

## HEMATOLOGICAL AND IMMUNOLOGICAL ASPECT THAT INFLUENCED BY DIETARY FENUGREEK OR/AND ALFA-ALFA IN THE BROILERS

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### Abstract

This study, carried out to study the effect of dietary fenugreek, alfalfa and their mixture in broiler diet on some physiological traits and immunity of broilers. The experiment was conducted in special field during a period for 35 days. The study involved 120 one-day unsexed Ross 308 chicks, that were divided into four groups (30 birds/group) with three replicated (10 birds/replicate), the first group (control): chicks were fed on a basal diet without addition (G1), the second group fed on a basal diet with 2.5 gm fenugreek powder/Kg diet (G2), the third group fed on a basal diet with 2.5 gm alfalfa powder/Kg diet (G3), the fourth group was fed on a basal diet with 2.5 gm fenugreek and 2.5 gm alfalfa powder/Kg diet (G4). Blood samples were collected for biochemical parameters at 17<sup>th</sup> day and 35<sup>th</sup> day of age. The result revealed there were significant increase ( $P \leq 0.05$ ) in total protein, globulin and albumin with a significant decrease ( $p \leq 0.05$ ) in the cholesterol, triglycerides and low density lipoprotein (LDL) while the high density lipoprotein (HDL) recorded a significant increase ( $p \leq 0.05$ ), also significant decreases of aspartate transaminase (AST) and alanine transaminase (ALT) that present in the addition groups as compared with the control, the anti-body titer against New castle disease and infection bursal disease showed a significant increase ( $P \leq 0.05$ ) in addition group as compared with the control.

Key words: fenugreek, alfalfa, immunity, broiler.

**Introduction:** *Poultry industry is one of the most dynamic and growing divisions in the world, especially in growing countries (Alkhalaf et al., 2010). The global poultry sector is expected to grow continuously ever since the demand for meat is continuously increasing by the populations increase (Mottet and Tempio, 2017). 801*

Consumers' growing interest in bioactive components of natural origin has resulted as use of herbal plants, as beneficial ingredients in the industries of feed and food production (Sacchetti et al., 2005) 899

*Fenugreek (Trigonella foenum graecum) is one of the herbs having multi-functional characteristics such as antimicrobial, anti-inflammatory, hypoglycemic, hypolipidemic, hypocholesteremic, antipyretic, and antioxidative properties on animals, It contains several vital compounds, including flavonoids, alkaloids, saponins, vitamins, minerals, carbohydrates, and proteins (Srinivasan, 2006). (711) other roles of fenugreek including anti-atherosclerotic effect, and immunity stimulation have been confirmed ( Zadehet et al., 2015). 757*

*Alfaalfa* (*Medicago sativa* L.) is one of the cheapest sources of protein from the aspect of high yields and low production costs (Radović *et al* 2009). It is feedstuff with high fiber and with low metabolizable energy (Donalson *et al.*, 2005). It is a readily available, high protein, high fiber feedstuff with one of the slowest rates of passage through the avian system (Garcia *et al.*, 2000)(835). 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) 801. Alfalfa is also a natural source of xanthophylls, which, when deposited in the skin and shanks give poultry carcasses a desirable yellow color (Dansky, 1971). 802

So the objectives of this study were to evaluate the effects of fenugreek, alfalfa and their mixture on blood parameters and immune response of broiler chickens.

**Material and method** This study was carried out in a private breeding hall, from 22 Jan. to 26 Feb. / 2022. A total of 120 day-old broiler chickens was taken and randomly allocated into 4 groups with 3 replicates per group (10 chicks per replicate). G1 was used as a control, while the chicks of G2 fed basal diet with 2.5 gm of dry fenugreek leaves /kg diet, G3 fed basal diet with 2.5 gm dry alfalfa leaves powder/kg diet, G4 fed basal diet with 2.5 gm/kg dry fenugreek leaves and 2.5 gm dry alfalfa /kg diet.

Standard broiler diets for starter (0-21 day) and finisher (21-35 day) showed in table (1). The broiler chicks were housed in a litter system and provided *ad libitum* feed and water throughout the trial period. The temperatures were 33°C, 30°C, 28°C, 24°C and 22°C during the 1st, 2nd, 3rd, 4th and 5th weeks of age respectively. The lighting regime was 23:1 light-dark cycle. Table (2) showed the vaccination program.

Blood was collected from each group via brachial vein at days 17 and 35 of age, serum were separated, labeled and stored at -20°C until analysis, antibody titers against Newcastle Disease Virus and Infectious Bursal Disease virus in serum samples were detected at 35 days of age by using Enzyme Linked Immunosorbent Assay (ELISA technique) for different groups (Spalatin *et al.*, 1973).

Data obtained from the present study were analyzed as one-way analysis of variance (ANOVA) using Complete Randomized Design (CRD) procedure to SPSS 22.0 software (Corp, 2011). Four treatment means were separated by using a Duncan's analysis in level (0.05).

**Table ( 1). Ingredients and ofchemical analysisexperimental diets.**

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

**Chemical Analysis\*of basal diet**

Gross energy k cal/kg	3078	3125.2
Crude protein %	22.74	20.16
Energy/protein ration	135.35	155.07
Calcium %	0.97	1.0
Available Phosphate %	0.41	0.48
Methionine + cystein %	0.83	0.75
Lysine%	1.02	0.95
Methionine %	0.78	0.51

Chemical analysis according to(NRC, 1994).

**Table (2) program of vaccination**

Age of chicks (days)	Disease	Type of vaccine	Origin	Rout of vaccination.
1 <sup>st</sup>	Newcastle + Avian influenza+ bronchitis+ Infectiousbursal disease	Killed vaccine +MA5 +trans immune	Holland	Spray + injection
10 <sup>th</sup>	Newcastle disease	Clone 30 strain	Holland	Via drinking water
20 <sup>th</sup>	Newcastle disease	Clone30 strain	Holland	spray

**The results:-** Table (3) cleared the effect of fenugreek, alfalfa and their mixture on total protein, Albumin and Globulin at 17<sup>th</sup> days of age, the results showed a significant increase( $P \leq 0.05$ ) in

addition groups as compared with control, The same results were achieved at the 35<sup>th</sup> day of age in table (4), table (5) showed a significant decrease ( $P \leq 0.05$ ) in the serum cholesterol, triglyceride and LDL concentration of the addition groups at 17<sup>th</sup> days of age as compared with the control, since G4 had a lowest value as compared to other groups and G3 lower than G2, while the control recorded higher value, HDL recorded a significant increase ( $P \leq 0.05$ ) in addition groups, especially G2, as compared to control, also this results match with that at 35<sup>th</sup> day of age table (6),

Table (7) revealed a significant difference ( $P \leq 0.05$ ) in AST and ALT in all groups, G2, G3 and G4 groups showed a significant decrease ( $P \leq 0.05$ ) in AST and ALT as compared with the control group. Table finding also revealed that there were no significant differences between G2 and G3 groups, while there were a significant decrease ( $P \leq 0.05$ ) in value of AST and ALT at G4 as compared with other experimental groups, It could be noted that there is a statistical similarity in the results of AST and ALT among the groups, the antibody titers against Newcastle disease and infectious bursal viruses at 35<sup>th</sup> of age after adding fenugreek, alfalfa and their mixture in the diet of broilers that cleared in table (8), in present study there is a significant increase of G2, G3 and G4 on antibody titer against ND and Gumboro if compared with control group

**Table (3) Effect of fenugreek, alfalfa and their mixture on Protein profile of broilers at 17<sup>th</sup> days of age (Mean±SE):**

Total protein, albumin and globulin (gm/L)				
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 ( $p < 0.05$ ), G1(Control): basal diet only, G2: 2.5 gm/kg diet Fenugreek, G3: 2.5 gm/kg diet alfalfa, G4: 2.5 gm/kg diet Fenugreek + 2.5 gm/kg diet alfalfa.

**Table (4) Effect of fenugreek, alfalfa and their mixture on total Protein, albumin and globulin of broilers at 35<sup>th</sup> days of age (Mean±SE):**

Total Protein ,albumin and globulin (gm/L)				
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 ( $p < 0.05$ ), G1(Control):basal diet only,G2: 2.5 gm/kg diet Fenugreek, G3:2.5 gm/kg diet alfalfa, G4: 2.5 gm/kg diet Fenugreek +2.5 gm/kg diet alfalfa .

Table (5):Effect of fenugreek, alfalfa and their mixture on lipid profile of broilers at 17<sup>th</sup> days (Mean±SE):

Lipid profile (mg/dl)				
Group parameter	G1	G2	G3	G4
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 ( $p \leq 0.05$ ), G1(Control):basal diet only,G2:2.5 gm/kg diet Fenugreek, G3:2.5 gm/kg diet alfalfa , G4: 2.5 gm/kg diet Fenugreek +2.5 gm/kg diet alfalfa .

Table (6)Effect of fenugreek, alfalfa and their mixture on lipid profile of broilers at 35<sup>th</sup> days (Mean±SE):

lipid profile (mg/dl)				
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 ( $p \leq 0.05$ ), G1(Control):basal diet only,G2:2.5gm/kg diet Fenugreek, G3:2.5 gm/kg diet alfalfa , G4:2.5gm/kg diet Fenugreek +2.5 gm/kg diet alfalfa .

Table(7) Effect of fenugreek and alfalfa and their mixture on liver function enzymes of broilersat 35<sup>th</sup> days(Mean±SE):

AST, ALT (U/100ml)				
Group parameter	G1	G2	G3	G4
AST (U/100ml)	655.2 ±2.06 a	537.8 ±4.16 b	526.0 ±6.3 b	423.2 ±4.39 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 ( $p < 0.05$ ), G1(Control):basal diet only,G2:2.5gm/kg diet Fenugreek ,G3:2.5 gm/kg diet alfalfa , G4:2.5gm/kg diet Fenugreek +2.5 gm/kg diet alfalfa .

Table (8) Effect of fenugreek, alfalfa and their mixture on humeral immunity of broilerchicks at 35<sup>th</sup> days of the study (Mean± SE).

ND,IBD antibody titers				
Group parameter	G1	G2	G3	G4

NDv Antibody titter	9000 ±35.64 D	9800 ±35.04 c	10100 ±14.62 b	10990 ±17.73 a
IBDv Antibody titter	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 ( $p < 0.05$ ), G1(Control):basal diet only,G2:2.5gm/kg diet Fenugreek ,G3:2.5 gm/kg diet alfalfa , G4:2.5gm/kg diet Fenugreek +2.5 gm/kg diet alfalfa ,(ND )Newcastle disease ,(IBD) Gumboro.

## DISCUSSION :

The results of the current study showed a significant increase in total protein, globulin and albumin in the addition group (G2,G3and G4) as compared with control, the increment in total serum proteins might be attributed mainly to that fenugreek can stimulate the thyroid gland directly of serum increased significantly and led to increase serum protein content(Azoua, 2001), these results agree with Hassan, (2000) who reported that the total protein and globulin of serum increased significantly by feeding broiler chicks on additive diets with fenugreek seeds.

An increased total protein content in the blood plasma of the alfalfa groups may be due to the positive effect of the xanthophyll protein content of the alfalfaon protein metabolism in the animal body(Grela *et al.*,2008) is as other authors suggest attribute to biologically active substances, such as saponins with their beneficial properties that promote effective utilization of dietary proteins(Ender *et al.*, 1996). this result agrees with Elkomy and Elghalid, (2014) who found that the plasma total protein and albumin were progressively increased in alfalfa groups as compared to the control.

Our study revealed a significant decreases ( $P \leq 0.05$ ) in TC and triglyceride in G2, G3and G4 as compared with control. HDL values also increased significantly in addition groups as compared with control, particularly G2, while, LDL values presented adverse result to HDL in trial groups that were significantly decrease ( $P \leq 0.05$ ) speciallyatthe G4 as compared with control,a reduction in the serum cholesterol level when dietary supplements of fenugreek broiler diets could be due to the presence of saponins and resins in fenugreek such as hemicelluloses, mucilage, tannin and pectin which inhibit bile acid, help lesser 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).

These results agree with Abed El Latif and Enas,(2021)who reported that feeding broiler chicks on a diet containing fenugreek lowered total plasma lipids and cholesterol levels. El-Hack *et al.*, (2015) explained that a decrease in chicken serum total cholesterol concentration and an increase

in high density lipoprotein cholesterol concentration duo to fenugreek seed extract supplementation improvement in HDL led to reduce in LDL-cholesterol.

Our study showed that there were significantly decrease in the TC, TG and LDL in G3 and G4, which supplement with alfalfa as compared with the control, the best value recorded in G4 that received mixture of fenugreek and alfalfa, numerous researchers explain that high saponin levels (approximately 2–3%) were found in the groups additive with alfalfa which are known for the hypocholesterolemic (Francis *et al.*, 2002), saponins which are involved in the reduction of total cholesterol levels through the formation of insoluble complexes with cholesterol, which inhibits their absorption. Besides, they increase secretion of bile, gastric, and intestinal juices (Khaleel *et al.*, 2005).

These results consistent with Mansoub and Myandoab, (2012) who reported that serum total cholesterol and triglycerides levels were significantly reduced in group fed alfalfa compared to the control group, Guiwen *et al.*, (2021) reported that the TG was moderately lower in the Alfalfa supplementation groups at the level 50 gm /kg diet than in the control.

AST or ALT levels of blood are very important as they are indicators of liver status, current, present study indicated that there were a significant decrease ( $P \leq 0.05$ ) in AST and ALT of G2, G3 and G4 groups as compared with the control, alkaloids, including trigonelline, carpine, and gentianine compounds are the most important alkaloids of fenugreek, it seems vitamins A and B1 component of fenugreek are effective in liver function and might decrease ALT and AST enzyme levels (Moradi *et al.*, 2013).

Consistent with the results of Jafar *et al.*, (2021) who indicated all groups that fed on fenugreek showed a significant improved hepatic function with significantly decreased in the AST and ALT level, which explained that, liver and its normal functioning is very vital in broilers where Saponin, vit. A, B1, C, nicotinic acid, and alkaloids are active ingredient which can act as immunomodulators and liver tonic components. Guiwen *et al.*, (2021) showed that alfalfa supplementation groups at the ratio 25 gm/kg was significantly lower the level of AST than that in the other groups.

Antibody titer against NDV and IBDV at 35 day of broilers age indicated that the G2, G3 and G4, which supplemented with 2.5 gm fenugreek, 2.5 alfalfa and combination of these two herbs respectively, revealed achieved higher and best titer against two studied diseases, because it have higher antibody titer, but the control recoded lowest titer against these viruses, the cause of these increases might be immunomodulating ability of these herbs to improve immunity by active components such as flavonoids, steroid saponin, or by raising the weight of lymphatic tissue (Abid *et al.*, 2011) these results approve with Bin-Hafeez *et al.*, (2003) who recorded that the fenugreek increase the immunity of broiler at 24 and 34 day, thus These herbs increase the cellularity of the thymus gland as well as the bone marrow, they also noticed increasing the action of macrophage

and humeral response by plaque forming cell assay , as just as Abed and Fateh, (2014) showed that adding of 1% fenugreek in the broiler chicken diet recorded a high antibody titer against ND<sub>v</sub> and IBD<sub>v</sub> .

In current study saponins and flavonoids have the function of immune improvement, which has been found in alfalfa polysaccharides (Jiang and Yu, 2005). Polysavone, a compound containing the three active ingredients., exhibited obvious activity in improving immunity, may be as a result of synergism among its active ingredients or the simple combination of the functions of each component.

Another explanation might be that the plant with feed additive properties can decrease the pathogenic microorganisms, motivating the immune system, and then strengthen the immune system of broilers. Newly it was resumed that, the supplementation with alfalfa 4%, 6%, and 7.3% in the diet of broiler chickens revealed increasing the proliferation of useful bacteria, thus immune strengthening against pathogenic bacteria (Gulizia and Downs, 2020; Pliego *et al.*, 2020).899

**Conclusion** In the present study some of beneficial acts toward using fenugreek and alfalfa on biochemical and some immune parameters on broiler chicks were demonstrated. We may conclude it will be possible that the fenugreek and alfalfa concentration at 2.5 gm/kg diet had a good immunomodulatory ,liver tonic effect,improve total protein and it also could reduce the risk of hyperlipidemia.

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