



## Effect of different levels of Fenugreek powder supplementation on performance, Influenza, Sheep red blood cell, New Castle diseases anti-body titer and intestinal microbial flora on Cobb 500 broiler chicks

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**Abstract.** The aim of this study was to investigate the effect of using fenugreek powder on performance, some immune parameters and intestinal micro-flora on broiler chicks. A total of 320 one day Cobb 500 broiler chicks were divided and assigned into 4 groups and 5 replicates of 16 birds each. Chicks were fed by basal diet as control, 3 levels (0.7, 1.4 and 2.8 percentage) of fenugreek powder respectively. During the experimental period feed intake (FI), body weight gains (BWG) and feed conversion ratio (FCR) were calculated. Also, after 30 and 38 days last, the blood samples were taken from wing vein to evaluate the sheep red blood cell (SRBC) and anti-body against Influenza and New castle disease vaccine (ND). For evaluation carcass traits 4 birds of the same weight in each group were slaughtered, separated and weighed. The result obtained from this study showed that the highest FI related to the fenugreek powder and highest BW was seen in the groups that the fed by 2.8 % powder. Also, there were significant differences between treatments about FCR ( $p \leq 0.05$ ). The SRBC, ND and Influenza titer as an immune system was for 2.8 % fenugreek powder respectively. The carcass evaluation mentioned that the highest carcass percentage was for 2.8 % fenugreek powder and there were significant differences between groups about intestine and gizzard percentage. The results showed that different levels of fenugreek powder used in experimental broilers had significant effects on intestinal microbial population. We also concluded that fenugreek powder at the present levels can enhance body performance, some carcass visceral percentage, some immune system parameters titer in Cobb 500 broiler chicks.

**Keyword:** Broiler, Fenugreek, Performance, Immune system, Intestinal microbial population.

### Introduction

The effect of herbals as phytogetic and feed additives on broiler nutrient and digestibility has been well mentioned by many researchers, herbal feed additives are plant derived products used in poultry feeding to improve the consumption and conversion of food, and the digestibility and weight gain of broiler chickens [WINDISCH, 2008, PENTEA *et al.*; 2016].

Fenugreek (*Trigonella foenum-graecum* L.) a well-known medicinal plant is cultivated in Iran, India and Pakistan [ABBAS, 2010, VARDANIAN *et al.*; 2018].

It is a good source of dietary protein for consumption by human and animals. It is having properties of lowering blood sugar level, anthelmintic, antibacterial, anti-inflammatory, antipyretic, and antimicrobial.

It contains minerals, B complex, iron, Phosphates, (Para Amino Benzoic Acid), A and D vitamins, lecithin and choline that help to dissolve cholesterol and fatty substances [DIXIT *et al.*, 2005, CAUNII *et al.*; 2015; IANCULOV *et al.*; 2004].

It also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system [AHMADIANI *et al.*, 2001, SAMFIRA *et al.*; 2014; BUTNARIU; 2012; BUTU *et al.*; 2014a].

Gacche and collab. reported moderate level of anti-proteolytic activity in fenugreek [GACCHE *et al.* 2010].

Fenugreek contains coumarins and other constituents that might affect platelet aggregation, but this might not be significant clinically. It contains different alkaloids, flavonoids and saponins but out of all these, saponins are found to be in



maximum concentration in the fenugreek [TARIQ *et al.*, 2016; KUMARI *et al.*, 2012].

Elmahdi Elbushra noted that significant improvements in efficiency of energy utilization values in average feed consumption were recorded for the groups fed diets with 0.5 and 1.5 % during the experiment period [ELMAHDI ELBUSHRA, 2012].

Also, Dixit and collab. reported that fenugreek seeds powder improved broiler metabolism [DIXIT *et al.*, 2005].

Gomez and collab. showed that the improvement in body weight gain may be due to antibacterial related to flavonoids in fenugreek that led to maintaining normal intestine microflora population [GOMEZ *et al.*, 1998, BUTNARIU and SAMFIRA, 2012; IANCULOV *et al.*; 2005].

Weerasingha and Atapattu conclude that use of fenugreek powder improved the (FCR) by 13.8 % [WEERASINGHA and ATAPATTU 2013], compared control group.

Also, they showed that dietary fenugreek linearly increased the relative

length of the small intestine and the weight of the pancreas.

Several researchers reported that supplementation of poultry diets with fenugreek seed powder reduced plasma total lipids and total cholesterol in broiler chicks [AZOUZ, 2001; FAESTE *et al.*, 2009] and improve the performance of broiler breeders [TAHA, 2008, PUTNOKY *et al.*; 2013, BUTNARIU *et al.*; 2014; BUTNARIU and GIUCHICI, 2011].

To the best of our knowledge the aqueous solution of fenugreek seeds on the carcass quality has not been reported.

The present study was conducted to investigate the effect of fenugreek powder on performance, some immune response and intestinal microbial population on Cobb 500 broiler chicks.

## Material and methods

### Birds, diets and the management

A total 320 one day Cobb 500 broiler chicks were divided and assigned into 4 groups and 5 replicates of 16 birds each.

**Table 1.**

Composition of the experimental diets for experimental chicks

Ingredients %	0–14 (days)	14–21 (days)	21–42 (days)
Corn grain	54.8	59.4	65
Soybean meal	39.6	35.5	30
Soybean Oil	1.45	1.50	1.70
Di Calcium Phosphate	1.70	1.45	1.30
Oyster shells	1.05	0.95	0.86
Methionine – D–L	0.310	0.250	0.230
Lysine –L	0.22	0.10	0.10
Edible Salt	0.3	0.3	0.25
Vitamin Premix*	0.25	0.25	0.25
Mineral Premix*	0.25	0.25	0.25
Threonine –L	0.09	0.04	0.04
<b>Calculated nutrient content</b>			
ME(Kcal/Kg)	2850	2900	2940
CP (%)	21.8	20.4	18.4
Ca (%)	0.91	0.82	0.74
Available Phosphorus (%)	0.46	0.41	0.37
Lysine (%)	0.180	0.120	0.130
Methionine+Cystine (%)	0.92	0.84	0.75

\*Supplied Per Kilogram of Feed: 7.500 IU of vitamin A, 2000IU vitamin D3, 30 Mg vitamin E, 1.5 µg vitamin B12, 2Mg B6, 5 Mg. Vitamin K, 5 Mg vitamin B2, 1 Mg vitamin B1, 40 Mg nicotinic acide, 160 µg vitamin Biothine, 12 Mg Calcium pantothenate, 1 Mg Folic acid 20 Mg Fe, 71 Mg Mn, 100 µg Se, 37 Mg Zn, 6 Mg Cu, 1.14 Mg I, 400 µg Cu.

Chicks were fed by basal diet as control, 3 levels (0.7, 1.4 and 2.8 %) of fenugreek powders. During the aviculture period FI, BWG and FCR were measured.

Feed and water were given ad libitum. Control and treated groups received diet that they formulated according to NRC, 1994 (Table 1).



Chicks were reared in cages in an open sided house, provided with feeders, drinkers, electric bulbs and sand was used as bedding material.

Fenugreek seeds were purchased from vegetables market and milled and was prepared according to the method described by [MOMENI *et al.*, 2010, BUTNARIU *et al.*; 2012].

#### Evaluation of carcass yield

Carcass yield was calculated as eviscerated carcass with neck, feet, and abdominal fat pad removed, as percentage of live body weight at the time of feed withdrawal.

#### Determine Influenza, Sheep red blood cell and New castle diseases

For determine the SRBC test after washed sheep red blood cells injection to broilers at 30 days old and influenza vaccine at 38 days old, the samples of blood via wing veins were taken.

Using hemagglutination inhibition test (HI) according to the method described by [BEARD, 1989] with chicken red blood cells and four units of ND and SRBC antigen, and then geometric mean titers were calculated.

The internal organs were removed after slaughter.

About 8 cm from the length of the ileum was sampled to determine the microbial population [DIMITRIU *et al.*; 2016; GEORGIEVA *et al.*; 2018; BUTNARIU and CAUNII, 2013].

Also 1 g of ileum content was used to make 10-fold dilution using buffered peptone water and then 0.1 mL of the

appropriate ileum dilution was spread on *Lactobacillus* MRS1 Agar-Hi Media Laboratories to detect lactic acid bacteria and violet red bile agar to detect *Escherichia coli* and *Lactobacillus* colonies form.

The cultures of *Lactobacillus* and *Escherichia coli* bacteria were made an aerobically form. The plates were incubated at 37.5 °C for 48 h.

After counting the number of colonies in each plate, the number so obtained was multiplied by inverse of the dilution and the result was stated as the number of colonies forming unit (CFU) in 1 g of the sample described by Downes and Ito [DOWNES and ITO, 2001].

Also, ileocecal intestinal microbial population of *Escherichia coli* and *Lactobacillus* bacteria were investigated as described by Dumonceaux and collab. [DUMONCEAUX *et al.*, 2006].

#### Statistical analysis

Data analysis was performed by using the general linear model procedure and the comparison of means was made through Duncan's (1995) multiple range test by using SAS 9.1 software [SAS, 2001].

#### Results and discussion

Data on feed intake, body weight, feed conversion ratio and Pre-slaughter weigh are shown in (Table 2). Mean feed and was none significant in control and treated groups.

**Table 2.**

The effect of experimental diets on broilers performance

Treatments	FI (g.d)	BW (g.d)	FCR	Pre-slaughter weigh (g)
Control	94.3	47.3 <sup>a</sup>	1.99 <sup>ab</sup>	2276.4 <sup>a</sup>
0.7% Fenugreek	90.4	46.3 <sup>ab</sup>	1.97 <sup>b</sup>	2250.5 <sup>ab</sup>
1.4% Fenugreek	91.1	41.4 <sup>b</sup>	2.21 <sup>a</sup>	2064.5 <sup>b</sup>
2.8% Fenugreek	95.4	45.9 <sup>ab</sup>	2.09 <sup>ab</sup>	2243.3 <sup>ab</sup>
SEM	2.42	1.84	0.086	76.2

\*Means within column with no common on letter are significantly different ( $p \leq 0.05$ ).

The body weight and feed conversion ratio were significantly heavier ( $P \leq 0.05$ ) in treated compared to control.

The results of this experiment show clearly a positive effect of fenugreek on the body weight gain and Pre-slaughter weigh of broiler chicks ( $p \leq 0.05$ ).

Also mean feed intake was higher but none significant in control and treated groups.

This positive effect of fenugreek powders in feed consumption could be evaluated on the basis of different perspectives, that fenugreek as natural



feed additives improved diet palatability and lead to higher feed intake [ALLOUI *et al.*, 2012].

Using of fenugreek powder levels lead to FCR differences.

This might be related to the development of the broiler chicks gut morphological changes of gastrointestinal tissues can be induced by differences in

gut fluid of microbial content including their metabolites.

Result showed that supplementation of fenugreek powder in various levels improved significantly ( $p \leq 0.05$ ) body weight and Pre-slaughter weigh [table 3](#).

**Table 3.**

The effect of experimental diets on some visceral organs percentage

Treatments	Intestine	Liver	Gizzard	Carcass
Control	5.56 <sup>ab</sup>	2.29	1.79 <sup>a</sup>	69.5 <sup>a</sup>
0.7 % Fenugreek	6.09 <sup>a</sup>	2.58	1.52 <sup>ab</sup>	68.7 <sup>b</sup>
1.4 % Fenugreek	5.63 <sup>ab</sup>	2.34	1.57 <sup>ab</sup>	66.3 <sup>b</sup>
2.8 % Fenugreek	5.21 <sup>b</sup>	2.36	1.48 <sup>b</sup>	70.9 <sup>a</sup>
SEM	0.30	0.28	0.090	1.30

\*Means within column with no common on letter are significantly different ( $p \leq 0.05$ ).

This could be due to the presence of the fatty acids, or due to stimulating effect on the digestive system of broilers [HERNANDEZ *et al.*, 2004] and may be attributed to increase of feed intake or to the fenugreek contents of active compounds such as anti-bacterial, antifungal, anti-inflammatory, carminative and antioxidant activities.

The findings of current study are also in agreement with those of Alloui and collab. who noted that addition of fenugreek seed in broiler diets increased live body weight [ALLOUI *et al.*, 2012] and El-Kloub, who indicated that fenugreek seeds at level of 0.05 % revealed no significant effect on feed consumption compared to the control group [EI-KLOUB, 2006].

In this experiment, we obtained better carcass weight percentage in experimental group having treated with of 2.8 % of fenugreek powder. Also, the better intestine weight percentage in treated

group with of 0.7 % fenugreek powder ( $p \leq 0.05$ ).

There were no significant differences between liver weight percentages among treated and control group.

Weerasingha and Atapattu noted that, the relative length of the small intestine was significantly higher for birds given 2, 4 or 5 % fenugreek than those fed 0 or 1% fenugreek [WEERASINGHA and ATAPATTU, 2012].

Khan and collab. reported that fenugreek seed extract had no impact on visceral organs (liver, heart, gizzard, and intestines) of broiler chicks [KHAN *et al.*, 2011].

There was a significant linear increase in relative length of the small intestine with increasing dietary fenugreek levels.

Feeding fenugreek powder significantly decreased gizzard weight, and significant effect on intestine weight and liver. These results were in line with results of [GUO *et al.*, 2004; MUKHTAR *et al.*, 2013].

**Table 4.**

The effect of experimental diets on some Immune response

Treatments	Influenza (Log <sup>2</sup> )	ND (Log <sup>2</sup> )	SRBC (Log <sup>2</sup> )
Control	4.12 <sup>b</sup>	5.75	6.62
0.7 % Fenugreek	4.87 <sup>a</sup>	6.75	7.75
1.4 % Fenugreek	4.87 <sup>a</sup>	5.85	8.25
2.8 % Fenugreek	4.37 <sup>ab</sup>	5.78	8.38
SEM	0.24	0.15	0.52

\*Means within columns with no common on letter are significantly different ( $p \leq 0.05$ ).



Table 4 indicated that feeding different type fenugreek extracts supplementation significantly ( $p \leq 0.05$ ) affected Influenza titer.

Broiler that they fed by fenugreek powder had the higher and significant values ( $p \leq 0.05$ ) compared to the control.

Fenugreek reported to have anti-diabetic, anti-fertility, anti-cancer, anti-microbial, antiparasitic, hypo-cholesterolaemic effects and has been reported to have antimicrobial hypoglycemic, hypolipidemic, and antioxidant effect on animals [BASCH *et al.*, 2003, GROZEA *et al.*; 2017].

Abid and collab. demonstrated that the fenugreek increasing the immunity of birds at 24 and 34 day and because fenugreek increases the cellular ties of

thymus gland and bone marrow [ABID *et al.*, 2011, BUTU *et al.*; 2014c, SAMFIRA *et al.*; 2015, BUTNARIU *et al.*; 2015b; BUTU *et al.*; 2014b].

Awad and collab. results suggest that the fenugreek seed [AWAD *et al.*, 2015], specially the highest dosage used in the present work could be considered a good food supplement to improve the immune status and increase the production of gilthead sea bream.

Abed and collab. showed that treatment that supplemented with 1 % fenugreek recorded high antibody titer against Newcastle disease virus and Gumboro disease virus at 21 and 35 day of broilers age [ABED *et al.*, 2014].

The effects of experimental diets on intestinal micro-flora are shown in Table 5.

**Table 5.**

The effect of experimental diets on Intestinal micro-flora

Treatments	<i>Escherichia Coli</i> (CFU. g)	<i>Lactobacillus</i> (CFU. g)
Control	4.91 <sup>a</sup>	5.62 <sup>d</sup>
0.7 % Fenugreek	4.57 <sup>b</sup>	5.95 <sup>c</sup>
1.4 % Fenugreek	4.12 <sup>c</sup>	6.25 <sup>b</sup>
2.8 % Fenugreek	4.00 <sup>d</sup>	6.38 <sup>a</sup>
SEM	0.06	0.14

\*Means within columns with no common on letter are significantly different ( $p \leq 0.05$ ).

Data showed that there were significant differences between treatments about intestinal micro-flora by using fenugreek powder.

Faghani and collab. concluded that the use of turmeric extract enhanced useful microbial population in and decrease the *Escherichia coli* in broiler chicks [FAGHANI *et al.*, 2014, BAGIU *et al.*; 2012; BUTNARIU and CORADINI, 2012].

Valiollahi and collab. found that the inhibitory action of sumac on *Escherichia coli* population [VALIOLLAHI *et al.*, 2014].

Also, Nasar Abbas and Halkman reported that water extract of sumac at the rate of 0.1, 0.5, 1.0, 2.5 and 5% showed bacteriostatic /bactericidal effects against gram positive and negative bacteria [NASAR ABBAS and HALKMAN, 2004, PETRACHE *et al.*; 2014, BUTNARIU *et al.*; 2014, BARBAT 2013, BUTU *et al.*; 2015].

Zomorodian and collab. showed that the use of ajwain could inhibit *Escherichia coli* in intestinal tract [ZOMORODIAN *et al.*, 2011]. The findings of the present study agree

with Nayaka and collab. who observed that inclusion of turmeric [NAYAKA *et al.*, 2013] at 1g/kg diet lowered microbial colony in the ileal content of broiler chickens.

### Conclusions

We could conclude that some beneficial acts toward using fenugreek powder on performance, some immune response and intestinal microbial population on broiler chicks. In conclusion it can be said that (0.7, 1.4 and 2.8 %) of alcoholic and aqueous extract of fenugreek produced positive results in broiler chicks.

This improvement may be due to the biological functions to improve growth, digestibility and other activities of fenugreek. Although the beneficial effects on health and growth are believed to be mediated by effects on gastrointestinal macrobiotic, the underlying mechanisms remain to be discovered. It was concluded from this study that though the fenugreek



has beneficial effect on the weight of the studied parameters in broiler chicks.

It may be possible that the higher levels of fenugreek powder concentration may produce better result in term of weight of these studied parameters in broilers chicks. More research is needed on the other microbial and other biochemical parameters of fenugreek treated broilers.

Also, further studies are needed to more detail explanation.

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