could limit growth of the common intestinal pathogens. A phytogenic feed additive is improving efficiency of feed conversion thereby enhancing the intestinal availability of essential nutrients for absorption. The dietary supplementations with eucalyptus leaves powder on quail diet resulted increases in the intestinal weight and length (15), the increase in the (duodenum goblet cells, villus height and crypt depth) all the above were led to the perfection of the duodenum digestive enzymes secretion, so no increased risks of mortality due to higher early live weights (37). Thus the EO and volatile oils motivated the sensory of the thigh and breast meat especially juiciness and flavor of them whereas decreased the hardness of meat in the groups of EuP and EuO compared with the control except 2% EuP because high levels it in dietary due the diet more bitter which depressed bids performances. The essential oil (EO) in eucalyptus is able to confirming its antiseptic and anti-inflammatory properties, the monoterpe-noids in eucalyptus EO changes among the different species, for immune regulatory agents useful against infectious disease and microbiological pathogens *E. Coli* and *Staphylococcus aureus* (31). The EO of Eucalyptus showed antibacterial activity against Gram-positive and Gram-negative bacteria (4 and 24). The EO of E. *camaldulensis* leaves have potency as an antimicrobial agent especially against pathogens, so E. globulus has shown a higher antibacterial activity against influenza (6). Cold stress can disrupt the balance of the oxidant/antioxidant system and cause oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status (29). The misbalance of oxidant and antioxidant systems leads to oxidative damage and influences tissue function (23). The EOs of eucalyptus being also able of scavenging free radicals may play an important role in some disease prevention such as immune system decline, increasing evidence has aromatherapy and act as pharmacological suggested that these diseases may result from cellular damage caused by free radicals (19), it has been use in activities like anti-inflammatory, antibacterial, anti-oxidative, antiviral properties (36). The strong anti-oxidant properties of EOs are due to their phenolic constituents, such as 1, 8-cineole, which has moderate DPPH radical scavenging activity (9). Heat shock proteins are a family of constitutive proteins of all pro- and eukaryotic cells that play different physiological roles, including promotion of the folding (acquisition of tertiary structure) assembly, translocation and secretion of newly synthesized poly-peptides and participa-tion in the removal or repairing of denatured proteins

acting as molecular chaperons. Most of the Hsp interacts with other proteins in cells and alters their function. When the cells are subjected to stress such as hyper or hypo thermic shock are overexpressed. In this way, they exert a cyto-protective effect, protecting the cells against harmful insults, thus making the cells resistant to apoptosis (7). In a heat-shocked cell, the HSP may bind to the heat-sensitive proteins and protect them from degradation or may prevent damaged proteins from immediately precipitating and permanently affecting cell viability (10). In chronic cold stress groups, the expression of Hsps was increased. The results showed that effect of stress on the animal immune system is complicated (32). Hsp40 cooperates with Hsp70 to facilitate protein folding (22). This study was concluded the active material in eucalyptus leaves and oil (1.8-cinole and phenolic constituents) due as antioxidant which cause scavenging free radicals lead to improving body performance when chicks exposed to cold temperature also improved BWG and FCR, moreover decreased the mortality which reflected positively on the profit of the study project. As well as more development of small intestine morphology especially duodenum, and enhanced the secretion of duodenum digestive enzymes and lactobacillus. The volatile oils and nutrient values of eucalyptus leaves and oil dietary addition increased bird appetite which affirmatively in the perfection of meat juicy and favor, also improved antioxidant statues more vit. E and reduced TBARS in blood and meat. Though EuP and EuO were decreased the action of cold stress influenced significantly reduce HSP expression the damage of intestinal mucosa, which could effectively scavenge oxygen free radicals, improve body oxidation reduction system imbalance, reduce oxygen free radical damage to cells in mucosal tissues, and improve the permeability of intestinal mucosa, thus effectively maintaining the structure and function of the intestinal barrier. Finally the uses of 1.0% EuP and all levels of EuO in this study play an imported role to elevated cold stress. The best age for marketing is 35 days.

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