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Effect of adding leave powder of fenugreek, alfalfa and their mixture in broiler chicken's diet on some productive and physiological aspect

Thesis

Submitted to the Council of the College of Veterinary Medicine at University of Kerbala as a Partial fulfillment of the Requirement for the Degree of Master in the Sciences of Veterinary Medicine in Veterinary/Public Health

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بسم الله الرحمن الرحيم

﴿ وَفِي ٱلۡأَرۡضِ قِطَعٞ مُّتَجَٰوِرَٰتٞ وَجَنَّٰتٞ مِّنۡ أَعۡنَٰبٖ وَزَرۡعٞ وَنَخِيلٞ صِنۡوَانٞ وَغَيۡرُ صِنۡوَانٖ يُسۡقَىٰ بِمَآءٖ وَٰحِدٖ وَنُفَضِّلُ بَعۡضَهَا عَلَىٰ بَعۡضٖ فِي ٱلۡأُكُلِۚ إِنَّ فِي ذَٰلِكَ لَأٓيَٰتٖ لِّقَوۡمٖ يَعۡقِلُونَ ﴾

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We certify that this thesis **(Productive, Biochemical and Immunologic Aspects that Influenced by Dietary Supplementation of Fenugreek or/and Alfalfa in the Broilers)** has been prepared by ***Fatimah Abbas Majeed*** under my supervision at the college of Veterinary Medicine, University of Kerbala in partial fulfillment of the requirements for the Degree of Master in the Sciences of Veterinary Medicine in Veterinary Public health.

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I hereby declare that this thesis is my origin work except for equations and citations which have been fully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University of Kerbala or other institutions.

Fatimah Abbas Majeed

26/2/2022

Dedication

Imam Mahdi, may God hasten his reappearance Oh hope of the weak on earth.

.... To my beloved mother's soul, I miss you so much

….To my dear father's soul …

….To my husband, Mr.Haider Al-Janabi I am greatly thankful for your tolerance and your kind support to successful complete my research.

….To my beloved sisters ,brothers and my wonderful nephew Haider Al-Nasrawi thankful for your tolerance and your kind support to successful complete my research

....My family …..My husband and My kids,who have been constant source of help and encouragement .

....your presence in my life is a grace….

I owe a lot to you

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Fatimah

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Table of Abbreviations

G1	Group one
G2	Group two
G3	Group three
G4	Group four
BW	Body weight
WG	Weight gain
FI	Feed intake
FCR	Feed conversion ratio
TP	Total protein
TC	Total cholesterol
TG	Triglyceride
HDL	High density lipoprotein

LDL	Low density lipoprotein
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
ND	Newcastle disease
IBD	Infectious bursal disease
ELISA	Enzyme linked immunosorbent assay
FSP	Fenugreek seed powder

Abstract

This study was carried out to evaluate the effect of dietary fenugreek , alfalfa and their mixture on productive and some physiological traits of broiler chickens. The experiment was conducted in private field for 35 days from 22 Jan. to 26 Feb./2022. One-hundered twenty unsexed one day old broiler chicks (Ross308), that were divided into four groups (30chick/group) with three replicates each group (10birds/replicate). The first group (control G1): chicks were fed on a basal diet without any addition.The second group(G2) fed on a basal diet with 2.5 gm fenugreek/Kg diet. The third group(G3) fed on a basal diet with 2.5 gm alfalfa/Kg diet. The fourth group(G4) was fed on a basal diet with 2.5 gm fenugreek + 2.5 gm alfalfa /Kg diet.

The productive parameters icluding body weight, weight gain, feed intake and feed conversion ratio were measured weekly throughout the experimental period. Blood samples were collected for biochemical and immunological parameters at 17 and 35 day of the experiment. Hedonic tasts were conducted on breast and thigh muscles of carcasses at the end of the study.

The result revealed the following:

1.There was a significant improvement ($p \leq 0.05$) in body weight, weight body gain, feed intake and feed conversion ratio in the G4, G3 and G2 as compared with the control.

2.. At 17^{th}day old G2, G4 and G3 showed a significant increase ($P \leq 0.05$) in total protein as compared with the control, albumin also showed a significant increase ($P \leq 0.05$) at G4 and G2 as compared with the control while, G3 had not significant difference as compared with control, globulin showed a significant increase ($P \leq 0.05$) in G4, G2 and G3 as compared with control. At 35^{th} day old ,there were a significant increase ($P \leq 0.05$) in total protein in G3, G4 and G2 as compared with the control. Albumin showed a significant increase ($P \leq 0.05$) in treated groups as compared with the control, globulin had a significant increase ($P \leq 0.05$) in G3,G4 and G2 as compared with the control too.

3. There were a significant decreases ($p \leq 0.05$) in the total cholesterol, triglycerides and low density lipoprotein (LDL) while, high density lipoprotein (HDL) increased significantly in all treated groups compared with control.

4.Liver function enzymes such as aspartate transferase and alanine tranferase were measured at the end of the period and showed a significant decrease ($p \leq 0.05$) in the addition groups compared with control .

5.There were a significant increase ($p \leq 0.05$) in antibody titer against Newcastle disease and Infectious bursal viruses at 35^{th}day old in G4, then G3 and G2 as compared with the control .

6. As a sensory test, the results showed that flavor, palatability, juiciness, tenderness and color in the breast and thigh muscles were improved in addition groups as compared with control, except that moderate juiciness and tenderness in fenugreek group.

According to the current results, fenugreek and alfalfa can be used as a good natural addition in broiler diets, and the results also showed that using of alfalfa alone is better than fenugreek alone, but using of mixture with fenugreek gave better results in improving the productive and healthy condition of broiler chickens.

Chapter One: Introduction

Introduction

Poultry industry is one of the most dynamic and growing sectors in the world especially in developing countries. The global poultry sector is continuously growing since the demand for meat is continuously increasing by the growing populations. Broilers efficiently convert feed into body mass, as a consequence of intensive selection and low input required to produce high-quality meat protein. For breeders it is crucial to maintain a high feed efficiency, being also a major challenge, since poultry production is frequently affected by infectious diseases (Diaz Carrasco *et al*., 2019). Thus,the challenge is to produce poultry with high productivity.

Phytogenics are a group of natural growth promoters derived from plants, seeds, or herbs that have biologically active compounds, and posses several biological functions. In comparison with antibiotics, phytogenics are residue free, safe and less toxic ,it is thought to be ideal growth promoters in poultry production and gained much interest in poultry production as alternative to antibiotics to stimulate the growth by increasing the efficiency of feed utilization and to enhance the immunity and the public health status (Saleh *et al*., 2018).

Fenugreek (*Trigonella foenum graecum*), is one of the spices having multi-functional characteristics such as antimicrobial, hypoglycemic, antiinflammatory, hypolipidemic, hypocholestoremic, antipyretic, and antioxidative properties in animals, It contains several vital compounds, including flavonoids, alkaloids, saponins, vitamins, minerals, carbohydrates, and proteins (Srinivasan, 2006). Fenugreek is also a good source of dietary protein for consumption by human and animals, and it characterized by its content of fatty acids, which are predominantly linoleic, linolenic, oleic and palmitic acids (Schryver, 2002). In a study conducted by Yassin *et al*.(2020) it was suggested that fenugreek stimulates appetite and predicted feed intake which in turn might lead to improved body weight and over all performance.

Alfalfa (*Medicago sativa L.*) is one of the cheapest sources of protein, which described by high yields and low production costs (Radović *et al*., 2009). It is a feedstuff with high fiber and low metabolizable energy (Donalson *et al*., 2005).it contains many active components such as flavonoids, β-carotene, tocopherol (Ouyang *et al*., 2016). Due to the high content of saponins (2–3% of dry matter), alfalfa meal has hypocholesterolaemic, anticarcinogenic, anti-inflammatory, and antioxidant activities (Englmaierova *et al*., 2019).It is also a natural source of xanthophylls,

which when deposited in the skin and shanks give poultry carcasses a desirable yellow color (Dansky, 1971). in a recent study by Guiwen *et al*.(2021) it was revealed that the different levels of alfalfa inclusion in broiler diet effects on feed intake to consume more feed which led to significant higher body weight.

The aim of the study was to:

Evaluate the effect of fenugreek or/and alfalfa on the productive performance **,** biochemical and immunological aspects of broiler chickens Ross 308.

Chapter Two: Review of The Related Literature

2.1.Fenugreek (*Trigonella foenum graecum*)

Is an annual plant belongs to the family *Leguminosae* and it is well known as famous spices in human food. The seeds and green leaves of fenugreek are used in food as well as in medicinal application.Fenugreek has been used to increase the flavor and color, and modifies the texture of food materials.It has many medicinal properties such as antibacterial, antidiabetic, hepato-protective effects with hypocholesterolemic and anticancer influences with improve lactation in women, , relieves anorexia and has a positive influence on digestion. The antidiabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the intrinsic dietary fiber constituent which have a promising nutraceutical value (Srinivasan, 2006).

Scientific classification of fenugreek plant

Kingdom /*Plantae*

Division */ Magnoliophyta*

Class / *Magnoliopsida*

Order /*Fabales*

Family / *Fabaceae*

Genus / *Trigonella*

Species /*T. foenum graecum*

(Verma *et al*.,2013)

Bioactive compounds like saponins, flavonoids, alkaloids, and steroids are the major classes of compounds in fenugreek .The chemical analysis of fenugreek plant are trigonelline which constitutes to (91.20 mg/gm) , 4-hydroxyisoleucine (90.10 mg/gm) , pinitol (45.80 mg/gm) , isovitexin (3.18 mg/gm), sarsasapogenin (1.35 mg/gm) and isoorientin (1.12 mg/gm) (Singh *et al*., 2020).

2.1.1.Chemical composition of fenugreek

Fenugreek is used in foods as a herb (the leaves) and as a spice (the seed) to improve flavor, pungency and color. The main bioactive compounds of fenugreek are saponins, flavonoids and polysaccharides that fixed oils and some identified alkaloids viz. trigonelline and choline (Yoshikawa *et al*., 1997).

Fenugreek leaves contain 86.1% moisture, 6% carbohydrates, 4.4% protein, 1.5% minerals and 1.1% fiber. The mineral and vitamins in fenugreek leaves embrace calcium, phosphorous, iron, zinc, thiamine, riboflavin, niacin, carotene , and vitamin C.The saponins of fenugreek leaves are glycosides of diosgenin (Rao, 2003).

Yadav and Sehgal (1997) found that fresh leaves of fenugreek contain vitamin C about 220.97 mg per 100 gm and b-carotene is present about 19 mg/100 gm. On the other hand, about 84.94% and 83.79% of ascorbic acid were definitely reduced in sun-dried and oven-dried fenugreek leaves, respectively.

Another notice that fenugreek seed contains approximately 4-10% moisture, 6-8 fat, 18-30% protein and 48-55% fibers depending on ecological factors. In additions, it was reported that mature seeds (100g)contained fiber (50gm), protein (30gm), fat (7.5gm) , sapogenins, diosgenins, , gitogenin, yamogenin, neogitogenin, yuccagenin, sarsasapogenin, tigogenin and smilagenin (2gm), trigonelline (380 mg) , Ca (160) mg, Mg (160mg), P (370mg), Na (19mg) ,Fe (14mg), K (530mg), Cu (33mg), CI (165mg), S (16mg), Mn (1.5mg) , Zn (7.0mg) , Cr (0.1mg) , choline (50mg), B-carotene (90mg) , vitamin C (50mg), thiamine (340mg) , riboflavin (290 mg) , nictoinic acid (1.1mg), folic acid (84mg) (srinivasan , 2006). The study of Yadav *et al*.(2011) cleared that were many constituents present in fenugreek as indicated in table(2-1):

Table (2-1) Chemical composition of fenugreek

Substance	Chemical constituents of fenugreek
Alkaloids	Trimethylamine, Neurin, Trigonelline, Choline,
	Gentianine, Carpaine and Betain
Amino acids	Isoleucine, 4-Hydroxyisoleucine, Histidine,
	Leucine, lysine, L-tryptophan, Arginine
Saponins	Graecunins, fenugrin B, fenugreekine,
	trigofoenosides A–G
Steroidal	Yamogenin, diosgenin, smilagenin
Sapinogens	sarsasapogenin, tigogenin, neotigogenin,
	gitogenin, neogitogenin, yuccagenin, saponaretin
Flavonoids	Quercetin, rutin, vitexin, isovitexin
Fibers	Gum, neutral detergent fiber
Lipids	Triacylglycerols, diacylglycerols,
	monoacylglycerols, phosphatidylcholine
	phosphatidylethanolamine, phosphatidylinositol,
	free fatty acids.
Other	Coumarin, lipids, vitamins, minerals. 28%
	mucilage; 22% proteins; 5% of a strongerswelling, bitter fixed oil

(Yadav *et al*.,2011) ; (Sowmya and Rajyalakshmi ،1999)

2.1.2.Health benefits of fenugreek

Fenugreek remained a key ingredient in a 19th century medicine for dysmenorrheal and postmenopausal symptoms. Fenugreek also is one of the oldest medicinal plant dating back to Hippocrates and ancient Egyptian times (Jensen,1992), where it has been used for over two thousand years as a medicinal plant in various parts of the world (Srinivasan, 2006).It was found

that there was a better retention of nutrients in the leaves of fenugreek and thus was used in the folk medicines for the treatment of cellulitis, boils, and tuberculosis (Madar and Stark, 2002**)** .

Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicine (Basch *et al*., 2003). It may considered as the oldest medicinal plant, using of fenugreek is associated with a wide range of therapeutic applications including carminative (prevents flatulence) and aphrodisiac (Chopra *et al*., 1982). Fenugreek seeds are regarded as an appetizer and helps indigestion also it has antioxidant, antiviral and anti-carcinogenic activities (Mazur *et al*.,1998).In traditional medicine, fenugreek is used to aid digestion, treat gastrointestinal inflammation and promote milk production in lactating women (Petruzzello, 2016).

Fenugreek also maintains mucus conditions of the body, typically the lungs, by assisting to clear congestion and it acts as a throat cleanser and mucus solvent that also eases the cough. Fenugreek has been used to relieve colds, bronchial complaints, influenza, asthma, catarrh, constipation, sinusitis, pleurisy, pneumonia, sore throat, laryngitis, hay fever tuberculosis and emphysema (Home remedies guide).

Michael and Kumawat (2003) thought supposed therapeutic effects of fenugreek belong to its contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical components such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are play important role in this action.

2.1.3. Bioactivities of fenugreek

2.1.3.1. Antioxidant activity

Antioxidants already in low concentrations significantly delay or prevent oxygenation of oxidizable constituents (Halliwell and Gutteridge, 2001). Fatty acids that deposited in animal tissues are derived from various sources such as endogenous synthesis or directly from the feed, and from microbial synthesis or modification in the digestive tract. In non-ruminants such as pigs and poultry the dietary fatty acids more directly influence the body fat composition, making nutrition an effective tool to manipulate animal lipid composition (Scheeder, 2006).

Bhatia *et al*. (2006) reported protective effect of fenugreek, on lipid peroxidation and on enzymatic antioxidants.Bukhari *et al*.(2008) reported that fenugreek seed extract with methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate has a radical scavenging activity.

The chemical composition of fenugreek represented by seeds, husk and cotyledons, showed that endosperm had the highest (4.63 gm/100 gm) saponin and (43.8 gm/100 gm) protein content, where as husk contains higher total polyphenols. The extracts of endosperm, husk, and fenugreek seed at about 200 µg exhibited antioxidant activity 72% ,64%, and 56%, respectively by free-radical scavenging method (Naidu *et al.*, 2010). From that study it was indicated that separating fenugreek seeds into husk and endosperm can achieve practical efficiency for effective separation to biologically active components in terms of selective fractionation.

In another study, it was observed that *Aegle marmelos* has the highest phenolic content followed by fenugreek and *Coriander sativum* and the flavonoids contents are higher in fenugreek. Antioxidant property was checked by reducing power, a quantitative nitroblue tetrazolium (NBT) assay and hydrogen peroxide(H_2O_2) scavenging . Fenugreek showed the highest superoxide and free radical scavenging (Joglekar *et al*., 2012).

Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical. Because of the high reactivity of the hydroxyl group of the flavonoids, radicals are made inactive, according to the following equation):

Flavonoid(OH) + R· > flavonoid(O·) + RH

Where R· is a free radical and O· is an oxygen free radical (Hanasaki *et al*.,1994).

2.1.3.2. Antimicrobial activity of fenugreek

Microorganisms are the hidden enemies to the mankind. They are small but cause a very deep damage in the human body. In addition, to other living organisms. The agents, which have the ability to kill the microbes or stop their multiplication are named the antimicrobial agents or drugs. Some of these antimicrobial drugs are discovered and some are hidden in the nature (Priya,2012).

Antibacterial and antifungal role of fenugreek is recently being shown. The effectiveness of extracts obtained from fenugreek against *Helicobacter pylori* has been reported by several studies (O'Mahony *et al*., 2005; Randhir and Shetty, 2007). In a study of honey samples collected from different plant sources, including fenugreek, to find out the antibacterial activity of these plants, pollen based on fenugreek showed the highest antibacterial activity against *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli* compared to other plants (Mercan *et al*., 2007). Along with Qureshi *et al*.(2015) who investigated the *in vitro* antibacterial activity of fenugreek and reported the 2.1 mm of inhibition zone for the concentration of 0.05 mg/ml of extract against *E. coli* on the Mueller Hinton agar. Similarly, another *invitro* antibacterial activity of methanolic extract of fenugreek against *E. coli* has been noted by (Dash *et al*., 2011) .These antimicrobial activities of fenugreek may be due to the alkaloid ,flavonoids, saponins and phenols present in it (Schryver, 2002) which may inhibit enzymes necessary for amino acids biosynthesis or interact with enzymes and proteins of the microbial cell membrane causing inactivation of protons in the direction outside the cell led to cell death (Gill and Holley, 2006). Verma *et al*. (2010) concluded that fenugreek seed oil and aqueous extract have a strong antibacterial activity against *Salmonella typhi, Escherichia coli,* and *Staphylococcus aureus.*

Haouala *et al*. (2008) showed in a study ,that an aqueous extracts from different plant parts of fenugreek in different solvents which include petroleum ether, methanol and ethyl acetate fractions of the aerial parts had their action against fungal strains such as *Botrytis cinerea, Fusarium graminearum* , *Alternaria sp* and *Rhizoctonia solani* . Furthermore, It was revealed that all parts of the fenugreek plant showed antifungal potential and the degree of effect varies with plant parts and species of fungus therefore it could be suggested that fenugreek is an important source of biologically active compounds beneficial for developing better and new antifungal drugs. Additionally, the methanol soluble fraction of fenugreek extract showed nematicidal activity and caused significant mortality of *Meloidogyne javanica* larvae, pointing to the potential use of fenugreek against nematodes (Zia *et al.,* 2001).

2.1.3.3. Anti-inflammatory activity of fenugreek

Fenugreek stimulates the body to produce mucus which allows the removal of allergens from the respiratory system and toxins from the urinary tract (Hossain *et al.,* 2018**;** Wani and Kumar,2018) .

Many studies have shown that fenugreek reduced pro-inflammatory cytokines, including tumor necrosis factor alfa (TNF-α), interleukin-one beta (IL-1β), and Interleukin-six(IL-6)which released in response to induced inflammation (Zhou *et al*., 2020; Nagamma *et al*., 2021). Furthermore, a clinical trial found that 15 gm/day supplementations of fenugreek seed powder decreased C-reactive protein(CRP) which is a general measure of inflammation levels in type 2 diabetes patients However, there was no effect on IL-6 and TNF-α (Tavakoly *et al.*, 2018). Anti-inflammatory cytokines were found to be increased with fenugreek supplementation, specifically interleukin- ten (IL-10), interferon type II (IFN-γ), and transforming growth factor beta TGF-β (Piao *et al*., 2017; Liu, 2019). Fenugreek has been found to inhibit c-Jun N-terminal kinases and nuclear factor kappa-light-chain-enhancer of activated B cells(NF-κB) activation (Zhou *et al*., 2020) as well as cyclooxygenase enzymes (COX-1) and (COX2) (El-Taib *et al*., 2020). Moreover , fenugreek has been found to be effective for reducing *Mycobacterium tuberculosis*-induced paw edema (Cheurfa *et al.*, 2021).

For these reasons, fenugreek has shown promising results in ameliorating inflammation. It should be noted that the clinical trial found no change in two major pro-inflammatory cytokines, inconsistent with many animal studies. Thus more research should be conducted to confirm the effects of fenugreek on pro-inflammatory cytokines (Garnier and Shahidi, 2021).

2.1.3.4. Hypocholesterolemic activity of fenugreek

Abnormal deficiency of cholesterol level in the blood is known as hypocholesterolemic problem. Fenugreek seeds contain the largest amount of fiber galactose and mannose ,the latter compounds are associated with reduced cholesterolemia (Robert, 2011). Fenugreek may lower lipid level because it contains saponins which are converted to sapogenins in the gastrointestinal tract (Basch *et al.*, 2003) ,in experimental rats were administrated with fenugreek extract, and Metformin HCl for six weeks , rats treated with fenugreek had lower triglycerides, total cholesterol, and higher HDL in a dose-dependent manner (Xue *et al*., 2007).

A study of Petit *et al.*(1995) showed that reduction in the serum cholesterol level might be due to the presence of saponins and resins in fenugreek such as hemicelluloses, mucilage, tannin and pectin which inhibit bile acid and cholesterol absorption from intestine, thereby, decreasing cholesterol level in blood ,and hence have a potential role in prevention of arteriosclerosis

(Mullaicharam *et al*., 2013) .Also, Sharma (1986) demonstrated that fenugreek administration increased excretion of bile acid in feces, thus depleting the cholesterol stores in the body of experimental rats, in the same trend Awal *et al.*(1999) studied the effect of fenugreek and Karela on lipid profile in hypercholesterolemia diabetic patients and showed that fenugreek significantly reduced the lipid levels .

Mamoun *et al.*(2014) reported that feeding commercial broiler chicks on diet containing fenugreek seed powder lowered total plasma lipids and cholesterol levels.Untill recently, there are several researchers reported that supplementation of poultry diets with fenugreek seed powder reduced plasma total lipids and total cholesterol in broiler chicks (Faeste *et al.,* 2009; Abbas, 2010). While , Weerasingha and Atapattu (2013) suggested that is none of the serum parameters including serum cholesterol was significantly reduced due to fenugreek supplementation in broiler chicks diet.Contrary Basch *et al*.(2003) suggested the anti-hyperlipidemic properties of fenugreek seed powder given orally.

2.1.4.Effect of fenugreek in productive performance in poultry

Scientists recently observed that the stimulating and appetizing activity of herbs and plant extracts on poultry digestive and immune system could be a dvantagous for health and performance of farm poultry . In many studies, the different active components of herbal plants including fenugreek reported the different influences on poultry performance. They are identified to possess desirable characteristics like thermos-tolerance, resistance to diseases, better egg production, and hard eggshell, high fertility, high hatchability rates, and improved meat flavor .

Gaikwad *et al*.(2019) noticed that 0.5% ,1%and 1.5% of fenugreek seed powder (FSP) led to highest feed intak ratio and increased cumulative weight over control in broiler chickens .Also, Yassin *et al*.(2020) indicated that supplementation of 1% ,2% and3% fenugreek seed powder in broiler diets improved feed consumption which could be due to the improvement of palatability of the feed containing fenugreek also he reported that the average daily gain for 3% and 2% was higher than those fed 0% diets while 1% had an intermediate value during the growing phase. Besides,based upon findings of Qureshi *et al*.(2015) it was noted that the cumulative feed consumption was improved in supplemented groups with 1% of fenugreek seeds also showed that FCR was significantly enhanced in the birds fed diets containing fenugreek seeds.

Bhale (2015) also noticed that inclusion 1% germinated FSP in broiler ration improved feed intake and that resulted into better weight. Moreover, Hind *et al*.(2013) reported an increased daily gain and feed intake due to the stimulating effect of FSP on the digestive system of broilers.While a study of Al-Habori and Roman (2002) attributed that to fenugreek content of neurine, biotin and trimethylamine which tended to stimulate appetite through their action on the nervous system. Abo El- Nor (1999) recommended that the inclusion of fenugreek seed in the basal diet might have an effect on hypothalamus gland to stimulate hunger center in the brain and increase appetite.

Ali *et al*. (2021) showed that 0.5%, 1% and 1.5% fenugreek seed supplementation of broiler diets resulted in increase in live body weight. The broiler of 1.5 and 1% fenugreek supplemental group was significantly higher in average of weekly live weight gain. During the finisher and the entire period, the highest average daily gain was for groups fed 3% FSP while the lowest was for control one. Rahimian *et al*.(2018) showed that supplementation of various levels of fenugreek seed powder improved significantly body weight and feed conversion ratio 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.Another study implemented by Abid and Fateh(2014) noticed that beneficial use of fenugreek at 1% in broiler chicken diets to increase body weight, and it had improvement on feed conversion ratio compared with control group , as well as it can be used as growth promoters and a dietary natural supplement.

Mamoun *et al*.(2014) reported that 1% and 1.5% level of dietary fenugreek inclusion was useful for improving live body weight, body weight gain, feed conversion ratio, protein efficiency ratio, feed consumption and efficiency of energy utilization. Alloui *et al*.(2012) reported that the palatability of feedstuffs containing fenugreek seeds in diet were improved because of the presence of high levels of the carbohydrate fraction(galactomannan) which led to increased average daily gain and significantly affect on FCR during the age of 42 days due to the development of the broiler chicks' gut and advanced morphological changes in gastrointestinal tissues that can be induced by alterations in gut load of microbial content including their metabolites.

Another study found that fenugreek seed powder addition at various levels in broiler diets enhanced the productive performanc including body weight and feed conversion ratio of

broiler chickens and that was probably due to the improvement of palatability of FSP containing feed (Yassin *et al*., 2020). Hamden *et al*.(2010) reported that addition of FSP improved feed conversion efficiency of broiler chicks. Moreover, Weerasingha and Atapattu(2013) indicated that the birds fed 1% fenugreek recorded the best FCR, which was achieved by 13.8% enhancement in FCR value in comparison to control group.

Those results were contradictory to Awadein *et al*.(2010) who reported that fenugreek seeds had no significant effect on body weight .Also Weerasingha and Atapattu (2013) reported that the birds fed with 5% fenugreek seed powder consumed less amount of feed .In additions, in a study performed by Duru *et al*.(2013) revealed that feeding of broiler chicks on diets containing 5, 10, 20 and 40gm fenugreek seed powder per kg of commercial broiler diet has decreased body weight and breast weight compared with untreated one with decreased feed intake with 5gm fenugreek seed, while 40gm fenugreek treatment has decreased feed efficiency.

Wahab *et al*.(2019) showed that fenugreek seeds supplementation at the level of 0.5% decreased feed intake and improved egg production of spent layers by 10%. In fact, the highest percentage of egg production in group of fenugreek seeds (0.5%) perhaps due to phytoestrogen which may have a stimulatory effect on the egg production while the lower egg production in high levels fenugreek supplemented groups may be belong to reduced feed intake in these groups(Awadein *et al*., 2010).

2.1.5.Effect of fenugreek in blood biochemistry of poultry

Evaluation of blood biochemistry in birds is crucial for the identification of metabolic alterations due to many endo- and exogenous factors including the genetic type, husbandry conditions, sex, season and age (Rajman *et al*., 2006). Furthermore, the biochemical blood parameters offer valuable information on the health state and are often helpful in revealing health disorders already in the preclinical stage.

Fenugreek has a beneficial effect on cleansing the blood and as a diaphoretic.It is able to bring on a sweat and to help in detoxification the body (Home remedies guide). Fenugreek has properties of reducing blood sugar level (Raghuram *et al*.,1994) and incorporation of dietary fenugreek seeds in broilers at the 1 % level has significantly decreased the blood cholesterol

(Safaei *et al*., 2013) and reduction in blood cholesterol levels by supplementation of fenugreek seeds at 40 gm/kg in the diet of broiler chicken has also been confirmed by Duru *et al*. (2013) .

Abdouli *et al*.(2014) stated that ground fenugreek seeds given to laying hens at 6 gm/hen/day resulted in reduction of serum cholesterol level and the reduction in the serum cholesterol level might be due to the presence of saponins and resins in fenugreek which inhibit bile acid and cholesterol absorption from intestine. Thereby, decreasing cholesterol level in blood was found by fenugreek and hence had a potential role in the prevention of arteriosclerosis (Petit *et al*., 1995).

Total plasma proteins are common parameter investigated to estimate the avian body condition. It is generally known that blood plasma proteins play key roles in the maintenance of colloid osmotic pressure, rapid substitute for indispensable amino acids, assuring glucose through gluconeogenesis, transport of minerals and hormones, enzyme and supporting immune system in the organism. Therefore, blood plasma proteins have an exceptional significance in homeostasis maintenance. Moreover, albumin, one of the main serum proteins, serves as the most favorable source of amino acids for the synthesis of tissue proteins in the period of quick somatic growth of birds, especially under feed restricted conditions (Yaman *et al*., 2000; Filipovic *et al*., 2007). It is known that the change in albumin levels reflects the change in the liver function, since the liver is the site of albumin synthesis, but globulin is formed by lymphatic tissues (Jones and Bark, 1979) . The addition of fenugreek seeds to chick diet increased serum content of total protein and albumin. In this respect, Azoua (2001) found that total protein and globulin of serum increased significantly by feeding Hubbard broiler chicks on diets supplemented with fenugreek seeds. The increment in total serum proteins may be attributed mainly to the fenugreek seeds which may stimulate the thyroid gland directly for groups fed on fenugreek diet (Hassan, 2000) .

AL-Habori *et al*.(1998) cleared that fenugreek and its extracts reduced the levels of cholesterol, triglycerides and LDL-cholesterol with no effect on HDL-cholesterol. This selective reduction in LDL-cholesterol resulted in the improvement of HDL-cholesterol to LDL-cholesterol ratio.

Reduction of serum cholesterol is usually accompanied with its decrease in a tissue or meat, this conclusion is very valuable for broiler meat consumers, particularly those with atherosclerosis. The improvement of the plasma lipid profile by fenugreek treatment is further supports the use of fenugreek seeds as a hypolipidaemic agent through improvement of lipid disorders (Khadr and Abdel Fattah, 2007).

2.1.6.Effect of fenugreek in liver function enzymes

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes are found inside hepatocytes and are released when the liver is damaged. In hepatocyte injury,during damage to the membranes of cells and their organelles allows intracellular enzymes to leak into the blood, where the elevated concentrations can be measured(Simon,2019).

Despite being widely used, fenugreek has not been implicated in cases of clinically apparent liver injury and, in prospective studies, had no effect on serum enzyme levels. *Invitro* studies have demonstrated hepatoprotective activity of fenugreek extracts in several animal models. Because of the high fiber content, estrogenic and coumadin-like effects of fenugreek, it has a potential to cause herb-drug interactions particularly if taken in high doses with antiplatelet drugs and warfarin(Zimmerman,1999).

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 and ALT levels , in all supplementary groups compared to the control group that might be to presence of vitamins A and B1 component of seeds which were effective in liver function and could decrease ALT and AST enzyme levels.

2.1.7. Effect of fenugreek in immunity

The immune system is a network of cells, tissues, and organs that work together to inhibit bodily infections and diseases**,** The main parts of immune system consist of the immunocyte (circulating cells) and lymphoid organs and vessels both central and peripheral. Some supplements and remedies boast the ability to enhance the immune system, while some supplements alter components related to immune system function, there is debate on if they are effective in protecting the consumer from infection and disease. Nevertheless, the immune system may benefit from supplemental consumption that affects components of the immune

system. Spices and herbs have grown in popularity as immune enhancers. Immune-enhancing properties of fenugreek supplementation have varying effects on the immune system, however, results are inconsistent and less impressive than other plants. The IgM levels were increased in Nile tilapia (*Oreochromis niloticus*) (Moustafa *et al*., 2020) and juvenile blunts snout bream (*Megalobrama amblycephala*) (Yu *et al*. 2019) supplemented with fenugreek at 0.3 and 0.16% in diets, respectively.

Seleem *et al*.(2008) recorded that supplementation of 0-3% fenugreek to rabbit diet revealed a great role in improving the immune system .

Abid *et al*. (2011) demonstrated that the fenugreek increased the immunity of birds at 24 and 34 days because of fenugreek mechanism to increase the cellularties of thymus gland and bone marrow. Awad *et al*. (2015) results suggested that the fenugreek seed, especially the highest dosage used could be considered a good feed supplement to improve the immune status and increase the production of gilthead sea bream. Abid and Fateh(2014) noticed that diet supplemented with 1% fenugreek exerted to produce high anti-body titer against Newcastle disease virus and infectious bursal disease virus in broiler chickens.

The presence of immunoglobulins especially IgA and IgG in chicken sera enhances the immunity of the birds. According to the research reports, FSE can promote Ig secretion in animals, 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 of fenugreek (Huang *et al.,* 2022). Additionaly , the results of Safaei *et al*.(2013) showed that the use of fenugreek extract in drinking water of broiler chickens had a significant effect on the immune system response compared to the control group.

Conversely, fenugreek supplemented in diet of gilthead seabream (*Sparus aurata L.)* at 1, 5, and 10% had no change in IgM levels (Guardiola *et al*., 2018)

2.1.8. Effect of fenugreek in carcass traits

Poultry meat is suitable for the production of so-named functional foods for human consumption, which is currently at the heart of agricultural and food research (FAO, 2002; Gueye, 2009). Sensory evaluation is analysis of product attributes perceptible by the

human senses of smell, taste, touch, sight, and tenderness. Volunteers (consumers or users of the product) are used to assess the sensory characteristics and providing a response. There are two general types of sensory methods. Laboratory/analytical methods which depend on using a small number of panelists to determine if a difference exists between samples and the nature, direction, and intensity of the difference. Consumer affective methods involve a larger number of panelists and include tests that measure how consumers feel or react to the product to provide a measure of preference and acceptance .Color, appearance, and texture are important factors that consumers will consider before making a decision to buy poultry product (Liu *et al*., 2004).

The study of Abdalla *et al*.(2018) recommended using 1% fenugreek seed powder in the feed to improve the quality of chicken meat (color, flavor, juiciness and tenderness).

The meat was white and beige in the fenugreek group which was moderately tender with low juiciness as compared with the control group . It was also spicier as compared to the control group(Belaid-Gater *et al*., 2021).

2.2. Alfalfa *(Medicago sativa L.)*

Alfalfa which called also lucerne, is one of the earliest cultivated plants and most common perennial legume forage (Tufarelli *et al*., 2018; Zheng *et al*., 2018), The name alfalfa cames from the Arabian al-fac-facah for "father of all foods" .Alfalfa meal is a commercially available feed stuff, as it is moderately rich in protein, well balanced in amino acids and a rich source of minerals as well as vitamins (Jiang *et al*., 2012).It provides an excellent protien-rich food source for cattle, horses, sheep and other animals species .

Alfalfa has been reported as high quality forage with high protein percentage and energy generally higher than pasture, particularly when pasture is not freshly growing. Correct management of alfalfa will provide top quality herbage (Tremblay *et al.*, 2001).

Alfalfa has great potential as a feedstock for the production of fuel, forage and industrial materials. Despite this, the refining of alfalfa remains undeveloped. Unlike other major field crops such as corn and soybeans, which are usually refined to produce fuels and industrial materials. Instead, alfalfa is primarily processed and used on-farm in the form of dried hay,

silage, and fresh forage known as “green chop” or are grazed by animals in pastures (Samac *et al*.,2006).

In many countries, including the United States, alfalfa is used as a basic component in feeding programs for dairy cattle and is an important feed for beef cattle, horses, sheep, and other livestock. Alfalfa is known as the “Queen of the Forages” which provides highly nutritious forage in terms of protein, fiber, vitamins, and minerals for ruminant animals. If alfalfa is developed to its full potential as a feedstock for biorefining, a major shift may occur in the manner in which alfalfa is produced and used for feeding farm animals (Samac *et al*.,2006) .

2.2.1. Chemical composition of alfalfa

Alfalfa is one of the best sources for protein(17.5%) and crude fiber (24.1%) while exhibiting a relatively low metabolizable energy (1,200 kcal/kg) , it is a rich source of minerals as well as vitamins (NRC, 1994).Recent studies on alfalfa revealed that its fiber concentrations could be reduced, and its valuable ingredients and protein heightened, depending on the cultivation, harvesting and processing methods (Sommer and Sundrum, 2014)

Alfalfa is a good source of some amino acids such as arginine, lysine, tryptophan and a reasonably good source of methionine with the glycine level remaining questionable, other valuable amino acids are also present (Conney *et al*, 1948).

Main vitamins of alfalfa are A, D, E, K, C, thiamin , riboflavin, pyridoxine B6, cobalamin B12, niacin, panthothanic acid, biotin, and folic acid, alfalfa also contain the following minerals: phosphorus, calcium, potassium, sodium, chlorine, sulfur, magnesium, copper, manganese, iron, cobalt, boron, and molybdenum. Functional components such as polysaccharides, flavonoids, xanthophylls, β-carotene, tocopherol also contained in alfalfa (Ouyang *et al.*, 2016) .

Due to the high content of saponins (2–3% of dry matter) of alfalfa, it has many bioactivities such as hypocholesterolaemic, anticarcinogenic, anti-inflammatory, and antioxidant activities (Englmaierova *et al*., 2019).

Alfalfa is reported to contain citric, malic, oxalic and malonic acids, which are present in minor quantities.Enzymes reported in alfalfa are amylase, emulsion , coagulase, peroxidase, erepsin, lipase, invertase, and pectinase. Tannin concentrations of alfalfa between 2.7 and 2.8%

have been reported (Waldrourp, 1997).The study of Al-shami *et al*. (2011)cleared that were many constituents present in alfalfa as indicated in table (2-2):

Table(2-2)Chemical composition of dried alfalfa leaves

Item	Percent
Dry matter	96.1
Crude protein	22.75
Ether extract	1.14
Crude fiber	13.26
Ash	5.81
Nitrogen free extract	53.14
Calculated metabolizable Energy (kcal/kg)	2300

2.2.2. Medical uses of alfalfa

In addition to being used of alfalfa as feed, it has a long history of use as a medicinal herb for humans .Its seeds or dried leaves can be taken as a supplement, or the seeds can be sprouted and eaten in the form of alfalfa sprouts. Extracts of alfalfa produce antibacterial activity against gram positive bacteria. Powdered alfalfa is used as a diluent to adjust the strength of digitalis powder, and the root has been used as an adulterant of Belladonna root. Seeds yield (8.5-11%) of a drying oil suitable for making paints and varnish. Seed screening is ground and used to a limited extent in feeds for ruminants. The seeds also contain a yellow dye (Waldrourp, 1997).

An aqueous extract of alfalfa in turn has an antibacterial and antifungal effect and stimulates the immune system (Aziz *et al*., 2006).Additionally, alfalfa proteins have great functional properties and thus many applications in the pharmaceutical sector for example, their antioxidant properties have been reported to be helpful in diabetes by acting on damaged cells (Sadeghi *et al.,* 2016), atherosclerosis cardiac disorders, hypercholesterolemia (Reilly, 1989) and a variety of central nervous system disorders such as anxiety (Bora & Sharma, 2011).

Coumestrol has long been known as the phytoestrogenic compound in alfalfa, the practical importance of the phytoestrogens lies in their ability to alter the biological response to endogenous estrogen. Estradiol receptors will bind to the adverse group of chemical compounds, including other steroids, isoflavons and phytoestrogens. When phytoestrogens bind to estrogen receptors on cells,they translocate to the nucleus and stimulate cell growth in a manner similar to

estrol. Despite the apparent weak relative binding capacity of phytoestrogens, they can have significant hormonal effects. This is due to their lower affinity for the serum estrogen binding proteins, this resulting in a net effect of enhancing the concentration of available phytoestrogen at the target tissue sites.

In condition of hypoestrogenism , phytoestrogens will bind directly to estrogen receptors and provide a mild estrogenic effect. This is enhanced by the tendency of phytoestrogens to concentrate in the reproductive tissue, in preference to serum proteins. This implies a useful role for the phytoestrogens as adjuncts in the treatment of hypoestrogenic conditions, including hot flashes, menopasusal vaginal atrophy and treatment or prevention of osteoporosis, but In conditions of hyperestrogenism, the relatively weak-acting phytoestrogens will compete for binding sites, thus reducing the number of receptors available to stronger estrogenous estrogens and reducing the net estrogenic stimulation. This is most useful in estrogen excess conditions such as pre-menstrual syndrome, fibrocyticbreast, uterine leiyomyomas, and estrogen-responsive cancer of the breast and uterus (Martin,1978). Bickoff *et al*.(1964) reported that alfalfa extract has been found to contain several other biologically active compounds like tricin, which has the ability to relax smooth muscle, also has weak estrogenic activity.

2.2.3. Antioxidant activity of alfalfa

Oxidation is an essential biological process central to the functioning of living organisms for energy production. However, it also leads to the production of reactive oxygen species (ROS), which may result in lipid oxidation, protein damage, and even cell death (Apel and Hirt,2004). Antioxidant materials plays an important role in delaying, preventing, and removing peroxide products and has been used to lessen the impact on oxidative damage (Ruhe and McDonald,2001)**.** Lipid oxidation is a major cause of quality deterioration of processed and unprocessed foods. Secondary oxidation products include aldehydes, ketones, hydrocarbons, and alcohols, among others. Secondary products of oxidation are generally odor-active, whereas primary oxidation products are colorless and flavorless (Akoh and Min, 2002).

Investigations of antioxidant substances of plant origin indicate that they exert antioxidant effects through free radical scavenging, reduce the levels of LDL and DNA susceptibility to

oxidative stress, and increase the activity and expression of antioxidant enzymes (Erba *et al.*, 2012).

Alfalfa flavonoids have been usually used as an additive added to animal feed to promote the antioxidant activity in serum , liver and meat quality. Alfalfa contains significant amounts of bioactive substances that exhibit antioxidant properties. Antioxidant is targeted against oxidation. The role of antioxidants is to protect lipids against radical peroxidation (Lauro, 1991).

Aziz *et al*. (2006) showed that flavonoids, β-carotene, xanthophylls, vitamin E, as well as copper and zinc, which are present in alfalfa are responsible for the high antioxidant properties of this plant .

The most important alfalfa flavonoids were the flavones: tricin and apigenin glycosides (Goławska *et al*.,2010)which they possess a higher antioxidant activity than the conventional antioxidants such as butylated hydroxytoluene. Flavonoids, coumestrol and apigenin in particular, have shown good antioxidant properties in a variety of low-density lipoprotein oxidation systems, and to work synergistically with ascorbic acid and other antioxidants (Hwang *et al*.,2001).

Jing *et al*.(2015) reported that the flavonoids derived from alfalfa has exhibited very strong antioxidant activity. The result of Zhan *et al*.(2017) has investigated the effects of alfalfa flavonoids on productive performance, immunity, and ruminal fermentation which revealed that the flavonoids could sharply reduce methane dicarboxylic aldehyde concentration, and the superoxide dismutase enzyme activity presented a significant increasing tendency. Ouyang *et al*.(2016) reported that alfalfa flavonoids enhanced the *in vivo* antioxidant activities such superoxide dismutase (SOD) and decreased the level of malondialdehyde (MDA) in serum of broilers.

2.2.4. Effect of alfalfa in productive performance of poultry

The microbiota within the gastrointestinal tract (GIT) plays an important role in overall health status and productivity in chickens (Wei *et al.*, 2013) by promoting digestion and absorption of nutrients, modulating their immune system, and enhancing resistance to infection (Wang *et al.*, 2016). Optimal gut health can be assured by the bacteria populations that are

present in the small intestine (Rinttilä and Apajalahti, 2013). The digestion and absorption of nutrients can be influenced by the microbiota from the small intestine, being an important factor of growth rate .The composition of GIT microbiota can be changed by dietary manipulation, being a successful approach in preventing gut health disorders and promoting animal performance(Gong *et al.*, 2008).

Scientists recently observed that the stimulating and the appetizing activity of herbs and plant extracts on poultry digestive and immune system could be beneficial for health and performance of poultry.They are identified to possess desirable characteristics like thermo tolerance, resistance to diseases, better egg production, and hard eggshell, high fertility, hatchability rates, and meat flavor and also have a high carcass percentage (Yemane *et al*., 2014).

Dong *et al*. (2007)reported that the higher daily weight gains are resulted by the high protein content of alfalfa which constitutes up to (21.58%), and the amino acid composition which was advantageous to poultry, this is due to the fact that it is rich in tryptophan, lysine and threonine. In spite of alfalfa is fibrous but has the potential to be used as feed for poultry because of its complete nutritional content, including essential amino acids.

Alfalfa is an important source of vitamins such as β-carotene and another 10 vitamins (Sen *et al*., 1998) with various microelements too, the animals need these nutrients for normal growth and development (Marković *et al*., 2007) . Alfalfa meal must be used in feed mixtures in limited quantities for maintaining a high production of broiler chickens (Guenthner *et al*., 1973) .

Khaleel *et al*.(2005) suggested that dried alfalfa or its water extract can be used in poultry diets as alfalfa is rich in proteins, vitamins and mineral elements, as well as in various substances impact on secondary metabolism (Wang *et al*., 2008). It was observed that a dried alfalfa additive improved nutrient absorption and assimilation, stimulated growth of a beneficial microflora and regenerated the mucous membrane of the alimentary duct in poultry.

Alfalfa meal at levels below 5% appears to improve early growth occasionally, but, as the levels increase above 5%growth declines proportionately. Fiber content and bulk of this product are probably important factors (Wilgus *et al*.,1954). Zheng *et al.*(2019) observed that supplementation different levels of alfalfa meal (5 %, 8 % and 10 %) decreased feed conversion ratio and mortality compared to the control in chickens.

On the contrary, higher feed conversion than control was observed during using in a high level up to 7.3 % of alfalfa meal included in the basal diet of broilers (Gulizia and Downs, 2020). This increase in the feed conversion ratio of the alfalfa meal group can be attributed to the anti-nutritional factors present in alfalfa, several detrimental effects have been associated with the ingestion of high levels of saponins by poultry (Ponte *et al*., 2004). Moreover, the indigestible fiber contained in alfalfa meal can decrease the digestibility of feed and increase the negative impact on the gastrointestinal tract (Gulizia and Downs, 2020).

2.2.5. Effect of alfalfa in biochemical parameter

2.2.5.1. Effect of alfalfa in total protein

Serum Proteins act as transport substances for hormones, vitamins, lipids, minerals, and other materials.They also help in balance the osmotic pressure of the blood and tissue. Osmotic pressure is part of what keeps water inside a special compartment of the body (Ma and Yin,2012).

A higher total protein content in blood plasma may be attributed to the positive effect of alfalfa protein concentration supplementation on protein metabolism in the animal body (Kundan and Anupam, 2011**).** Some studies have shown that with advancing age and development, total protein and globulin (especially gamma globulin) concentrations increase (Kaneko *et al*., 2008). El-Kelawy *et al*.(2018) suggested that all supplemented groups of alfalfa seeds and vitamin E increased total protein and globulin in serum.

While a study of Eldamarawy *et al*.(2021) who found that the supplementation of alfalfa seed oil to broilers diet did not affect on Plasma total protein, albumin and globulin also the same result in another study by Guiwen *et al*.(2021) was noted there were no changes worth mentioning in total protein of broiler that fed in different levels of alfalfa which inclusion in diets.

2.2.5.2.Effect of alfalfa in lipid profile

Dehydrated alfalfa contains the hypocholesterolemic compounds such as saponins,that have the capacity to link to cholesterol and steroids, impeding its absorption and consequently

reducing cholesterol plasma concentrations (Sidhu and Oakenfull,1986). Alfalfa consumption has been shown to decrease the cholesterol content of broiler meat (Ponte *et al*.,2004) .Activity of saponins is well documented, with clearly defined molecular mechanisms. Some saponins form insoluble complexes with cholesterol in the digesta and inhibit the intestinal absorption of endogenous and exogenous cholesterol. In addition, saponins can affect the enterohepatic circulation of bile acids by forming mixed micelles, which directly affect the reabsorption of bile acids from the terminal ileum (Oakenfull and Sidhu, 1990). Guclu *et al.*(2004) observed that the addition of 9% alfalfa meal into laying quail diets reduced serum triglycerides and cholesterol without any adverse effect on performance.

The flavonoids at low level may enhance cholesterol metabolism and reduce its accumulation in the chicken meat , thus improving meat quality (Chen *et al*., 2016).

Kwiecien *et al*.(2020) showed that the supplementation of 30 gm/kg diet of alfalfa protein concentration caused a decrease in the content of total cholesterol, triglycerides, and low density lipoprotein and an increase in the level of high density lipoprotein,this may indicate that the bioactive substances contained in alfalfa protein concentration play an important role in modulation of lipid metabolism. Another study reported by Ouyang *et al*. (2016) showed a decline in serum total cholesterol and low density lipoprotein levels with a simultaneous increase in high density lipoprotein levels. Alike (Chen *et al*. 2016) demonstrated that different amounts of alfalfa flavonoids added to the diet reduced the levels of triglycerides, total cholesterol ,and low density lipoprotein while improved the high density lipoprotein content in serum .

2.2.6.Effect alfalfa in liver enzymes of poultry

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are commonly measured clinically as a part of a diagnostic liver function test to determine liver health. The levels of serum AST and ALT generally increase during liver or muscle damage(Simon,2019).

In the rise of the activity of certain muscular tissue-specific enzymes, i.e. lactate dehydrogenase (LDH) and AST,they penetrated from the damaged muscle cells into the extracellular fluid and blood. Moreover, an increased LDH activity can reflect deficiency of B12 vitamin or folic acid. Whereas, enhanced ALT activity in the blood plasma can be considered as

a sensitive index of pathological liver changes (Kaneko, 2008). However, due to the unavailability of perceptible changes in the anatomopathological examination of this organ, it should be stated that alfalfa additive evoked changes in the functioning of the liver.

El-Kelawy *et al*.(2018) reported that the bird fed diet containing alfalfa in a level 0.5 and 1% showed a decreased in serum AST, ALT compared to control group.

A study carried out by Krauze and Grela (2010) had revealed that alfalfa additive significantly modified the activity of certain enzymes such as aspartate aminotransferase, γ-glutamyl transpeptidase and alkaline phosphatase. Higher AST activity was identified only in the control group, while, in treated groups the activity of this enzyme was lowered by around 31%. However, the alfalfa additive did not significantly affect ALT and LDH activities.

In broiler chicks, ALT activity and AST/ALT ratio decreased significantly in alfalfa group compared to control group (Karimi *et al* ., 2013) .However Guiwen *et al*.(2021) indicated that the AST and ALT levels were similar among the treatment groups with various level of alfalfa meal which indicated that use of alfalfa may not be harmful for broiler organs .

Another study done by pours (2015) showed the methanol extract of alfalfa has not significantly increased AST and ALT enzymes in broilers .

2.2.7. Effect of alfalfa in the immune status

The thymus, bursa of Fabricius, and spleen are the primary immune organs of chickens. According to Yan *et al*. (2017) the assessment and estimation of the weight of immune organs considers an excellent model for determining the protection rate provided by vaccines against some diseases .

Das *et al*.(2012) suggested that there is evidence that saponins within alfalfa may stimulate the immune system of animals by increasing the uptake of antigens from the gut and other membranes .

Another study of Zemin Li *et al*. (2021) has reported that alfalfa supplements caused a significant increase in the IgG and IgA levels in serum compared to the control, suggesting that alfalfa supplementation improved immune status of broilers.

A research done in broiler chickens by El-Kelawy *et al*. (2018)showed that feeding diet with 0.5 and 1% supplementations of alfalfa increased total protein , globulin, β-globulin, γ-globulin, IgA, IgM, IgG,phagocytic activity and phagocytic index compared to control group, and this was attributed to the presence of natural polyphenol in alfalfa which improve immune response of broiler chicks, contrary Liu *et al*. (2010) showed that supplementation of chicken diets with alfalfa meal had no significant effect on the development of immune organs .

2.2.8. Effect of alfalfa in sensory character

Sensory evaluation may be described as a scientific discipline which measures the properties of a product with the use of human senses (Gengler,2009). A normal person uses the five senses (sight, smell, hearing, taste and touch) and involves all of these in different ways and with various intensities to establish quality of food products (Wadhera and Capaldi, 2014). Taste is the strongest sense of human sensations concerning food (Breslin, 2013).

The use of high percentages of young harvested alfalfa conserved as silage in the feed of broiler chickens can affect the resulting meat quality in a positive way. This refers to reduced cholesterol content due to the saponins contained in alfalfa and to a higher amount of polyunsaturated fatty acid in the meat. Moreover, the yellowness and chroma of the meat can be increased by the xanthophyll and carotenoids present in alfalfa (Carrasco *et al*.,2017).

In a study of using high and moderate levels of alfalfa implemented by Ponte *et al*.(2005) showed that alfalfa lowered cholesterol and lipid contents meat of broiler chickens. Consumers might be less satisfied with meat derived from broilers consuming high levels of alfalfa. It may be due the lower fat content of meat or from the presence of no-flavor caused by the higher percentage of alfalfa in the diet of chickens (Ponte *et al*.,2004). Furthermore, alfalfa consumption did not create an acceptable-flavors in poultry meat compared with meat derived fed on diets containing fish products (Lopez-Ferrer *et al*., 1999).

Chapter Three: Methodology

3. Materials and Methods

3.1. Experiment design

This study was carried out in a private poultry house, from 22 Jan. to 26 Feb. / 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 to four groups (30/group) with 3 replicates,.Each replicate involved 10 birds/ pen, the experimental groups as follows:

G1:(Control) basal diet without any addition.

G2 :basal diet with dried fenugreek leaves at 2.5 gm/kg of diet.

G3: basal diet with dried alfalfa leaves at 2.5 gm/kg of diet.

G4: basal diet with dried fenugreek leaves at 2.5 gm/kg of diet and dried alfalfa leaves 2.5 gm/kg of diet at figure (3-1).

The experimental period was lasted for five weeks.Feed and water were provided *ad Libitum* along the study.Special programs were used for vaccination of birds and health care as recommended in the broiler guide. The chicks were fed at 1 to 21 days age on a starter diet, afterwards were fed on a finisher diet until the age of 35 days. Table (3-1) shows the diets composition which fed to birds in starter,grower and finisher stages. Chicks were kept in floor cages under similar management and hygienic system in a close house. The brooding temperatures were 36ºCduring first and second days, then lower it to 34 ºC, 32 ºC, 30ºC, 28ºC and 26ºC during the 1st , 2nd , 3rd , 4th and 5th weeks of age respectively . The lighting regime was 23:1 of light-dark cycle.

Experimental Design

One day old (Ross-308)chicks n=120

G 1	G2	G3	G4
Control Basal diet without any addition (n=30 chicks)	Basal diet + Fenugreek leaves 2.5gm/kg of diet (n=30 chicks)	Basal diet + Alfalfa leaves 2.5gm/kg of diet (n=30 chicks)	Basal diet + Fenugreek leaves 2.5gm/kg of diet + Alfalfa leaves crumble 2.5gm/kg of diet (n=30 chicks)

Studied parameters

Productive performance	Biochemichal parameter	
Body weight and Body weight gain	Total protein, albumin and globulin	Aspartate aminotransferase (AST)
Feed intake	Total cholesterol and triglyceride	Alanine aminotransferase (ALT)
Feed conversion ratio	High density lipoprotein and low density lipoprotein	

Study of Ab titer by using of ELISA test at 35thday age against

Newcastle & infections bursal disease vaccine

Sensorial traits of meat

Figure (3-1) Experimental Design of the Study

Table (3-1) Ingredients and chemical analysis 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

Chemical Analysis

Gross energy k cal/kg	3078	3100	3125.2
Crude protein (%)	22.74	21.5	20.16
Energy/protein ratio	135.35	145.25	155.07
Calcium (%)	1.1	0.95	0.9
Available Phosphorus (%	0.5	0.47	0.44
Methionine + cystein (%)	0.78	0.70	0.65
Lysine (%)	1.02	1.02	0.95
Methionine (%)	0.48	0.4	0.36
Fiber	0.1	0.09	0.11

According to NRC, (1994).

3.2.The dietary additives used in the experiment

A. Fresh fenugreek leaves were bought from the local market in Karbala city. The fresh fenugreek leaves were visually sorted and trimmed .The clean leaves were then dried in a tray in an open shady place with constant stirring, then ground to powder using a blender.

B. Fresh alfalfa leaves were bought from Algader Market in karbala city, fresh Alfalfa leaves were spread on the floor of an open shady place , turned up-side down once or twice daily. The collected, dried alfalfa leaves were milled into powder by using a blender.

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).

3.3.Preparation of poultry house

After cleaning the walls, floor and ceiling by clean water and carrying out the disinfection by formalin and potassium permanganate.After 2 days all windows were opened and fans were switched on for ensuring removal of toxic gases completely before chicks has been entranced.All feeders and waterers were cleaned and disinfected as well. Chicks were distributed randomly to the groups. All experiment groups were provided with suitable litter (wood shaving), experimental 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 deprived from feed and water for 2 hours before vaccination .Vitamin C was used routinely in the ratio of 1 gm/liter of water after each vaccination protocol (Table 3-2).

Table (3-2) program of vaccination

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

3.5.Parameters studied

3.5.1.Productive performance

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

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

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

Weekly mean body weight gain for each replicate was calculated depending upon the following formula:

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:

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

3.5.2. Blood sampling

Blood sera were used to determine biochemical parameters and determine ELISA antibody titer against ND, IBD viruses at 17 ^{the} and35th days. All blood samples were collected from each replicate randomly (3 sample from each replicate)and obtained from the wing vein in a test tube without anticoagulant. Serum tubes were immediately separated and kept overnight at 4ºC (in the refrigerator) ,after that they were put in centrifuge for 10 minute/ 3000 rmp, and stored in deep freeze (-20ºC) until analysis.

3.5.3. Biochemical parameters

3.5.3.1.Total protein concentration (gm/L)

Total protein was estimated using the kit as a colorimetric to reagent estimate total protein, which depends on the interaction of copper ion with the protein of the sample in alkaline medium forming a colored complex that could be measured by a spectrophotometer (Biuret method) (Burtis *et al* ., 1999).

3.5.3.2.Total albumin concentration (gm/L)

Total albumin was estimated according to colorimetric albumin in the presence of bromocresol green (BCG) at a slightly acid pH , 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 (Rodkey, 1964).

3.5.3.3.Total globulin concentration (gm/L)

Total globulin was measured as follows:

Total serum globulin (gm/L)=total serum protein(gm/L) –total serum albumin (gm/L) in the sample.

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. Estimation of Triglyceride concentration (mg/dl)

Triglyceride concentration was estimated by Cormay triglyceride kit hydrolyzed to glycerol enzymatically (Fossati & 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. Liver function enzymes

3.5.3.8.1.Aspartate aminotransferase (AST) (U/100ml)

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

3.5.3.8.2.Alanine aminotransferase (ALT) (U/100ml)

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

3.5.4. Immunological test

Antibody titers against Newcastle virus disease and infectious bursal disease virus in broiler chicks serum samples were detected at 35 days of age by using enzyme linked immunosorbent assay (ELISA) for different groups based on (Spalatin *et al*., 1973).

3.5.5. Sensory tests

3.5.5.1. Carcass preparation

At the end of the experiment, two chicks from each replicate were randomly selected and slaughtered.After bleeding, the slaughtered chickens were cleaned and feathers plucked automatically, then washed after evisceration sawed into two halves. One side divided into the commercial cuts (thigh and breast). The carcasses were chilled at 4ºC for 24 hours for carcass characteristics for team taste.

3.5.5.2.Evaluation of sensory tests

Sensory evaluation was done by taking meat slices from breast and thigh with a size of 2 and 3 cm cooked in an electric oven at a temperature of 177 ° C then lowered to the sensory evaluation. In the sensory evaluation share five adults persons, the sensory evaluation was done for flavor, Tenderness, juicy, color and palatability according to the five degree hedonic scale where 1,2,3,4,5, for the best respectively .Table (3-3) (Al–Fayadh *et al.*, 2005).

3.6.Statistical analysis

Data obtained from the present study were analyzed as one-way analysis of variance (ANOVA) using general linear model (GLM) procedure using SPSS 22.0 software (Corp, 2011). The Duncan`s test was used to find out the significant difference among group at level Four (0.05).

Table (3-3) Point hedonic scale showed evaluation of sensory tests

Evaluation	Tenderness	Juicy	Color	Flavor	Palatibility
1	not tenderness	very dry	very light brown	very undesirable flavor	very unpalatible
2	less tenderness	dry	light brown	undesirable flavor	unpalatible
3	moderate tenderness	moderate	moderate brown	moderate flavor	moderate palatible
4	tenderness	Juicy	dark brown	desirable flavor	palatible
5	very tenderness	very Juicy	very dark brown	very desirable flavor	very palatible

The laboratory chemicals and kits were used in this study are listed in table (3-4) with their company

Table (3-4) Kits and their suppliers that used in study

	Chemical	**company**	**origin**
I	Total serum protein kit	Spanish company spinreact	Spain
II	Albumin kit	Spanish company spinreact	Spain
III	Serum total cholesterol Kit	PZ CORMAY S.A company	Poland
IV	Serum triglyceride Kit	PZ CORMAY S.A company	Poland
V	Serum HDL-Cholesterol Kit	PZ CORMAY S.A company	Poland

VI	Serum LDL-Cholesterol Kit	PZ CORMAY S.A company	Poland
VII	Serum aspartate aminotransferase (AST) Kit	PZ CORMAY S.A company	Poland
IIX	Serum alanine aminotransferase (ALT) Kit	PZ CORMAY S.A company	Poland
IX	Newcastle disease virus antibody test kit	Synbiotics	USA
X	IBD disease virus antibody test kit	Synbiotics	USA

Chapter Four: Results and Analysis

4.The Results

4.1.Productive performance

4.1.1.Body weight (BW)

Means of live body weight of broiler chicks were stated in table (4-1).The results showed that there were not any significant differences between experimental groups during first and second weeks .But there were a significant increase (P≤0.05) in live body weight during 3 to 5 weeks of age of addition groups as compared with control group. At the third week , G3 and G4 groups recorded a significant increase (P≤0.05) as compared with control and G2, the G2 recorded a significant increase (P≤0.05) as compared with control.

Table (4-1) Effect of fenugreek, alfalfa and their mixture on weekly live body weight (gm/bird) of broiler chickens (mean±standard error).

Group / Age	G1	G2	G3	G4
Week1	217.3 ± 1.01 a	218.5 ± 0.66 a	218.0 ± 0.72 a	218.5 ± 0.71 a
Week2	564.0 ± 0.63 a	565.8 ± 3.65 a	564.8 ± 2.59 a	565.8 ± 2.90 a
Week3	1064.0 ± 3.16 c	1090.0 ± 2.98 b	1104.2 ± 7.88 a	1118.0 ± 2.50 a
Week4	1668.0 ± 7.18 c	1802.0 ± 11.78 b	1804.0 ± 6.36 b	1860.2 ± 1.28 a
Week5	2289.2 ± 3.79 c	2579.2 ± 8.68 b	2578.0 ± 5.15 b	2689.4 ± 2.58 a

Different letters in the same row showed a significant difference at (P≤0.05).

At the fourth and fifth week of the experiment, the groups(G2, G3 andG4)showed a significant increase (P≤0.05) as compared with control.Also, there were no significant differences between G2 and G3 groups.The fourth group recorded the significant increase in live body weight as compared to other groups.

4.1.2. Weight Gain (WG)

Means of body weight gain of broiler chicks were shown in table (4-2). The results were similar with respect to body weight results as there were no significant differences among experimental groups in the first and second weeks .

At the third week of age, results revealed that presence of significant increase ($P \leq 0.05$) in G2,G3 and G4 as compared with control. Although there were no significant differences between G2 and G3groups and between G3and G4 groups but, there was a mathematical difference between them .

Table (4-2) Effect of fenugreek, alfalfa and their mixture on weekly body weight gain (gm/bird) of broiler chickens (mean± standard error).

Group / Age	G1	G2	G3	G4
Week 1	**176.8 ±0.80 a**	**177.3 ±1.15 a**	**177.2 ±1.50 a**	**177.1 ±1.15 a**
Week 2	**346.7 ±0.96 a**	**347.3 ±3.20 a**	**346.8 ±2.93 a**	**347.3 ±3.20 a**
Week 3	**500.0 ±3.35 c**	**524.2 ±5.95 b**	**539.4 ±8.27 ab**	**552.2 ±2.73 a**
Week 4	**604.00 ±5.23 c**	**712.0 ±14.45 b**	**699.8 ±8.33 b**	**742.2 ±3.18 a**
Week 5	**621.2 ±10.87 c**	**777.2 ±17.57 b**	**774.0 ±9.84 b**	**829.2 ± 3.37 a**
Cumulative (gm)	**2248.8 ±3.76 c**	**2538.0 ±8.40 b**	**2537.2 ±2.10 b**	**2648.0 ± 2.09 a**

-Different letters in the same row showed a significant difference at ($P \leq 0.05$) .

The findings of the fourth and fifth week showed a significant increase ($P \leq 0.05$) among G2, G3 and G4 groups as compared to control group, the fourth group recorded the highest increase in weight gain compared to other groups, there were no significant differences between G2 and G3 groups at the same weeks of age .

A cumulative weight gain recorded a significant increase (P≤0.05) in addition groups as compared to control , also G4 showed a significant increase (P≤0.05)as compared to all groups of experiment.

4.1.3. Feed Intake (FI)

The result in table (4-3) showed no significant differences among experimental groups during the first week. Whereas there were a significant difference (P≤0.05) in feed intake between the addition groups and control during the trial period from 2-5 weeks of age.

At the second week of age, the results showed no a significant difference among G1, G2 and G3 also there is no a significant difference among G1,G2and G4 .The fourth group (G4) recorded a significant increase (P≤0.05) in feed intake as compared to other groups at the third week of study.Besides G3 group recorded a significant increase as compared to G2 and control groups at the same week

Table (4-3) Effect of fenugreek, alfalfa and their mixture on weekly feed intake (gm/bird) of broiler chickens (mean± standard error).

Group / Age	G1	G2	G3	G4
Week 1	200 ± 2.09 a	197 ± 1.14 a	200 ± 2.90 a	200 ± 1.41 a
Week 2	447 ± 2.49 ab	445 ± 2.19 ab	448 ± 2.30 a	440 ± 2.77 b
Week 3	670 ± 1.41 d	698 ± 5.15 c	710 ± 2.77 b	720 ± 1.79 a
Week 4	1051 ± 8.99 b	1035 ± 2.77 bc	1025 ± 3.22 c	1067.6 ± 4.64 a
Week 5	1106 ± 2.00 d	1292 ± 1.79 b	1268 ± 2.61 c	1327 ± 2.19 a
Cumulative	3474 ± 11.13 c	3667 ± 6.66 b	3651 ± 7.40 b	3754.6 ± 8.52 a

Different letters in the same row showed a significant difference at (P≤0.05) .

At the fourth and fifth week of age, our results showed a significant increase (P≤0.05) at the fourth group as compared to other groups, and the third group showed a significant decrease (P≤0.05) as compared to other groups.

The result of accumulative feed intake revealed a significant increase (P≤0.05) in addition groups as compared to control , with a significant increase of G4 as compared to other groups, besides there were no significant differences between G2 andG3.

4.1.4.Feed conversion ratio (FCR)

The result of feed conversion ratio was recorded in table (4-4)**,** it showed there were no significant differences among experimental groups at the first, second and third week of age, although there were a mathematical differences among groups.

Table (4-4) Effect of fenugreek, alfalfa and their mixture on weekly feed conversion ratio of broiler chickens (mean± standard error).

Group / Age	G1	G2	G3	G4
Week 1	**1.13 ±0.03 a**	**1.11 ±0.01 a**	**1.13 ±0.19 a**	**1.12 ±0.01 a**
Week 2	**1.29 ±0.01 a**	**1.28 ±0.13 a**	**1.29 ±0.01 a**	**1.27 ±0.01 a**
Week 3	**1.34 ±0.01 a**	**1.33 ±0.01 a**	**1.32 ±0.02 a**	**1.30 ±0.004 a**
Week 4	**1.74 ±0.02 a**	**1.46 ±0.03 b**	**1.47 ±0.02 b**	**1.44 ±0.005 b**
Week 5	**1.78 ±0.03 a**	**1.67 ±0.04 b**	**1.64 ±0.02 b**	**1.60 ±0.01 b**
FCR Cumulative	**1.46 ±0.004 a**	**1.37 ±0.005 b**	**1.37 ±0.002 b**	**1.35 ±0.003 c**

-Different letters in the same row showed a significant difference at (P≤0.05).(FCR)feed conversion ratio.

The results during the fourth and last weeks of trial period revealed a significant decrease (P≤0.05) in G2,G3and G4 groups as compared to control.The fourth group showed better FCR as compared to other groups of study .

There was also a significant difference (P≤0.05) among addition groups in FCR mean as compared to control. There were no significant differences between G2 and G3, while there were a significant improve noted in G4 which recorded the lowest value as compared to other groups

4.2. Biochemical parameters

4.2.1. Concentrations of serum total protein, albumin and globulin (gm/L)

Table (4-5) declared the effect of fenugreek, alfalfa and their mixture on serum total protein, albumin and globulin at 17th days of age, the result of total protein recorded significant differences(P≤0.05) among groups, the results showed a significant increase(P≤0.05) in G2, G3 and G4 groups as compared with control.

Table (4-5) Effect of fenugreek, alfalfa and their mixture on serum protein profile of broiler chickens at 17th days of age of broilers (mean± standard error).

Group / Parameter	G1	G2	G3	G4
Total protein (gm/L)	**1.80 ±0.05 c**	**2.56 ±0.07 a**	**2.24 ±0.05 b**	**2.33 ±0.06 a**
Albumin (gm/L)	**0.64 ±0.02 b**	**0.88 ±0.04 a**	**0.68 ±0.04 b**	**0.98 ±0.04 a**
Globulin (gm/L)	**1.12 ±0.04 c**	**1.68 ±0.06 ab**	**1.56 ±0.05 B**	**1.74 ±0.04 a**

Different letters in the same row showed a significant difference at (P≤0.05) .

Albumen and globulin showed a significant difference (P≤0.05) among addition and control groups, they showed a significant increase (P≤0.05) in G2, G3 and G4 groups compared to the

control group with the exception the presence of non- significant differences in albumin value between the control and G3 group. Fourth group recorded the highest increase in albumin and globulin levels as compared to other groups of study.

Table (4-6) recorded the effect of fenugreek, alfalfa and their mixture on serum total protein, albumin and globulin at 35th day of age.The result of total protein showed a significant increase (P≤0.05) in groups (G2,G3 and G4) as compared to control group.While there was no significant difference between G2and G4 groups.

.**Table (4-6) Effect of fenugreek, alfalfa and their mixture on serum total protein profile of broiler chickens at 35th days of age (mean± standard error).**

Group / Parameter	G1	G2	G3	G4
Total protein (gm/L)	**1.94 ±0.05 c**	**2.68 ±0.04 b**	**2.92 ±0.05 a**	**2.74 ±0.02 b**
Albumin (gm/L)	**0.64 ±0.02 b**	**0.98 ±0.04 a**	**0.96 ±0.05 a**	**0.90 ±0.05 a**
Globulin (gm/L)	**1.30 ±0.04 d**	**1.70 ±0.03 c**	**1.96 ±0.02 a**	**1.84 ±0.02 b**

Different letters in the same row showed a significant difference at (P≤0.05) .

The result of albumin concentration showed a significant difference(P≤0.05) in G2,G3 andG4 groups as compared to control. There were significant differences (P≤0.05) among all experimental groups, G3 recorded the highest value in globulin level followed by groups (G2 and G4)as compared to control

4.2.2.Concentration of serum lipid profile

Table (4-7)revealed the effect of fenugreek, alfalfa and their mixture on serum lipid profile of broilers at 17th days**.**There was a significant decrease (P≤0.05) noticed in the serum cholesterol concentration of the addition groups as compared with the control, and there was a significant

decrease (P≤0.05) in the value of G4 as compared to other groups, also a significant decrease (P≤0.05) found in G3 as compared to G2 and control group.

There was a significant decrease (P≤0.05) in triglyceride concentration for G2, G3 and G4 groups as compared with control group.These results were similar to results of total cholesterol, The G4 had the lowest value as compared to other groups and G3 was lower than G2, while the control recorded higher group in the parameter.

Table (4-7) Effect of fenugreek, alfalfa and their mixture on serum lipid profile of broiler chickens at 17th days (mean± standard error).

Group / parameter	G1	G2	G3	G4
Total cholesterol (mg/dl)	175.60 ± 0.75 a	137.00 ± 1.18 b	122.6 ± 1.25 c	116.80 ± 1.62 d
Triglyceride (mg/dl)	75.00 ± 1.00 a	56.40 ± 0.51 b	47.60 ± 1.50 c	36.90 ± 3.28 d
HDL (mg/dl)	52.60 ± 0.51 d	86.00 ± 0.32 a	61.80 ± 0.80 c	75.80 ± 0.80 b
LDL (mg/dl)	68.40 ± 0.68 a	54.60 ± 0.81 b	49.00 ± 0.55 c	45.40 ± 0.93 d

Different letters in the same row showed a significant difference at (p≤0.05),(HDL) high density lipoprotein,(LDL)low density lipoprotein.

High density lipoprotein value was increased significantly (P≤0.05) in addition groups as compared to control, especially G2,that showed a significant increase (P≤0.05) as compared to other groups with higher value.Also G4 showed a significant increase (P≤0.05) as compared to G3and control groups.

Low density lipoprotein values in serum showed adverse result to HDL in experimental groups that were significantly decreased (P≤0.05) especially for the G4 as compared with the other groups, also G3 was significantly decreased as compared to G2 and control.

Table (4-8) showed the effect of fenugreek, alfalfa and their mixture on serum lipid profile at 35th days. There were significant differences(P≤0.05) among experimental groups as compared with control, there were a significant decrease (P≤0.05) in serum cholesterol for G4 as compared

with control and other groups.Also, there were significant decrease (P≤0.05) in G2 and G3 groups as compared with control, and there was a significant decrease (P≤0.05) for G3 as compared with G2.

Table (4-8) Effect of fenugreek, alfalfa and their mixture on serum lipid profile of broiler chickens at 35th days (mean± standard error).

Group / Parameter	G1	G2	G3	G4
Cholesterol (mg/dl)	188.8 ±0.66 a	177.8 ±1.43 b	115.4 ±1.54 c	81.8 ±1.02 d
Triglyceride (mg/dl)	85.8 ±0.66 a	60.4 ±0.51 b	53.8 ±0.73 c	45.2 ±0.37 d
HDL (mg/dl)	51.2 ±1.16 c	66.2 ±0.66 a	58.0 ±0.95 b	65.6 ±1.03 a
LDL (mg/dl)	80.0 ±1.58 a	55.8 ±0.66 b	52.0 ±0.89 bc	50.0 ±1.67 c

Different letters in the same row showed a significant difference at (p≤0.05), (HDL) high density lipoprotein,(LDL)low density lipoprotein.

The value of triglyceride recorded significant differences(P≤0.05) among experimental groups and there was a significant decrease (P≤0.05) in groups (G2,G3 and G4) as compared to control. Besides, a significant increase (P≤0.05)was in HDL value for G2,G3 and G4 groups as compared to the control while there were not significant differences between G_2 and G4.

There was also a significant difference (P≤0.05) in LDL value of G2, G3 and G4 groups compared to control.Moreover, there were no significant differences between G2 and G3 or betweenG3 and G4 groups.

4.2.3.Values of liver function enzymes

Table (4-9) showed the effect of fenugreek, alfalfa and their mixture on serum liver function enzymes of broilers at 35th days.The result revealed a significant difference(P≤0.05) in AST and ALT in all groups, G2, G3 and G4groups showed a significant decrease (P ≤0.05) in AST and ALT as compared with the control group.

Table (4-9) Effect of fenugreek and alfalfa and their mixture on serum liver function enzymes of broiler chickens at 35th days (mean± standard error).

Group / parameter	G1	G2	G3	G4
AST (U/100ml)	65.52 ± 0.20 a	53.78 ± 0.41 b	52.60 ± 0.63 b	42.32 ± 0.43 c
ALT (U/100ml)	14.8 ±0.37 a	10.0 ±0.45 b	9.8 ±0.49 b	7.4 ±0.68 c

Different letters in the same row showed a significant difference at ($p \leq 0.05$).

The findings also revealed that there were no significant differences between G2 and G3 groups. While there was a significant decrease ($P \leq 0.05$) in value of AST and ALT for G4 as compared with other experimental groups.

4.3. Immunological Parameters

Table (4-10) declared the antibody titers against Newcastle disease and infectious bursal viruses at 35th of age after adding fenugreek, alfalfa and their mixture in the diet of broilers. There were significant differences ($p \leq 0.05$) among experimental groups. Humeral immunity titer against Newcastle (ND) and infectious bursal disease (IBD) were improved significantly ($P \leq 0.05$) in the G2,G3 and G4groups as compared with the control, there were significant increases($P \leq 0.05$) for G4 group as compared to other groups, also there were significant increases ($P \leq 0.05$) in the titer of G3 as compared with G2 and control .

Table (4-10) Effect of fenugreek, alfalfa and their mixture on serum humeral immunity of broiler chickens at 35th days of the study (Mean± standerd error).

Group / Parameter	G1	G2	G3	G4
ND Antibody titer	9000 ±35.64 d	9800 ±35.04 c	10100 ±14.62 b	10990 ±17.73 a
IBD Antibody titer	6000 ±17.50 d	7502 ±17.94 c	7803 ±5.95 b	9004 ±34.76 a

Different letters in the same row showed a significant difference at ($p \leq 0.05$), (ND)Newcastle disease,(IBD) infectious bursal disease.

4.4. Sensory Evaluation

4.4.1.Sensory evaluation of thigh meat

Table(4-11) depicted the influence of fenugreek , alfalfa and their mixture on the sensory evaluation of thigh muscle meat. The results showed that significant improvement ($P \leq 0.05$) was found in tenderness, juiciness, color, flavor and Palatability in addition groups as compared with control, except that was no significant differences between G2 and G1 with respect to tenderness and juiciness.

There was also a significant improvement ($P \leq 0.05$) found in thigh muscle regarding color between addition groups as compared to control.While there was no significant difference between G2 and G3 groups.There was a significant improvement ($P \leq 0.05$) of flavor in G2 and G4 as compared to G3 and control .Moreover, all experiment groups recorded the best palatability compared to G1

Table(4-11) Effect of fenugreek, alfalfa and their mixture on the sensory evaluation of thigh muscle of broiler chickens (mean± standard error).

Group / Parameter	G1	G2	G3	G4
Tenderness	**3.2 ± 0.4 b**	**3.2 ± 0.2 b**	**4.4 ± 0.2 a**	**4.6 ± 0.2 a**
Juiciness	**3.4 ± 0.2 b**	**3.4 ± 0.2 b**	**4.6 ± 0.2 a**	**4.4 ± 0.2 a**
Color	**1.4 ± 0.2 c**	**3.2 ± 0.2 b**	**3.6 ± 0.2 b**	**4.6 ± 0.2 a**
Flavor	**2.6 ± 0.2 c**	**4.6 ± 0.2 a**	**3.6 ± 0.2 b**	**4.4 ± 0.2 a**
Palatability	**3.2 ± 0.2 b**	**4.4 ± 0.2 a**	**4.4 ± 0.2 a**	**4.8 ± 0.2 a**

-Different letters in the same row showed a significant difference at ($P \leq 0.05$).

4.4.2. Sensory evaluation of the breast meat

As shown in table (4-12) the influence of fenugreek, alfalfa and their mixture as dietary addition on the sensory evaluation of breast muscle meat was recorded.The results of this study revealed that were significant differences in tenderness between G3 and G4 groups as compared to second group, while there were no significant difference in tenderness as compared to control .There were also a significant improvement in juiciness parameter of G3 and G4 groups as compared to control and G2.Whereas, there were no significant differences between G2 as compared to control group in this parameter.

The results also showed significantly improve ($P \leq 0.05$) in color for G3 and G4 as compared to G2 and control,but there were no significant differences between G3 and G4, which registered the best improvement compared to G1 .

In case of flavor there were significantly improve ($P \leq 0.05$) in G2 andG4 groups as compared to G3 and control , there were a significant improvement ($P \leq 0.05$)in flavor for G3 as compared to control the Palatability of all addition groups showed significant improvement($P \leq 0.05$) as compared to control group, while there were no significant differences among all dietary addition groups.

Table (4-12) Effect of fenugreek, alfalfa and their mixture on the sensory evaluation of breast muscle of broiler chickens (mean± standard error).

Group / Parameter	G1	G2	G3	G4
Tenderness	**3.6 ±0.24 a**	**2.6 ±0.24 b**	**3.8 ±0.2 a**	**4.2 ±0.2 a**
Juiciness	**3.2 ±0.2 b**	**2.8 ±0.2 b**	**4.0 ±0.32 a**	**4.4 ±0.25 a**
Color	**1.0 ± 0.0 c**	**1.8 ±0.2 b**	**3.0 ±0.32 a**	**3.6 ±0.25 a**
Flavor	**2.6 ±0.25 c**	**4.4 ±0.25 a**	**3.4 ±0.25 b**	**4.4 ±0.25 a**
Palatability	**3.0 ±0.32 b**	**4.2 ±0.2 a**	**3.8 ±0.2 a**	**4.4 ±0.25 a**

Different letters in the same row showed a significant difference at ($P \leq 0.05$).

Chapter Five: Discussion

5.Discussion

5.1.Productive performance

5.1.1. Body weight and weight gain

Several researchers reported that the use of herbal medicines in broiler feeding improved productive performance in broilers (Azoua, 2001; Abdel-Azeem, 2006; Farman *et al.*, 2009).

The results of the current study illustrated that significant differences among all experimental groups, addition groups recorded a significant increase of BW, BWG as compared with control, Improved body weight might be due to antimicrobial activity of fenugreek because of flavonoids, saponins and phenols present within it (Schryver, 2002) which have strong antioxidant and anti-microbial properties which reported to inhibit bacteria growth, such as *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus* in the poultry gut. Thus, these compounds enhance performance and health by modulating the gut ecosystem of poultry birds (Iqbal,2020) .The stabilization of the gut microbiota ecosystem and the stimulation of digestive enzymes secretion are the two well-accepted mechanisms that play a leading role in improving feed utilization and inhibiting the growth-depressing elements related to metabolism and digestion (Bento, 2013). These positive impacts has been confirmed to improve the nutrient digestibility and absorption of nutrients (Jang et al.,2007).Our results were in agreement with those of Azoua (2001) who found that adding fenugreek to the broiler chicken diet increased body weight.

It was documented that broiler chicks fed diets supported by fenugreek seeds, has increased live body weight means at 21^{st} to 35^{th} days of age which might be attributed to the presence of fatty acids in fenugreek (Murray *et al.*,1991). Also, it can be attributed to high-quality proteins present in fenugreek or because of its mechanism for high availability of nutrients (Hernandez *et al*., 2004 ; Murlidhar and Goswami, 2012). EL-Mallah *et al*. (2005) noted that 2% fenugreek seeds added in diet of chickens caused a significant increase in digestibility and absorption of nutrients, and a significant increase in body weight gains, because of a stimulating effect on the digestive system of broiler chickens (Hernandez *et al*., 2004).

Mamoun *et al*. (2014) stated that inclusion levels of fenugreek at 1% and 1.5% are useful in improving body weight and weight gain. Also,Abo El-Nor (1999) suggested that fenugreek might have an impact on the hypothalamus gland to stimulate the hunger center in the brain, thus increase the desire for eating which that Improved appetite and feed intake which might in turn lead to enhanced body weights and performance.Our results are in line with Rabia (2010) reported that 3 gm/kg of feed as the best inclusion level of fenugreek for enhancing the performance and body weight of broiler chickens.Moreover Abid and Fateh (2014) showed that there was high significant difference among the experimental groups in live body weight for the first week until the end of the trial after fenugreek inclusion at level of 1%.The result of this study also matched with those of Motlaq and Sadik (2011) who indicated that level of 0.5% of fenugreek leaves involved in diet improved live body weight .

In contrast to several studies that report that the inclusion of fenugreek did not significantly improve the live weight of the chickens (Belaid-Gater *et al*., 2021; Awadein *et al*., 2010) .

In such studies, like results of Weerasingha and Atapattu (2013) noted that the addition of 5% fenugreek tended to reduce body weight and feed intake at 38 days of age of chickens, and the optimal level of dietary fenugreek that induced positive effects on growth performance was 1%. Increasing the concentrations of fenugreek leads to a decrease in body weight and increases the feed conversion (Duru *et al*.,2013).

Over the past few decades, alfalfa has gained considerable attention, as an alternative feed ingredient for chickens and other poultry diet because of its content of amino acids, vitamins, saponins,carotenoids and dietary fibres (Sen *et al.,* 1998). The results of the current study showed that the dietary alfalfa resulted in a significant improvement in the growth performance of chickens. The addition of alfalfa leaves may also cause a significant increase in the average weight body and the cumulative weight gain , which can be attributed to the natural nutritional content of this plant. The alfalfa, especially in its leafy part, is a distinguished source of many vitamins, minerals and other important compounds. It also contains estrogen , which plays a role in increasing body weight and depositing proteins and fats in tissues. Farthermore, it has high protein content in the leaves, thus it comes in the second rank after soybeans as a source of protein (Edminster *et al*.,2001). Additionaly, the enzymatic content of the alfalfa leaves including phytase is a great important in digestion, which might be an additional reason in the

results of this group which outperformed the control group in terms of weight gain(Edminster *et al*.,2001).

The result of the present study was agreed with the results of Tkáčová *et al.*, (2011), who revealed that the addition of 2% alfalfa increased body weight gain of broilers as compared with control birds,which may be due to inclusion of fibres within alfalfa that improved the BWG and FCR of broilers (Jiménez-Moreno *et al*., 2010).

Escarcha *et al.*(2012) found that alfalfa supplementation could reduce the pathogen populations in the poultry gastrointestinal tract, due to the fact that bacterial fermentation of alfalfa could produce volatile fatty acids, being toxic to some pathogenic bacteria. Due to high level of fibres in alfalfa, Nassar *et al*.(2019) reported that providing a high-fiber diet increased chicken body weight gain, followed by an increase in feed intake. Svihus and Hetland (2001) also showed that broilers can maintain an adequate BWG when fed diets supplemented with high levels of insoluble fiber.

Kwiatkowska *et al*.(2017) showed that variety of biologically active compounds of alfalfa could improve digestion and utilization of feed nutrients. In a recent study by Guiwen *et al*.(2021) revealed that the final body weight was significantly higher ($P < 0.05$) in the different levels of alfalfa inclusion in broiler diet group than in the control group, also the weight gain was linearly higher in alfalfa groups than in the control. Ouyang *et al.*(2016) reported that the inclusion of alfalfa in broiler chickens could improve the growth performance when compared with the control group, especially when the inclusion level was 15 mg/kg diet.

Different results were reported by Jiang *et al*.(2018) found that were no significant differences in body weight gain when 4% and 8 % alfalfa meal were added to broiler diets. Also Tkáčová *et al*. (2015) discovered that the addition of 4 percent alfalfa meal did not have any effect on body weight gain while 6% alfalfa meal had a reducing effect on body weight gain .

On the other hand, the supplementation of diet with alfalfa meal at 4% and 6% showed lower BW and FI while increasing the proliferation of beneficial bacteria and inhibiting potential pathogens were found in boiler chickens **(**Gulizia *et al*., 2020**;** Pliego *et al*., 2020).

5.1.2.Feed intake

Addition groups with fenugreek and alfalfa showed a significant increase in feed intake as compared with control which started from the third week of age, this result of high feed intake in G2 and G4 can be attributed to the presence of steroid saponins (appetite-stimulating materials) in fenugreek leaves which increased feed consumption and motivation to consume more feed (Khadr and Abdel Fattah, 2007) .Also,Yassin *et al*.(2020) suggested that fenugreek seeds might have an effect on the hypothalamus gland to stimulate appetite and feed intake, which in turn might lead to improved body weight .

Feeding of fenugreek supplemented diet significantly affected feed intake value during the 42 days of age, while there were not significant differences when broiler chicks fed fenugreek during 21 days of age as compared with the control and that improvement could be attributed to the carbohydrates and their chief component (galactomannan) of fenugreek, which stimulated the appetite and digestion (Steiner, 2009).Moreover, Alloui *et al*.(2012) noted that feeding broilers with a fenugreek seed at a level of 3 gm/kg diet significantly increase feed intake. Fenugreek contains neurin, biotin, tri-methylamine which tend to stimulate appetite through their mode of action on the nervous system (Ahmadiani *et al*., 2001). Inclusion of fenugreek seeds in broiler diet resulted in increase of feed consumption, which might be due to the presence of fatty acids or to the relaxing impact on the digestive system due to fenugreek diets (Ali *et al*., 2021).

In contrast, the report of Awadein *et al*. (2010) found that fenugreek seeds had no significant effect on feed consumption compared to the chickens fed control diet. Also, Abbas (2010) found that feed intake of broiler chicks fed fenugreek seed diets decreased significantly over 42 days of age, although differences were not significant over 21 days of age.

The inclusion of alfalfa in broiler chicken diet showed a greater consumption of feed than the control group, which may be attributed to the role of the alfalfa phytase enzyme content which led to an increase in the utilization of food in general, and thus obtaining a good amount of protein, minerals and vitamins that have an appetite-stimulating effect (Naher *et al*., 2012).

Feed intake depression has been mentioned in several studies, with broiler diets containing 10% alfalfa or higher, resulting in a depressed feed intake, the adverse effects of saponins have been attributed to depressed feed consumption because of the bitter taste (Milgate and Roberts, 1995). Therefore, it was concluded that the effects of alfalfa may depend on its level in the diets .

5.1.3. Feed conversion ratio

The feed conversion ratio is regarded as an important economic indicator on the ability of the bird to converted the diet to live body weight, the natural feed additive (G2,G3 and G4) helped in improving the efficiency of feed consumption and ensuring more net return with minimizing the feed cost. The result of the current study revealed significant decrease in dietary addition groups since compared with control, FCR was improved significantly as fenugreek has stimulatory effect on secretion of digestive enzymes and intestinal mucous to stabilize microbial balance and digestion of feed which can improve feed conversion ratio in broilers ((Bin-Hafeez *et al.*, 2003). Similar results were obtained by Elbushra (2012) who noted that supplementation of 0.5% or 1.5% fenugreek had a significant positive effect on the feed conversion ratio in broilers.

Dixit *et al*. (2005) indicated that fenugreek seed powder improved the metabolism in broilers which led to enhance feed conversion efficiency, also Yassin *et al*. (2020) reported that the inclusion of fenugreek seed powder improved feed conversion efficiency of broiler chickens, Fenugreek seeds significantly affected feed conversion ratio at 42 days of age, which probably associated with the development of gut morphological changes in gastrointestinal tissues or might be induced by differences in intestinal microbial content, including their beneficial metabolites .

Alloui *et al*. (2012) reported that feeding fenugreek seeds at 3 g/kg of feed in broiler chicken significantly improved feed conversion ratio due to the beneficial effect on gut microflora.The results of this trial are consistent with the findings of Hamden *et al*. (2010)and Safaei *et al*. (2013) who recorded that the inclusion of fenugreek powder enhanced the feed conversion efficiency of broilers.

These results agreed with the finding of El-Gendi *et al.*, (1994) which indicated that there was an improvement in feed conversion with feeding herbal products as feed additives that could be attributed to their effect on improving the digestibility of dietary protein in the small intestine.

Ali *et al*.(2021) indicated that improvement in feed efficiency of fenugreek seed group may be linked to the modification of gastrointestinal tissue morphology in broiler chick gut, Yadav and Jha (2019) also mentioned in their study that gut microbiota a and their metabolic products improved absorption and nutrient utilization in poultry which led to improved feed conversion ratio.

Al-Kerwi *et al*. (2020) reported that the value of the feed conversion ratio with feeding fenugreek during the period from 8 to 35 days of the experiment were no significant differences between the treatments during this period as compared with control.

The current study showed that the group of chicks that were fed alfalfa leaves had improved in feed conversion ratio when compared to the control group, the explanation for this result may be due to the increasing to the real benefit of the ingested feed and increasing of its metabolite, which was evident by the increase in body weight (Kwiatkowska *et al.*,2012).

Zheng *et al*. (2019) observed that supplementation different levels of alfalfa meal (5 %, 8 %, and 10 %) decreased feed conversion ratio and mortality compared to the control in Beijing-you chickens. Also Guiwen *et al*.(2021) found there was a declining trend in FCR than the control of the alfalfa group at level 75 gm/kg diet.

Unlike results, for example Paredes and Risso (2020)noted dietary inclusion of alfalfa meal (5 % and 10 %) in broiler diets showed no significant effect in feed consumption, feed conversion and carcass yield.Moreover, higher feed conversion than control was observed when a 7.3% alfalfa meal was included in the basal diet of broilers (Gulizia and Downs, 2020).

5.2.Biochemical Parameters

5.2.1. Concentrations of total protein, albumin and globulin

The results of the present study obtained a significant increase in total protein, globulin and albumin in all addition group of experiment (G2,G3and G4) as compared with control.The increase in total serum proteins may be attributed mainly to that fenugreek effect which stimulate the thyroid gland directly and led to increase serum protein content (Azoua, 2001) .These results also agreed with Hassan (2000) who found that the total protein and globulin of serum increased significantly by feeding broiler chicks on diets supplemented with fenugreek seeds.

It is known that the change in albumin levels reflects the liver function, since the liver is the site of albumin synthesis, but globulin is formed by lymphatic tissues (Jones and Bark,1979). It was concluded that broiler chickens fed diet supplemented with 1% fenugreek seeds powder had a significantly higher value of serum total protein while serum albumin, globulin and albumin\globulin ratio were not significant (Abd El Latif and Enas, 2021).

In contrary to study of Belaid-Gater *et al*. (2021) who noted that was not improvement in the serum total protein of the chickens fed fenugreek in diet as compared with control.

A higher total protein content in blood serum in G3 may be attributed to the positive effect of alfalfa protein (xanthophylls) on protein metabolism in the animal body(Grela *et al*.,2008) or due to biologically active substances, like saponins in alfalfa with their beneficial properties for promoting efficient activity protein in serum(Ender *et al.,* 1996).

Elkomy and Elghalid, (2014) found that the plasma total protein and albumin were gradually increased in alfalfa groups compared to the control.Also alfalfa feed additive added to diet at 3% during the fattener phase increased the total protein level in blood plasma during the first and second fattening phases (Pietrzak and Grela, 2015) .

Different results to us,it was found that the levels of total protein, albumin and globulin, did not differ among groups that supplementation with alfalfa as compared with control (Karimi *et al.*,2013). Also, Guiwen *et al.(*2021) indicated that dietary alfalfa leaf meal had a no significant effect in total protein, globulin and albumin.

5.2.2.Concentration of lipid profile

The results of the current study showed a significant decrease ($P \leq 0.05$) in cholesterol and triglyceride in G2, G3and G4 as compared with control. High density lipoprotein(HDL) values also increased significantly in addition groups as compared with control, especially in G2, while, LDL values showed adverse result to HDL in experimental groups that was significantly decreased especially in G4 as compared with control. These results agreed with Mamoun *et al*. (2014) who reported a reduction in the serum cholesterol level when supplements of fenugreek added to broiler diets, This result could be due to the presence of saponins and resins in fenugreek seeds such as hemicelluloses, mucilage, tannin and pectin that inhibit bile acids, help

to lower LDL-cholesterol and inhibit intestinal cholesterol absorption, thereby reducing blood cholesterol levels which might have reduced the bile acid and cholesterol absorption from the intestine (Petit *et al*., 1995).

Raghuram *et al*.(1994) indicated that fenugreek seeds increased bile acid excretion and reduced serum cholesterol content due to the presence of unsaturated fatty acids in seeds. Lanksy *et al.*(1993) attributed this effect to steroid saponins, that may either compete with cholesterol-binding sites or interfere with cholesterol bio-synthesis in the liver. Also soluble fibers and mucilage content of fenugreek can block cholesterol absorption from the intestine, thus reduced its level in the serum.

Abdul-Rahman (2012) and Safaei *et al*. (2013) indicated that inclusion of dietary fenugreek seeds in broilers at 1% level significantly decreased the blood cholesterol levels. Reduction in blood cholesterol levels by supplementation of fenugreek seeds at 40 g/kg in the diet of broiler chicken has also been reported by Duru *et al*. (2013). Broiler chicks fed diet supplemented with 1% fenugreek seeds powder has significantly lowered values of cholesterol concentration compared to other dietary treatments (Abd El Latif and Enas,2021).

Similarly, Khadr and Abdel Fattah (2007) concluded that supplementation of broiler diet with 1% fenugreek modulated cholesterol profile in the serum which might be reflected in the meat.Similary,Safaei *et al.*(2013) reported that feeding commercial broiler chicks on a diet containing fenugreek seed powder lowered total plasma lipids and cholesterol levels. Also El-Hack *et al.*(2015) revealed that a decrease in serum total cholesterol concentration and an increase in high density lipoprotein cholesterol concentration due to fenugreek seed extract supplementation in diet and improvement in HDL led to reduce in LDL-cholesterol.

In a study of Belaid-Gater *et al.*(2021) observed that fenugreek infusion in drinking water of broilers did not improve serum biochemical parameters of the chickens, especially total cholesterol and triglycerides as compared with control.

The results in the our study showed that there were significantly decreased in the total cholesterol, triglyceride and LDL in G3 and G4, which supplementation with alfalfa as compared with the control, the best value recorded in G4 that received combination of fenugreek and alfalfa. Francis *et al.*(2002) attributed that high saponin levels (approximately 2–3%) contained

in the groups supplemented with alfalfa were known for the hypocholesterolemic effect. Alfalfa contains saponins, which are involved in the reduction of total cholesterol levels through the formation of insoluble complexes with cholesterol, which inhibits their absorption. Besides, saponins increase secretion of bile, gastric, and intestinal juices (Khaleel *et al.,* 2005) .These results agreed with Liu *et al*.(2016)who found that alfalfa saponins reduced hepatic cholesterol by inhibiting β-Hydroxy β-methylglutaryl-CoA(HMG-CoA) activity and the formation of insoluble complexes between saponins and cholesterol, which eventually transform into bile acid thus the triglyceride decreased in the alfalfa diets compared to the control diet (Jiang *et al*., 2012).

In previous study was reported that alfalfa meal showed a lower total cholesterol, triglycerides, low density lipoproteins ,while not significantly lowering in the HDL,and this mechanism exerted to the reduction intestinal absorption of the both endogenous and exogenous cholesterol and an increase in fecal biliary excretion (Molgaard *et al.*,1987).

Elkomy and Elghalid (2014) found that plasma total lipids were gradually and significantly decreased in alfalfa groups compared to the control. Also, Mansoub and Myandoab, (2012) reported that serum total cholesterol and triglycerides levels were significantly reduced in group fed alfalfa compared to the control group. Besides the main reason of cholesterol and triglyceride reduction in blood of chicks group fed alfalfa might be due to substances like carvacrol and thymol present in herbs**,** these substances were reported to have positive effects on cholesterol and triglyceride and decrease their concentration in blood (Zargari, 2001).

Guiwen *et al.* (2021) found that the triglyceride level was comparatively lower in the alfalfa supplementation group at the rate 50 gm /kg diet than in the control, while the alfalfa at 75 gm/kg and 25 gm/kg diet groups did not show any obvious changes.

5.2.3. Values of liver function enzymes

The AST or ALT levels of blood are very important as they are indicators of liver health status. Therefore, the levels of AST and ALT in the blood are directly related to the extent of tissue and liver damage (Huang *et al*.,2006). The current study indicated that there were a significant decrease ($P \leq 0.05$) in AST and ALT of G4, G3 and G2 groups respectively as compared with the control. Our results indicated that the use of natural supplementation might

be useful for broiler organs which is consistent with the results of Jafar *et al.(*2021) who indicated that ALT and AST in all groups that fed on fenugreek seed powder and extract showed a significant difference with the control group. In other words,these groups revealed improved hepatic function with significantly decreased in the AST and ALT level, which explained that, liver and its normal functioning is very vital in broiler chickens.In fenugreek Saponin, vitamins A, C, B1, nicotinic acid, and alkaloids are active ingredient that can act as immunomodulators and liver tonic ingredients. Alkaloids, including trigonelline, carpine, and gentianine compounds are the most important alkaloids of fenugreek (Moradi *et al*., 2013). It seems that vitamins A and B1 of fenugreek are effective in liver function and could decrease ALT and AST enzyme levels.

Another study of Khadr and Abdel-Fattah (2007) indicated that liver function enzymes showed no significant change in level of AST and ALT compared to the control in dietary supplementation with fenugreek of broiler.

Guiwen *et al.* (2021) indicated that alfalfa supplementation groups at the rate 50 and 75 gm /kg of broiler chickens showed similar levels in AST and ALT enzymes among the groups compared with the control, except for alfalfa at the rate of 25 gm/kg diet in which the ALT level was the highest and the AST level was significantly lower than that recorded in the other groups.

5.3. Antibody titer against ND and IBD viruses

Antibody titer against Newcastle disease and infectious bursal disease viruses at 35^{th} day of broiler chickens age of the current study showed higher and significant values in G4, G3 and G2 respectively as compared with the control. The reason of these increases may be due to the immunomodulating ability of these dietary additives to improve immunity by its active ingredients such as flavonoids, steroid and saponin, or by raising the weight of lymphatic tissue (Abid *et al.*,2011) .These feed additives increased the cellularities of thymus gland and bone marrow and also increased the action of macrophage and humeral response (Bin-Hafeez *et al*.,2003). These results agreed with Abid and Fateh (2014) found that diet supplemented with 1% of fenugreek for broiler recorded high antibody titer against Newcastle and infectious bursal disease viruses.

In a study performed by Khadr and Abdel-Fattah (2007) showed that the value of antibody titer against Newcastle disease increased significantly by the addition of fenugreek seed in

broiler diet up to 2% as compared with the control. This improvement in antibody titer may be due to galactomannan in fenugreek seed, while Jafar *et al*.(2021) noted that fenugreek seed was effective in immune response at 0.2% and 0.3% regarding antibody response against IBD, whereas groups with low IBD antibody levels showed a high ND antibody level.

In present study,it could be reasoned that saponins, polysaccharides and flavonoids compounds present fenugreek and alfalfa pocessed their functional role in activation of immune system (Jiang and Yu, 2005). Another explanation could be belonged that these feed additives decreased the pathogenic microorganisms stimulated the immune system and therefore strengthened the immune system of broilers. Recently, it was resumed that the supplementation with alfalfa meal at 4, 6, and 7.3% in the diet of broiler chickens showed increasing in the proliferation of beneficial bacteria and thus boosting of immune system against pathogenic bacteria was resulted (Gulizia and Downs, 2020; Pliego *et al.*, 2020).

The proliferation of T and B lymphocytes was significantly greater in the groups supplemented with 3, 6 and 9% of alfalfa meal than the unsupplemented control (Jiang *et al*., 2014).

Moreover, it was reported that the difference between feeding single herbal plants versus herbal mixtures might be due to some antimicrobial components present in the ingredients of the herbal plants used in the present study (Saleh *et al*., 2018). This could explain the reason of various studies which have reported that herbal plants and leaf meals used alone or in different combinations had positive, negative, or unchanged effect on broiler performance.

5.4.Sensory evaluation and meat quality

The results of current study showed that significant improvement were found in tenderness, juiciness, color, flavor and palatability of thigh and breast muscls in dietary addition groups as compared with control and these positive effect might be due to fenugreek and alfalfa contents of significant amounts of bioactive substances (flavonoid) that exhibit antioxidant properties, The role of antioxidants is to protect lipids against radical peroxidation (Lauro, 1991). Lipid peroxidation caused by high levels of free radicals can cause deterioration of the meat (Kim *et al*., 2012). Moreover, the lipid oxidation is closely related to meat color and the accumulation of antioxidant substances may increase the color stability of the meat

(Faustman and Cassens, 1990). Thus, improving the antioxidant properties of the muscle is a great importance to improving meat quality and sensory trait (Wang *et al*., 2017).

These results agree with the study of Abdalla *et al*.(2018) who recommended that using 1% fenugreek seed powder in the feed is beneficial to improve the quality of chicken meat. Also Belaid-Gater *et al*.(2021) found that the addition of fenugreek to the drinking water of broiler chickens showed white and beige meat color with medium tenderness, low juiciness but there was a spicy smell.

Yassin *et al*.(2020) indicated that the inclusion up to 3% of fenugreek powder in the diet of broilers did not affect the palatability.Also indicated that the dry matter, ash and protein content of the pectoral muscles (P major and P minor) were higher in the broilers receiving fenugreek supplemented diet. Mukhtar *et al*., (2013) found that supplementation of fenugreek seed powder in broiler diet produced moderate meat quality due to anti fat properties of the fenugreek.

Feldhusen *et al*. (1995) determined that the color of meat depends on both the amount and the degree of oxidation of heme pigments .In the present data, fenugreek supplemented in diet had a significant effect on color.

Palatability is the most important attribute affecting consumer selection and tenderness is usually assumed to be the most important organoleptic characteristic of meat (Seabra *et al*., 2001). In our study the result showed improve in an addition group with alfalfa. Ponte *et al.*(2005) reported that consumers might be less satisfied with meat derived from broilers consuming high levels of alfalfa.

In present study groups, with alfalfa additive(G3 and G4) showed significant improve in color might be due to the presence of saponins and pigments found in alfalfa that improved meat quality (Liu *et al.,* 2013). The color improvement of the breast and thigh muscles was the most influenced characteristic due to the dietary alfalfa addition, it has been reported to be a natural source of xanthophylls which make poultry carcases have a desirable yellow color (Ponte *et al*., 2004).

The color of meat largely depends on the composition of the muscle fibres (Ryu *et al*.,2008). Hence, the light color of muscles is related mainly to the high proportion of fibres in the muscle,

which are characterized by a low content of myoglobin and a high content of myofibrils (Ruusunen and Puolanne, 2004).Also carotenoids of plants are essential for improving skin pigmentation (Olgun and Yıldız, 2015).

Batkowska and Brodacki (2011) having analyzed the impact of the feeding system and the maintenance of turkeys on the color of meat and showed that turkeys fed with feed additives such as alfalfa characterized by a higher color index

These results are in contrast with those reported by Ouyang *et al.* (2016) who showed that alfalfa supplemental diet did not affect the meat color of the breast meat of broiler chickens.

Flavor is another important factor that determines meat quality ,in the current study the results revealed improvement in flavor in all dietary addition groups compared with control. These results are matched with Zheng *et al.*(2019) suggesting that alfalfa meal could improve the flavor in chicken meat through significantly increase the inosine monophosphate and delicious amino acid contents in breast muscle, which represent the active flavor components in chicken meat as compared to control.

Chapter Six: Conclusions and Recommendations

Conclusions and Recommendations

6.1. Conclusions

According to the results of this study, it can be concluded as follows :

1. Addition of fenugreek or / and alfalfa at 2.5 gm/kg diet led to high improvement in productive performance and health status.
2. Our study proved that adding fenugreek + alfalfa alone or in combination resulted in improve in all biochemical traits as comparing with control.
3. Antibody titers against Newcastle Disease and infectious bursal disease viruses were improved as compared with control.
4. A clear improvement in a quality of chicken meat in terms of tenderness, juiciness, color, palatability and flavor according to sensory trait tests.

6.2. Recommendation

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

1. . Fenugreek and alfalfa leaves can be effecting to use it as alternatives to the growth promoter or some ingredients of basal diet of broiler diets.
2. Study the effect of higher or lower level than 2.5 gm/kg of dried fenugreek leave or alfalfa leaves and their combination on physiological traits, carcass quality and immune system of chicken.
3. Use seeds of these herbs instead of leaves to study.
4. Inclusion fenugreek and alfalfa leaves into laying hens diets, to investigate their effect on egg yolk weight and color and other laying hens productive traits .
5. Using the active substances extracted from them to reduce the quantities added so as not to affect the design of the diet.

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Appendices

Appendix I

Estimation of serum cholesterol concentration (mg/dl):

Principle:

Easter of cholesterol+H2O *Chol. estease* Cholesterol + Fatty acids

Cholesterol +O2 *Chol. oxidase* Cholest-4-en-one+H2O2

H2O+4-Aminophenazone + phenol *peroxidase* Quinonimine

Reagent:

Reagent (1) Buffer solution: pipes PH 6.9 mmol/L, phenol 26 mmol/L

Reagent (2) vial of enzyme: cholesterol oxidase 300 U/L, peroxidase 1250 U/L, cholesterol esterase 300 U/L, 4-aminophenazone 0.4 mmol/L

Reagent (3): cholesterol standard 200 mg/dl

1. Manual procedure: Cholesterol concentration in serum samples was measured according to the following

a. Reagent and serum samples were brought to room temperature

b. Serum sample, blank and standard were treated as follow:

c. Tube contents were mixed and left to stand for 5 minutes at 37℃ before reading.

d. the absorbance of the standard was measured and sample was read via spectrophotometer at wavelength 505 nm against the blank

Tubes	Blank	Standard	Sample
Cholesterol standard (s)	-	- 10 ml	-
Sample	-	-	- 10 ml
Working Reagent	1 m	1 ml	1 ml

Appendices

Calculation:

Result were calculated according to the following equation:

Total Cholesterol concentration = (O.D sample)/ (O.D/ standard)× nn = 200 mg/dl

Appendix II

Estimation of serum triglyceride concentration (mg/dl):

Principle:

Triglyceride $\xrightarrow{lipoprotein\ lipase}$ Glycerol + fatty acid

Glycerol + ATP $\xrightarrow{Glycerol\ kinase, Mg++}$ Glycerol-3-phosphate+ADP

Glycerol-3-P+O2 $\xrightarrow{3-G-P-oxidase}$ Dihydroxyacetone ne-p+H2O

H2O2+4-Aminophenazone+p+Chlorophenol $\xrightarrow{peoxidase}$ Quinonimine+ H2O

Reagent:

Reagent (1) buffer solution: pipes buffer PH 7.2, 50 mm0l/L, p- chlorophenol 2 mmol/L

Reagent (2) Enzyme: lipoprotein lipase 150 000 U/I, glycerol kinase 800 U/I, glycerol-3-phosphate oxidase 4000 U/I, peroxidase 440 U/I, 4-aminophenazone 0.7 mmol/L, ATP 0.3 mmol/L.

Reagent (3) triglyceride Standard (S): Glycerol 200mg/dl.

Procedure:

Triglyceride concentration in serum samples was measured according to the following:

a. Wave length/filter. 505nm (Hg546nm)/green

b. Temperature 37℃/R.

c. Light path 1 cm

Pipette into clean dry test tubes labelled as Blank (B), standard (S), and Test

(T).

Mix well and incubated at 37℃ for 5 min or at R. T (25℃) for 15min.

measure the absorbance of the standard

Calculation:

Results were calculated according to the following equation:

Triglyceride concentration mg/dl = (O.D sample) / (O.D standard) × n = 200

mg/dl

Additive sequence	**Blank**	**Standard**	**Test**
Working reagent	**1.0**	**1.0**	**1.0**
Distilled water	**0.01**	-	-
Triglyceride standard	-	**0.01**	-
Sample	-	-	**0.01**

Appendix III

Estimation of serum HDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol esters + H2O *Chol.esterase* Cholesterol + fatty acid

Cholesterol +½O2 + H2O *Chol.oxidase* Cholestenone + H2O2

2 H2O2 + 4-Aminoantipyrine + DCFS *peroxidase* Quinoneimine + 4H2O

Reagent

Reagent (1) Good's buffer (pH 6.6)100 mmol/l, cholesterol esterase 1400 U/l, cholesterol oxidase 800 U/l, catalase 600 kU/l, N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) 0.6 mmol/l

Reagent (2) Good's buffer (pH 7.0) 100 mmol/l peroxidase 3 kU/l 4–aminoantipyrine (4-AA) 4 mmol/L

Appendices

Additive sequence	Blank	Standard	Test
Distilled water	50 ml		
Triglyceride standard		50 ml	-
Sample supernatant	-		50 ml
Reagent (B)	1.0 ml	0 ml	1ml

Procedure:

HDL-Cholesterol concentration in serum sample was measured according to the following steps: serum sample 40 – 60 mg/dl 1.04 1.55mmol/l, wavelength 600 nm,

temperature 37°C CORMAY HDL DIRECT is intended for automated analysers.

a. Reagent (A, B) and serum sample were brought to room temperature.

b. Serum sample, blank and standard were treated as followed:

c. 0.2 ml of sample was mixed with 0.5 ml of reagent (A) in centrifuge tube and let stand for 10 minute at room temperature.

d. Centrifuged at a minimum of 4000 r.p.m. for 10 minutes.

e. The temperature was collected carefully.

f. Sample supernatant, blank, standard and reagent (B)were treated as follows:

g. Tubes contents were mixed thoroughly and incubated for 10 minute at 37℃.

h. the absorbance (A) of the standard was measured and sample was read via spectrophotometerat wave length 500 nm against the blank.

Tubes Blank Standard Sample

Distilled water 50 ml - -

Appendices

Cholesterol standard (S) - 50 ml -

Sample supernatant - - 50 ml

Reagent (B) 1.0 ml 1.0ml 1.1ml

Calculation: results were calculated according to the following equation:

HDL-cholesterol concentration in the sample (mg/dl) = (Absorbance of the sample/Absorbance of standard) × concentration of standard × sample dilution factor (1.7).

Appendix IV

Estimation of serum LDL-Cholesterol concentration (mg/dl):

Principle:

Cholesterol ester $\xrightarrow{chol.esterase}$ chol. + fatty acid

Cholesterol + O2 $\xrightarrow{Chol.oxidase}$ chol. H2O2

2H2O2 $\xrightarrow{catalase}$ H2O + O2

Reagent:

Reagent (1) Good's buffer (pH 7,0) 50 mmol/l, cholesterol esterase 600 U/l, cholesterol oxidase 500 U/l, catalase 1200 kU/l, ascorbate oxidase 3 kU/l, TOOS [N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline] 2.0 mmol/l

Reagent (2) Good's buffer (pH 7,0) 50 mmol/l, peroxidase 5 kU/l, 4–aminoantipyrine (4-AA) 4 mmol/l.

Procedure:

wavelength 600 nm, temperature 37°C, CORMAY LDL DIRECT is intended for automated analysers. serum/plasma < 100 mg/dl < 2.59 mmol/l.

As LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges for local population.

Appendices

Calculation:

A comparison between LDL cholesterol values determined at Biolis 24i Premium (y) and at COBAS INTEGRA 400 (x) using 52 samples gave following results:

y = 0.9642 x - 0.8968 mg/dl;

R = 0.9762 (R – correlation coefficient)

Appendix V

Serum Aspartate aminotransferase (AST) activity determination:

Aspartate aminotransferase activity is (ASAT, AST, GOT) Measured by cormay GOT kit produced by PZ CORMAY S.A. company (Tietz, 1995).

Principle:

L-aspartate + 2-oxoglutarate <ASAT> oxalacetate + L-glutamate

oxalacetate + NADH + H+ <MDH > malate + NAD+

The rate of absorbance changing at λ=340 nm is directly proportional to aspartate aminotransferase activity.

	(24-TRAY)	(36-TRAY)
1-Reagent	6 x 40 ml	8 x 23 ml
2- -Reagent	6 x 12.5 ml	8 x 7.5 ml

Reagent:

Tris (pH 7.8) 80 mmol/l, L-aspartate 240 mmol/l, MDH > 10 μkat/l, LDH > 20 μkat/l, 2-oxoglutarate 15 mmol/l, NADH 0.18 mmol/l

Procedure:

These reagents may be used in automatic analysers Prestige 24i, Biolis 24i and Sapphire 400.

Appendices

1-REAGENT and 2-REAGENT are ready to use.

Parameter	Liquick Cor-ALAT 500	Liquick CorALAT "bulk"
1-ALAT	3 x 400 ml	-
2-ALAT	1 x 300 ml	-

1-Reagent put on basic position in reagent tray.

2-Reagent put on start position in reagent tray. For reagent blank deionized

water is recommended. Reagent blank is required each day

Calculation:

A comparison between ASAT values for samples obtained on

Prestige 24i (y) and obtained on COBAS INTEGRA 400 (x) using

100 samples gave following results:

y = 1.1501 x - 2.8845 U/l;

R= 0.9972 (R - correlation coefficient)

Appendix VI

Serum Alanine aminotransferase (ALT) activity determination:

Principle:

L-alanine + 2-oxoglutarate $ALAT$ pyruvate + L-glutamate

pyruvate + NADH + H+ LDH lactate + NAD+

The rate of absorbance changing at l=340 nm is directly proportional

to alanine aminotransferase activity.

Reagent:

Tris (pH 7.5) 100 mmol/l, L-alanine 500 mmol/l, LDH > 36.7 µkat/l 2-

oxoglutarate 15 mmol/l, NADH 0.18 mmol/l PROCEDURE

These reagents may be used both for manual assay (Sample Start and

Reagent Start method) and in several automatic analysers.

Applications for them are available on request.

Appendices

Simple start method:

Pipette into the cuvette. working reagent 1000µl, bring up to the temperature of determination. Then add simple 100µl, Mix and incubate at adequate temperature. After about 1 min. read the absorbance against air or water.

Repeat the reading after exactly1, 2 and 3 minutes. Calculate the mean absorbance change per minute (DA/min.).

Calculation:

ALAT activity [U/l] = DA/min. x F

Appendix VII

Procedure of determination immunity of ND and IBD by ELISA test

ELISA Kit (Synbiotics–USA)

The procedure used in this test was performed according to the manufacturer instructions listed in the ProFLOK® (ND and IBD) ELISA Kit (Synbiotics–USA), which is a rapid serologic test for the detection of IBV antibody in chicken serum samples. It was developed primarily to aid in the detection of post-vaccination (ND and IBD) antibody levels in chickens.

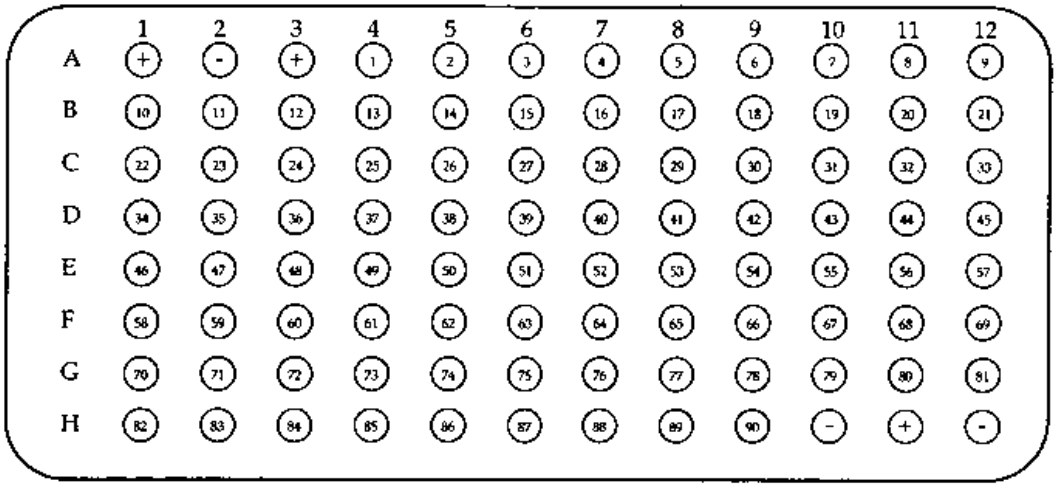

Figure Diagram explains microtitre plate test kit

Appendices

ELISA was performed as the following:-

Each test sample 1:500 was diluted by adding 1 µl to 0.5 ml of sample diluents.

1. Fifty µl of diluents was added to each plate that contained coated antigen.
2. Fifty µl of positive control was added in wells (H11, A3, and A1) for the plate.
3. Fifty µl of negative controls was added in wells (A2, A10, and H12) for the plate.
4. Fifty µl of diluted serum was added to the suitable wells for the presided plate.
5. Plate was incubated for 30 minutes at room temperature.
6. Plate holes were washed by using washing solution.
7. Hundred µl of diluted antiserum was added at ratio of 1:100 and conjugated with (horseradish peroxides) for each hole.
8. Plate was incubated for 30 minutes at room temperature.
9. All the plate holes were washed by using washing solution in amount of 300 µl twice with good drying.
10. Hundred µl of substrate was added to expose the color, and then the plates were left at room temperature in a dark place.
11. Hundred µl of stop solution (H_2SO_4) was added.
12. Results were read through recording the optical density absorbance of the control and the samples antibody titers were calculated automatically, by using ELISA reader using profile flock software.

6. أظهرت نتائج الاختبار الحسي أن النكهة ، الاستساغة ، العصيرية ، الطراوة واللون في عضلات الصدر والفخذ قد تحسنت في مجموعات الإضافة مقارنة مع مجموعة السيطرة ، باستثناء العصيرية والطراوة اللتان كانتا معتدلتان في مجموعة الحلبة مقارنة مع مجموعة السيطرة.

وفقا للنتائج الحالية،تبين انه يمكن استخدام الحلبة والجت كإضافة غذائية طبيعية جيدة في دجاج اللحم.كما أوضحت النتائج ان استخدام الجت بمفرده أفضل من استخدام الحلبة بمفردها لكن استخدامه مع الحلبة أعطى نتائج أفضل في تحسين الحالة الإنتاجية والصحية لفروج اللحم.

الخلاصـــــــــة

اجريت هذه الدراسة لتقييم تأثير الاضافة الغذائة للحلبة، الجت وخليطهما على الاداء الانتاجي وبعض الصفات الفسلجية في فروج اللحم. أجريت التجربة في حقل خاص لمدة 35 يوماً للفترة الممتدة من 22 كانون الثاني ولغاية 26 شباط 2022 م ،شملت الدراسة تربية 120 افراخ لحم (روز 308)غير مجنس وبعمر يوم واحد نوع تم تقسيمها إلى أربع مجاميع (30طير / مجموعة) بثلاث مكررات (10 طيور / مكرر) لكل مجموعة.المجموعة الأولى (G1): تغذت على العليقة الاساسية بدون اي إضافة . المجموعة الثانية(G2) تم تغذيتها على العليقة الاساسية + اوراق الحلبة بمستوى 2.5 غرام / كغم علف . المجموعة الثالثة(G3)) تم تغذيتها على العليقة الاساسية + 2.5 غرام من اوراق الجت/ كغم علف . المجموعة الرابعة(G4)) تم تغذيتها على العليقة الاساسية مضافا لها 2.5 غرام مناراق الحلبة + 2.5 غرام من اوراق الجت / كجم علف.

تم قياس معايير الأداء الإنتاجي اسبوعياً مثل وزن الجسم ، الزيادة الوزنية ، كمية العلف المستهلك ومعامل التحويل الغذائي طوال فترة التجربة. جمعت عينات الدم لقياس المعايير البايوكيميائية والمناعية بعمر 17 و 35 يوم من عمر التجربة .كما تم إجراء اختبار التذوق الحسي على عضلات الصدر والفخذ للذبائح في نهاية الدراسة

كشفت النتائج ما يلي:

1. هناك تحسن معنوي($p\leq0.05$) في وزن الجسم و الزيادة الوزنية وكمية العلف المستهلك ومعامل التحويل الغذائي في المجاميع G2, G3 ,G4 مقارنة مع مجموعة السيطرة.
2. في اليوم السابع عشر من العمر أظهرت المجاميع G2 و G4 وG3 زيادة معنوية($P\leq0.05$) في البروتين الكلي مقارنة مع السيطرة. كما أظهر الألبومين زيادة معنوية ($P\leq0.05$)في المجموعتين G2 و G4 مقارنة مع مجموعة السيطرة بينماG3 لم تظهراي اختلاف معنوي عند مقارنتها مع مجموعة السيطرة . أظهر الجلوبيولين زيادة معنوية($P\leq0.05$)في المجموعات G4 و G2 وG3 مقارنة مع مجموعة السيطرة. في عمر 35 يوم كانت هناك زيادة معنوية($P\leq0.05$) في البروتين الكلي في المجموعات G3 و G4 وG2 مقارنة مع مجموعة السيطرة كما أظهر الألبومين زيادة معنوية($P\leq0.05$) في مجموعات الاضافة مقارنة مع مجموعة السيطرة. الكلوبيولين اظهر زيادة معنوية($P\leq0.05$)في المجموعات G3 و G4 وG2 على التوالي بالمقارنة مع السيطرة.
3. كان هناك انخفاض معنوي($p\leq0.05$) في جميع مجاميع الاضافةG4, G2, G3 في الكوليسترول الكلي والدهون الثلاثية والبروتين الدهني منخفض الكثافة (LDL) بينما زاد البروتين الدهني عالي الكثافة(HDL) مقارنة مع مجموعة السيطرة.
4. تم قياس قيم إنزيمات وظائف الكبد مثل AST و ALT في نهاية التجربة وأظهرت انخفاضًا معنويا($P\leq0.05$) في مجاميع الإضافة مقارنة مع السيطرة.
5. بالنسبة لقياس معيار الأجسام المضادة ضد فايروسات مرض نيوكاسل ومرض الجراب المعدي كانت هناك زيادة معنوية($P\leq0.05$) في المجاميع G4 ، ثم G3 وG2 مقارنة بمجموعة السيطرة عند عمر 35 يوم.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الطب البيطري

تأثير إضافة مسحوق أوراق الحلبة والجت وخليطهما في عليقة فروج اللحم على بعض الصفات الأنتاجية والفسلجية

رسالة مقدمة الى مجلس كلية الطب البيطري / جامعة كربلاء وهي جزء من متطلبات نيل درجة الماجستير في اختصاص علوم الطب البيطري/الصحة العامة

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2022م **1444 هـ**