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Effects of Dried Peppermint & Fenugreek Leaves Under Heat Stress of Broilers

A thesis Submitted to the Council of Faculty of Veterinary Medicine, the University of Kerbala in Partial Fulfillment of the Requirements for the Degree of Master in Public Health

By

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بسم ٱلله الرَّحْمٰنِ الرَّحِيـمِ

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Ghadheer Abbas Hassan

/ / 2023

Dedication

To almighty Allah Creator of the heaven and the earth.

This work might not have been written without a number of people, to whom I owe a great deal of gratitude.

To Father and mother whom are gone but never forgotten.

To my Father-in-law and Mother-in-Law, who supported me and give me the power to keep going forward.

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ALP	Alkaline Phosphatase
ALT	Alanine transaminase
AST	aspartate aminotransferase
BW	Body weight
FCR	Feed conversion ratio
FI	feed intake
HDL	high density lipoprotein
HS	heat stress
HSF	heat shock factor

HSP	heat shock proteins
LDL	low density lipoprotein
RBCs	Red blood cells
ROS	reactive oxygen species
VLDL	Very low density lipoprotein
WG	Weight gain

Abstract

This study was carried out to evaluate the effect of dietary Peppermint leaves, fenugreek leaves and their mixture on some productive and some biochemical traits of broilers under heat stress. The experiment was conducted in privet farm for 35days. It involved 120 unsexed one day Ross 308 broiler chicks that were divided into four treatments (30 birds /group) with three replicates (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/ Kg. Peppermint leaves (T2), the third treatment fed on a basal diet with 1.5 gm. fenugreek leaves Kg diet (T3), the fourth treatment was fed on a basal diet with 1.5 gm. fenugreek leaves + 1.5 gm. Peppermint leaves /Kg diet (T4).

The productive parameters calculated weekly throughout the experimental period. Blood samples were collected for biochemical and immunological parameters at 17^{th} and 35^{th} day of the experiment.

The result revealed that was a significant improvement ($p \leq 0.05$) in all productive traits in the addition treatments as compared with the control, while there was a significant decrease ($p \leq 0.05$) in the total cholesterol, triglycerides and low density lipoprotein (LDL) however the high density lipoprotein (HDL) has been a significantly increased of addition treatments compared with control, data of total protein recorded no significant differences among treatments.

Liver function enzymes (AST, ALP & ALT) were measured at the end of the experimental period and showed a significant decrease ($p \leq 0.05$) in the addition treatment comparing with control. the result also appeared a significant ($P \leq 0.05$) increase in addition treatment as compared to control treatment in value of catalase , Superoxide dismutase and Glutathione peroxidase at 17^{th} and 35^{th} days of experiment also the result appeared significant ($P \leq 0.05$) decrease in addition treatments as compared with control treatment in heterophil/lymphocyte of broilers at 17^{th} and 35^{th} days. There was a significant ($P \leq 0.05$) increase in anti-body titer against Newcastle disease and Infectious bursal viruses in addition treatments as compared with the control. In conclusion according to current results Peppermint leaves and fenugreek leaves can be used as good feed additive to reduce heat stress(HS) in broiler and enhancement of productive and biochemical traits of broilers.

Chapter One: Introduction

1. Introduction

Heat stress is a major obstacle to coping 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).

Recent studies have found that modern poultry species are very sensitive to chronic heat stress.. When birds living 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 livability, morbidity and death, and altered meat quality that characterized by increased adiposity and reduced skeletal muscle mass in broilers (Song *et al.*, 2015).

Recent studies have found that heat stress can increase mitochondrial reactive oxygen species (ROS) generation and lead to oxidative stress in muscles (Shehata *et al.*, 2020). The focus of poultry nutrition researchers on the study of certain non-nutritional additives has been of great importance to the physiological and productive state of birds, these additives are plant species with a medicinal effect. So the trend began to use medicinal herbs that have many effective compounds with few side effects especially true for diseases that accompany progress made in the poultry industry (Abaza, 2001).

Herbal medicine 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 and meet its needs for nutrients (Rabia, 2010) Medicinal plants are

known for their antimicrobial properties, natural antioxidants, and ability to stimulate the immune system too.

Scientific study have shown that the products from these plants have the ability to cure many diseases and eliminate their symptom , Seeds of the fenugreek leaves 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 leaves increased the birds' demand for food, resulting in an increase in the body's average weight (Rabia, 2010). It was noted that the use of fenugreek leaves in the diet of laying hens has led to an improvement in productivity (Abaza, 2007).

Peppermint leaves is a member of the *labiate* 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 *et al.*, 2012). Peppermint leaves 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 mint depend on the abundant menthol which is the main phenolic component which has antibacterial activities. Also, Peppermint leaves contains poly phenolic compounds hence could possess strong antioxidant properties (Schuhmacher *et al.*, 2003).

The aim of study using of Peppermint leaves, fenugreek leaves and combination as feed additive to ameliorating the heat stress in broiler chicken.

Chapter Two: Literatures Review

2. Literatures Review

2.1. Heat stress

Heat stress results from a negative balance between the net amount of energy flowing from animal's body to its surrounding environment and the amount of heat energy produced by the animal. This imbalance may be caused by variations of a combination of environmental factors (e.g., sunlight, thermal irradiation, and air temperature, humidity and movement), and characteristics of the animal (e.g., species, metabolism rate, and thermo regulatory mechanisms). Environmental stressors, such as heat stress, are particularly detrimental to animal (Renaudeau *et al.*, 2012).

The environmental stress has quickly become a great point of interest in animal particularly due to public awareness and concerns. The importance of animal responses to environmental challenges applies to all species. However, poultry seems to be particularly sensitive to temperature-associated environmental challenges, especially heat stress. It has been suggested that modern poultry genotypes produce more body heat due to their greater metabolic activity (Deeb *et al.*, 2002). Understanding and controlling environmental conditions is crucial to successful poultry production and welfare.

A generalized stress response occurs at the cellular level to maintain homeostasis. The cellular stress reaction depends on the integrity and role of proteins. The production of heat shock proteins (HSPs) in response to stressors that threaten the cell's life is one of the most common characteristics of the cellular stress response. Heat shock proteins profoundly modify the physiological stress response and encourage stress tolerance (Chen *et al.*, 2018) Heat shock or stress induced proteins are a group of phylogenetically conserved proteins distributed among diverse

cellular organisms. Because HSPs have been linked to a critical role in cellular resistance to physical and chemical stresses it has been proposed that the levels of HSP production or accumulation can help to identify if an organism finds a specific environment stressful.

During stress, the HSP–heat shock factor (HSF) complex dissociates to reach an active state in the cytosol. The HSF–phosphorylated trimer complex enters the nucleus and activates the HSP gene by binding to the promoter's heat shock element (HSE). This leads to the proliferation of HSPs in the cell to help refold the damaged proteins. Furthermore, the close relationship between the accumulation of HSPs and the organism's physiological state suggests that the HSP response can be more sensitive than the existing stress indicators. Heat shock proteins are activated in response to thermal and non-thermal stressors (Etches *et al.*, 2008).

HSPs are classified systematically based on molecular weight. HSPs with a large molecular weight include families HSP110, HSP90, HSP70, and HSP60, while small HSPs include HSPB1–HSPB10 with a molecular weight of 8–30 k Da (Shende *et al.*, 2019). In chickens, the *HSP70* gene is located on the fifth chromosome and has a 1905bp coding region length with only one exon. This phenomenon contributes to the ability of chickens to tolerate stressors differently. Generally, HSPs have proven their protective role in various cellular processes, ensuring cells' survival during stress (Shende *et al.*, 2019).

Moreover, HSPs are well-known to participate in protein secretion, assembly, maintaining the integrity of structural proteins, folding, trafficking, protein degradation, and regulating transcription factors as a molecular chaperone, HSPs are responsible for maintaining cell homeostasis by preventing cell apoptosis and promoting cell survival (Madkour *et al.*, 2022). To achieve this, HSPs are actively involved in cytoprotective mechanisms, restricting protein aggregation and

preventing protein misfolding (Hartl and Hayer., 2002). Heat shock proteins are also involved in the refolding of stress denaturing proteins in a cell. In general, HSPs reside at the frontier of cellular defense, and become overexpressed and overproduced under stress, assuming cytoprotective roles to maintain cellular integrity and gain stress tolerance in cells, when exposed to stress for a certain period of time (Madkour *et al*., 2022).

2.1.1. Effect of heat stress on poultry production

Heat stress is a hazard that influences the growth performance (Sun *et al.*, 2015), immune responses (Hirakawa *et al.*, 2020), nutrients digestibility, meat quality and cellular oxidant/antioxidant system (Habashy *et al.*, 2018) in broiler chickens and laying performance, Moreover, reduces the consumption and retention of protein and fat (Habashy *et al.*, 2017). However, previous studies indicated that lowered FI was not the only aspect that reduced broiler performance and body weight (BW) in hot weather (Daghir, 2008).

Exposure to high ambient temperature disrupts the metabolic/ endocrine responses. Either acute or chronic heat stress causes serious molecular, cellular and immune dysfunction. Nutrient transporters play a key role in the gastrointestinal tract for dietary nutrient intake. Alteration in the function of these transporters may lead to impairing the nutrients availability and growth performance has been demonstrated that cyclic heat exposure causes changes in jejunum glucose and lipid transporters (Sun *et al.*, 2015).

The study of Habashy *et al.* (2017), who noted that heat stress altered the relative mRNA expression of oligopeptide, sugar, and fatty acid transporters in ileum and Pectorals, reduction in nutrient intake and alters nutrient digestibility which might illustrate the deterioration in weight gain and performance of chicken raised under

heat stress conditions. Moreover, protein and fat retention in the *pectorals muscles* were reduced in broilers subjected to heat stress as a result of the reduction in protein synthesis or increase breakdown.

2.1.2. Effect of heat stress on oxidative status in poultry

Exposure to heat stress conditions elevated the body temperature and accelerated the metabolic rates leading to raising free radicals (in particular reactive oxygen species) concentration. Free radicals destroy cell phospholipid membranes and attack the vital components within the cell such as DNA, mitochondria, lysosomes, which is consequently related to troubles, such as deteriorating muscle membrane integrity, apoptosis and several diseases, therefore, it is verified that heat stress is one of the major causes of oxidative stress (Akbarian *et al.*, 2016). It's reported that heat stress can induce oxidative damage indifferent species of livestock, such as broilers (Belhadj *et al.*, 2016). During the past few decades, the consumption of poultry meat increased consecutively in global market. Owing to its relatively low cost and high nutritive value .Heat stress-induced oxidative damage is complicated in impairing intestinal mucosal barrier integrity and increasing the intestinal barrier permeability, leading to high absorption of toxic agents into body.

Therefore, maintaining a normal intestinal barrier function has an important role in body homeostasis (Banan *et al.*, 2001). Oxidative stress can also influence protein function leading to protein carbonylation levels of protein carbonyl were elevated in liver of broilers exposed to acute heat stress. However, broilers exposed to acute and chronic heat stress showed high protein oxidation levels in pectoralis *muscle*. Protein oxidation and lipid peroxidation were showed in the muscles and livers of chickens exposed to heat stress (Sultana *et al.*, 2013).

Protein and fatty acids oxidation impair growth performance of chickens raised under high ambient temperature (Habashy *et al.*, 2019). Oxidative stress can affect all parts of the body; however, mitochondria are the most vulnerable to oxidative damage. It has been noticed that acute heat stress down-regulates the avian form of mitochondrial uncoupling protein (avUCP) and protein concentration in skeletal muscle mitochondria (Del Vesco and Gasparino, 2013). Down regulation of avUCP mRNA expression upon acute heat stress appears to increase ROS generation, which leads to mitochondrial dysfunction. In contrast, chronic heat stress confers up-regulation of avUCP in an attempt to reduce ROS generation (Akbarian. *et al.*, 2016).

Oxidative stress is the starting point of the intestinal permeability dysfunctional process. Under heat stress conditions, increased concentrations of (ROS) occur leading to increased intestinal permeability, which in turn facilitates the translocation of bacteria from the intestinal tract. In fact, increased inflammation and translocation of *Salmonella Enteritis* in broilers subjected to heat stress has been reported (Quinteiro Filho *et al.*, 2012), resulting in increased levels of the pathogen in spleen samples. It is reasonable to speculate that high environmental temperature would not only affect the bacterial levels in the feces of birds, but also the duration and level of contamination in the environment where feces are deposited, potentially leading to increased dissemination. However, heat stress did not result higher levels or longer survival of *Salmonella* shed in feces (Quinteiro Filho *et al.*, 2012).

Oxidative injury can directly or indirectly induce the bio macro molecules (DNA, proteins, and lipids) damage and further because cell dysfunction and even tissues injury, When broilers are subjected to heat stress, the free radicals increase whereas the activities of antioxidant enzymes and free radical scavenging ability decrease. Nuclear factor elytroid 2-related factor 2 (Nrf-2) has been recognized as a critical

regulator of cellular redox homeostasis, which can protect cells against oxidative damage. Upon oxidative stress(Miao *et al.*, 2020). the adapter protein sequestosome 1(Sqstm1/p62) interacts with the Nrf2, then modulating the expression and function of Nrf2.Nrf2 in the nucleus can bind to conserved antioxidant response elements in the promoter regions of target genes, and regulate the activities of antioxidant enzymes such as glutathione peroxidase (GPx) , The Nrf2/Kelch associated protein1 (Nrf2 Keap1) pathway is involved in cellular responses to environmental and oxidative stress. Activation of the Nrf2 Keap1 pathway increases antioxidant response element-related molecules, including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Zhang *et al*, 2018).

2.1.3. Effect of heat stress on immune responses of poultry

Heat stress impairs humeral and cellular immune responses and causes immune dysfunction Heat stress can suppress the innate immune responses and induce immune disorders via altering the spleen functions, of innate immunity. Heat stress elevated the atrophy the bursa of fabricius (Jahanian and Rasouli, 2015), decreased both blood and bursal B lymphocytes (Tang, 2016), and reduced bursa follicle size and even circulating antibody levels (Chen, 2016). It was found that broilers subjected to chronic heat stress (35C during 15–42 day of age) had a significant suppression in T helper lymphocytes (CD4) proportion and activation in cytotoxic T lymphocytes (CD8), which result in reduced CD4/CD8 ratio in peripheral bloodstream (Jahanian and Rasouli, 2015).

A recent study showed that heat stress in broiler chickens suppresses innate immunity and triggers cell death through several pathways (Ma *et al.*, 2019). Besides, growing evidence has indicated that heat stress reduces IgG and IgM levels and synthesis of leukocyte protein (Kamel *et al.*, 2017) increases pro inflammatory cytokines and splenocyte apoptosis rate and decreases the levels of anti-

inflammatory cytokine (Ma *et al.*, 2019). These findings provide a potential target for mitigating the negative impacts of heat stress on the immune system. Further, it was shown that exposure of broilers to chronic heat stress 34.5C^{o} for 14days led to multiple immune dysfunctions by disturbing the T and B cells maturation in the different lymphoid organs (Hirakawa *et al.*, 2020).

2.1.4. Effect of heat stress on meat quality of poultry:

Acute or chronic heat stress could impair meat quality characteristics of broiler chickens, such as decreasing pH, water-holding capacity, meat color, and tenderness (Azad *et al.*, 2010). Ante-mortem high ambient temperature induces various hormonal alterations in poultry, in particular thyroid hormones. Changed concentration of thyroid hormones impacts Ca^{2+} regulation of skeletal muscle and then affects its expression in the myo fibrillary proteins. Increased level of Ca^{2+} during muscle contraction results in muscle hyper metabolism, leading to myopathy and/or rhabdo myolysis (damage of skeletal muscle fibers) (Chiang *et al.*, 2008).

The hyperthermia-associated myopathy is initiated by the disrupted functions of lactate dehydrogenase, aspartate transaminase, and elevated activity of creatine kinase , Ca^{2+}-mediated changes in muscle membrane integrity. It has been reported that thermal stress-induced ante-mortem changes in membrane integrity of muscle may disrupt post-mortem meat quality (Sandercock *et al.*, 2001). Additionally, exposure to heat stress may result in pale, soft and exudative meat and reduce muscle pH and water holding capacity, motivating consumers to reject meat and subsequently economic loss (Fouad *et al.*, 2016).

Heat stress during the growth period of broilers has been associated with undesirable meat characteristics and quality loss (Zhang *et al.*, 2012). Additionally, transportation of broilers from farms to processing facilities under high temperature

conditions have also been shown to cause meat quality losses. In laying hens, heat stress has been shown to negatively affect egg production and quality (Bozkurt *et al.,* 2012). More recently, food safety has become a major issue to the poultry and egg production industry worldwide. In fact, food safety is increasingly being considered an important part of the modern food quality concept.

Colonization of birds by foodborne pathogens, such as *Salmonella* and *Campylobacter*, and their subsequent dissemination along the human food chain are a major public health and economic concern in poultry and egg production. In fact, consumption and handling of undercooked poultry products constitutes one of the most commonly implicated sources of foodborne illness (Dadgar *et al.*, 2010).

There is increasing evidence to demonstrate that stress can have a significant deleterious effect on food safety through a variety of potential mechanisms. However, while there is evidence linking stress with pathogen carriage and shedding in farm animals, the mechanisms underlying this effect have not been fully elucidated (Rostagno, 2009). Environmental stress has been shown to be a factor that can lead to colonization of farm animals by pathogens, increased fecal shedding and horizontal transmission, and consequently, increased contamination risk of animal products , it was reported that heat stress facilitated muscle glycogen breakdowns, which decreases meat pH, and therefore, lowering meat quality. (Gu *et al.*, 2012).

2.1.5. Effect of heat stress on intestinal integrity and gut microbiota of poultry

Considerable evidence has shown that heat stress injures intestinal integrity and morphology, Exposure of broilers to cyclic chronic heat stress (33C for 10 h/d) for 20 consecutive days from 22 to 42-day old resulted in altering villus height, crypt depth, tumor necrosis factor alpha interleukin-10 and D lactic acid and diamine

oxidase activity, soluble intercellular adhesion molecule-1 level, and tight junction proteins (occluding, claudin-1 and zonula occludens-1) expression levels (Zhang *et al.*, 2017)

Furthermore, it was demonstrated that digestive enzymes activity in broilers is inversely connected with high ambient temperature. It is well known that the appropriate ambient temperature for chickens is 16C° – 25C° , In this respect, evidence has shown that appetite was reduced by 1.5% for every increase in ambient temperature by 1C° between 21 C° and 30 C°, and was reduced by 4.6% for the same increase pattern between 32C° and 38C° (Song *et al.*, 2012). The intestinal mucosal barrier represents a highly dynamic interface that separates the inner and outer environments.

The intestinal mucosal barrier has the potential for robust and accurate morphological and functional development according to genetics, environment and nutrition (Julian, 2005). Increase of enteric permeability in intestinal segments of heat-stressed chickens is involved in intestinal immune disorders (Hirakawa *et al.*, 2020). Altogether these findings indicate that heat stress can cause an intestinal epithelial barrier dysfunction, leading to reduce the efficiency of nutrient absorption and increase bacterial translocation and the potential of disease outbreaks. Poultry intestinal microbiota is highly diversified, very complex, utilize the diet as substrate and interact extensively and directionally with the host. This intricate and close relationship explains the high susceptibility of gut microbiota to a multitude of factors related to the host or the environment, including heat stress (Julian, 2005) .

Several studies reported specific alterations in microbiota of birds subjected to heat stress condition, including higher counts of total *coliforms* and *Clostridium* and lower populations of *Bifido bacterium* and *Lactobacillus*. Nevertheless, the exact mechanisms underlying the influence of such stressors on community structure and

activity of intestinal microbiota are still being unraveled. Several biological pathways have been proposed to deliberate the direct and indirect impacts of heat stress on gut microbiota. Although most of these basic researches have been conducted on humans or animals other than poultry, this acquired knowledge applies equally to poultry (Kers *et al.*, 2018).

One of these mechanisms is the increased water consumption and decreased feed intake of heat-stressed birds which, on one hand, affect the nutrients' availability to be utilized by the microbiota in the intestinal segments and, on the other hand, alter the intestinal microenvironment such as mobility, digesta viscosity, and secretory activity patterns (Kers *et al.*, 2018) . Moreover, heat stress causes a serious damage to the intestinal mucosa due to the reduction in blood flow directed to the gastrointestinal tract and hypoxia, leading to the incidence of inflammation and oxidative stress, and the following disturbance in the intestinal integrity, heat stress triggers the hypothalamus-pituitary axis that alters the immune system and consequently leads to alteration in the interactions between gut microbiota and the host (Lara and Rostagno, 2013).

2.2. Peppermint

Peppermint is a perennial, aromatic, and curative herb which has extensive global distribution. Belongs to the family *Lamiaceae* and comprises 25–30 known species. Peppermint leaves grows vigorously at low temperatures but could undergo a wide range of environmental conditions. Normally, it can reach a height of 10 to 20 cm or more. This genus emerged from Midland countries and progressively expanded worldwide by either artificial or natural genesis (Salehi *et al.*, 2018).

They are now predominantly found in Asia, Australia' South Africa, and North America. According to various studies, Peppermint leaves plants have superabundant ingredients of phenolic compounds distinctly phenols, flavonoids, terpenes, quinines, and polysaccharides (Bouyahya *et al.*, 2020). These phytochemicals paved the way for significant utilization in the production of pharmaceuticals food and beverage industry, numerous species of *Mentha* are used as spices and for herbal teas. Generally, every part, for instance, the leaves, stems, and roots of Peppermint leaves, have been used in tribal and traditional medicines (Asghar *et al.*, 2022). Economically, highly important species are *Mentha aquatica* L. (*M. aquatica*), *Mentha longifolia* L. (*M. longifolia*), *Mentha* × *piperita* L. (*M.* × *piperita*), *Mentha spicata* L. (*M. spicata*), and *Mentha arvensis* L. (*M. arvensis*). All these species possess potential phytochemicals, such as isomenthol, isomenthone, cineol, limonine, piperitone, carvacrol, dipentene, linalool, thujone, piperitenone oxide, and phellandrene, which play an important role in pharmacy, food, flavor, ointment, and associated industries (Kalakoti *et al.*, 2014) taxonomical classification of Peppermint leaves in figure (2-1).

Taxanomical Position
Kingdom: Plantae plants
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: Mentha
Species: Piperita

Table **(**2-1): Taxonomical classification of Peppermint leaves (Kanakis *et al., 2012)*

2.2.1. Chemical constituent and bioactive compounds of Peppermint leaves:

The chemical makeup and numerous biological functions of Peppermint leaves essential oils have been examined (Tafrihi *et al.*, 2021). There have been reports that contains a variety of secondary metabolites, like as phenolic substances, including (alkaloids, tannins, phenolic acids, flavonoids, and steroids, terpenoids, resins, coumarins, and their glycosides), among others. Anti-inflammatory and antioxidant properties are linked to its high non-volatile phenolic component concentration, including flavonoids Due to the abundance of essential oils, such as menthol, carvone, and limonene, found in many Peppermint leaves species, this plants are of great value. Mentone, alphapinene, betapinene, and geraniol and pharmaceutical substances that work well. However, according to Schuhmacher (2003), menthol is the primary flavonoid present in Peppermint leaves. Along with the essential oils,

Peppermint leaves also includes tannins, glycosides, saponins, and other bioactive substances (Edris *et al.* 2003).

According to studies, medicinal plants' antioxidant and flavonoid contents may be crucial in enhancing liver and cardiovascular health (Pouramir *et al.,* 2006) Menthol, menthone, 1, 8cineole, methylacetate, methofuran, isomenthone, limonene, bpinene, apinene, germacrened, Tran's sabinene hydrate, and pulegone are the chemical components of Peppermint leaves. The primary phenolic substance in Peppermint leaves oil that possesses antibacterial properties is menthol. This herb has antibacterial, spasmolytic, and disinfecting qualities, according to studies. Widely used as a herbal remedy, Peppermint leaves is thought to boost immune system response, combat secondary infections, and successfully treat irritable bowel syndrome and gallbladder inflammation. Additionally, it possesses antispasmodic and antiseptic properties. The chemical components of Peppermint leaves in table (2-2) according to Loolaie *et al.*, (2017)

Table (2-2): Chemical Components of Peppermint leaves (Loolaie *et al.*, 2017)

Polyphenols	Rosmaric acid, eriocitrin, cinnamic acid, caffeic acid etc**.**
Flavonoids	Glycosides –Narirutin, luteolin-7-o-rutinoside, isorhoifolin, hesperidin etc.
Limonene	1-methyl-4-(1-methylethenyl)-cyclohexene
Cineole –	1,3,3 -Trimethyl-2-oxabicyclo [2.2,2] octane
Methonl	(2S,5R)-2-isopropyl-5-methylcyclohexanone
Menthofuran	3,6-Dimethyl-4,5,6,7-tetrahydro-1-benzofuran

Isomenthone	(2R,5R)-5-methyl-2-propan-2-ylcyclohexan-1-one
Carvone	2-Methyl-5-(prop-1-en-2-yl) cyclohex-2-en-1-one,
Pulegeone	p-Menth-4(8)-en-3-one
Menthyl -acetate	Acetic acid [(1R,2S,5R) -2-isopropyl-5- methylcyclohexyl] ester

2.2.2. Effect of Peppermint leaves on productive trait of poultry

Productive performance in broiler poultry production was increased faster than other animal Production like beef (Al-Kassie, 2010). This due to efficiency of the feed conversion and lower production costs related with intensive poultry production. Broiler performance including body weight gain, feed intake and feed conversion ratio were measured at day 10, 24 and 35. (Amasaib *et al.*, 2013) who showed that the addition of different levels of Peppermint leaves improved feed intake. Due to this narcotic plant, broiler chickens fed Peppermint leaves may have increased feed intake and improved growth. Because Peppermint leaves contain active substances like menthol, they can boost feed efficiency and increase hunger in broiler chicks.

The active ingredients (cineole, citral, geraniol, linalool, and menthol) have been demonstrated to have antibacterial and antioxidant properties as well as to enhance nutrient digestion and absorption. (Bupeshet *et al.*, 2007) that could have enhanced the broiler chicks' growth performance. Additionally, menthol addition in the broiler diets boosted feed consumption as compared to the control diet. Menthol is an appetizer drug, this increase may in part be caused by its activity (Akbari *et al.*,

2016). Therefore, an increase in feed consumption may result in an improvement in growth performance. According to Ocak *et al.* (2008), Peppermint leaves leaves appear to have a good effect on increasing daily body weight gain by lowering the effects of gastrointestinal diseases, which strengthens the digestive system and increases feed efficiency.

Al-Kassie, (2010) noted the all Peppermint leaves additive except 1% in water had significantly improved feed conversion ratio at day 35 This result is similar to Gurbuz and Ismael (2016) found that the Peppermint leaves supplementation had a significantly effect on FCR compared with (Mustafa *et al.*, 2013) study who reported that (1% betony+ 1% Peppermint leaves) had significant effect on feed conversion ratio in which have better when compared with control at age 25- 42day. Most of medical plants consist of different essential oil in which stimulate digestive enzyme secretion and the effect on microbial that present in gut through the balance of microbial (Cross *et al.*, 2007).

The Peppermint leaves supplementation either in water or feed have effect on weight gain on day 24 and 35. The result was agreement with finding (Witkowska *et al.*, 2019) study who reported the addition Peppermint leaves oil mist had increase the mean BW and WG than control and birds.

The higher body weight gain observed in broilers fed the Peppermint leaves diet may be related to the reported properties of menthol. The active principles of essential oils act as a digestibility enhancer, balancing the gut microbial system and stimulating the secretion of endogenous digestive enzyme and thus improving growth performance in poultry(Cross *et al.*, 2007) was variety of active components which affect process of digestion in which stimulate saliva secretion Therefore, the main compound of Peppermint leaves may probably improve the digestibility of diet as a digestion stimulant, and hence increase the nutrient entry rate at an early

stage of bird's life without affecting feed conversion(Toghyani *et al.*,2010), diets containing Peppermint leaves leaves enhanced growth performance due to improved trypsin and amylase activities (Jang *et al.*, 2004), as well as bile acid secretion (Amad *et al.*, 2011).

Feeding broilers with Peppermint leaves led to significant improvements in daily weight gain in the grower and finisher periods. This result was in accord with the results of (Ocak. *et al* 2008). It seems that the positive effect of different levels of Peppermint leaves on increasing average daily weight gain was due to its decreasing effects on gastrointestinal disorders, thus strengthening the digestive system and improving feed efficiency. Moreover, the antiseptic property of Peppermint leaves prevents harmful bacterial growth in the digestive system that led to better digestion and absorption. The anti sepproperty of Peppermint leaves results from the presence of menthol (Movaseghi, 1990).

Use of Peppermint leaves increased feed consumption, feeding efficiency and bird's survival. Peppermint leaves to have antimicrobial properties so prevent the growth of harmful bacteria in digestive system thus improves digestion and absorption and increase the body weight (Aridogan *et al.*, 2002). Also, Narimani-Rad *et al.* (2011) reported that dietary supplementation of medicinal plants mixture (1% Oregano, 0.5% Ziziphora and 0.5% Peppermint leaves) caused performance and carcass quality improvement via more weight gain increase in carcass yield and then decreases abdominal fat deposition. And, they investigated 0.3 % ethanolic extract of Peppermint leaves to drinking water seem to have a positive influence on broiler performance productive via more carcass yield and decrease abdominal fat deposition.

Galib and Al-Kassi, (2010) shows an improvement in BWG and FCR under dietary treatment with Peppermint leaves powder. Also Ocak *et al.* (2008) reported

that adding 0.2 percent mint to basal diet help increase growth and reduce mortality in chickens. The Peppermint leaves with antimicrobial properties prevent the growth of harmful bacteria in the digestive tract, thereby improving the digestion and absorption as well as body weight gain (Cross *et al.*, 2007).

2.2.3. Effect of Peppermint leaves on biochemical parameter:

Blood biochemical analysis is widely used to assist the diagnosis and characterization of diseases in most animal species, but it is seldom used in avian species. However, it is an important tool, as some metabolic disorders are difficult to detect only by clinical signs (Andreasen *et al.*, 1996). It can also assist the monitoring of poultry health, the diagnosis and treatment of diseases, and to assess their health status, Toghyani *et al.*, (2010) who showed that the addition of different levels of Peppermint leaves had no significant effect on protein and albumin.

Dietary supplementation of Peppermint leaves powder by up to 2% in a layer hen diet decreased serum cholesterol significantly and increased calcium and phosphorous (Abo-Ghanima *et al.*, 2020). Peppermint leaves extract reduced the blood urea level, triglyceride level and cholesterol in broilers when fed in drinking water (Roozbeh *et al.*, 2013). Supplementation of Peppermint leaves extract in drinking water decreased plasma total cholesterol, triglyceride, low density lipoproteins (LDL), and liver synthesis of lipid concentration in laying hen (Rahim *et al.*, 2012).

Fallah *et al.* (2013) who reported that Peppermint leaves had increased albumin, total protein, and HDL cholesterol and significantly reduced total cholesterol, triglycerides, LDL-cholesterol in broilers. It seems that some components of Peppermint leaves, including menthol and menthone, have a potential to decrease blood lipids in broilers, Peppermint leaves extract significantly decreases

triglycerides, total cholesterol, LDL- cholesterol, VLDL-cholesterol and increases HDL-cholesterol in blood serum. The menthol and thymol, which are components of essential oils of Peppermint leaves , at the level of 200 mg per kg in broilers fed with diets containing cholesterol or no cholesterol, reduced serum total cholesterol and triglyceride concentrations

Al-Harthi *et al.* (2004) reported that the addition of some herbs such as Peppermint leaves extract to broiler diets reduces the concentration of serum total cholesterol and triglyceride. This would increase the total protein in the blood of broiler chickens. Abdolkarimi and Mirzaaghazade (2010) found that Peppermint leaves extract reduces levels of cholesterol, triglycerides and serum LDL-cholesterol in broilers. Positive response of birds to thyme and Peppermint leaves oils (or their combination) supplementation to diets. On the other hand, the reduction in serum total cholesterol and triglyceride could be attributed to the increased digestive enzymes secretion or better bile acids release (Amad *et al.*, 2011).

Liver cells contain a high concentration of ALT, AST and ALP enzymes. The ALT is present in the cytoplasm, while AST is present in the mitochondria of liver cells. Destruction of the liver cells leads to the leakage of these enzymes into the bloodstream and an increase in their concentration (Drotman & Lawhorn, 1978).

According to study of (Measem *et al.*, 2020) showed a substantial decrease in liver enzyme (AST, ALT and ALP) in treatment which was complemented by 2 % Peppermint leaves leave powder 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 which have antioxidants and antiperoxidant characteristics (Twegh *et al.*, 2020).

It can be nutritional situation and compounds of herbs and also there are many other factor, like age, sex, type of bird, and environmental. Al-Kassie (2010) observed the lowest H/L ratio in the group supplemented with a minimum dose of Peppermint leaves. The heat stress increases the H/L ratio. Stress factors could stimulate the adrenal gland to produce hormones such as estrogen, which could influence lymphatic cell counts and increase the H/L ratio.

2.2.4. Effect of Peppermint leaves on immunity of poultry

Peppermint leaves essential oils blend proved to have an immune stimulant effect on the humoral and cell mediated immune response against Newcastle disease in chickens, Their utility in modulating the immune response of immune compromised birds after infection with infectious bursal disease virus (IBDV) and/or vaccination against IBDV as compared with untreated control groups was also evident (Nahed *et al.*, 2019).

Barbour *et al.* (2013) who evaluated the impact of eucalyptus and Peppermint leaves essential oils on immune modulation and production of broiler chickens challenged with a molecularly characterized velogenic Newcastle disease. Nahed *et al.*, (2020) who stated that Eucalyptus and Peppermint leaves 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 leaves improved the homogeneity of immune responses and performance in Mycoplasma gallisepticum/H9N2 virus-infected broilers.

Peppermint leaves oil maintains the structural integrity of immune cells due to its strong antioxidant action which protects cell membrane from free radical oxidants, there by resulting in an improved immune response, Administration of Peppermint oils blend evoked the immune response in chickens. Enhancement of the immune

system by herbal products and oils has been reported by many investigators (Nahed *et al.*, 2020).

Arab Ameri.*et al* (2016) showed the birds fed a diet supplemented with 1% Peppermint leaves powder had higher total antibody titer T Ig, IgM and IgG compared to other treatments on day 42 of the experiment significant interaction were observed between diet and sex on IgG at 35 days. Also Khaligh *et al.* (2011), reported that polysaccharides extracted from a number of plants such as Peppermint leaves stimulate the growth of organs such as the spleen, thymus and bursa of fabricius and also increase the number of immune cells such as T- cells and lymphocytes and macrophages resulting in humoral and cellular immune responses.

2.3. Fenugreek leaves:

Fenugreek leaves *(Trigon Ella foenum-graecum L.)* is a leguminous herb in the *Fabaceae* family that is used to treat common colds, coughs, bronchitis, sore throats, arthritis, menstrual pain, and to stimulate intestinal digestion in traditional medicine (Herrera *et al.*, 2019). Fenugreek leaves seeds are high in total polyphenols and flavonoids, both of which have physiological benefits (Khlifi *et al.*, 2016). Diosgenin, a chemical molecule utilized in the manufacture of steroids such as sexual hormones, oral contraceptives, and corticosteroids, has been discovered in seeds (Bahmani *et al.*, 2016). Standardized extracts have recently been accessible as capsules or tablets and are being used in the management of many diseases and are being employed in a variety of applications scientific classification of fenugreek leaves showed in table (2-3).

Scientific classification

Table (2-3) scientific classification of fenugreek leaves Watson *et al* (2007)

Kingdom:	Plantae
Clade:	*Tracheophytes*
Clade:	*Angiosperms*
Clade:	*Eudicots*
Clade:	*Rosids*
Order:	*Fabales*
Family:	*Fabaceae*
Subfamily	*Faboideae*
Genus:	*Trigonella*
Species:	*T. foenum-graecum*

2.3.1 Chemical constituents and bioactive compounds of fenugreek leaves :

Fenugreek leaves is an annual spicy herbal legume native to Mediterranean regions. It is now cultivated in other parts of the world. It has been reported that the major bioactive compounds found in fenugreek leaves include Alkaloids (pyridine, trigonellin), polyphenols (choline, luteolin, quercetin), steroidal saponins (diosgenin, protodioscin, yamogenin), coumarin, lipids, anon-proteinogenic amino acid (4-hydroxyisoleucine), vitamins,galactomannans, and minerals. The Fenugreek leaves seeds have 51.5 % carbohydrate, 25.0 % protein, and 4-6 % lipids, but the leaves have 20-30 % protein, 22-30 % starch, 12.5 % neutral detergent fiber, 4.0 % gum, 10.6% ash, and 4-6 % lipids (Basu and Srichamroen, 2010) . Trigonelline is

the main alkaloid in fenugreek leaves decomposed into nicotinic acid and pyridines after roasting, giving the seeds their distinctive flavor (Ouzir *et al*., 2016), and has a great therapeutic potential and minimal toxicity. 4-hydroxyisoleucine makes up about 80% of the total free amino acid concentration in seeds (Fuller and Stephens, 2015).

In addition to dietary fiber (both insoluble and soluble), fenugreek leaves contain galactomannan ,Fenugreek leaves seeds contained fourteen bioactive components, according to a GCMS (Chromometry-Mass Spectrometry) analysis (Srinivasan, 2019). Premanath *et al.* (2011) found that fenugreek leaves leaves have the highest concentration of phenolic chemicals and flavonoids Diosgenin, trigonelline, galactomannan, 4-hydroxyisoleucine quercetin, and scopoletin are the main active components responsible for fenugreek leaves 's varied pharmacological qualities found a wide range of pathogenic microorganisms, including *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Premanath *et al.*,2011).

Discovered that an ethanolic extract of fenugreek leaves has strong antibacterial activity against *Staphylococcus aureus and Pseudomonas aeruginosa*. Similarly, antibacterial activity of a methanolic extract of stem leaves was discovered against *Staphylococcus aureus and E. coli* (Premanath *et al.*, 2011).

2.3.2 Health benefit of fenugreek leaves

Fenugreek leaves is a prominent herb in the practices of Ayurvedic medicine (The Ayurvedic Pharma copoeia of India). Fenugreek leaves , with a diversity in its forms and modes of use, has elicited its potential health benefits as a functional food in recent years, which until now has been a part of the traditional system of curing skin conditions and many other diseases .Furthermore, fenugreek leaves seeds are currently used as an antidiabetic agent, antibacterial, anti lithogenic,antiulcer, anthelmintic, immune modulatory effect, enzymatic pathway modifier, gastric

stimulant, hypo cholesterolemic ,hypoglycemic, antioxidant, antiulcer, antifertility, and anti-anorexia agent (Basch *et al.*, 2003).

It has various potential pharmacological effects in modern medicine such as antidiuretic, antilipidemic, antioxidant, hypocholesterolemic, hepatoprotective, antifungal,anti-inflammatory,antibacterial,ant-carcinogenic,antiulcer, antilithigenic and neuroprotective effects in both clinical trials in humans as well as in experimental animals (Neelkanthan *et al.*, 2014).

Fenugreek leaves contains 20-25% protein, 45-50% dietary fiber, 20-25% mucilaginous soluble fiber, 2-5%steroidal saponins, 6-8% fixed fatty acid, and essential oils. Initial research suggests that fenugreek leaves was conventionally advised for increasing milk production in lactating women (Vishwakarma *et al.*, 2022).

2.3.3 Pharmacological actions of fenugreek leaves

This herb contains gastro protective (Pandian *et al.*, 2002), hypoglycemic effect diabetes mellitus is a chronic metabolic condition defined by persistent hyperglycemia due to insufficient insulin production by the pancreas or inability of peripheral target tissue to respond to normal insulin concentrations, It is a leading source of illness and mortality in the United States, with an ever-increasing prevalence and the world's fastest-growing disease, Diabetes is a major public health concern, and its prevalence is rising over the world. In the treatment of diabetes, herbal medicine plays an essential role (Jarald *et al.*, 2008). This hypoglycemic action may be due to the presence of galactomannan in the fenugreek leaves powder. Galactomannan, a water soluble fiber, can inhibit glucose absorption and delay gastric emptying (Hannan *et al.*, 2007).

Anti-carcinogenic properties: Cancer is one of the most common causes of death worldwide. Fenugreek leaves have been demonstrated to have a protective effect in experimental cancer models utilizing cell lines in a number of studies (Amin *et al.*,

2001). Fenugreek leaves consumption was linked to a reduction in polyamine concentration in tumor tissue, Fenugreek leaves seed extract prevented and reduced the incidence of 7,12-dimethylbenz(a)anthracene-induced mammary hyperplasia in rats, suggesting that its anti-breast cancer protective action could be attributable to enhanced apoptosis (Verma *et al.*, 2010). According to the study, fenugreek leaves extract therapy inhibited the proliferation of breast, pancreatic, and prostate cells, but immortalized prostate cells were unaffected. Fenugreek leaves's capacity to induce cell death, despite concomitant activation of growth stimulatory pathways in normal cells, is related to its ability to inhibit cancer cell proliferation (Shabeer *et al.*, 2009).

Hypocholesterolemic activity: Hypocholesterolemia is a condition in which the blood cholesterol level is abnormally low. In mice, oral administration of methanolic and aqueous extracts of seeds at a concentration of 1gm/kg body weight caused hypoglycemia, The major components of gum are galactose and mannose, which are abundant in fenugreek leaves. The latter chemicals have been linked to lower cholesterol levels (Weerasingha *et al.*, 2013).

The effects of fenugreek leaves extract on blood lipids and diabetes in experimental rats have been studied. The streptozotocin-induced diabetic rats were given low, intermediate, and high doses of fenugreek leaves extract, as well as Metformin HCl, through oral intragastric intubation for roughly one and a half months (6 weeks). Rats given fenugreek leaves extract exhibited lower blood sugar levels than diabetic rats it has antibacterial and antifungal properties. Have just been discovered. Antibacterial and antifungal substances derived from plants have been found and reviewed (Ahmad and Beg, 2001). Various aqueous preparations of fenugreek leaves plant components in various Determine the action of methanol, petroleum ether, and ethyl acetate fractions of aerial parts in a solvent .the

antifungal potential of the fenugreek leaves plant was found in all portions of the plant, and the magnitude of the action varies depending on the fungus type.

The antimicrobial properties of fenugreek leaves dried seed are well-known. Fenugreek leaves contains a number of metabolites with antibacterial activity, including tannins, alkaloids, flavonoids, terpenoids, and glycosides (Khorshidian *et al.*, 2016) .fenugreek leaves extracts have also been shown to be effective against Helicobacter pylori by a number of researchers (Randhir *et al.*, 2007).

Antilipidemic action Dyslipidemia, obesity, diabetes, cardiovascular disease, inflammation, and their related illnesses are all linked to abnormalities in lipid metabolism (Lee *et al.*, 2003) Various medicines are currently used to treat dyslipidemia, obesity, and other metabolic disorders, all of which have negative side effects and cost money (Garg and Simha, 2007). Seeds of fenugreek have long been used to cure a variety of chronic human illnesses. This idea is supported by studies on the use of fenugreek seeds in the treatment of diabetes, dyslipidemia, and obesity (Basch *et al.*, 2003). Fenugreek leaves was found to have lower levels of serum triglyceride, total cholesterol, and hepatic lipids, its efficacy in the prevention and management of dyslipidemia and obesity-related problems has yet to be studied using a mechanism-based approach. Fenugreek seeds are used as a condiment and are believed to offer health advantages. A new thermos table extract of fenugreek seeds has been developed. 3T3-L1 cells and HepG2, a human hepatoma cell line were employed as in vitro models to determine the hypolipidemic effect of the novel thermos table extract of fenugreek leaves seeds (Gregoire,2001).

2.3.4 Effect of fenugreek leaves on productive traits of poultry:

2.3.4.1 Effect of fenugreek leaves on body weight and weight gain:

the Productive performance is defined as the capacity of a system to meet a specified demand for deliveries or performance The system configuration, reliability, maintainability, maintenance support ability and functional capacity of its items.

Ali *et al.* (2021) showed that 0.5%, 1% and 1.5% fenugreek leaves seed supplementation of broiler diets resulted in increase in live weight than 0% fenugreek leaves seed in broiler. The broiler of 1.5 and 1% fenugreek leaves supplemental group was significantly ($P \leq 0.05$) higher in average of weekly weight gain compared to 0.5% and non-supplemented group,(Significant difference ($P \leq 0.05$) were found at 14 days, 21 days and 28 days of age). The highest body weight gain was observed in 1.5% fenugreek leaves group, followed by 1, 0.5 and 0% supplemental groups, respectively.

Rahimian *et al.* (2018) showed that supplementation of various levels of fenugreek leaves seed powder improved significantly ($P \leq 0.05$) body weight and Preslaughter weight of broiler chicks. This may be due to the presence of the fatty acids, or due to stimulating effect on the digestive system of broilers. Yassin *et al.* (2020) reported that the average daily weight gain for T4 (3%) and T3 (2%) was higher ($P<0.05$) than those fed T1 (0%) diets while T2 (1%) had an intermediate value during the growing phase. During the finisher and the entire period the highest ($P \leq 0.05$) average daily gain was for T4 groups fed 3% fenugreek leaves while the lowest ($P \leq 0.05$) was for T1.

Gaikwad *et al.*, (2019) showed that 1.5% Fenugreek seed powder supplemented feed diet increases cumulative weight ($P \leq 0.05$) over control and other supplemental groups. Alloui *et al.* (2012) who found that addition of fenugreek leaves seed in broiler diets increased average daily gain. Magda (2012) inclusion1% level and 1.5% levels useful for improving live body weight, body weight gain, feed

conversion ratio, protein efficiency ratio, feed consumption and efficiency of energy utilization. Hind *et al.* (2013) reported increased daily weight gain and feed intake due to the stimulatory effect of fenugreek leaves on the digestive system of broilers.

Inclusion of Fenugreek leaves in the diet significantly improves the body weight of broiler chicken, Further, it improves the feed efficiency with reduction in feed cost when used as natural feed additive in broiler chicken diets (Azoua, 2001). Rabia (2010) however reported 3 g/kg of feed as best inclusion level for enhancing the performance and body weight of broiler chickens. EL-Mallah *et al.* (2005) noted that fenugreek leaves seeds at 2% in the diet of turkey chicks caused a significant increase in digestibility and absorption of nutrients and there was a significant improvement in body weight gains.

The development in live body weight in fenugreek leaves addition may be by reason of beneficial influence on digestion and ability to modify feed texture (Murlidar and Goswami, 2012), or attributable to existence of the fatty acids (Murray *et al.*, 1991). Elkhider (2013) who's found that adding of fenugreek leaves seeds in poultry feed improved live body weight. The improvement in weight gain may be due to the fenugreek leaves substances of affect composites for example antifungal, anti- bacterial, and anti-inflammatory and antioxidant actions.

The improvement in live body weight and body weight gain of broilers fed diet supplemented with 1.0% fenugreek leaves seeds may be due to that the fenugreek leaves seeds contain of fat soluble, unidentified factors (Murray *et al.*, 1991), The existence of important fatty acids and high-quality proteins has a stimulating impact on the villus height of the broiler digestive system and the contents of active compounds such as antibacterial, antifungal, anti-inflammatory, carminative and antioxidant activities that have improved the body gain(Mamoun *et al.*, 2014). Morsy (1995) reported significant improvement in body weight gain and dressing percentage with broiler fed diets containing 500g fenugreek leaves .

2.3.4.2 Effect of fenugreek leaves on feed intake

Feed intake has been suggested as the single-most important factor determining the growth rate of broilers (Ferket and Gernat, 2006). Higher FI increases weight gain and consequently reduces the proportion of energy used for bird's maintenance in relation to gain (Svihus *et al.*, 2004).

Gaikwad *et al.* (2020) concluded that with highest level of fenugreek leaves seed powder had highest feed consumption rate in the broiler birds. Yasin *et al.* (2020) also indicated that supplementation of Fenugreek leaves in broiler diets improved feed consumption, which can be due to the improvement of palatability of the feed containing fenugreek leaves . Qureshi *et al.* (2015) noted that the cumulative feed consumption was significantly improved ($P \leq 0.05$) in fenugreek leaves seeds supplemented groups than compared with the control group. Bhale (2015) also noticed that inclusion of 1% fenugreek leaves seed powder in broiler ration improved feed intake and that resulted into better weight than without fenugreek leaves seed powder. Tariq *et al.* (2014) also reported that the birds fed on diet containing 1, 2 and 3% fenugreek leaves seed significantly ($P \leq 0.05$) increased the feed intake. Alloui *et al.* (2012) who reported that the palatability of feedstuffs containing fenugreek leaves seeds are improved because of the presence of high levels of the carbohydrate fraction, galactomannan, in the fenugreek leaves seeds. Moreover, Hind *et al.* (2013) reported increased daily gain and feed intake due to the simulative effect of fenugreek leaves on the digestive system of broilers.

2.3.4.3 Effect of fenugreek leaves on feed conversion ratio

Fenugreek leaves powder addition at various amounts in broiler diets improved feed conversion efficiency, according toYasin *et al.* (2020) concluded that fenugreek

seed powder addition at various levels in broiler diets improved the feed conversion efficiency which is in agreement with the findings of Hamden *et al.* (2010) who reported that inclusion of fenugreek leaves improved feed conversion efficiency of broiler chicks. Rahimian *et al.* (2018) showed that the body weight and feed conversion ratio were significantly highest ($P \leq 0.05$) in treatment group that fed on fenugreek leaves supplemented feed compared to control group.

Qureshi *et al.* (2015) reported that feed conversion ratio was significantly ($P \leq 0.05$) improved in the birds fed diets containing either raw or enzyme treated fenugreek leaves, alone or in combination, when compared with the control group. Among the different treated groups, best FCR of 1.88 was observed in the group fed combination of enzyme treated fenugreek leaves, followed by enzyme treated fenugreek leaves group.

Alloui *et al.* (2012) showed that fenugreek leaves seeds significantly ($P<0.05$) affect Feed Conversion Ratio during the 42 days of age due to the development of the broiler chicks' gut and progressive morphological changes in gastrointestinal tissues that can be induced by differences in gut load of microbial content including their metabolites. Weerasingha and Atapattu (2013) reported that a 13.8% improvement in FCR value in comparison to control group, the birds fed 1% fenugreek leaves reported the best FCR. Broiler chicks fed diet supplemented with 1.0 % fenugreek leaves powder had substantially improved feed conversion. (Medina, *et al.*, 2020) showed Fenugreek leaves powder addition at various levels in broiler diets improved the feed conversion efficiency which is in agreement with the findings of Hamden *et al.* (2010) who reported that inclusion of fenugreek leave improved feed conversion efficiency of broiler chicks.

2.3.4.4 Effect of fenugreek leaves on biochemical parameter of poultry

Blood in animal's body serves as a medium of transporting nutrients absorbed from the digestive system or released from storage in adipose tissues or in liver. The blood picture changes with advancement of animal with age and with certain conditions such as nutrition. Fenugreek leaves has a beneficial effect on cleansing the blood and as a diaphoretic, it is able to bring on a sweat and to help detox the body (Anone, 2013).

Mamoun *et al.* (2014) reported that incorporation of dietary Fenugreek seeds in broilers at 1% level significantly decreased the blood cholesterol and glucose levels. The reduction in the serum glucose levels may be related to direct α-cell stimulation by amino acid (4-hydroxy isoleucine) which increases insulin secretion thus improves glucose tolerance when Fenugreek leaves seeds are fed (Schryver, 2002). Fenugreek leaves also regulates blood sugar through the enzymes sucrase and alpha-amylase. By inhibiting these two enzymes, fenugreek leaves can help slow the breakdown of carbohydrates into sugar further reducing blood sugar levels in the body, Reduction in blood cholesterol levels by supplementation of fenugreek leaves seeds at 40 g/kg in diet of broiler chicken has also been reported by Duru *et al.*(2013), the hypoglycemic effect of fenugreek leaves seeds could be attributed also to the alkaloid trigonelline present in fenugreek leaves seeds that may improve peripheral glucose utilization, Similar results have been explained by Hannan *et al.*(2007) which attributed the antidiabetic effects through inhibition of carbohydrate digestion and metabolism and enhancement of peripheral insulin action. It also aids in lowering blood glucose absorption and controlling sugar

levels, making insulin action easier. Galactomannans are a type of soluble fiber found in seeds that helps to reduce glucose absorption in the body (Meghwal and Goswami, 2012).

El-Hack *et al.* (2015) discovered that fenugreek leaves extract supplementation resulted in a decrease in laying hens serum total cholesterol concentration and an increase in high-density lipoprotein cholesterol concentration. . According to Mamoun *et al.* (2014), the presence of saponins and resins in fenugreek seeds may have rendered bile acid and cholesterol absorption from the intestine according to Weerasingha *et al.* (2013) Fenugreek leaves includes bioactive components such as minerals, Vitamins, lecithin, and choline are all chemicals that aid in the dissolution of cholesterol and fatty compounds When fenugreek seeds were employed in turkey poults, Possessions (Bash *et al.*, 2003). It also includes lecithin and choline, which aid in the breakdown of cholesterol and substances that are fatty it also contains neurin, biotin, and other proteins.

There is increase in RBC count, PCV percentage, and Hb concentration in broiler breeder chickens supplemented with Fenugreek leaves at a dose of 10 g/kg of diet was due to an increase in antioxidant activity in RBCs, which inhibits the production of free radicals that destroy Hb and cause RBC hemolysis. It's possible. fenugreek leaves extract contains steroid saponins, which lower serum cholesterol levels(Faste *et al.*, 2009) observed that supplementing broiler meals with fenugreek leaves seed powder lowered plasma total lipids and total cholesterol in broiler chicks Its seeds contain a variety of medicinal properties, including hypoglycemic, anti-helminthic, anti-inflammatory, and antibacterial properties Bhaisare and Thyagarajan (2014) found that some bioactive principles in fenugreek leaves seeds have a favorable influence on the body's haemopoietic process, resulting in a substantial ($P \leq 0.05$) increase in haemoglobin content.

Oral consumption of ethyl acetate extract of fenugreek leaves seeds has been shown to lower triglycerides and LDL cholesterol (LDL-C) , while increasing high-density lipoprotein cholesterol (HDL-C); as a result, it had a significant impact Antioxidant and cholesterol-lowering properties (Belguith-Hadriche, 2013). It also protects cellular organelles from oxidative damage by scavenging free hydroxyl radicals (-OH) and discouraging hydrogen peroxide-induced peroxidation in liver mitochondria (Kaviarasan *et al.*, 2007).

Flavonoids, phenols, saponins, alkaloids, and other bioactive substances abound in fenugreek leaves (Akbari *et al.*, 2020). It possesses a variety of bioactivity qualities, including antibacterial, antioxidant, antifungal, antiviral, digestive stimulation, and immunomodulation (Srinivasa and Naidu, 2020). Recent research in broiler have demonstrated that fenugreek leaves supplementation lowers blood cholesterol and Glycemia levels, improves immunological response, and increases plasma total protein and globulin levels (Al-Homidan *et al.*, 2020).

Maha *et al.* (2021) that found Broiler chicks fed diet supplemented with 1.0% fenugreek seeds powder had significantly the highest value of serum total protein and the lowest value of cholesterol concentration compared to other dietary treatments. Birds fed control diet without addition recorded the highest value of cholesterol the increase in total serum proteins can mainly be due to the fact that fenugreek leaves seeds can directly stimulate the thyroid gland as serum T3 and T4 are significantly increased and contribute to increased serum protein content (Hassan, 2000).

Azouz (2001) found that total protein and globulin of serum increased significantly by feeding broiler chicks on diets supplemented with fenugreek leaves.

In *vitro* studies have demonstrated hepatoprotective activity of fenugreek leaves extracts in several animal models. Because of the high fiber content, estrogenic and Coumadin-like effects of fenugreek leaves, it has a potential to cause herb-drug interactions particularly if taken in high doses with antiplatelet drugs and warfarin. Jafar *et al.*(2021) reported that the bird fed on diet containing FSP in a level of 0.1% , 0.2% and 0.3% showed significantly decrease in AST ,ALT and ALP levels , in all supplementary groups compared to the control group that may be to vitamins A and B1 component of seeds are effective in liver function and could decrease ALT ,and AST enzyme levels.

According to Al-Nuaimmi and Abdul-Rahman (2018), treatment with fenugreek seeds alone (10 g/kg of feed) or with H2O2 0.5% (which causes oxidative stress) in quail females from 7–42 days of age resulted in a significant decrease in glucose and triglyceride levels as well as a reduction in the activity of AST and ALT, but an increase in the level of heart tissue glutathione.

2.3.4.5 Effect of fenugreek leaves on immunity

The immune system is amazingly complex. It can recognize and remember millions of different enemies, and it can produce secretions and cells to match up with and wipe out each one of them. The secret to its success is an elaborate and dynamic communications network. Millions and millions of cells, organized into sets and subsets, gather like clouds of bees swarming around a hive and pass information back and forth. Once immune cells receive the alarm, they undergo tactical changes and begin to produce powerful chemicals. These substances allow the cells to regulate their own growth and behavior, enlist their fellows, and direct new recruits to trouble spots. (Abid *et al.*, 2011) Show that fenugreek leaves administration significantly increases antibody titer against ND at 24 and 34 days when compared to the control group. The capacity of these herbs to boost immunity

through active components (flavonoids, steroid saponin) or by increasing the weight of lymphatic tissue may be the cause of these increases.

The findings of Abed *et al.*(2014) demonstrated, supplemented with 1% fenugreek leaves , strong anti-body titers against Newcastle disease virus and Gumboro disease virus were seen. effect of fenugreek leaves on the immune system of Swiss albinos at three doses (50, 100, and 200 mg per kg) of body weight for ten days (as evidenced by assay, phagocytosis, cellularity of lymphoid organs of the body, late type of hypersensitivity response, plaque forming cell assay, lymph proliferation, and significant increase in phagocytic index and phagocytic capacity of macrophages) (Meghwal and Goswami, 2012).

The supplementation of 0-3% fenugreek leaves to rabbit diet revealed a great role in improving the immune system .As Abid and Fateh,(2014),noticed supplemented with fenugreek leaves reported high anti-body titter against Newcastle disease virus and infectious bursal disease virus.

The presence of immunoglobulins especially IgA and IgG in chicken sera enhances the immunity of the birds. According to the research reports, fenugreek leaves can promote Ig secretion in animals. We observed that every level of FSE dietary supplementation had a positive regulatory effect on the serum IgG, IgM, and IgA of broilers, and among them 100 mg/kg (Huang *et al.*, 2022), it shows that fenugreek leaves could significantly increase the level of immunoglobulin in broilers, which is beneficial to enhance the immunity of broilers. The main reason why fenugreek leaves affect immunoglobulin secretion is that fenugreek leaves contains 50% polysaccharides, and polysaccharide compounds can enhance the immune modulatory activity of macrophages in animal bodies, have the ability to stimulate the production of serum immunoglobulins, and have the potential to regulate innate and adaptive immunity (Huang *et al.*, 2022).

Chapter Three: Materials and Methods

3. Materials and Methods

3.1. Experiment design

This study was carried out in a private brooding 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 Ross 308 were divided randomly into four treatments (30 chick/treatment) with 3 replicates each treatment, each replicate involved 10 birds/ pen, the experimental treatments as follows:

T1 :(Control) basal diet without any additive.

T2: basal diet with dry Peppermint leaves at 1.5 gm. /kg diet.

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

T4: basal diet with dry Peppermint leaves at 1.5gm/kg and 1.5 gm. /kg of dry fenugreek leave.

The experimental period was five weeks. Feed and water provided *ad Libitum* along the study, Special programs were used for vaccination of birds and health care as recommended in the broiler The chicks were fed at 1 to 10 days age on a starter diet and grower diet from 11-24 days afterwards were fed on a finisher diet from 24 days until the age of 35 days. The figure (3-1) showed the experiment design and studies parameters. 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 3C^{o}above the normal that starting at 12pm to 4pm o'clock by using automatic heating incubator .

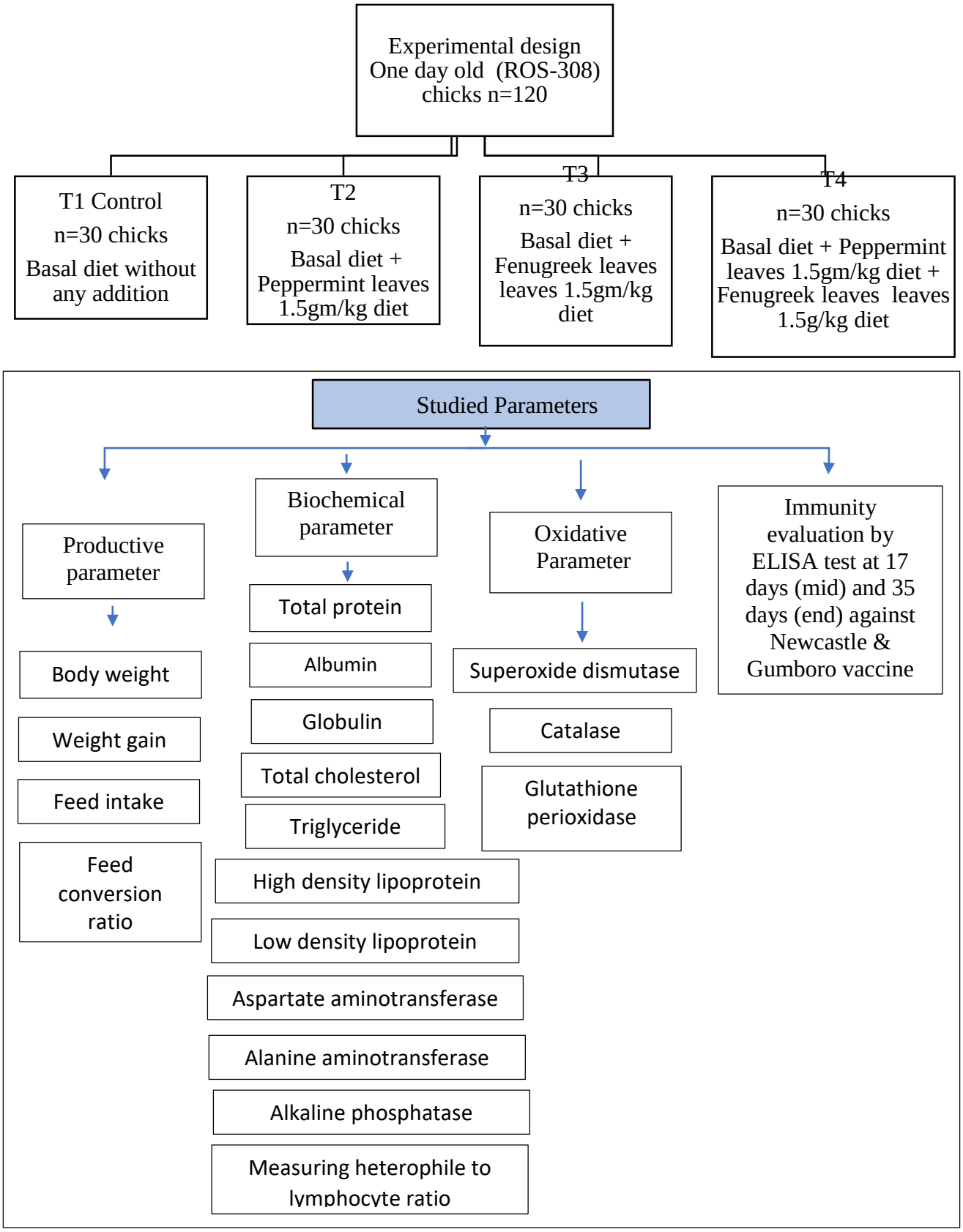

Figure (3-1): experimental design and studied parameters

Basal diet: basal diets for all experimental groups was formulated according to NRC, (1994) recommendations, based on corn and soybean meal for 1 to 35 days of age the composition and chemical analysis of the basal diet is presented in Table (3-1) and table (3-2).

Table (3-1) Ingredients of experimental diets

Ingredient %	Starter (1-10 day)	Grower (11-21 day)	Finisher (22-35 day)
Corn	35.5	37.8	39
Soya bean meal(44% protein)%	28	26	24
Wheat%	27.8	27.8	28.8
Animal Protean (40%)	5	5	5
Oil%	2	2	2
Salt%	0.2	0.2	0.2
Limestone%	1.5	1.2	1
Total	100	100	100

Table (3-2): chemical analysis of experimental diets

	Starter (1-10 day)	Grower (11-21 day)	Finisher (22-35 day)
Gross energy k cal/kg	3078	3100	3125.2
Crude protein %	21.3	20,57	19.76
Energy/protein ration	135.35	145.25	155.07
Calcium %	1.1	0.95	1.0
Available Phosphate %	0.5	0.47	0.48
Methionine + cystein %	0.78	0.70	0.75
Lysine%	1.02	1.02	0.95
Methionine %	0.48	0.4	0.51
Fiber	0.1	0.09	0.11

3.2. The dietary additives used in the experiment

A. Fresh Peppermint leaves were bought from Amil District Market in Karbala, fresh Peppermint leaves an open, shaded area's floor was covered in leaves. Turn over once or twice a day .The collected dried Peppermint 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.

3.3. Preparation of poultry house

After cleaning the walls, floor and ceiling by clean water disinfection carried out by formalin and potassium permanganate, then all windows were opened and ventilation was 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 were provided with suitable litter (wood shaving), ventilation and lighting were controlled according to the Aviagen guide (Aviagen, 2022) for broiler chickens Ross 308.

3.4. Vaccination programs

All vaccines opened and mixed in free chlorine water, the chicks were prevented from water for 2 hours before vaccination .Vitamin C was used routeinly at the ratio of 1 gm/Litter water after each vaccination to relive the stress .table (3-3) show the vaccination programs

Table (3-3) Program of vaccination:-

Age of chicks (days)	Disease	Type of vaccine	Origin	Rout of vaccination.
1st	Newcastle + Avian influenza	Killed vaccine +MA5+traunsmune	MSD	Injection
	Infection bronchitis			Spraying
	Gumboro			Injection
10^{th},20^{th} ,30^{th}	Newcastle disease	Clone 30 strain	Holland	Via drinking water

3.5. Parameters studied

3.5.1. Productive performance

Weekly production traits were measure by (Al-Fayadh and Naji, 1989).

3.5.1.1. Weekly mean body weight (BW) (gm/birds)

Weekly mean body weight was calculated from the total weight of all chicks in each replicate divided on the number of chicks by sensitive scale.

3.5.1.2. Weekly mean weight gain (WG) (gm/birds)

Weekly mean body weight gain for each replicate was calculated by substrate recording the Body weight gain at the end of the week and depending on the following equation:

Mean weekly weight gain= mean body weight at the end of the week- mean body weight at the beginning of the week.

3.5.1.3. Weekly feed intake (FI) (gm/bird):

The feed intake was calculated every week depending on weighting the remaining feed at end of the week from the total feed that offered at the beginning of the same week with into consideration taking the number of the dead chicks and number days of feeding **.**

3.5.1.4. Feed conversion ratio (FCR)

Weekly feed conversion ratio was calculated for each group until the end of the experiment upon the following formula:

$$\textbf{Feed conversion ratio} = \frac{\boldsymbol{mean\ weekly\ feed\ intake\ (gm)}}{\boldsymbol{mean\ weekly\ body\ weight\ gain\ (gm)}}$$

3.5.2. 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 plasma to measure heterophil\lymphocyte ratio, test tube without anticoagulant, serum tubes were immediately separated and kept overnight at 4ºC (in the refrigerator), after that putted in centrifuge for 3000pmr\10 minute,and stored in deep freeze (-20ºC) until analysis.

3.5.3. Biochemical parameters

3.5.3.1. Serum protein concentration (gm /dL)

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)

3.5.3.2. Serum Albumin Concentration (gm /dL)

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 (Tietz , 1990).

3.5.3.3. Serum Globulin Concentration (gm/dL)

Total globulin can be measured as follows:

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

3.5.3.4. 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).

3.5.3.5 .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).

3.5.3.6 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).

3.5.3.7. Estimation of LDL-Cholesterol concentration (mg/dl)

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

3.5.3.8. Estimation of VLDL-Cholesterol concentration (mg/dl)

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

3.5.4. Liver function enzymes

3.5.4.1. AST (U/dl)

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

3.5.4.2. ALT (U/dl)

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

3.5.4.3. ALP (U\dl)

Alkaline phosphatase was determined by the kinetic method of Klin , 1980

3.5.5. Antioxidant enzyme

3.5.5.1Measurement of serum Glutathione Peroxidase concentration (GPX)

Measurement of serum Gpx was done by using ELISA kit.

3.5.5.2Measurement of serum Superoxide dismutase concentration (SOD)

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

3.5.5.3 Measurement. Of serum catalase (CAT) :

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

3.5.6. Immunological test

Antibody titters against Newcastle Disease vaccine (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).

3.5.7. 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.

3.6. Statistical analysis

Data obtained from the current study were analyzed as one-way analysis of variance) ANOVA) using general linear model (GLM) procedure to SPSS 22.0 software (Corp, 2011). Four groups means were analyzed by using a Duncan`s analysis in level (0.05).

Chapter Four: Results

4. The result

4.1. Productive performance

4.1.1. Body weight

Means of live body weight of broiler chicks were listed in table (4-1), there were a significant increase (P≤0.05) in live body weight during1-5 weeks of age of addition treatments as compared with control ,wherever T4 treatment recorded highest weight as compared with control and T2,T3. The second and third treatments record a significant increase (P≤0.05) as compared with control treatment

Table (4-1) Effect of Peppermint leaves , fenugreek leaves and their mixture on weekly live body weight (gm/bird) of broilers. (Mean ±SD).

Age / group	Week1	Week2	Week3	Week4	Week5
T1	165.3 ±4.26 B	440.0 ± 2.26C	822.60 ±1.12C	1335.20±13.26C	1899.00 ±5.73C
T2	168.5 ± 0.73A	460.20 ± 1.74B	873.20 ± 7.88B	1438.80 ± 3.08B	2074.60 ±4.80B
T3	166 ± 3.33A	464.20 ± 4.44B	868.40 ± 2.37B	1497.80 ± 3.56B	2081.40±21.35B
T4	169.5 ± 2.18A	509.40 ± 4.34A	914.80 ±10.42A	1743.80±10.62A	2212.80±23.31A

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 leaves , T3:1.5gm\kg diet fenugreek leaves , T4:1.5gm\kg diet Peppermint leaves +1.5gm\kg diet fenugreek leaves , WK1, 2,3,4,5 weeks of experiment.

At the third, fourth and fifth week of experiment the treatment T2, T3, T4 showed significant increase (P≤0.05) as compared with control, in spite of, there were no

significant differences among them, the fourth treatment recorded the high significant increase in live body weight as compared to other treatments.

4.1.2. Weight Gain (WG)

Means of body weight gain of broiler chicks were listed in table (4-2) The results showed decrease means in control as compared with addition treatments but there were a significant increase($P \leq 0.05$) in T2,T3,T4 and high significant increase in weight gainT3,T4as compared with T2 during first week .At second , third ,fourth weeks that showed no a significant difference($P \leq 0.05$) in all addition treatments but high significant increase in T2,T3,T4 as compared with control treatment. At the fifth week the result showed significant decrease in control treatment as compared with addition treatment , showed significant increase ($P \leq 0.05$) in T2, T3, T4 but the fourth treatment highest significant increase in weight gain as compared with T2, T3 treatment.

Table (4-2) Effect of Peppermint leaves, fenugreek leaves and their mixture on weight gain (gm/bird) of broilers. (Mean ±SD).

Age / group	Week1	Week2	Week3	Week4	Week5
T1	143.60 ± 2.46 C	243.8.8 ± 1.12 B	477.00 ± 1.58 B	531.60 ± 1.40 C	661.00 ± 0.44 C
T2	163.60 ± 2.78 B	266.00 ± 1.87 A	585.00 ± 6.29 A	583.60 ± 10.70 B	711.20 ± 1.85 B
T3	160.8 ± 4.43 B	268.60 ± 17.40A	578.00 ± 3.56 A	585.40 ± 1.20 B	717.60 ± 23.76 B
T4	170.40 ± 2.58 A	318.00 ± 2.21 A	579.40 ± 7.20 A	615.00 ± 23.20 A	860.00 ± 5.26 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 leaves ,T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet Peppermint leaves + 1.5 gm/kg diet fenugreek leaves , wk1,2,3,4,5:weeks of experiment

4.1.3. Feed intake (FI)

The result in table (4-3) showed a significant difference ($P \leq 0.05$) in feed intake between the addition and control treatments in first week ,there were a significant increase in addition treatment as compared with control ,at second ,third ,fourth week significant difference in experimental treatment, there were a significant decrease in control as compared with addition treatments but highest significant increase in feed intake in T4 as compared with T2,T3 treatments .At fifth week the result showed a significant increase ($P \leq 0.05$) in feed intake in T2,T3,T4 as compared with control treatment, the highest significant increase in T4 in all week.

Table (4-3) Effect of Peppermint leaves , fenugreek leaves and their mixture on weekly feed intake (gm)/bird of broilers (Mean ±SD).

Age / group	Week1	Week2	Week3	Week4	Week5
T1	228.80 ± 4.31 B	387.00 ± 4.80 C	784.60 ± 10.15B	772.40 ± 7.32 C	1111.80 ± 27.16B
T2	241.80 ± 1.80 A	414.60 ± 5.16 AB	824.80 ± 2.65 A	799.60 ± 2.76 B	1177.20 ± 12.40A
T3	245.20 ± 3.18 A	385.20 ± 1.93 B	865.20 ± 1.65 A	815.40 ± 6.70 B	1191.20 ± 9.38 A
T4	253.20 ± 1.24 A	444.60 ± 6.23 A	846.80 ± 4.89 A	861.40 ± 3.24 A	1223.40 ± 4.76 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 leaves ,T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet Peppermint leaves +2.5 gm/kg diet fenugreek leaves , wk1,2,3,4,5:weeks of experiment.

4.1.4. Feed Conversion Ratio (FCR)

The result of feed conversion ratio recorded in table (4-4)**,** showed there were significant differences among experimental treatment, there were a significant decrease in addition treatment as compared to control, improved FCR for addition treatments as compared control the T3 treatment showed better FCR as compared to other treatment.

Table (4-4) Effect of Peppermint leaves, fenugreek leaves and their mixture on weekly feed conversion ratio of broilers (Mean ±SD).

Age / group	Week1	Week2	Week3	Week4	Week5
T1	1.59 ± 0.01 A	1.58 ± 0.02 A	1.64 ±0.02 A	1.45 ± 0.02 A	1.68 ± 0.01 A
T2	1.47 ± 0.02 B	1.55 ± 0.06 A	1.4 ± 0.01 B	1.37 ± 0.05 B	1.59 ± 0.02 B
T3	1.52 ± 0.05 B	1.43 ± 0.10 B	1.49 ± 0.01 B	1.39 ± 0.01 B	1.59 ± 0.05 B
T4	1.48 ± 0.01 B	1.39 ± 0.11 C	1.46 ± 0.02 B	1.4 ± 0.03 B	1.44 ± 0.04 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 leaves ,T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves , wk1,2,3,4,5:weeks of experiment, FCR: feed conversion ratio.

4.2. Biochemical parameters

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

Table (4-5) cleared the effect of Peppermint leaves , fenugreek leaves 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 ($P\leq0.05$) among treatments.

Table (4-5) Effect of Peppermint leaves, fenugreek leaves 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 leaves ,T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves .

4.2.2. Concentration of lipid profile

Table (4-6) showed the effect of Peppermint leaves, fenugreek leaves and their mixture on lipid profile of broilers at 35th days under heat stress**,** there were a significant decrease (P≤0.05) that was noticed in the serum cholesterol concentration of the addition treatments as compared with the control, and there were a significant decrease (P≤0.05) in the value of T2 as compared to other treatments, also a significant decrease (P≤0.05) existed in T4 as compared to T3 and control treatment. There were a significant decrease (P≤0.05) in triglyceride concentration at T2, T3 and T4 groups as compared with the control treatments, these results were similar to results of total cholesterol, since T2 had a lowest value as compared to other treatments.

High density lipoprotein values increased significantly (P≤0.05) in addition treatments as compared to control, so T4 recorded high value. Low density lipoprotein values showed significant increase (P≤0.05) in control treatment as compared with addition treatments there were significantly increase (P≤0.05) in T4 as compared with T3, and significant decrease (P≤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≤0.05) in control treatment as compared with addition treatment. Were T1 control treatment high value and T3 low value**.**

Table (4-6) 2Effect of Peppermint leaves , fenugreek leaves 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 ± 8.16A	87.64 ± 0.88 B	76.87 ± 2.63 A	25.27 ± 0.35 A
T2	147.11 ± 5.76 B	102.49 ± 1.52B	103.31 ± 2.93A	47.33 ± 2.20 C	20.84 ± 0.96 B
T3	153.73 ± 4.96 B	104.44 ± 9.93B	106.04 ± 3.84A	57.76 ± 2.08 BC	18.69 ± 1.00 B
T4	150.46 ± 3.61 B	103.30 ± 5.51B	102.14 ± 2.16A	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 leaves , T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves .

4.3. Values of liver function enzymes

Table (4-7) showed the effect of Peppermint leaves , fenugreek leaves and their mixture on liver function enzymes of broilers at 35th days under heat stress, the result revealed a significant difference ($P\leq0.05$) in AST, ALT and ALP in all groups, T2, T3 and T4 treatments showed a significant decrease ($P \leq0.05$) in AST, ALT and ALP as compared with the control .

Table (4-7) Effect of Peppermint leaves and fenugreek leaves 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 leaves , T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves .

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 were significant decrease (P≤0.05) in T3 and T2 as compared with T4 and control treatments.

4.4 values of antioxidant enzymes

Table (4-8) cleared the effect of Peppermint leaves , fenugreek leaves and their mixture on antioxidant enzymes of broilers at 17th days under heat stress, the result showed a significant increase (P≤0.05) in addition treatments as compared with control in value of CAT ,SOD ,GPX ,although there were no significant differences among addition treatments.

Table (4-8) Effect of Peppermint leaves and fenugreek leaves and their mixture on antioxidant enzymes of broilers at 17th day's 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 leaves , T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves

Table (4-9) showed the effect of Peppermint leaves , fenugreek leaves 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 in value of CAT,SOD,GPX. The value of CAT appeared significant decrease

(P≤0.05) in T1 as compared with T2, T3 and T4, there were significant increase (P≤0.05) in T2 as compared with T3 and T4.

The value of SOD also appeared 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 appeared significant decrease (P≤0.05) in T1 as compared with T2, T3 and T4.

Table (4-9) Effect of Peppermint leaves and fenugreek leaves 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 leaves , T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves .

4.5. Values of heterophil/lymphocyte ratio

Table (4-10) showed the effect of Peppermint leaves , fenugreek leaves and their mixture on heterophil/lymphocyte ratio of broilers at 17th and 35th days of broilers under heat stress , the result appeared significant difference between addition treatment as compared with control but no significant difference among addition treatments, were the result appeared a significant increase(p<0.05) in control as compared with addition treatments .At 17th days of age the result appeared 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 andT4

Table (4-10) Effect of Peppermint leaves and fenugreek leaves and their mixture on heterophil\lymphocyte ratio of broilers at 17th and35th days under heat stress (Mean ±SD).

Parameter group	**at 17th days**	**at35th 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 leaves , T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves .

4.6 Immunological parameters

Table (4-11) cleared the antibody titers against Newcastle disease and infectious bursal viruses at17th and35th of age after adding Peppermint leaves , fenugreek leaves and their mixture in the diet of broilers under heat stress that measured by ELISA test. There were a significant differences ($p\leq0.05$) among experimental treatments. Humeral immunity titer against Newcastle (ND) and Gumboro (IBD) were improved significantly ($P\leq0.05$) in the T2, T3 and T4 as compared with the control , there were a significant increase ($P\leq0.05$) at T4 treatment as compared to other treatment.

Table (4-11) Effect of Peppermint leaves , fenugreek leaves 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	2769 ± 33 C	1957 ± 101 C	2606 ± 214 C
T2	2454 ± 106 B	2909 ± 184 B	2480 ± 161 B	2897 ± 264 B
T3	2677 ± 170 B	2962 ± 160 B	2510 ± 136 B	2903 ± 283 B
T4	2937 ± 166 A	3158 ± 125 A	2931 ± 64 A	3835 ± 129 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 leaves , T3:1.5 gm/kg diet fenugreek leaves , T4:1.5gm/kg diet pepermint+1.5 gm/kg diet fenugreek leaves , (ND) Newcastle disease, (IBD) Gumboro.

Chapter Five: Discussion

5. Discussion

5.1. Productive performance

5.1.1. Body weight and weight gain

The results of the current study illustrated that significant differences among all experimental treatments, addition treatment recorded a significant increase of BW, BWG as compared with control, Results of this study are in agreement with some previous researches that indicated herbs, plant extracts, essential oil and / or the main components of essential oil did affect body weight, feed intake and feed efficiency in broilers because they have appetizing and

This improvement may be attributed to supplementations of Peppermint leaves to broiler diet that significantly increased body weight and daily-body weight gain compared to control under heat stress, proving that Peppermint leaves has an imperative effect on the conversion of digested feed into body weight gain. Also the Peppermint leaves with antimicrobial properties prevent the growth of harmful bacteria in the digestive tract, thereby improving the digestion and absorption as well as body weight gain (Cross *et al.*, 2007).

Also the active components in Peppermint leaves (cineole, citral, geraniol, linalool, and menthol) are shown to possess antimicrobial and antioxidant activities as well as to improve digestion and absorption of dietary nutrients (Bupesh *et al.*, 2007) that might have improved the growth performance of broiler chicks in this study. It seems that the presence of active compounds such as menthol in Peppermint leaves leaves stimulate appetite and increase feed efficiency in broiler chicks.

It seems that the positive effect of Peppermint leaves leaves on increasing daily body weight gain was due to its decreasing effects on gastro intestinal disorders, thus strengthening the digestive system and improving feed efficiency (Ocak *et al.*, 2008). In confirming these mentioned studies, Toghyani *et al.* (2010) reported that diets containing Peppermint leaves improved the growth performance of broiler chicks at early stages of life. Similarly,

In a study by Arab-Ameri *et al.* (2016), broilers whose diets were supplemented 1% with Peppermint leaves powder had higher BW on day 42 of the experiment. Al-Kassie (2010) analyzed the performance of broilers whose diets were supplemented with different doses of dry Peppermint leaves and observed higher weekly WG and lower FCR in chicks fed a lower (0.5%) than a higher (1.5%) Peppermint leaves dose.

Also the result of current study may be attributed to use of fenugreek leaves such as improve in body weights has been attributed to the presence of essential fatty acids and high quality proteins in the Fenugreek leaves seeds (Murray *et al.*, 1991) and stimulating effect on the digestive system (Hind *et al.*, 2013) and improvement in the gut microflora (Aksa *et al.* 2012).

Also the result may due to that isolated steroidal saponin fraction of fenugreek leaves seeds increases feed intake and motivation to eat in normal rats(Petit *et al.* ,1995)

The result of current study record the fourth treatment best result that high significance increase in body weight and weight gain indicated that the Improvement in production performance could be attributed to the antioxidant

prosperities of herbs (Peppermint leaves and fenugreek leaves) that may stimulate protein synthesis by bird enzymatic system as well as to antimicrobial components which act as growth promoter (Al-Ankari *et al.*, 2004).

Weerasingha and Atapattu (2013) and Mamoun *et al.* (2014) reported 1% level and 1.5% inclusion levels of fenugreek leaves useful for improving live body weight, body weight gain .Alloui *et al.* (2012) who found that addition of fenugreek leaves in broiler diets increased weight gain.

The report of Rabia (2010) who found that 3% of fenugreek leaves in the feed as best inclusion level for enhancing the performance and body weight of broiler chicken .Also EL-Mallah *et al.* (2005) noted that fenugreek leaves seeds at 2% in the diet of turkey chicks caused a significant increase in digestibility and absorption of nutrients and there was a significant improvement in body weight gains.\)

Ali *et al.*, (2021) showed that 0.5%, 1% and 1.5% fenugreek leaves seed supplementation of broiler diets resulted in increase in live weight and significantly (P<0.05) higher in average of weekly live weight gain

Yassin *et al.*, (2020) reported that the average daily gain for (3%) and (2%) was higher (P≤0.05) than those fed T1 (0%) diets while T2 (1%) had an intermediate value during the growing phase. During the finisher and the entire period the highest (P≤0.05) ADG was for T4 groups fed 3% fenugreek seed powder while the lowest was for (P≤0.05) T1. Rahimian *et al.* (2018) showed that supplementation of various levels of fenugreek seed powder improved significantly (P≤0.05) body weight and Preslaughter weight of broiler chicks.

5.1.2. Feed intake

The result of current study showed a significant difference ($P \leq 0.05$) in feed intake between the addition and control treatments in first week ,there were a significant increase in addition treatment as compared with control ,at second ,third ,fourth week significant difference in experimental treatment, there were a significant decrease in control as compared with addition treatments but highest significant increase in feed intake in T4 as compared with T2,T3 treatments .At fifth week the result showed a significant increase ($P \leq 0.05$) in feed intake in T2,T3,T4 as compared with control treatment, the highest significant increase in T4 in all week.

This improvement in the feed intake may be it seems that the presence of active compounds such as menthol in Peppermint leaves leaves stimulate appetite and increase feed efficiency in broiler chicks. The Peppermint leaves leaves and menthol significantly increased feed intake and improved feed-conversion ratio and this could be related to increased efficiency of feed utilization and appetite (Akbari *et al.*, 2016), Subsequently an increase in feed intake may cause an increase in growth performance .Also this improvement due to use fenugreek leaves could be attributed to the carbohydrates and their main component (galactomannan) which stimulated the appetizing and digestive process of broilers (Aksa *et al.* 2012) .

The fourth treatment record the best result due to herbs(mixed Peppermint leaves and fenugreek leaves) develop their initial activity in the feed of animals as flavor and can therefore influence the eating pattern, secretion of digestive fluids and total feed intake (Wenk, 2002).

Our results are in concordance with the results of Magda (2012), Yatoo *et al.* (2012) who reported that inclusion of fenugreek leaves seeds at 1% level in the diet of

broiler chicken significantly (P<0.05) increases the feed intake. Gaikwad *et al.* (2020) concluded that with highest level of fenugreek leaves seed powder had highest feed consumption rate in the broiler birds. Yasin *et al.* (2020) ,also indicated that supplementation of fenugreek leaves in broiler diets improved feed consumption, which can be due to the improvement of palatability of the feed containing fenugreek leaves

In harmony of these above findings Bhale (2015) also noticed similar that inclusion of 1% germinated fenugreek leaves seed powder in broiler ration improved feed intake and that resulted into better weight than without germinated fenugreek leaves seed powder. Tariq *et al.,* (2014) also reported that the birds fed on diet containing 1, 2 and 3% fenugreek leaves seed significantly (*P*<0.05) increased the feed intake , Alloui *et al.* (2012) who reported that feeding fenugreek leaves seeds at 3% of the feed in broiler chickens significantly increased feed intake due to the presence of galactomannans and neurin which stimulate the appetite.

5.1.3. Feed conversion ratio

The feed conversion ratio is an indicator of how much feed is used and converted into live weight the result of feed conversion ratio that showed there were significant differences among experimental treatment, there were a significant decrease in addition treatment as compared to control, improved FCR for addition treatments as compared control the T4 treatment showed better FCR as compared to other treatment.

The improvement in FCR may be due to the treatment of chicks that were fed Peppermint leaves leaves powder and menthol significantly increased feed intake and improved feed-conversion ratio and this could be related to increased efficiency of feed utilization and appetite (Akbari *et al.*, 2016).Also the current study may be

to the improvement in gut microflora due to fenugreek leaves seeds (Aksa *et al.* 2012). The improvement in the FCR could also be due to the good nutritive composition of fenugreek leaves seedsthere was an improvement in FCR with feeding of herbal products which they attributed to their effect on improving the digestibility of dietary protein in the small intestine there for the T4 better FCR(Schryver, 2002).

Result agreed with a study by Arab-Ameri *et al.* (2016), birds whose diets were supplemented with 1% Peppermint leaves were characterized by lower FCR values than birds administered flavomycin or control group birds. Hasan and Sadeq (2020) found at the 24 day the Peppermint leaves supplementation either in feed and water had improve feed conversion ratio. Also, the all Peppermint leaves additive except 1% in water had significantly improved feed conversion ratio at day 35.

the results were agreed with Mustafa *et al* .(2013) , study who reported that (1% Peppermint leaves) had significant effect on feed conversion ratio in which have better when compared with control at age 25- 42 day.

Similar results were obtained by Rabia (2010) and Raziq (2012) reported that supplementation of fenugreek leaves seed in broiler chicken significantly ($P<0.05$) improved FCR. Qureshi *et al.* (2015) reported that feed conversion ratio was significantly ($P<0.05$) improved in the birds fed diets containing either raw or enzyme treated fenugreek leaves seeds, alone or in combination, when compared with the control, Alloui *et al.* (2012) showed that fenugreek leaves seeds significantly ($P<0.05$) affect Feed Conversion Ratio due to the development of the broiler chicks' gut and progressive morphological changes in gastrointestinal tissues that can be induced by differences in gut load of microbial content including their metabolites.

Yasin *et al.* (2020) concluded that fenugreek leaves powder addition at various levels in broiler diets improved the feed conversion efficiency which is in agreement with the findings of Hamden *et al.* (2010) who reported that inclusion of FSP improved feed conversion efficiency of broiler chicks. Weerasingha and Atapattu (2013) reported that a 13.8% improvement in FCR value in comparison to control group, the birds fed 1% fenugreek leaves reported the best FCR. Rahimian *et al.* (2018) showed that the body weight and feed conversion ratio were significantly highest (P≤0.05) in treatment group that fed on fenugreek leaves supplemented feed compared to control group, Mamoun *et al.* (2014) noticed improvement in FCR on inclusion of Fenugreek leaves seed powder in diet of broilers .

5.2. Biochemical parameters

5.2.1. Concentrations of total protein, Albumin and Globulin:

The results of the present study obtained recorded no significant differences (P≤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 leaves as food additive. Khursheed *et al* .(2017) who reported that the supplementation of Peppermint leaves leaves with or without enzyme in both 1or 2% levels were not observed any significant effect on serum total protein when compared with control.

5.2.2. Concentration of lipid profile

The results of the current study showed a significant decreases (P≤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 leaves

It seems that some components of Peppermint leaves , including menthol and menthone, have a potential to decrease blood lipids in broilers (European Scientific Cooperative on Phytotherapy, 2003), due the activity of some of the compounds in the volatile oil of Peppermint leaves (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 leaves extract is the presence of volatile phenolic compounds such as essential oils: menthol, menthone, mentyl acetate, methofuran, limonene, polygon, cineole and azolen.

On the same side, the active ingredients in Peppermint leaves by increasing the activity of liver cells, give rise to the con-centration 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. Mimica Dukic *et al.* (2003) during a trial showed that Peppermint leaves , 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 ($P \leq 0.05$) in triglyceride in T2, T3and T4 as compared with control agreed with the study of Akbari and Torki (2014) supplementation of essential oil of Peppermint leaves 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. .Fallah *et al.* (2013) who reported that Peppermint leaves 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 leaves extract to broiler diets reduces the concentration of serum total cholesterol and triglyceride. This would increase the total protein in the blood of broiler chickens. Abdolkarimi and Mirzaaghazade (2010) found that Peppermint leaves 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 leaves 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 leaves 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).

Weerasingha *et al.* (2013) indicated that fenugreek leaves 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 leaves 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 leaves inhibits the absorption of cholesterol and thereby lower hepatic cholesterol concentration and increases biliary cholesterol excretion, ultimately lowering the serum cholesterol concentration (Adil *et al.*, 2015).

Raghuram *et al.* (1994) stated that fenugreek leaves 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 saponins which may either compete with cholesterol at binding sites or interfere with cholesterol biosynthesis in the liver. Fenugreek leaves can control blood lipids and lower serum total cholesterol. The results of Belguith-Hadriche *et al.* (2010) showed that the use of fenugreek leaves 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 leaves has a significant increase in serum HDL-C concentration.

The reason why fenugreek leaves reduced the content of TC and LDL-C may be related to the alkaloids contained in fenugreek leaves extract (Cheng *et al.*, 2020) . According to research, alkaloids can reduce blood TC and LDL-C levels, while beneficially increasing HDL-C levels (Wang *et al.*, 2018). Trigonelline in fenugreek leaves 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.

5.3. Values of liver function enzymes

The current study indicated that there were a significant decrease ($P \leq 0.05$) in AST, ALT and ALP of T2, T3, and T4 as compared with the control under heat stress. ALT, AST and ALP are three important biochemical markers indicating the

normal function of liver. They are intracellular enzymes and their concentration increase by cellular injuries such as hepatocyte necrosis and cell membrane permeability dysfunction. AST is found in liver, cytoplasm, and mitochondria of skeletal and cardiac muscles (Rocha *et al.*, 2013).

The result of this study showed a substantial decrease in this enzyme in additive treatments 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 leaves which have antioxidants and anti-per oxidant characteristic. The important role of essential oil Peppermint leaves in controlling the liver function (Twegh, 2020), the result of the current study agreed with Abo-Ghanima *et al.* (2020) reported that the addition of essential oils of Peppermint leaves reduced AST, and ALT values.

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

The aqueous extract of fenugreek leaves inhibits ethanol and prompts toxicity in the liver by decreasing the activities of serum AST, ALT and ALP. Kaviarasan *et al.* (2006) have demonstrated that the ethanolic extract of fenugreek leaves powder inhibited ethanol-caused toxicity in change liver cells and its preventive effect is similar to the standard hepatoprotective agent silymarin. Fenugreek leaves Polyphenolic extract was found to prevent against ethanol-caused liver damage through normalizing the markers of liver damage (ALT, AST, and ALP), enhancing hepatocyte viability and decreasing the apoptotic nuclei (Kaviarasan *et al.*, 2007).

5.4 Values of antioxidant enzyme

The result shoewd significant increase (P≤0.05) in addition treatment as compared to control in value of CAT, SOD, and GPX. Olennikov and Tankhaeva (2010) reported that Peppermint leaves contained phenolic and flavonoid compounds of approximately 2.70 to 5.52 and 3.02 to 6.32%, respectively. The essential oil components of Peppermint leaves that relate to antioxidant properties are 1, 8-cineole, dihydrocavone, limonene, phytol, linalool, thymol, carveol, piperitenone, and eugenol (Pudpila et *al.*, 2011) this supports the potential antioxidant activity of Peppermint leaves.

The result of current study agreed with(Khempaka .*et al.*(2013) that showed beneficial effects of Peppermint leaves at level 0.5,1,0,1.5and2% on antioxidant properties .Also the result of current study may due to Fenugreek leaves contains fairly high amount of flavonoids, alkaloids, saponins and other antioxidants. (Rababah *et al.*, 2011). Fenugreek leaves has antioxidant effect and 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).

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, (Silvaa *et al.*,2014)The presence of these compounds along with palmitic acid could be a possible reason for antioxidant activity of fenugreek leaves seed oil (Kozłowska *et al.*,2016). These results support the good antioxidant capacity of fenugreek leaves oil. High content of phenolic and flavonoid compounds means higher antioxidant activity of the plants Also the dietary fenugreek leaves can influence the oxidative stability of muscle and liver of broiler chicks. The

antioxidant effect of fenugreek leaves may be attributed to the presence of phytoestrogens and vitamin C (Kaviarasan *et al.*, 2007)

5.5 Values of heterophil/lymphocyte Ratio

The results showed significant increase($p<0.05$) in control treatment as compared with addition treatments .At 17^{th} days of age the result appeared significant decrease($p<0.05$) in T2 as compared with T3 and T4 and the age of 35^{th} days the result appear significant decrease ($p<0.05$) in T2 as compared with T3 and T4 under heat stress fenugreek leaves 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. 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 appeared increased in H\L ratio 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(Davis *et al.*,2008).

Al-Kassie (2010) observed the lowest H/L ratio in the additive treatment supplemented with a minimum dose of Peppermint leaves. this was in agreement with Alkattan (2006) in laying hens,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 leaves treatment .Also the result agreed with (Abdul-Rahman,2012) showed a significant decrease in the Hetrophils % , a significant increase in lymphocytes %

and a significant decrease in the Hetrophils : Lymphocytes in broiler supplemented of 10gm of fenugreek leaves \Kg diet.

5.6 Antibody titters 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 leaves , 1.5fenugreek leaves 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 leaves 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).

Our obtained findings are in agreement with those reported by Barbour *et al.* (2013) who evaluated the impact of eucalyptus and Peppermint leaves essential oils on immune modulation and production of broiler chickens challenged with a molecularly characterized velogenic Newcastle disease virus .also Peppermint leaves has a potent immune modulatory effect that confirm findings of that Peppermint leaves 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 leaves improved the homogeneity of immune responses and performance in *Mycoplasma gallisepticum*/H9N2 virus-infected broilers).

Arab-Ameri *et al.* (2016) observed that the addition of Peppermint leaves powder to broiler diets increased total Ig, IgM and IgG titres against sheep RBC. 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 leaves as an immune stimulating factor. Studies have shown that Peppermint leaves 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 leaves 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 leaves has an obvious immune stimulating effect, which is responsible for inducing macrophages, and improve immunity. Also, Motamedi *et al.* (2014) showed that fenugreek leaves 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 leaves increasing the immunity of birds at 24 and 34 day and because fenugreek leaves increases the cellular ties of thymus gland and bone marrow. As (Abed *et al.*, 2014) showed supplemented with 1% fenugreek leaves recorded high anti-body titter against Newcastle disease virus and Gumboro disease virus

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

In a diet with 1% fenugreek leaves 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 leaves (Abed and Kadhim, 2014). Also the result of study might be attributed to the immunomodulatory effects of fenugreek leaves 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 and Saif 2020).

Chapter Six: Conclusions and Recommendations

6. Conclusions and Recommendations

6.1. Conclusions

As for mentioned results, we conclude the following

1- The beneficial use of Peppermint leaves and fenugreek leaves powder at 1.5gm/kg in broiler chicken diets, as it increases production performance.

2- Improvement in biochemical parameter.

3-Improvenment in immunity against Newcastle Disease and infectious bursal disease vaccine were improved also improve LH ratio as compared with control.

4-Important role of Peppermint leaves and fenugreek leaves to decrease heat stress in adttion to decrease oxidative stress in broiler.

5- It can be an alternative to antibiotic growth promoters and is highly recommended as feed supplement

6.2. Recommendations

From the results of the present study, it can be recommended the following**:**

1- Study the effect of higher or lower level than 1.5 gm/kg of dried Peppermint leaves or fenugreek leaves and their combination on physiological traits, carcass quality and immune system of chicken.

2- Study the apoptotic factor before and after supplementation of Peppermint leaves and fenugreek leaves

3-use Peppermint leaves and fenugreek leaves to improve oxidation stability of broiler meat modulate the cholesterol profile in the serum which can be reflected in the meat and make an advantage for human diets.

4- Study the effect of Peppermint leaves and fenugreek leaves on laying hens diet and their egg production.

5-Use seeds of Peppermint leaves or fenugreek leaves instead of leaves and study there effect.

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الخلاصة

اجريت هذه الدراسة لتقييم تأثير الاضافة الغذائية للنعناع والحلبة وخليطهما على الاداء الانتاجي وبعض الصفات الكيموحيوية للدجاج اللحم المجهد بالحرارة.اجريت التجربة في حقل قطاع خاص لمدة 35 يوم وشملت تربية 120 افراخ لحم (روز 308) غير مجنس بعمر يوم واحد. تم تقسيمها الى اربع مجاميع (30 طير \ مجموعة) بثلاث مكررات (10 طير \ مكرر). المعاملة الاولى (T1) (مجموعة السيطرة) تغذت على العليقة الاساسية بدون اي إضافة . المعاملة الثانية (T2) تم تغذيتها على العليقة الاساسية + اوراق النعناع بمستوى 5. 1غرام / كغم علف . المجموعة الثالثة (T3) تم تغذيتها على العليقة الاساسية + اوراق الحلبه بمستوى 5. 1غرام / كغم علف. علف . المجموعة الرابعة (T4) تم تغذيتها على خليط من اوراق النعناع + اوراق الحلبه بمستوى 5. 1غرام / كغم لكل مكون مع العليقة الاساسية.

تم قياس معايير الأداء الإنتاجي بشكل اسبوعي طوال فترة التجربة , كما جمعت عينات الدم للمعلمات البيوكيميائية والمناعية في اليومين السابع عشر والخامس والثلاثين من التجربة.

أظهرت النتائج وجود تحسن معنوي ($p\leq0.05$) في جميع الصفات الإنتاجية في معاملات الإضافة مقارنة مع مجموعة السيطرة. كان هناك انخفاض معنوي ($p\leq0.05$) في الكوليسترول الكلي والدهون الثلاثية والبروتين الدهني منخفض الكثافة (LDL) بينما زاد البروتين الدهني عالي الكثافة (HDL) بشكل كبير في معاملات الإضافة مقارنة مع معامله السيطرة، لم تسجل بيانات البروتين الكلي الالبومين والكلوبيولين,أي فروق ذات دلالة إحصائية بين المعاملات المختلفة.

تم قياس إنزيمات وظائف الكبد مثل AST,ALTوALPفي نهاية الفترة وأظهرت انخفاضًا معنويًا ($p\leq0.05$) في معاملة الإضافة مقارنةً بالسيطرة كما أظهرت النتائج زيادة معنوية ($P\leq0.05$) مع معاملة الإضافة بالمقارنة مع السيطرة في قيمة CAT و SOD و GPX في اليومين السابع عشر والخامس والثلاثين من التجربة ،كما اظهرت النتائج انخفاضًا معنويًا ($p <0.05$) في مجموعات الاضافة اذا ماتم مقارنتها بمجموعة السيطرة في الخلايا الليمفاوية من دجاج اللحم في اليومين السابع عشر والخامس والثلاثين. كما كان هناك زيادة معنوية ($p\leq0.05$) في معيار الاجسام المضاد لمرض نيوكاسل والكمبوروفي مجموعة الاضافات اذا ماتم مقارنتها بمجموعة السيطرة. في الختام ، يمكن استخدام النعناع والحلبة كا ضافات علفية جيدة لتقليل الاجهاد الحراري في دجاج اللحم وتحسين الصفات الإنتاجية والكيموحيويه

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب البيطري
فرع الصحة العامة

تأثير اوراق النعناع والحلبه المجففة على الاداء الانتاجي لفروج اللحم تحت تأثير الاجهاد الحراري

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء وهي جزء من متطلبات نيل درجة

الماجستير في اختصاص علوم الطب البيطري/الصحة العامة

بواسطة

غدير عباس حسن علوان

بأشراف

ا. م. د. علي رضا عبد

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