Influence of Peppermint, Fenugreek and Their Mixture on Biochemical Status of Heat Stressed Broiler Chickens

Ghadeer Abbass
Department of Public Health, College of Veterinary Medicine, University of Kerbala, Iraq

Ali Redha Abid
Department of Public Health, College of Veterinary Medicine, University of Kerbala, Iraq

Abstract

This study carried out to evaluate the effect of dietary peppermint , fenugreek and their mixture on biochemical traits of broilers under heat stress The experiment was conducted in special house for 35days from 6 March to 10 April / 2022 .It involved 120 unsexed one day Ross 308 broiler chicks , that were divided into four groups (30 /group) with three replicated (10 birds/replicate), the first treatment (control): chicks were fed on a basal diet without addition(T1) ,the second treatment fed on a basal diet with 1.5 gm peppermint / Kg diet(T2) ,the third treatment fed on a basal diet with 1.5 gm fenugreek Kg diet (T3)/,the fourth treatment was fed on a basal diet with 1.5 gm fenugreek + 1.5 gm peppermint /Kg diet(T4) .), Blood samples were collected for biochemical parameters at 17thday and 35thday of age, the result revealed there were a significant decrease ($p \leq 0.05$) in the total cholesterol, triglycerides and low density lipoprotein (LDL) while high density lipoprotein (HDL) has been a significantly increased of addition treatments as compared with control. The result of total protein, albumin and globulin that recorded no significant differences($P \leq 0.05$) among treatments.

Liver function enzymes such as aspartate transaminase, alanine transaminase and alkaline phosphatase were measured at the end of the period showed a significant decrease ($p \leq 0.05$) in the addition treatment comparing with control. the result appear significant increase ($P \leq 0.05$) in addition treatment as compared to control treatment in value of catalase , Superoxide dismutase and Glutathione peroxidase at 17 and 35 days of experiment also results appeared a significant increase($p<0.05$) in control treatment as compared with addiction treatment in heterophil/lymphocyte ratio of broilers at 17th and 35th days under heat stress .There were also a significant increase ($p \leq 0.05$) in anti-body titer against Newcastle disease and Infectious bursal viruses in addition treatment as compared with the control.

Keywords: *fenugreek, peppermint, broiler, heat stress.*

INTRODUCTION

Poultry products can be considered as one of the most important sources of cheap protein as compared with the red meat (cow meat).So the price of one kg of animal meat is equivalent to the price of 3–4 kg of poultry meat. Poultry production is characterized be a higher conversion rate of feed to meat in comparison with other animals, where the production of one kg of poultry meat needs from 2 to 2.5 kg of feed meanwhile the production of one kg of red meat needs more than seven kg of feed (Anonymus. 2017). High economic return due to its short production cycle, where the production cycle of poultry production takes 7–8 weeks, the capital cycle can be repeated 7 times a year (Anonymus. 2016) . Poultry production needs small area in comparison with other animals.

Heat stress consider a major barrier to coping with poultry farming in hot climate areas, leading to major economic losses in the poultry industry. Heat stress begins when the ambient temperature rises above the comfortable zone for poultry species (Diarra and Tabuaciri, 2014) When birds reared in hot, tropical or subtropical environments are kept constantly warm (over 30°C), they experience increased stress behaviors such as increased respiratory rate, panting, loss of appetite, and altered metabolism. Poor production performance is also associated with a number of negative outcomes, including poor fertility, dehydration, low liveability, morbidity and death, and altered meat quality characterized by increased adiposity and reduced skeletal muscle mass in broilers. (Raza et al., 2021).

Herbals has a stimulating effect on the digestive system of animals and poultry by increase in digestive enzymes that help the body to use food more effectively (Maheshwari et al., 2013). Medicinal plants are known for their antimicrobial properties, natural antioxidants, and ability to stimulate the immune system, too.

Peppermint is a member of the Labiatae family and one of the world's oldest medicinal herbs, and is used in both eastern and western traditions. It is widely used in herbal medicine and believed to be particularly beneficial in building of the immune system and fighting secondary infections (Nanekarani,S et al.,2012). peppermint is an important raw material that has been used as a carminative, antispasmodic, diuretic, and used as flavorings in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gum, desserts and candies. The main medicinal action of the leaves and flowers of the peppermint depend on the abundant menthol which is the main phenolic component which has antibacterial activities.Its also,contains polyphenolic compounds hence could possess strong antioxidant properties(Schuhmacher,A.et al.,2003).

Fenugreek plant, which is rich in protein, phosphorous, and starchy materials, have been used in the diet of laying hens since ancient times due to its pharmacological effectiveness, also fenugreek increase the Birds' demand for food increases, resulting in an increase in the body average weight. Rabia, (2010) was noted that the use of fenugreek seeds in the diet of laying hens has led to an improvement in productivity (Abaza, M. 2007) The aim of study using of peppermint, fenugreek and combination as feed additive to ameliorating the heat stress in broiler chicken.

Materials and Methods

This study was carried out in a private breeding house, from 6 March to 10 April / 2022. The chicks were obtained from a commercial hatchery of Karbala province. A total of 120 unsexed one-day broiler chicks (Ros308) were divided randomly into four treatments (30/birds) with 3 replicates each treatment, each replicate involved 10 birds/ pen, the experimental treatments as follows:

T1:(Control) basal diet without any addition.

T2: basal diet with dry peppermint leave at 1.5 gm/kg diet.

T3: basal diet with dry fenugreek leave at 1.5 gm/kg diet.

T4: basal diet with peppermint leave 1.5gm/kg and 1.5 gm/kg of fenugreek leave.

The experimental period was five weeks. Feed and water provided ad Libitum along the study,The chicks were fed at 1 to 21 days' age on a starter diet, afterwards were fed on a finisher diet until the age of 35 days. Table (2)

show basal diet and chemical analysis. Chicks were kept in floor cages under similar management and hygienic system in a close house.,. The lighting regime was 23:1 light-dark cycle. The chick exposure to heat stress starting in the second week by increase the temperature up to 31-34Co at 12pm to 4pm o'clock by using automatic heating incubator.

Preparation of poultry house

After cleaning the walls, floor and ceiling by clean water and disinfection by formalin and potassium permanganate then all windows were opened and ventilation were switched for ensuring removal of toxic gases completely before chick's entrance, all feeders and waterers were cleaned and disinfectant, too The experimental house was divided using wire mesh into 12 equal sized pens (2 m x 2 m). All experiment treatment was provided with suitable litter (wood shaving), experimental ventilation and lighting were controlled according to the Aviagen guide (Aviagen, 2022) for broiler chickens (Ros308).

The dietary additives used in the experiment

A. Fresh pepermint leaves were bought from Amil District Market in karbala, fresh Papermint An open, shaded area's floor was covered in leaves. Turn over once or twice a day .The collected dried Papermint leaves were crushed to reasonable size using a hammer mill.

B. Fresh fenugreek leaves was bought from local market in karbala , the fresh fenugreek leaves were visually sorted and trimmed ,the cleaned leaves was then dried in a normal tray of an open shady place with constant stirring then ground to crumble using a blender

Statistical Analysis

The data was analyzed with SPSS (16.0 for Windows) by using a one-way analysis of variance (ANOVA). Differences between means were determined using Tukey's test in which the significance level was designated at ($P<0.05$).

Vaccination programs

All vaccines opened and mixed in free chlorine water, the chicks were prevented from feed and water for 2 hours before vaccination. Vitamin C was used routinely at the ratio of 1 gm/Litter water after each vaccination to relive the stress. Table (1) show the vaccination programs

Table (1): Program of Vaccination

Age of chicks (days)	Disease	Type of vaccine	Origin	Rout of vaccination
1st	Newcastle + Avian influenza+ Infection bronchitis+ Infectious bursal disease	Killed vaccine +MA5 +trans immune	Holland	Spray+ injection
10th,20th ,30th	Newcastle disease	Clone 30 strain	Holland	Via drinking water

Table (2): Ingredients, and nutrient composition of experimental diets.

Ingredient %	Starter (1-21 day)	Finisher (22-35 day)
Corn%	30	30
Soya bean meal (44% protein)	28	20
Wheat%	27.5	35.5
Animal Protean (50%)	3	10
Oil%	3	3
Salt%	0.3	0.3
Limestone%	1	1.2
Total	100	10

Chemical Analysis*of basal diet

Gross energy	3078	3125.2
Crude protein %	22.74	20.16
Energy/protein ratio	135.35	155,07
Calcium %	0.97	1.0
Available Phosphate %	0.41	0.48
Methionine +cysteine%	0.83	0.75
Lysine%	1.02	0.95
Methionine%	0.78	0.51

chemical analysis according to NRC. (1994).

parameters studied

Blood sampling

Blood sera were used to determine biochemical parameters and determine ELISA antibody titer against ND, IBD viruses, liver enzymes at 17 the and35th days . All blood samples were collected from each replicate randomly and obtained from the wing vein in a test tube with anticoagulant to obtain plama to measure heteroplil\lymphocyte ratio and a test tube without anticoagulant , serum tubes were immediately separated and kept overnight at 4ºC (in the refrigerator) ,after that ,puted in centrifuged for 10 minute/ 3000 rmp, and stored in deep freeze (-20ºC) until analysis.the number of blood sample taken from each treatment is nine

Biochemical parameters

Serum protein concentration (gm/L)

Total protein was estimated using the kit as a colorimetric to reagent estimate total protein, which depends on the interaction of copper ion with the protein of the sample in alkaline medium forming a colored complex that could be measured by a spectrophotometer (Biuret method) (Burtis et al ., 2005).

Serum Albumin Concentration (gm/L)

Total albumin was estimated according to colorimetric albumin in the existence of bromocresol green at a slightly acid PH ,that produces a color convert indicator from yellow-green to green-blue color. The intensity of color formed is proportional to the albumin concentration in the sample was determine by spectrophotometer (Rodkey, 1964).

Serum Globulin Concentration (gm/L)

Total globulin can measured as follows:

serum globulin (gm/L)=total serum protein(gm/L) – serum albumin (gm/L) .

Estimation of serum total Cholesterol concentration (mg/dl)

Cholesterol concentration was estimated by using Cormay cholesterol kit after enzymatic hydrolysis and oxidation, the cholesterol is determined in the presence of phenol and peroxidase, the hydrogen peroxide and 4-aminoantipyrine forming quinoneimine the indicator (Fasce, 1982).

Estimation of Triglyceride concentration (mg/dl)

Triglyceride concentration was estimated by Cormay triglyceride kit hydrolyzed to glycerol enzymatically according to the following reaction (Fossati and Prencipe, 1982).

Estimation of HDL-Cholesterol concentration (mg/dl)

HDL-Cholesterol concentration was estimated by using Cormay HDL kit. The supernatant contains high density lipoprotein (HDL). The HDL-cholesterol is then spectrophotometrically measured by means of the coupled reaction described (Grove,1979).

Estimation of LDL-Cholesterol concentration (mg/dl)

LDL-C was Measured by using Cormay LDL kit (Alan, 2006).

Estimation of VLDL-Cholesterol concentration (mg/dl)

serumVLDL cholesterol was measured by dividing serum triglyceride concentration by five(Khaki et al.,2012)

Liver function enzymes

AST (U/dl)

Aspartate aminotransferase activity was determined by Cormay GOT kit (Tietz, 1995).

ALT (U/dl)

Alanine aminotransferase activity was determined by using Cormay ALT kit produced by PZ CORMAY S.A. Company (Burtis and Ashwood, 1999).

ALP(U\dl)

Alkaline phosphatase was determined by the kinetic method of Hausamen et al. (1967).

Antioxidant enzyme

Measurement of serum Glutathione Peroxidase concentration (Gpx)

Measurement of serum Gpx was done by using ELISA kit (Comhair et al., 1999)

Measurement of serum Superoxide dismutase concentration (SOD)

The procedure was done according to the instructions of the manufacture of ELISA Kit - Elabscience biotechnology (Elabscience,china)

measurement. of serum catalase (CAT)

using commercial assay kits, which were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Immunological test

Antibody titters against Newcastle Disease Virus (NDv) and Infectious Bursal Disease virus (IBDv) in broiler chicks serum samples were detected at 35 days of age by using Enzyme Linked Immunosorbent Assay (ELISA technique) for different groups (Spalatin et al., 1973).

Estimation of Heterophil / lymphocyte ratio

In the differential leucocyte counts, two drops of blood were collected from the branchial vein, and blood smears were made on duplicate glass slides. These smears were stained with Wright stain in 15 min. One hundred leucocytes, including heterophils, lymphocytes, were counted on each slide. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes. Both slides were counted and the means were calculated

The Result

Concentrations of total protein, Albumin and Globulin (gm/L)

Table (3) cleared the effect of peppermint, fenugreek and their mixture on total protein, albumin and globulin under heat stress at 35th days of age, the result of total protein recorded no significant differences($P \leqslant 0.05$) among treatments.

Table (3) Effect of peppermint, fenugreek and their mixture on Protein profile of broilers at 35th days of age (Mean±SD).

Parameter / group	protein	albumin	globulin
T1	3.50 ± 0.14 A	2.41 ± 0.05 A	1.01 ± 0.11 A
T2	3.60 ± 0.12 A	2.50 ± 0.09 A	1.13 ± 0.12 A
T3	3.27 ± 0.17 A	2.54 ± 0.15 A	1.15 ± 0.16 A
T4	3.28 ± 0.05 A	2.43 ± 0.06 A	1.61 ± 0.82 A

Different letters in the same column showed a significant difference at ($p<0.05$), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek, T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek.

Concentration of lipid profile

Table (4) showed the effect of peppermint, fenugreek and their mixture on lipid profile of broilers at 35th days under heat stress, there were a significant decrease ($P \leqslant 0.05$) was noticed in the total cholesterol concentration of the addition treatments as compared with the control , there was a significant decrease ($P \leqslant 0.05$) in the value of T2 as compared to other treatments, also a significant decrease ($P \leqslant 0.05$) existed in T4 as compared to T3 and control treatment. There was a significant decrease ($P \leqslant 0.05$) in triglyceride concentration at T2, T3 and T4 groups as compared with the control , these results were similar to results of total cholesterol, since T2 had a lowest value as compared to other treatments and T4 lower than T3, while the control recorded higher treatments.

Table (4): Effect of peppermint, fenugreek and their mixture on lipid profile of broilers at 35th days (Mean±SD).

Parameter / group	Cholesterol	Triglyceride	High density Lipoprotein	Low density Lipoprotein	Very Low Density Lipoprotein
T1	172.27 ± 2.91 A	135.37 ± 8.16 A	87.64 ± 0.88 B	76.87 ± 2.63 A	25.27 ± 0.35 A
T2	147.11 ± 5.76 B	102.49 ± 1.52 B	103.31 ± 2.93 A	47.33 ± 2.20 C	20.84 ± 0.96 B

T3	153.73 ± 4.96 B	104.44 ± 9.93 B	106.04 ± 3.84 A	57.76 ± 2.08 BC	18.69 ± 1.00 B
T4	150.46 ± 3.61 B	103.30 ± 5.51 B	102.14 ± 2.16 A	62.12 ± 3.69 AB	20.14 ± 1.01 B

Different letters in the same column showed a significant difference at ($p<0.05$), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek, T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek

High density lipoprotein values increased significantly ($P \leqslant 0.05$) in addition treatments as compared with control, so T3 recorded high value

Low density lipoprotein values showed significantly increase($P \leqslant 0.05$) in control treatment as compared with addition treatments, there were significantly increase($P \leqslant 0.05$) in T4 as compared with T3, and significantly decrease($P \leqslant 0.05$) in T2 as compared with T4 and control treatment. the heights value in control treatment and lowest value in T2.

Very low density lipoprotein values in serum showed the same result of cholesterol and triglyceride, so there were significantly increase($P \leqslant 0.05$) in control treatment as compared with addiction treatment. Were T1 control treatment high value and T3 low value.

Values of liver function enzymes

Table (5) showed the effect of peppermint, fenugreek and their mixture on liver function enzymes of broilers at 35th days under heat stress, the result revealed a significant difference($P \leqslant 0.05$) in AST, ALT and ALP . T2, T3 and T4 treatments showed a significant decrease ($P \leqslant 0.05$) in AST, ALT and ALP as compared with the control treatment.

Table (5) Effect of peppermint and fenugreek and their mixture on liver function enzymes of broilers at 35th days under heat stress (Mean±SD).

Parameter / group	AST	ALT	ALP
T1	139.97 ±5.08 A	5.68 ± 0.41 A	141.95 ± 3.98 A
T2	91.33 ± 1.57 B	3.79 ± 0.24 BC	112.04 ± 5.07 B
T3	96.92 ± 1.47 B	2.69 ± 0.21 C	113.97 ± 6.63 B
T4	102.82 ± 8.83 B	3.96 ± 0.35 B	123.75 ± 0.94 B

Different letters in the same column showed a significant difference at ($p<0.05$), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek,

T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek.

Table finding also revealed that there were no significant differences between T2, T3 and T4 treatments in AST value and ALP value even though presence of mathematical difference between them, but there was significant decrease (P⩽0.05) in T3 and T2 as compared with T4 and control treatments.

Values of antioxidant enzyme

Table (6) showed the effect of peppermint, fenugreek and their mixture on antioxidant enzymes of broilers at 17th days under heat stress, the result appear significant increase (P ⩽0.05) in addition treatment as compared with control in value of CAT, SOD, GPX, although there was no significance difference among addition treatments.

Table (6): Effect of peppermint and fenugreek and their mixture on antioxidant enzymes of broilers at 17th days under heat stress (Mean±SD).

Parameter / group	CAT	SOD	GPX
T1	0.46 ± 0.01 B	301.24 ± 20.10 B	465.08 ± 9.15 B
T2	0.58 ± 0.01 A	449.64 ± 8.39 A	520.22 ± 14.15 A
T3	0.62 ± 0.02 A	428.57 ± 9.81 A	520.64 ± 15.13 A
T4	0.63 ± 0.01 A	422.98 ± 16.33 A	518.67 ± 11.79 A

Different letters in the same column showed a significant difference at (p<0.05), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek, T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek.

Table (7) showed the effect of peppermint, fenugreek and their mixture on antioxidant enzyme of broilers at 35th days under heat stress, the result appeared significant increase (P⩽0.05) in addition treatment as compared to control treatment in value of CAT, SOD, GPX.

The value of CAT appears significant decrease(P⩽0.05) in T1 as compared to T2, T3 and T4, also there were significant increase (P ⩽0.05) in T2 as compared to T3 and T4.

The value of SOD also appears significant increase in T2 as compared to T3 and T4. And significant decrease (P⩽0.05) in T1 (control) as compared to T2, T3 andT4, the value of GPX appear significant increase (P⩽0.05) in T4 as compared with T2 and T3 but decrease significant in T1 as compared T2, T3and T4.

Table (7) Effect of peppermint and fenugreek and their mixture on antioxidant enzymes of broilers at 35th days under heat stress (Mean±SD).

Parameter / group	CAT	SOD	GPX
T1	0.57 ± 0.01 C	259.56 ± 11.68 C	482.77 ± 10.51 B
T2	0.85 ± 0.02 A	450.19 ± 9.85 A	533.78 ± 10.32 A
T3	0.77 ± 0.02 B	368.58 ± 11.32 B	531.71 ± 10.38 A
T4	0.80 ± 0.01 AB	346.38 ± 2.18 B	539.31 ± 12.25 A

Different letters in the same column showed a significant difference at ($p<0.05$), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek, T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek

Values of heterophil/lymphocyte ratio

Table (8) showed the effect of peppermint, fenugreek and their mixture on heterophil/lymphocyte of broilers at 17th and 35th days under heat stress. were the result appearing significant increase($p<0.05$) in control treatment as compared with addiction treatment. At 17th days of age the result appears significant decrease($p<0.05$) in T2 as compared with T3 and T4 and the age of 35th days the result appears significant decrease ($p<0.05$) in T2 as compared with T3 and T4

Table (8) Effect of peppermint and fenugreek and their mixture on antioxidant enzymes of broilers at 17th and35th days under heat stress (Mean±SD).

Parameter / group	At 17th days	At 35th days
T1	1.98 ± 0.15 A	1.96 ± 0.14 A
T2	0.65 ± 0.11 B	0.81 ± 0.03 B
T3	0.82 ± 0.16 B	1.01 ± 0.11 B
T4	0.80 ± 0.18 B	1.01 ± 0.16 B

Different letters in the same column showed a significant difference at ($p<0.05$), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek, T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek

Antibody-titter against ND and IBD viruses

Table (4-10) that cleared the antibody titers against Newcastle disease and infectious bursal viruses at17th and35th of age after adding

peppermint, fenugreek and their mixture in the diet of broilers under heat stress that measured by ELIZA test. There were a significant differences ($p \leqslant 0.05$) among experimental treatments. The improvement of humeral immunity titer against Newcastle (ND) and Gumboro (IBD) were improved significantly ($P \leqslant 0.05$) in the T4, T3 and T2 as compared with the control treatment, there were a significant increase($P \leqslant 0.05$) at T4 treatment as compared to other treatment, also there were a significant increase ($P \leqslant 0.05$) in the titter of T3as compared with T2 and control.

Table (9) Effect of peppermint, fenugreek and their mixture on humeral immunity of broiler chicks at17th and 35th days of the study (Mean± SD).

Parameter / group	ND at 17 days	ND 35th days	IBD at17th	IBD 35th days
T1	1810 ± 88 C	1852 ± 33 C	2657 ± 101 C	2206 ± 214 B
T2	2354 ± 106 B	2449 ± 184 B	3280 ± 161 B	3597 ± 264 A
T3	2677 ± 170 B	2462 ± 160 B	3312 ± 136 B	3167 ± 283 A
T4	3537 ± 166 A	3428 ± 125 A	3931 ± 64 A	3835 ± 129 A

Different letters in the same row showed a significant difference at ($p<0.05$), T1(Control): basal diet only, T2:1.5gm/kg diet peppermint, T3:1.5 gm/kg diet fenugreek, T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek, (ND) Newcastle disease, (IBD) Gumboro.

Discussion

Concentrations of total protein, Albumin and Globulin:

The results of the present study obtained recorded no significant differences($P \leqslant 0.05$) among treatments. Similar results were observed earlier by Abbas (2010) and Duru et al. (2013) that appear the serum protein content was not affected when using fenugreek as food additive.

(Khursheed.et al.,2017) who reported that the supplementation of peppermint leaves with or without enzyme in both 1or 2% levels were not observed any significant effect on serum total protein when compared with control.

Concentration of lipid profile

The results of the current study showed a significant decrease ($P \leqslant 0.05$) in cholesterol and triglyceride in T2, T3and T4 as compared with control. High density lipoprotein values also increased significantly in addition treatment as compared with control especially in T3. While, LDL values showed adverse result to HDL in experimental treatments that were significantly decrease especially at the T4 as compared with control. This result may be to use peppermint

It seems that some components of peppermint, including menthol and menthone, have a potential to decrease blood lipids in

broilers (European Scientific Cooperative on Phytotherapy, 2003) . Also the result due the activity of some of the compounds in the volatile oil of peppermint (menthol and thymol) decreases the enzymatic activity of hydroxymethyl glutaryl coenzyme A (HMG-COA) and hepatic reductase that regulates synthesis of cholesterol. It seems that one of the reasons for the decrease in total cholesterol in the presence of phenolic compounds such as peppermint extract is the presence of volatile phenolic compounds such as essential oils: menthol, menthone, mentyl acetate, menthofuran, limonene, polygen, cineole and azolen.

On the other hand, the active ingredients in peppermint by increasing the activity of liver cells, give rise to the concentration of bile acids. The high concentration of bile acids in the small intestine, facilitates digestion of fats and fat-soluble vitamins, because bile acids are essential for fat emulsion (Crossland, 1980). Mimica Dukic et al. (2003) during a trial showed that peppermint, due to its antioxidant and antibacterial properties, may increase the flow of bile in the gallbladder the reduction in serum total cholesterol and triglyceride could be attributed to the increased digestive enzymes secretion, better bile acids release (Amad et al., 2011).

The current result showed a significant decreases ($P \leqslant 0.05$) in triglyceride in T2, T3and T4 as compared with control agreed with the study of Akbari, M. and M. Torki.(2014)supplementation of essential oil of peppermint and chromium picolinate under heat stress decrease the concentration of triglycerides.The findings of present investigation corroborate with the previous study conducted by Abdel and Lohakare (2014) in which serum biochemical analyses in laying hens fed with various levels of peppermint leaves revealed that serum cholesterol linearly decreased with increasing experimental diet.(mint19) .Fallah et al. (2013) who reported that peppermint had increased HDL-cholesterol and significantly reduced total cholesterol, triglycerides, LDL-cholesterol in broilers

Al-Harthi et al. (2004) reported that the addition of some herbs such as peppermint extract to broiler diets reduces the concentration of serum total cholesterol and triglyceride. Abdolkarimi and Mirzaaghazade (2010) found that peppermint extract reduces levels of cholesterol, triglycerides and serum LDL-cholesterol in broilers. Stress increases synthesis of adrenocortical hormones that will be followed by blood glucose levels and body fat.

The result of current study may be to the presence of saponins and resins in fenugreek seeds which might have inhibited the bile acid and cholesterol absorption from intestine, thereby, decreasing cholesterol level in blood (Petit et al. 1995). Moreover, the steroidal saponins in fenugreek seeds (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and its synthesis, hence has a potential role in prevention of arteriosclerosis (Mullaicharam et al. 2013). Also the result due that fenugreek contains bioactive components such as minerals, vitamins, lecithin and choline that help to dissolve cholesterol and fatty substances.

Also these result due to Galactomannan from fenugreek seeds exerts hypolipidemic effect due to increased3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMG-CoA reductase), activity with additional bile acids and neutral sterols excretion in faeces (Ramulu et al., 2011) . Diosgenin, a furostanol saponin, in fenugreek inhibits the absorption of cholesterol and

thereby lower hepatic cholesterol concentration and increases biliary cholesterol excretion, ultimately lowering the serum cholesterol concentration (Sfar et al., 2018)

The reason why fenugreek reduced the content of TC and LDL-C may be related to the alkaloids contained in fenugreek extract (Cheng C.et al.,2020). According to research, alkaloids can reduce blood TC and LDL-C levels, while beneficially increasing HDL-C levels (Wang Y.et al.,2018). Trigonelline in fenugreek may control the absorption of intestinal cholesterol and affect the LDL cholesterol clearance mediated by LDL receptors, thereby controlling the serum cholesterol of broilers, affecting the lipid metabolism of broilers, and playing the role of lowering blood lipids

Our result agreement with El-Hack et al. (2015) revealed that a decrease in laying hens serum total cholesterol concentration and an increase in high-density lipoprotein cholesterol concentration duo to fenugreek seed extract supplementation Weerasingha et al. (2013) indicated that fenugreek contains bioactive components such as minerals, vitamins, lecithin and choline that help to dissolve cholesterol and fatty substances.(Raghuram et al.,1994) stated that fenugreek seeds or extracts increased the excretion of bile acids and so reduced cholesterol content of serum due to the presence of unsaturated fatty acids in the seed. On the other side(Lanksy et al.,1993)attributed this effect to steroid saponins which may either compete with cholesterol at binding sites or interfere with cholesterol biosynthesis in the liver . Fenugreek can control blood lipids and lower serum total cholesterol. The results of Belguith-Hadriche et al (2010) showed that the use of fenugreek ethyl acetate extracts significantly reduced the levels of TC, TG, and LDL-C in plasma while increasing the plasma levels of HDL-C in plasma Begum et al. (2016) found that Fenugreek has a significant increase in serum HDL-C concentration.

Values of liver function enzymes

The current study indicated that there was a significant decrease ($P \leqslant 0.05$) in AST, ALT and ALP of T2, T3, T4 as compared with the control treatment under heat stress. ALT, AST and ALP are three important biochemical markers indicating the normal function of liver. are intracellular enzymes and their concentration increase by cellular injuries such as hepatocyte necrosis and cell membrane permeability dysfunction (Cruz CEB.et al.,2018). AST is found in liver, cytoplasm, and mitochondria of skeletal and cardiac muscles (Rocha TM.et al.,2013) ALP activity is related to hepatobiliary damage and hepatic cholestasis (Singh et al., 2014). ALP is associated with the hepatic cell membrane (Cullen, 2005).

The result of this study showed a substantial decrease in this enzyme in additive treatment relative to control. The reason for this result was attributed to the presence of compounds such as eugenol, caffeic acid, Rosmarinus acid, flavonoids and ±-tocopherol in peppermint which have antioxidants and anti per oxidant characteristic The results of this study confirmed the important role of essential oil peppermint in controlling the liver function (Twegh, 2020)

The result of current study due to Saponin, vitamins A, B1, C, nicotinic acid, and alkaloids are nutritional ingredient found in fenugreek that may act as immunomodulators and liver tonic ingredients. Alkaloids, including trigonelline, gentianine, and carpine compounds are the most important alkaloids in fenugreek seeds. It seems vitamins A and B1 component of seeds are effective in liver function and could decrease ALT and AST

enzyme levels(Morad N. et al., 2013) Fenugreek Polyphenolic extract was found enhancing hepatocyte viability and decreasing the apoptotic nuclei (Kaviarasan et al., 2007). The result of current study agreed withAbo-Ghanima et al. (2020) reported that the addition of essential oils of pepermint reduced AST, and ALT values.

values of antioxidant enzyme

The result appears significant increase ($P \leqslant 0.05$) in addition treatment as compared to control treatment in value of CAT, SOD, GPX. these result due to use dried peppermint has the potential to be used as a feed additive for broilers because it shows beneficial results for antioxidant properties and ammonia reduction without having any detri mental effects on growth performance or digestibility (Khempaka, S.et al., 2009).

Peppermint is composed of phenolic and flavonoid compounds, which have been confirmed to possess strong antioxidant activities also Olennikov and Tankhaeva (2010) reported that peppermint contained phenolic and flavonoid compounds of approximately 2.70 to 5.52 and 3.02 to 6.32%, respectively. Also Baliga and Rao (2010) reported that the polyphenolic compounds of peppermint leaves, namely eriocitin, luteolin-7-O-rutinoside, diosmin, hesperidin, narirutin, isorhoifolin, rosma-rinic, and caffric acid, were studied for their activity in the 2,2-diphenyl-1-picrylhydrazy free radical scavenging activity (DPPH assay) and it was found that eriocitrin, luteolin-7-O-rutinoside, and rosmarinic acid possessed higher activity than the others. The essential oil components of peppermint that relate to antioxidant properties are 1, 8-cineole, dihydrocavone, limonene, phytol, linalool, thymol, carveol, piperitenone, and eugenol (Pudpila, U.et al.,2011) This supports the potential antioxidant activity of peppermint

The result of current study agreed with (S. Khempaka.et al., 2013) showed beneficial effects of peppermint at level 0.5,1,0,1.5and2% on antioxidant properties. Also the result of current study may due to Fenugreek contains fairly high amount of flavonoids, alkaloids, saponins and other antioxidants. (Rababah et al., 2011). (fenugreek13) fenugreek has antioxidant effect it exhibits scavenging of free hydroxyl radical (-OH) and discourages hydrogen peroxide induced peroxidation in liver mitochondria and protects cellular organelles from oxidative damage (Kaviarasan et al., 2007). (fenugreek13)

The compounds with similar biological activity such as pinene, linoleic acid methyl ester, pentadance and phytol have a wide range of pharmacological activities as an antioxidant, (R.O. Silvaa .et al.,2014)The presence of these compounds along with palmitic acid could be a possible reason for antioxidant activity of fenugreek seed oil(M. Kozłowska .et al.,2016). These results support the good antioxidant capacity of fenugreek seed oil. High content of phenolic and flavonoid compounds means higher antioxidant activity of the plants (N. Semmar.et al.,2017) Also the dietary fenugreek seeds can influence the oxidative stability of muscle and liver of broiler chicks. The antioxidant effect of fenugreek seeds may be attributed to the presence of phytoestrogens and vitamin C.

Values of heterophil/lymphocyte

Were the result appear significant increase($p<0.05$) in control treatment as compared with addiction treatment .At at 17th days of age the result appear significant decrease($p<0.05$) in T2 as compared with T3 and T4 and the age of 35th days the result

appear significant decrease ($p<0.05$) in T2 as compared with T3 and T4 under heat stress these result due to fenugreek seeds causes a reduction in heterophils % and an increase in the Lymphocyte % , and this will reflected in the improvement in the heterophils : Lymphocyte ratio (stress index) specially inT2 treatment, the H/L ratio method to measure stress based on established principles and its wide use, reviewed in Davis et al. (2008). stress alters homeostasis by affecting the adrenal-corticoid axis. Because of high hormone levels, leukopenia (lymphocyte) and leukocytosis (heterophil) result in a change of the H/L..the result appear increase in H\Lratio in control treatment due to heat stress could stimulate the adrenal gland to produce hormones such as oestrogen, which could influence lymphatic cell counts and increase the H/L ratio

Al-Kassie (2010) observed the lowest H/L ratio in the additive treatment supplemented with a minimum dose of peppermint. this was in agreement with Alkattan (2006) in laying hens and Taha , (2008) in broiler breeder males .Campbell (1995) showed a negative significant correlation between WBCs and lymphocytes % and Taha (2008) showed a positive significant correlation between WBCs and heterophils % , as a result , all these effect, were reflected in the improvement of stress index due to the fenugreek treatment .Also the result agreed with (S. Y. Abdul-Rahman.2012) showeds a significant decrease in the Hetrophils % , a significant increase in lymphocytes % and a significant decrease in the Hetrophils : Lymphocytes in broiler supplemented of 10gm\Kg of fenugeek

Antibody titter against ND and IBD viruses

Effect of different experimental treatments on antibody titter against Newcastle disease and infectious bursal disease viruses at 17and 35 day of broilers age .These results indicated that the T2, T3 and T4, which supplemented with 1.5 gm peppermint, 1.5fenugreek and combination of two herbs respectively, showed achieved higher and best titter against two studied diseases because it have higher antibody titter ,while the control treatment recoded lowest titter against these viruses This improvement in antibody titer may be attributed to the fact that peppermint oil maintains the structural integrity of immune cells due to its strong antioxidant action which protects cell membrane from free radical oxidants, thereby resulting in an improved immune response (Nickels,1996).

Also the current result observed that the addition of peppermint powder to broiler diets increased total Ig, IgM and IgG titres against sheep RBC. Arab-Ameri et al. (2016). Guo et al. (2000) reported that the use of medicinal plants has led to the increased weight of the lymphoid organs such as thymus, spleen and bursa of fabricius in broiler chickens, due to the role of peppermint as an immune stimulating factor.

Our obtained findings are also in agreement with those reported by Barbour et al. (2013) who evaluated the impact of eucalyptus and peppermint essential oils on immune modulation and production of broiler chickens challenged with a molecularly characterized velogenic Newcastle disease virus .also peppermint has a potent immune modulatory effect that confirm findings of Awaad et al. (2009a) who stated that eucalyptus and peppermint essential oils blend implement both innate-cell mediated and humoral immune response. Similar findings have been reported by Barbour and Danker (2005) who mentioned that essential oils of eucalyptus and peppermint improved the homogeneity of immune

responses and performance in Mycoplasma gallisepticum/H9N2 virus-infected broilers).

Studies have shown that peppermint extract prevented bacterial growth of organisms such as Shigella dysenteries, Bacillus cereus, and Salmonella typhi. Sefidcon et al (1996) showed that existing limonen in peppermint removed the germs producing pneumococ in 1 to 3 hours, Staphylococcus in 20 minutes, and Streptococcus in 12 hours.

Bin-Hafeez et al. (2003) reported that fenugreek has an obvious immune stimulating effect, which are responsible for inducing macrophages, and improve immunity. Also, Motamedi et al. (2014) showed that fenugreek powder can increase antibody titer and IgG content of traits related to immune system, and play an immunomodulatory role in broilers immunity Abid et al. (2011) demonstrated that the fenugreek increasing the immunity of birds at 24 and 34 days and because fenugreek increases the cellular ties of thymus gland and bone marrow. As (Abed et al., 2014) showed supplemented with 1% fenugreek recorded high anti-body titter against Newcastle disease virus and Gumboro disease virus. Dash et al., (2011) reported that in vitro antibacterial activity of methanolic extract of fenugreek against E. coli and attributed to the flavonoids, saponins and phenols present in it.

Fenugreek could significantly increase the level of immunoglobulin in broilers, which is beneficial to enhance the immunity of broilers. The main reason why Fenugreek affect immunoglobulin secretion is that fenugreek contains 50% polysaccharides, and polysaccharide compounds can enhance the immunomodulatory activity of macrophages in animal bodies (Tang C.et al.,2019) have the ability to stimulate the production of serum immunoglobulins, and have the potential to regulate innate and adaptive immunity (Zuo T.et al.,2017).

In a diet with 1% fenugreek premix, antibody level against ND at 24th and 34th day significantly increased, which is attributed to ingredients such as flavonoids, steroid and saponin found in fenugreek. Abed ARand Kadhim FO (2014). Also the result of study might be attributed to the immunomodulatory effects of fenugreek seed protecting bursa of Fabricius and a low level of challenge due to IBD vaccination. As described in IBD pathogenicity, with the increased challenges in bursa of Fabricius by either live vaccine or field virus, high amount of IgG against IBD was found in serum. Eterradossi Nand Saif YM (2020).

Reference

Abaza,M. 2007 . Effect of using Fenugreek , chamomile and radish as feed additives on productive performance and digestibility coefficient of laying hens . Egyptian poult. Sci., 27,(1):199-218

Abbas R J. 2010. Effect of using fenugreek, parsley and sweet basil seeds as feed additives on the performance of broiler chickens. International Journal of Poultry Science 9 (7): 278–82.

Abdel-Wareth AA, Lohakare JD. Effect of dietary supplementation of peppermint on performance, egg quality and serum metabolic profile of Hy-Line rown hens during the late laying period. Anim. Feed Sci. Tech 2014;197:114-120.

Abdolkarimi R. and Mirzaaghazade A. (2010). Effects of pepper-mint extract on performance, blood parameters, organ relative weight of abdominal fat in broiler cook. Pp. 167-170 in Proc. Cong. Anim. Sci. Karaj, Iran.

Abed AR. Effect of mint and fenugreek and mixture on production and immunity of broilers. Journal of Kerbala University 2014;12(2):139-147.

Abid AA, Wahab HM, Israa NA. Effect of fenugreek and rosemary on some blood parameters, bacterial counts and immunity after ND vaccination of broilers. Sci. Conf. Vet. Med 2011, 28-29.

Abo-Ghanima, M.M., Elsadek, M.F., Taha, A.E., Abd El-Hack, M.E., Alagawany, M., Ahmed, B.M., Elshafie, M.M. & El-Sabrout, K., 2020. Effect of housing system and rosemary and cinnamon essential oils on layers performance, egg quality, haematological traits, blood chemistry, immunity, and antioxidant. Animals 10(2), 245. https://doi.org/10.3390/ani10020245

Akbari, M. and M. Torki. 2014.Effects of dietary chromium picolinate and peppermint essential oil on growth performance and blood biochemical parameters of broiler chicks reared under heat stress conditions. International journal of biometeorology, 58(6): 1383-1391

Alan, H. B. (2006). Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 564.

Al-Harthi M.A. (2004). Efficiency of utilizing some spices and herbs with or without antibiotic supplementation on growth performance and carcass characteristics of broiler chicks. J. Poult. Sci. 24, 869-899

Al-Kassie, G.A.M., 2010. The role of peppermint (Mentha piperita) on performance in broiler diets. Agric. Biol. J. N. Am. 1, 1009-1013.

Alkattan., M. M.,(2006). Effect of Using Some Antioxidants on Production Performance and Some Physiological Characters in Laying Hens. PhD. Thesis, College of Agriculture and forestry, University of Mosul .

Amad, A. A., K. Männer, K. R. Wendler, K. Neumann,and J. Zentek. 2011. Effects of a phytogenic feedadditive on growth performance and ileal nutrientdigestibility in broiler chickens. Poult. Sci.90:2811-2816.

Anonymus. 2016. Latest Poultry, Egg Markets, Forecast available in 2016. WATT executive guide to World Poultry Trends. www.poultrytrends.com/2016.

Anonymus. 2017. https://www.statista.com/statistics/237637/production-of-poultry-meatworldwide-since-1990/

Aviagen, (2022). Ross broiler handbook USA. pp: 14-110.

Awaad MHH, Abdel-Alim GA, Sayed KSS, Ahmed KA, Nada AA et al., 2009a. The immunostimulant effects of peppermint and eucalyptus essential oils in chickens. Pak Vet J, 30: 61-66.

Baliga, M. S., and S. Rao. 2010. Radioprotective po- tential of mint: A brief review. J. Cancer Res. Ther. 6:255– 262

Barbour EK and Danker S, 2005. Essential oils of eucalyptus and peppermint improve the homogeneity of immune responses and performance in MG/H9N2-infected broilers. J Am Holistic Vet Med Assoc, 24: 23-27

Barbour EK, Shaib H, Azhar E, Kumosani T, Iyer A et al., 2013. Modulation by essential oil of vaccine response and production improvement in chicken challenged with velogenic Newcastle disease virus. J Applied Microbiol, 115: 1278-1286.

Begum M, Hossain MM, Kim IH. Effects of fenugreek seed extract supplementation on growth performance, nutrient digestibility,

diarrhea scores, blood profiles, faecal microflora and faecal noxious gas emission in weanling piglets. J Anim Physiol Anim Nutr. (2016) 100:1121– 9. doi: 10.1111/jpn.12496

Belguith-Hadriche O, Bouaziz M, Jamoussi K, El Feki A, Sayadi S, Makni- Ayedi F. Lipid-lowering and antioxidant effects of an ethyl acetate extract of fenugreek seeds in high-cholesterol-fed rats. J Agric Food Chem. (2010) 58:2116–22. doi: 10.1021/jf903186w

Bin-Hafeez, B., R. Haque, S. Parvez, S. Pandey, I. Sayeed, and S. Raisuddin. 2003. Immunomodulatory effects of fenugreek (Trigonella foenum graecum L.) extract in mice. Int. Immunopharmacol. 3:257–265.

Burtis, C. A. and Ashwood, E. R. (1999). Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: Moss D. W., Henderson A. R., 652.

Burtis, C. A. and Ashwood, E. R. (1999). Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: Moss D. W., Henderson A. R., 652.

Campbell . T . W .(1995) Avian Hematology and Cytology .2 nd . Ed. A Blackwell Publishing Company Lowa State Press

Cheng C, Li Z, Zhao X, Liao C, Quan J, Bode AM, et al. Natural alkaloid and polyphenol compounds targeting lipid metabolism: treatment implications in metabolic diseases. Eur J Pharmacol. (2020) 870:172922. doi: 10.1016/j.ejphar.2020.172922

Comhair, S. A., Bhathena, P. R., Farver, C., Thunnissen, F. B., and Erzurum, S. C. (2001) FASEB J. 15, 70 –78

Corp, I. (2011). IBM SPSS statistics for windows, version 22.0.

Crossland J. (1980). Lewiss Pharmacologi. Churchill Livingstone, London, United Kingdom.

Cruz CEB, Freitas ER, Braz N de M, Salles RPR, da Silva ING. Blood parameters and enzymatic and oxidative activity in the liver of chickens fed with calcium anacardate. Rev Cienc Agron. 2018;49:343-52.

Cullen JM (2005). Mechanistic classification of liver injury. Toxicol. Pathol., 33(1): 68.

Dash B.K., Sultana S., Sultana N. Antibacterial activities of methanol and acetone extracts of fenugreek (Trigonella foenum) and coriander (Coriandrum sativum) LSMR. 2011;27:1–8.

Davis A. K., Maney D. L., Maerz J. C. REVIEW: The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Funct. Ecol. 2008;22:760–772. 10.1111/j.1365-2435.2008.01467.x.

Diarra, S.S., Tabuaciri, P., 2014. Feeding management of poultry in high environmental temperatures. Int. J. Poultry Sci. 13, 657–661.

Duru M, Zeynep E and Asuman D. 2013. Effect of seed powder of a herbal legume Fenugreek (Trigonella foenum-graceum L.) on growth performance, body components, digestive parts, and blood parameters of broiler chicks. Pakistan Journal of Zoology 45 (4): 1007–14.

El-Hack MEA, Alagawany M. Performance, egg quality, blood profile, immune function, and antioxidant enzyme activities in laying hens fed diets with thyme powder. J Anim Feed Sci 2015;24(2):127-133.

European Scientific Cooperative on Phytotherapy. (2003). Thymi herba. Pp. 505-510 in Monographs on the Medicinal

Uses of Plant Drugs. M. Escop, Ed. Exeter, UK

Fallah R., Kiani A. and Azarfar A. (2013). Effect of artichoke levels meal and Mentha piperita extract on immune cells and blood biochemical parameters of broilers. Glob. Vet. 10(1), 99-102.

Fasce, C. F. (1982). Serum Cholesterol determined colorimetrically with enzyme. Clin. Chem., 18: 901.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28:2077-2080

Grove, T. H. (1979). Effect of reagent pH on determination of high density lipoprotein cholesterol by precipitation with sodium phosphotungstate- magnesium. Clin. Chem., 25: 560-564.

Guo F.C., Savelkoul H.F.J., Kwakkel R.P., Williams B.A. andVerstegen M.W.A. (2000). Immunoactive medicinal properties of mushroom and herb polysaccharides and their potential use in chicken diets. Poult. Sci. 59, 427-440.

Hausamen, TU et al (1967): Clin Chim Acta 15:241

Ibrahim, N., El-Sayed, M., Assi, H., Enab, A., Abdel-Moneim, A.-M.E.,2021.Genetic and physiological variation in two strains of Japanese quail. Journal of Genetic Engineering and Biotechnology 19, 15–26

Kaviarasan S and Anuradha CV (2007). Fenugreek (Trigonella foenum graecum) seed polyphenols protect the liver from alcohol toxicity: A role on hepatic detoxification system and apoptosis. Pharmazie., 62(4): 299-304

Khaki, Z., P. Khazraiinia, S. Chegini and S.K. Nia, 2012. Comparative study of serum lipid profile in chicken, ostrich, cattle, and sheep. Comp. Clin. Pathol., 21: 259–263.

Khempaka, S., W. Molee, and M. Guillaume. 2009. Dried cassava pulp as an alternative feedstuff for broilers: Effect on growth performance, carcass traits, digestive organs, and nutrient digestibility. J. Appl. Poult. Res. 18:487–493

Khursheed, A, M. B. and A. KhAn, S. Adil, A. GAnAi, I . Sheikh, and A. Sofi. 2017.Effect of mint leaves with or without enzyme supplementation on blood biochemistry, carcass characteristics and sensory attributes of broiler chicken. Adv. Anim. Vet. Sci, 5(11): 449-455

Lanksy, P.S., Schilcher, H.,Phillipson, J.D. and Loew, D. (1993). Plants that lower cholesterol. First world congress on medicinal and aromatic plants for human welfare (WOCMAP), Maastricht, Netherlands, 19-25 July 1992. Acta-Horticulturae. No.(332)131-136.

M. Kozłowska, E. Gruczyn´ ska, I. S´ cibisz, M. Rudzin´ ska, Fatty acids and sterolscomposition, and antioxidant activity of oils extracted from plant seeds, Food Chem. 213 (2016) 450–456, https://doi.org/10.1016/j.foodchem.2016.06.102.

Maheshwari N.,A.Khan and B.Chopade. 2013. Rediscovering the medicinal properties of Datura Sp.: A review. Journal of medicinal plants Research, Vol. 7(39): 2885-2897.

Mimica Dukic N., Bozin B., Sokovic M., Mihailovic B. and Matavulj M. (2003). Antimicrobial and antioxidant activities of three Mentha species essential oils. Plant. Med. 69, 413-419.

Moradi N, Didarshetaban MB, Reza H, Pour S. Fenugreek (Trigonella foenum-graecum L.) As a Valuable Medicinal Plant. Int J Adv Biol Biomed Res. 2013;1:922 -31

Mullaicharam A R, Geetali D and Uma M. 2013. Medicinal values of fenugreek - A review. Research Journal of Pharmaceutical, Biological and Chemical Sciences 4 (1): 1304.

N. Semmar, M. Farman, J.S.O. Mccullagh, Measurement of total phenolic content and antioxidant activity of aerial parts of medicinal plant Coronopus didymus, Asian Pac. J. Trop. Med. 10 (2017) 792–801, https://doi.org/10.1016/j.apjtm.2017.07.024.

Nickels CHF, 1996. Antioxidants improve cattle immunity following stress. Anim Feed Sci Tech, 62: 59-68.

Olennikov, D. N., and L. M. Tankhaeva. 2010. Quan- titative determination of phenolic compounds in Mentha piperita leaves. Chem. Nat. Compd. 46:22–25.

Petit P, Sauvair Y and Hillaire D. 1995. Steroid saponins from fenugreek seeds: Extraction, purification, and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids 60: 674–80.

Pudpila, U., S. Khempaka, W. Molee, and C. Hor - mta. 2011. Comparison of distillation methods of Mentha cordiforlia Opiz. essential oil on antibacterial activity for application use in animal feeds. J. Agric. Sci. Technol. A 1:1336–1340

R.O. Silvaa, N.S.C. Francisca Beatriz M. Sousaa, Samara R.B. Damascenoa, Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress Phytol inhibits the inflammatory response, Fundam. Clin. Pharmacol. 28 (2014) 455–464. doi:10.1111/fcp.12049.

Rababah, T.M., Ereifej, K.I., Esoh, R.B., Al-u'datt, M.H., Alrababah, M.A., Yang, W., 2011.Antioxidant activities, total phenolics and HPLC analyses of the phenolic compounds of extracts from common Mediterranean plants. Nat. Product. Res. 6, 596-605.

Rabia, J. A. 2010. Effect of using Fenugreek, Parsley and Sweet Basil seeds as feed additives on the performance of Broiler chickens. International. J. of Poult. Sci., 3: 278-282

Raghuram T.C., Sharma R.D., Sivakumar, B. and Sahay B.K.(1994) Effect of fenugreek seeds and intravenous glucose disposition in non-insulin dependent diabetic patients. Phytotherapy Research 8: 83-86.

Raghuram T.C., Sharma R.D., Sivakumar, B. and Sahay B.K.(1994) Effect of fenugreek seeds and intravenous glucose disposition in non-insulin dependent diabetic patients. Phytotherapy Research 8: 83-86.

Ramulu P, Giridharan NV, Udayasekhararao P. Hypolipidemic effect of soluble dietary fiber (galactomannan) isolated from fenugreek seeds in WNIN (GR-Ob) obese rats. J Medicinal Plants Res 2011;5:4804-4813.

Raza, S., Abdelnour, S., Dhshan, A., Hassanin, A., Noreldin, A., Albadrani, G., Abdel-Daim, M., Cheng, G., Zan, L., 2021. Potential role of specific microRNAs in the regulation of thermal stress response in livestock. J. Therm. Biol. 96, 102859

Rhee M., H.Park, and J.Cho. 2009. Salicornia herbacea : Botanical , Chemical and pharmacological review of haylophyte marsh plant. Journal of medicinal plants Research Vol.,3(8): 548-555.

Rocha TM, Andrade MA, Gonzales E, Stringhini JH, Santana ES, Gagno Pôrto RN, et al. Liver function and bacteriology of organs in broiler inoculated with nalidixic acid-resistant Salmonella

Typhimurium and treated with organic acids. Ital J Anim Sci. 2013;12:353-8.

Rodkey, F. L. (1964) .Determination of albumin in human plasma and serum. Clin. Chem. 10: 643.

S. Arab Ameri, F. Samadi, B. Dastar andS. Zerehdaran et al.,2016. Effect of Peppermint (Mentha piperita) Powder on Immune Response of Broiler Chickens in Heat Stress .Department of Animal Science, University of Agricultural Science and Natural Resources of Gorgan, Gorgan, Iran . Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

S. KhempakaU. PudpilaW. Mole Effect of dried peppermint (Mentha cordifolia)on growth performance, nutrient digestibility,carcass traits, antioxidant properties,and ammonia production in broiler .(2013) J. Appl. Poult. Res. 22:904–912 http://dx.doi.org/10.3382/japr.2013-00813

S. Y. Abdul-rahman (2010)effect of fenugreek seeds on some physiological characters in broiler breeders malesissn: 2224-9796 (online) mesoptamia j. Of agri issn: 1815 – 316 x (print)

Sefidcon F. Study of Qualitative and Quantitative of Two Species of Peppermint Essential Oil. Tehran, Iran: Research Institute of Forests and Rangelands (RIFR); 1996.

Sfar M, Jaouani A, Ben-Mahrez K, Skhiri HA, Rayana CB, Chemli R, et al. The effects of fenugreek on blood glucose, lipid parameters, insulin and resistin in obese women with Type 2 diabetes. Int. J Pharmacy Pharmaceutical Res 2018;11:108-123.

Singh M, Gupta S, Pandey R, Aggarwal H and Aggarwal S (2014). Oxidative stress and chronic alcohol liver disease: the current perspectives. IAJPR, 4(3): 1428- 1446

Spalatin, J., Hanson R. P. and Joes T. D. (1973). Edema of the eyelid and face of chickens exposed to visceroeropic type of Newcastle virus. Avian diseases. 17: 623-628.

Taha., A. T.(2008). The Role of Vitamins A ,C and Fenugreek Seeds in Lowering Oxidative Stress Effect on Physiological and Reproductive Performance of Males Broiler Breeder. PhD. Thesis, College of Agriculture and forestry, University of Mosul .

Tang C, Sun J, Liu J, Jin C, Wu X, Zhang X, et al. Immune-enhancing effects of polysaccharides from purple sweet potato. Int J Boil Macromol. (2019) 123:923–30. doi: 10.1016/j.ijbiomac.2018.11.187

Tietz, N.W. (1995). Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: W.B. Saunders, 76.

Wang Y, Zidichouski JA. Update on the benefits and mechanisms of action of the bioactive vegetal alkaloid berberine on lipid metabolism and homeostasis. Cholesterol. (2018) 2018:7173920. doi: 10.1155/2018/7173920

Weerasingha AS, Atapattu NSBM. Effects of Fenugreek (Trigonella foenum-graecum L.) seed powder on growth performance, visceral organ weight, serum cholesterol levels and the nitrogen retention of broiler chicken. Trop. Agri. Res 2013;24(3):289-295.

Zuo T, Zhao R, Lu S, Zhang N, Zhang Q, Xue C. Novel dietary polysaccharide SIP promotes intestinal secretory immunoglobulin A secretion in mice under chemotherapy. J Funct Foods. (2017) 37:379–89. doi: 10.1016/j.jff.2017. 08.012