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BIOCHEMICAL ROLE OF PEPPER- MINT AND SWEET BASIL IN BROILER CHICKEN NUTRITION

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ABSTRACT

To evaluate the efficacy and biochemical role of peppermint and sweet basil leaves powder as supplements of broilers diets a nutritional comparative study has been carried out. A total number of 60 rats were randomly assorted into five groups. Control group fed on basal diet and the other groups fed on diets fortified with dried peppermint or sweet basil leaves powder by 0.2 or 0.4%. The obtained results indicated that supplementations of broiler's diet with either peppermint or sweet basil reduce the mortality of broilers and the percentage of total body fat, subcutaneous fat and abdominal fat. Biochemical analysis of broiler's blood shows that feeding on diets containing peppermint or sweet basil was associated with increase in the level of plasma total protein, albumin and globulin. A/G ratio was reduced by feeding on peppermint and sweet basil. This reflects significant enhancement in chick's immunity by the investigated natural additives. Plasma total lipids, triglycerides, and total cholesterol were significantly reduced after feeding on diets supplemented with peppermint and sweet basil. There were no significant differences observed in plasma ALT, AST and ALP activities between different treatments. Peppermint and sweet basil significantly reduce the level of lipid peroxidation by induction of the antioxidant enzymes catalase and lactic dehydrogenase. Also the obtained results clearly indicated that dietary peppermint and sweet basil improve the digestibility by increasing the activities of amylase, lipase, trypsin and chymotrypsin in gastrointestinal tract of broilers. The results conclude that supplementation of broiler's diets with 0.2% or 0.4% of dried leaves powder of peppermint or sweet basil improves

the digestibility, reduces the fats in the carcass and has no significant effect on the rate of growth or feed conversion ratio.

Key words: Broiler Chicken – Albumin – Globulin – Cholesterol – Triglycerides – Bilirubin - Liver function – Liver weight - Antioxidant enzymes - Digestive enzymes – Mortality – Peppermint - Sweet basil.

INTRODUCTION

Traditional medicinal herbs, in particular aromatic plants have gained much attention in recent years. They have potentials as alternative to antibiotics for therapeutic purposes and application in food industry because of their content of essential oils (terpenoid compounds). On the basis of their *in vitro* antimicrobial activity, it is logical to consider essential oils application as prophylactic and therapeutic agents in animal productions and especially in poultry production (Lee *et al.*, 2004). They are considered to optimize productive performance and enhance health of animals and strengthen the birds' defense system against invasion by infectious organisms. They also possess many bioactive ingredients with anti-microbial activities, immune enhancement and stress reduction properties (Wang *et al.*, 1998).

In the field of nutrition, there are a very limited number of controlled studies on the effect of essential oils components on growth performance and digestive enzymes in broiler chickens. However, there is evidence that a blend of essential oil components stimulates secretion of digestive enzymes in chickens (Williams and Losa, 2001). In addition, there is evidence that dietary essential oils lower serum cholesterol in chickens (Case *et al.*, 1995).

Peppermint, *Mentha piperita* (Labaitae), is shown to have antioxidant properties due to the presence of eugenol, ferulic acid, *p*-comaric acid, caffeic acid, rosmarinic acid and α -tocopherol in addition to its two major terpenoid content; menthol and menthone (Sharma and Kumar, 2006). Peppermint, traditionally, has been used to treat a variety of digestive complaints and as a spasmolytic to reduce gas and cramping. Peppermint is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, biliary tract disorders and liver complaints (Shah PP, D'Mello, 2004).

Peppermint is carminative, antispasmodic, decongestant, a cholagogue and has anesthetic activity (Mabey, 1998).

Sweet basil, *Osimum basilicum* (Lamiaceae), is native to India and Asia and has been known for its medicinal value. It is shown to have antioxidant property, recommended for digestive complaints, stomach cramp, vomiting and constipation. Basil has been described as having a slight sedative, for headache and anxiety. One study shows that basil leaf are able to inhibit carcinogen induced tumors (Ray Sahelian, 2004). Basil has been used for medicinal purposes as a digestive stimulant and for treatment of insomnia and constipation. Some of its volatile components have been suggested as allelopathic agent (Amparo and Elizabeth, 2003).

The present study aims to evaluate the effect of broiler's diet supplementation with peppermint and sweet basil dried leaves powder on the mortality, growth performance of broiler chickens. The antioxidant potential of both natural products has been investigated by measuring their effects on the activities of catalase and lactate dehydrogenase and the degree of lipid peroxidation. Also their effect on digestive enzymes activities of broiers was determined.

MATERIALS AND METHODS

A- MATERIALS

1- Birds

A total number of 60 unsexed broiler chicks were randomly assorted into five equal groups; twelve per each group, four chicks per cage.

2- Diets

The different five groups fed on different diet as follows:

Group (1), Control, fed on the basal diet which formulated to meet the recommended requirements of the chicks. The composition of the basal diet is shown in table (1).

Group 2 Peppermint 1, fed on diet contains 0.2% dried peppermint leaf powder substitution for 0.2% of wheat bran of basal diet.

Group 3: Peppermint 2, fed on diet contains 0.4% dried peppermint leaf powder substitution for 0.2% of wheat bran and 0.2% of salt of basal diet.

Group 4: Sweet basil 1, fed on diet contains 0.2% dried sweet basil leaf powder substitution for 0.2% of wheat bran of basal diet.

Group 5: Sweet basil 2, fed on diet contains 0.4% dried sweet basil leaf powder substitution for 0.2% of wheat bran and 0.2% of salt of basal diet.

Table (1): composition of the basal experimental diet (g/100g diet).

Ingredient	Starter diet*	Finisher diet*
Yellow corn	56.10	67.20
Soybean meal (44%)	37.80	28.00
Vegetable oil	2.60	1.50
Dicalcium phosphate	1.60	1.70
Permixon	0.30	0.30
DL-Methionine	0.10	0.05
Salt	0.30	0.30
Limestone	1.00	0.75
Wheat bran	0.20	0.20
Total	100	100

*Starter diet was applied in the first 21 days of the experimental period and then finisher diet was used.

The herbs additives were added to basal diet, manually mixed and stored for less than one week. The birds were fed their diet *ad libitum* and had free access water for 6 weeks feeding period. All birds were housed in battery cages with similar hygienic and managerial conditions.

B- METHODS

1- Biochemical Analysis

After 6 weeks of experimental period, chicks were sacrificed in a horizontal position to reduce the antiperistalsis movement of intestinal segments and regurgitation of their contents. Liver of each chick was separated and its weight was taken. Blood samples were collected in heparinized tubes, centrifuged at 5000g for 15 min.

Plasma were decanted and stored at -20°C for subsequent biochemical analyses as follows:

- **Total Protein** (g/dl) determined according to Henry (1974).
- **Albumin** (g/dl) estimated based on the method described by **Doumas et al, 1971**.
- **Globulin** (g/dl) calculated as the different between plasma albumin and plasma total protein.
- **Total lipids** (g/l) were determined according to Knight *et al.* (1972).
- **Cholesterol** (mg/dl) was determined according to Richmond (1973).

- **Triglycerides** (mg/dl) were estimated by the method of Trinder (1969).
- **Total Bilirubin** (mg/dl) determined by method described by Jendrassik (1938).
- **ALP** (E.C.3.1.3.1) (Alkaline Phosphatase activity) by method of Brooks and Purdy, 1972.
- **AST** (E.C.2.6.1.1) (Aspartate Transaminase activity) by Reitman and Frankel (1957).
- **ALT** (E.C.2.6.1.2) (Alanine Transaminase activity) by Reitman and Frankel (1957).

Immediately after chicks were sacrificed liver tissue samples were washed with physiological saline solution. The liver weights were recorded. Four grams of liver tissue were sliced and then homogenized for 5 min in a homogenizer with 22.5 ml of 150 mM of cold KCl-Tris-HCL buffer (PH 7.2) and centrifuged at $5000 \times g$ for 10 min to give a supernatant as liver homogenate.

Total protein content, the degree of lipid peroxidation (LPO), the activity of catalase (CAT) (EC:1.11.1.6) and lactate dehydrogenase (LDH) (EC:1.1.1.27) were determined in liver homogenate as follows:

- **Total Protein** was determined in liver homogenate according to the method of Lowry *et al.* (1951).
- **The degree of lipid Peroxidation** was estimated through the measurement of the concentration of the major product of lipid peroxidation process, malondialdehyde (MDA), by using spectrophotometric method described by Varshney and Kale (1990). The degree of lipid Peroxidation was expressed as μ moles malondialdehyde (MDA) /g FW.
- **Catalase activity** was measured in liver homogenate according to the method of Aebi (1984). The activity has been expressed as moles of H_2O_2 reduced /min. mg protein.
- **Lactate Dehydrogenase Activity** was assayed by measuring the rate of oxidation of NADH at 340 nm, according to the method of Ree (1970).

Measurement of Pancreatic Enzymes Activities in digestive tract of broiler chicks:

The contents and the adjacent epithelial lining of stomach, duodenum, and upper ileum were taken and individually centrifuged

at 7500g for 20 min. The supernatants were collected and used for hydrolytic enzymes determinations as follow:

- **Amylase Enzyme Activity** was measured as described by Sklan *et al.* (1975).
- **Lipase Enzyme Activity** was measured as mentioned by Sklan and Helevy (1985).
- **Trypsin and Chymotrypsin Enzymes Activities** were measured as described by Pinchasov and Noy (1994).

2- Growth performance parameters of the Chicks

As the performance of the chicks under investigation is the end product of this study, the following growth performance parameters were determined as follow:

- **Body Weight** was recorded for each individual chick at the beginning and the end of the experiment
- **The Weight Gain** was calculated by subtract the initial weight from the final weight.
- **Feed Intake per Cage** was recorded at the end of experiment.
- **Overall feed consumption and feed conversion ratio** i.e. grams of feed that required to obtain one gram of body weight during a given period were determined.
- **% of fats in carcass** was determined by isolation of abdominal fat, subcutaneous fat, and total fat. The percentage of fat was calculated on a base of fat weight.
- **% of mortality** was calculated as percentage of mortal chicks.

3-Statistical Analysis

Differences between treatment means and between treatment means and control means for different parameters were carried out using ANOVA test. Multiple comparasion between different treatments were carried out by using Duncan's multiple range test. Statistical calculations were done using SAS statistical software (SAS 1994).

RESULTS AND DISCUSSION

To follow up the biochemical effect of peppermint and sweet basil leaf powders as additives and/or supplements to broiler chicken diets, traits with their responsible biochemical elements and enzyme

activities such as: plasma biochemical profile (protein, lipid and bilirubin), liver function and liver weight, antioxidant potentials (oxidative enzyme activities), digestion efficiency (digestive enzyme activities) and growth performance (growth performance parameters) in addition to the lean body fat and the mortality ratio were determined. The obtained results with their discussions concerning the effect of peppermint and sweet basil on the above mentioned traits which may appear their biochemical role in broiler chicken nutrition are summarized as follow:

1- Effect of Dietary Peppermint and Sweet Basil on Plasma Biochemical Profile (Protein, Lipid and Bilirubin)

1-1- Effect of Dietary Peppermint and Sweet Basil on Plasma Protein Profile

Data in Table (2) show the effect of both additives; peppermint and sweet basil on total protein (g/dl), albumin (mg/dl), globulin (mg/dl) and albumin/globulin ratio (A/G ratio) in plasma at the end of experiment. Generally, the obtained data showed significant increases in total protein, albumin and globulin in plasma by the addition of the two additives in all applied concentrations. With respect of the control opposite effect was found for the A/G ratio since it decreased significantly under the influence of all treatments with different additives. The significant reduction of A/G ratio reflects that globulin increase is higher than albumin increase. Thus feeding on diets supplemented with peppermint and sweet basil enhanced the level of immunity.

Table (2): Effect of dietary peppermint and sweet basil on plasma protein profile in broiler chick

Diet	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Control	2.85 ± 0.28 ^b	2.00 ± 0.03 ^c	0.85 ± 0.08 ^b	3.46 ± 0.13 ^a
Peppermint 1	4.40 ± 0.35 ^a	2.34 ± 0.12 ^{cd}	2.17 ± 0.43 ^a	1.15 ± 0.20 ^b
Peppermint 2	4.55 ± 0.26 ^a	2.15 ± 0.15 ^{ab}	2.50 ± 0.26 ^a	1.32 ± 0.23 ^b
Sweet basil 1	4.81 ± 0.22 ^a	2.59 ± 0.13 ^a	2.22 ± 0.19 ^a	1.20 ± 0.14 ^b
Sweet basil 2	4.95 ± 0.21 ^a	2.66 ± 0.03 ^a	2.30 ± 0.20 ^a	1.80 ± 0.10 ^b

• Value is a mean of four replicates. *Means followed by unlike letters differ significantly (p<0.05).

The increasing of total protein content and its fractions by feeding on diets containing the aromatic plants is indicative of enhancing the protein synthesis which could be evidenced by the increasing of the liver weight, since our obtained results showed a significant increase in the chicken liver weight by the treatment of both additives; peppermint and sweet basil (Table 4). These findings are in concomitant with those by Williams *et al.* (1999) and Sharma and Kumar (2006), which stated that the increase in the levels of microsomal protein at all doses of peppermint is indicative of induced protein synthesis and possibly that associated with increase the numbers of ribosomes in the endoplasmic reticulum, which could be responsible for the increase in chicken liver weight.

1-2- Effect of Dietary Peppermint and Sweet Basil on Plasma Lipid Profile

Data presented in Table (3) show that total lipids content (g/l) were significantly decreased by all levels of peppermint and sweet basil. The lowest value was recorded at the highest concentration of the sweet basil addition. The same trend of effect could observe in the case of triglyceride and cholesterol content since they showed significant decreases of their content in plasma and shows the lowest value by the addition of the lower concentrations of peppermint and sweet basil (peppermint 1 and sweet basil 1, respectively). This results led to the suggestion that peppermint and sweet basil leaf are hypolipidemic agents as the same as hypotriglyceridemic and hypocholesterolaemic agents when they used as food additives in chickens. These results are in agreement with the findings of Yu *et al.* (1994) and Elson (1995).

The hypolipidemic effect of essential oils especially the lowering of cholesterol content (hypocholesterolaemic effect) has been ascribed to suppressing of 3-hydroxy-3-methylglutaryl Co-A reductase (HMG CoA reductase) (Elson, 1995), the enzyme that is consider to be rate limiting enzyme in cholesterol synthesis (Goldstein and Brown, 1995). In detailed study, the pure cyclic monoterpenes such as menthol from peppermint and 1,8-cineole from sweet basil inhibited hepatic HMGCoA reductase by 50–60 % after 17 hr of oral administration of a single dose to rats, which resulted in 52% inhibition of sterol synthesis, 55% inhibition of fatty acid synthesis and finally significant inhibition of lipogenesis (Middleton and Hui, 1982). Also, peppermint

and sweet basil could stimulate bile acid secretion as reported by (Platel and Srinivasan, 2004)

Table (3): Effect of dietary peppermint and sweet basil on plasma lipid profile in broilers chicks

Diet	Total lipids (g/L)	Triglycerides (mg/dl)	cholesterol (mg/dl)
Control	10.18 ± 0.62 ^a	228.92 ± 22.02 ^a	152.75 ± 30.78 ^a
Peppermint 1	7.980 ± 0.96 ^b	97.93 ± 14.55 ^b	131.88 ± 15.30 ^{ab}
Peppermint 2	7.550 ± 0.44 ^b	98.83 ± 7.64 ^b	109.71 ± 5.76 ^b
Sweet basil 1	8.170 ± 0.37 ^b	173.06 ± 42.86 ^c	104.85 ± 25.93 ^b
Sweet basil 2	7.010 ± 0.22 ^b	104.50 ± 19.42 ^b	134.79 ± 26.62 ^{ab}

* Value is a mean of four replicates.

*Means followed by unlike letters differ significantly (p<0.05).

2- Effect of dietary peppermint and sweet basil on liver function and liver weight

Liver functions as they expressed by the activities of certain enzymes in the plasma such as Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP), in addition to the secretion of bile salts (total bilirubin) are presented in Table (4). Data obtained show no significant changes in the ALT and AST activities A significant decrease in ALP activity was observed when broilers fed on diets supplemented with 0.2% sweet basil. Bilirubin was significantly increased by all treatments and almost doubled by the low dose of sweet basil treatment. These obtained results are in agreement with those by Liem *et al.* (2003), which they observed that the use of peppermint increase significantly the bile salt secretion and the ALP activity in human especially when it is in a high dose. The observed significant increases in bilirubin do not reflect any harmful effect of the investigated diet additives because the bilirubin level in all treatments is in normal range.

Liver weight as shown in Table (4) was significantly increased by feeding on diets containing peppermint and sweet basil in a dose and a type dependant manner. The highest liver weight was observed in the high dose of sweet basil. These findings are in concomitant with those by Williams *et al.* (1999) which stated that the increase in the levels of microsomal protein by peppermint is indicative of induced protein

synthesis and possibly that associated with endoplasmic reticulum, which could be responsible for the increase in chicken liver weight.

In general, the dietary of sweet basil and peppermint have not shown any increasing effect on liver enzymes activities. These non-significant changes in liver enzymes activities reflects a high degree of safety of the peppermint and sweet basil leaves as additives to broiler's diets (Liem *et al.*, 2003). These findings lead to conclusion that supplementation with both; peppermint and sweet basil under the applied levels could enhance the protein synthesis without any toxicity of liver.

Table (4): Effect of dietary peppermint and sweet basil on liver enzymes activities, total bilirubin and liver weight in broilers chicks

Diet	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	T. bilirubin (mg/dl)	Liver weight (g)
Control	6.43±0.77 ^a	29.88±0.83 ^a	199.85±46.65 ^a	1.33±0.04 ^d	45.70±3.77 ^d
Peppermint 1	6.08±0.39 ^a	34.58±1.33 ^a	187.68±55.35 ^a	1.62±0.11 ^c	61.51±5.16 ^c
Peppermint 2	5.61±0.91 ^b	30.40±1.80 ^a	168.43±71.31 ^{ab}	1.81±0.04 ^b	68.73±6.45 ^c
Sweet basil 1	6.40±0.78 ^a	30.78±3.60 ^a	144.25±15.40 ^b	2.05±0.02 ^a	78.35±5.84 ^b
Sweet basil 2	6.27±0.50 ^a	33.58±2.10 ^a	169.42±46.22 ^{ab}	1.85±0.06 ^b	86.92±6.61 ^a

* Value is a mean of four replicates.

*Means followed by unlike letters differ significantly ($p < 0.05$).

3- Effect of Dietary Peppermint and Sweet Basil on Oxidative Enzymes (Antioxidant potentials)

The effect of the two additives; peppermint and sweet basil to broiler chicks' diet on the oxidative enzymes such as CAT (catalase) and LDH (lactate dehydrogenase) and the lipid peroxidation as malonaldehyde content in blood (LPO) are shown in Table (5). The obtained results reveal that the two levels of the two additives have the same trend of effect in general on the specific activities of oxidative enzymes, CAT, LDH. While the activities of CAT and LDH were increased significantly. The level of LPO was oppositely decreased with majority of sweet basil effect in general. The reduction of lipid peroxidation is clearly explained by the increasing activities of antioxidant defensive enzymes

CAT plays an important role in the detoxification of peroxide and hydrogen peroxide which induces the LPO. Also, catalase is a major source of protection against severe oxidative stress (Yan and Harding,

1997), its activity was significantly increased by the administration of the peppermint and sweet basil, also, they induced significantly LDH activity, which plays its important role via the electron flows from NADPH-H^+ to NADP^+ through a flavoprotein P_{450} reductase or cytochrome b_5 reductase to different isomorphous forms of cytochrome P_{450} and cytochrome b_5 (Gibson and Skett, 1994).

CAT can catalyze the reduction of lipid hydroperoxides to their corresponding alcohols via glutathione, whereas glutathione serves as a hydrogen donor to lipoxy radicals which resulting in conversion of lipoxy radicals to hydroperoxides again (Ozturk-Urek *et al.*, 2001).

By increasing the activity of CAT under the influence of the essential oils of peppermint and sweet basil, the conversion of hydroperoxides to their corresponding alcohols will increase, meanwhile, an increasing of glutathione GSH could occur because of its participating with CAT and resulting in accumulation of their corresponding alcohols, which prevent (feed back inhibition) their propagation of a lipid free radicals chain reaction, and thus, decreasing the lipid peroxidation level (LPO). From the other point of our view, as decreasing the level of LPO which means lowering the production of peroxides and hydroperoxides (oxidative stress) in a companying with increasing the activities of both CAT (protector against severe oxidative stress) and LDH (canceller the oxidative stress) with such low level of peroxides and hydroperoxides production under the influence of peppermint and sweet basil, thus, peppermint and sweet basil could neutralize lipid peroxidation (LPO) and cancel its harmful effect.

Based on our results it could emphasize that using peppermint and sweet basil as food additives induces specific activities of antioxidant defensive enzymes in the liver which strongly assure the antioxidant potential of peppermint and sweet basil as chemopreventive prophylaxis agents via their capability to induce the activity of enzymatic antioxidant defense components; CAT and LDH in liver.

Table (5): Effect of dietary peppermint and sweet basil on CAT and LDH activities and LPO levels in broilers chicken liver

Diet	CAT (catalase)	LDH (Lactate dehydrogenase)	LPO (Lipid peroxidation)
Control	2.03 ± 0.10 ^c	162.06 ± 11.32 ^d	52.54 ± 2.57 ^a
Peppermint 1	3.01 ± 0.09 ^b	224.10 ± 12.58 ^c	44.18 ± 0.68 ^a
Peppermint 2	1.90 ± 0.14 ^c	266.79 ± 21.24 ^c	50.99 ± 1.43 ^b
Sweet basil 1	6.33 ± 1.40 ^a	350.33 ± 40.83 ^b	35.05 ± 1.88 ^c
Sweet basil 2	6.12 ± 0.58 ^a	457.14 ± 16.50 ^a	28.71 ± 2.39 ^d

- CAT activity was estimated as: (Δ OD.min⁻¹.mg⁻¹protein x10⁻³)

- LDH activity was estimated as: (μ moles malondialdehyde/g FW)

- LDH activity was estimated as: (IU. Mg⁻¹ protein)

* Value is a mean of four replicates.

*Means followed by unlike letters differ significantly (p<0.05).

4- Effect of Dietary Peppermint and Sweet Basil on Digestion Efficiency

Digestion efficiency of broiler chicks fed on peppermint or sweet basil has been followed by measuring of the activities of the digestive enzymes throughout the intestinal tract; stomach, duodenum and upper ileum. The three main food elements enzymes activities were followed in intestinal tract are; Amylase for carbohydrates digestion, Lipase for lipids digestion, and Trypsin and Chymotrypsin for proteins digestion. The obtained data are presented in Table (6).

The activity of amylase enzyme, the data in general show significant increasing in all chicks fed either on peppermint or on sweet basil leaf powder at both levels of addition. A higher effect of the sweet basil in its two applied concentrations could recognize than the effect of peppermint with its two applied concentrations in general. The obtained results could emphasize that both additives; peppermint and sweet basil enhance the carbohydrates digestion in broiler chicks and the sweet basil has the major effect than the peppermint.

The activity of lipase enzyme shows the same trend of effect as amylase activity, where a significant increasing in its activity is gained by the two additives at the two applied levels and the sweet basil additive has better effect than the peppermint additive also. These results show that both additives; peppermint and sweet basil could

Table (6): Effect of dietary peppermint and sweet basil on digestive enzymes activities in intestinal tract of broilers chicks

Enzyme	Diet	Intestinal tract		
		Stomach (IU/g homogenate)	Duodenum (IU/g homogenate)	Upper ileum (IU/g homogenate)
<u>Amylase</u> (EC: 3.2.1.1)	Control	0.35 ± 0.04 ^{cd}	0.65 ± 0.04 ^c	0.98 ± 0.06 ^{cd}
	Peppermint 1	0.30 ± 0.02 ^d	0.59 ± 0.03 ^{cd}	0.86 ± 0.05 ^d
	Peppermint 2	0.49 ± 0.03 ^{ab}	0.46 ± 0.03 ^d	1.14 ± 0.07 ^{bc}
	Sweet basil 1	0.52 ± 0.03 ^a	0.92 ± 0.05 ^b	1.25 ± 0.07 ^{ab}
	Sweet basil 2	0.41 ± 0.02 ^{bc}	1.18 ± 0.07 ^a	1.39 ± 0.08 ^a
<u>Lipase</u> (EC: 3.1.1.3)	Control	2.63 ± 0.15 ^b	9.61 ± 0.55 ^{bc}	8.40 ± 0.48 ^c
	Peppermint 1	3.16 ± 0.18 ^{ab}	10.20 ± 0.59 ^{bc}	9.15 ± 0.50 ^c
	Peppermint 2	2.81 ± 0.16 ^b	8.36 ± 0.48 ^c	12.30 ± 0.71 ^b
	Sweet basil 1	3.56 ± 0.21 ^a	10.83 ± 0.63 ^b	13.82 ± 0.80 ^{ab}
	Sweet basil 2	2.94 ± 0.017 ^b	13.52 ± 0.78 ^a	15.60 ± 0.90 ^a
<u>Trypsin</u> (EC: 3.4.21.4)	Control	33.28 ± 1.92 ^b	32.54 ± 1.84 ^{bc}	18.36 ± 1.6 ^c
	Peppermint 1	29.60 ± 1.71 ^c	28.39 ± 1.64 ^c	21.20 ± 1.22 ^{bc}
	Peppermint 2	26.64 ± 1.84 ^d	37.16 ± 2.15 ^{ab}	24.15 ± 1.39 ^b
	Sweet basil 1	35.75 ± 2.06 ^a	35.64 ± 2.06 ^a	30.34 ± 1.75 ^a
	Sweet basil 2	32.12 ± 1.61 ^b	42.43 ± 2.45 ^a	34.65 ± 2.00 ^a
<u>Chymotrypsin</u> (EC: 3.4.21.1)	Control	5.85 ± 0.34 ^a	8.22 ± 0.47 ^c	10.80 ± 0.62 ^b
	Peppermint 1	6.42 ± 0.38 ^a	12.30 ± 0.71 ^{ab}	10.36 ± 0.60 ^b
	Peppermint 2	6.75 ± 0.39 ^a	13.43 ± 0.78 ^{ab}	12.40 ± 0.78 ^{ab}
	Sweet basil 1	4.28 ± 0.25 ^b	11.35 ± 0.66 ^b	12.55 ± 0.72 ^{ab}
	Sweet basil 2	6.19 ± 0.36 ^a	14.26 ± 0.82 ^a	14.35 ± 0.83 ^a

* Value is a mean of four replicates.

*Means followed by unlike letters differ significantly (p<0.05).

play an important role in elaborating the lipids digestion in broiler chicks and the sweet basil has the major effect than the peppermint.

In contrast to amylase and lipase, the trypsin activity was decreased significantly in stomach in case of feeding on peppermint by both levels. By following up the trypsin activity in the intestinal tract; duodenum and upper ileum a significant increasing in its activity was found under the effect of the two additives and still the sweet basil additive has the major effect. For the activity of the chemotrypsin enzyme, no significant changes could find in the stomach while a significant increasing could observe in the duodenum and the upper ileum. These results conclude that both additives; peppermint and sweet basil decreased significantly the digestion of protein in stomach and increased it significantly in duodenum and in upper ileum.

Based on the obtained results it might be reasoned that dietary peppermint and sweet basil with their essential oils content will positively affect food digestion and improve digestion efficiency (Anonymous, 1997) via increasing secretion of pancreatic enzymes (Lee *et. al.*, 2003) and stimulating the activities of digestive enzymes of intestinal mucosa and of pancreas (Platal and Srinivasan, 2000) in addition to the increasing of the bile salt secretion (Srinivasan and Sambalah, 1991).

These results are in agreement with those obtained by Lee *et al.* (2004) which stated that dietary essential oils such as thymol and cinnamaldehyde stimulate secretion of pancreatic digestive enzymes, i.e. amylase, lipase, trypsin and chymotrypsin in female broiler chicken.

5- Effect of Dietary Peppermint and Sweet Basil on Growth Performance

No general trend could detect concerning the effect of both additives; peppermint and sweet basil leaf powder in their two applied concentrations on the growth performance parameters of broiler chicks. Data in Table (7) shows alternative decreases and increases of the body weight, body weight gain, feed consumption and feed conversion ratio of the broilers chicks. Although the two applied concentrations of sweet basil showed significant increases in feed conversion ratio and feed consumption (high concentration only), they oppositely lowered the body weight and the body weight gain

significantly which led to the suggestion that sweet basil leaf powder could enhance the food metabolism of broiler chicks without increasing the body weight. For the peppermint leaf powder addition, the obtained data reveal that only the high concentration gave significant increases in body weight, body weight gain and feed consumption which gives attention that peppermint leaf powder addition in high dose to broiler chicks' diet could increase their body weight gain significantly in addition to the enhancement of the food consumption. Our observations concerning the effect of the two additives; peppermint and sweet basil on the growth performance are in line with the observations of Langhout (2000) and Botsoglou *et al.* (2002).

Table (7): Effect of dietary peppermint and sweet basil on growth performance parameters

	Body weight		Body weight gain (g/6 weeks)	Feed consumption (g/bird)	Feed conversion ratio (feed gain)
	Initial (g/start exp.)	Final (g/6 weeks)			
Control	42.35 ± 1.21	1688.92 ± 00.50 ^b	1646.51 ± 81.17 ^b	3309.69 ± 135.81 ^b	2.01 ± 0.05 ^{cd}
Peppermint 1	42.15 ± 1.34	1672.14 ± 85.28 ^b	1629.95 ± 80.54 ^b	3211.10 ± 136.20 ^c	1.97 ± 0.06 ^d
Peppermint 2	41.86 ± 1.28	1739.75 ± 80.34 ^a	1697.90 ± 78.62 ^a	3446.74 ± 148.17 ^a	2.03 ± 0.07 ^c
Sweet basil 1	42.63 ± 0.95	1618.43 ± 86.75 ^c	1575.72 ± 72.13 ^c	3308.95 ± 141.56 ^b	2.10 ± 0.12 ^b
Sweet basil 2	41.96 ± 1.43	1630.60 ± 66.40 ^c	1588.62 ± 69.82 ^{bc}	3463.16 ± 118.65 ^a	2.18 ± 0.08 ^a

* Value is a mean of four replicates.

*Means followed by unlike letters differ significantly ($p < 0.05$).

The observed effects of essential oils contained plants on growth performance in broiler chickens are either positive (Langhout, 2000) or non-significant (Botsoglou *et al.*, 2002). When the effect was positive, weight gain and feed consumption were increased whereas the feed gain ratio was decreased. The lack of effect on growth performance could be explained by the non-useful role of the essential oils of peppermint and sweet basil on growth performance since the chickens performance was already superior by carefully cleaned housing and disinfected cages for the birds, leaving no chance for

growth enhancing effect of the additives. This may indicate that when experimental conditions and diets are marginal for the birds, a growth enhancing effects of the additives (essential oils contained plants) will be seen (Anonymous, 1997). Indeed, this interpretation could be confirmed by the explanation of Allen *et al.* (1997) when reported that two dietary essential oil components; camphor (camphor) and 1,8-cineol (sweet basil) showed no clear effects on weight gain when birds were reared without coccidian challenge, but led to significant weight gains when the birds were infected with coccidian. In conclusion, our results supported by related literature indicates that the effect of the dietary terpenoid additives on growth performance become apparent when chickens are subjected to suboptimal conditions such as a less digestible diet and/or a less clean environment.

6- Effect of Dietary Peppermint and Sweet Basil on Lean Body Fat and Mortality Ratio

Data presented in Table (8) shows an important role of both food additives; peppermint and sweet basil on lean body fat of broiler chickens since they decreased the abdominal fat and the subcutaneous fat significantly by the treatment with their two concentrations. The total body fat decreased significantly too under the influence of the two additives with their two applied concentrations and the largest effect was observed at the high concentration of peppermint.

The mortality percentage of the broiler chickens under this investigation showed very good results, since it lowered to 66% and 47% by the application of the low and the high concentrations of sweet basil leaf respectively. The addition of peppermint leaf powder to chickens diets is more effective of mortality percentage than sweet basil leaf powder since it lowered the mortality percentage to 30% and 21% with the two concentrations; the low and the high respectively.

These results show that both dietary peppermint and sweet basil have great effect on reducing the mortality ratio among the chicken under investigation which may be consider to be as a results of improving the immunity system of the broiler chickens through enhancing of the immunity responsible factors; albumin and globulin in addition to albumin/globulin ratio (A/G ratio) in plasma (Table 2),

Table (8): Effect of dietary peppermint and sweet basil on lean body fat and mortality percentage of broilers chicks

Diet	Abdominal fat	Subcutaneous fat	Total body fat %	Mortality %
Control	55.80 ± 6.35 ^a	90.15 ± 8.42 ^a	7.61 ± 0.45 ^a	4.2 %
Peppermint 1	22.85 ± 2.41 ^b	20.92 ± 1.14 ^c	2.40 ± 0.13 ^c	1.3 %
Peppermint 2	16.65 ± 1.24 ^c	12.50 ± 1.43 ^d	1.63 ± 0.05 ^d	0.9 %
Sweet basil 1	26.34 ± 1.35 ^{bd}	48.10 ± 3.52 ^b	4.43 ± 0.25 ^b	2.8 %
Sweet basil 2	32.18 ± 2.46 ^d	39.41 ± 2.75 ^b	4.26 ± 0.65 ^b	2.0 %

* Value is a mean of four replicates.

*Means followed by unlike letters differ significantly ($p < 0.05$).

Conclusions

Based on the results from this study, the well-recognized improvement of broiler chickens are overall performance and the diminishing of their mortality ratio after feeding on basal diet incorporated with peppermint and sweet basil. These could be mediated through different modes (*i*) stimulation of enzyme activities that participate in digestion, both of pancreatic and intestinal origin, (*ii*) stimulation of the liver to increase protein biosynthesis and reduce lipogenesis. (*iii*) the antioxidant properties of the herbs that may stimulate the antioxidant defensive enzymes and reduce the peroxidation of cellular membrane lipids, and (*v*) enhancing the immunity of the birds that may resulted from the increasing of plasma globulin, and (*vi*) enhancing chicks quality by decreasing the percentage of abdominal and subcutaneous fats. Such stimulation and enhancement properties of the herbs under investigation lead to improve the broiler chicken performance and reduce their mortality ratio.

From economic point of view, the most important outcome of this investigation is the ability of these natural additives to diminish the mortality ratio to less than 50% compared to control. The total end product (chickens) will increased significantly via increasing the total number of the living chickens in general, which means that the dietary peppermint and sweet basil increase the total end product of chicken production.

Moreover, these treatments increased significantly the total protein and decreased significantly the total lipid, triglycerides and cholesterol in chicken blood plasma and resulted in high quality meat characterized with high protein, low fat, low cholesterol and even low skin fat which means high quality broiler chicken meat.

Thus the dietary peppermint and sweet basil leaf powders may play an important role as food supplements and/or food additives for broiler chickens in improving their final total production with enhancing their meat quality.

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الدور الكيميائي الحيوي لكلامن النعناع والريحان في تغذية كتاكيت اللحم

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- تم تقسيم ٦٠ دجاجة شواء لى ٥ مجموعات حسب طريقة التغذية المقدمة اليهم كالاتى:
- ١- مجموعة التغذية القياسية والتي تتغذى على الوجبة الأساسية (الكنترول)
 - ٢- مجموعة تتغذى على الوجبة الأساسية + ٠,٢% مسحوق ورق النعناع مستبدلة بـ ٠,٢% نخالة
 - ٣- مجموعة تتغذى على الوجبة الأساسية + ٠,٤% مسحوق ورق النعناع مستبدلة بـ ٠,٢% نخالة و ٠,٢% ملح
 - ٤- مجموعة تتغذى على الوجبة الأساسية + ٠,٢% مسحوق ورق الريحان مستبدلة بـ ٠,٢% نخالة
 - ٥- مجموعة تتغذى على الوجبة الأساسية + ٠,٤% مسحوق ورق الريحان مستبدلة ب ٠,٢% نخالة و ٠,٢% ملح
- أدت المعاملات السابقة الى أهم النتائج الآتية:
- ١- حدوث زيادة معنوية فى البروتينات الكلية فى بلازما الدم
 - ٢- حدوث زيادة معنوية فى تركيز كلا من الألبومين والجلوبيولين فى بلازما الدم
 - ٣- حدوث إنخفاض معنوى فى نسبة الألبومين الى نسبة الجلوبيولين فى بلازما الدم
 - ٤- حدوث إنخفاض معنوى فى الليبيدات الكلية الموجودة فى بلازما الدم
 - ٥- حدوث إنخفاض معنوى فى الجليسيريدات الثلاثية الموجودة فى بلازما الدم
 - ٦- حدوث إنخفاض معنوى فى نسبة الكوليستيرول الموجود فى بلازما الدم

- ٧- عدم حدوث أى تأثير معنوى على نشاط أنزيمات وظائف الكبد مثل: ALT, AST and ALP
- ٨- حدوث زيادة معنوية فى تركيز البيلروبين الكلى Total Bilirubin فى البلازما
- ٩- حدوث زيادة معنوية فى نشاط إنزيم الأميليز Amylase فى كلا من القناة الهضمية وبلازما الدم
- ١٠- حدوث زيادة معنوية فى نشاط إنزيم الليباز Lipase فى كلا من القناة الهضمية وبلازما الدم
- ١١- حدوث زيادة معنوية فى نشاط إنزيم التربسين Trypsin فى كلا من القناة الهضمية وبلازما الدم
- ١٢- عدم حدوث تغيير معنوي فى نشاط إنزيم الكيموتربسين Chemotrypsin فى كلا من القناة الهضمية وبلازما الدم
- ١٣- حدوث زيادة معنوية فى نشاط إنزيم الكاتاليز (Catalase) CAT
- ١٤- حدوث زيادة معنوية فى نشاط إنزيم اللاكتات ديهيدروجينيز LDH (Lactate dehydrogenase)
- ١٥- حدوث إنخفاض معنوى فى أكسدة الليبيدات (LPO (Lipid Peroxidation) لم يكن هناك إتجاه محدد لتأثير النعناع والريحان بتركيزيهما المستخدممين على الصفات الإنتاجية لدجاج الشواء حيث:
- ١- لم تحدث زيادة معنوية فى وزن الجسم بعد ٦ أسابيع من إستخدام النعناع بتركيزيه والريحان بتركيزه المنخفض
- ٢- حدث أنخفاض معنوى فى وزن الجسم بعد ٦ أسابيع من إستخدام الريحان بتركيزه المرتفع
- ٣- حدث أنخفاض معنوى فى الوزن المكتسب للجسم بإستخدام النعناع بتركيزيه والريحان بتركيزه المنخفض
- ٤- حدث إرتفاع معنوى فى الوزن المكتسب للجسم بإستخدام الريحان بتركيزه المرتفع
- ٥- لم يحدث تغيير معنوى فى كلا من معدل إستهلاك الغذاء أو معامل التحويل تحت تأثير كل المعاملات تحت الدراسة
- ٦- حدوث أنخفاض معنوي في نسبة الطيور النافقة بإستخدام النعناع و الريحان
- ٧- حدوث أنخفاض معنوي في نسبة الدهن في الذبيحة المغذاة على النعناع أو الريحان

إستنتجت هذه الدراسة: بناء على النتائج المتحصل عليها أن مسحوق أوراق كلا من النعناع والريحان عند إستخدامهم كمكملات أو كمضافات غذائية للوجبات الأساسية لدجاج الشواء يمكن أن تلعب دور مهم فى تحسين إنتاجهم النهائي الكلى (ناتج لحم) مع تحسين نوعية وجودة لحوم الدجاج الناتج.