

Article

## Performance, some immune parameters and intestinal microbial flora of Ross 308 broiler chicks fed by Fenugreek essential oil

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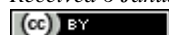
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### Abstract

This study was to investigate the effect of using fenugreek essential oil on performance, some immune parameters and intestinal micro-flora on broiler chicks. A total of 240 one day Ross 308 broiler chicks were divided and assigned into 4 groups and 5 replicate of 16 birds each. Chicks were fed by basal diet as control and 3 levels (100, 200 and 250 ppm) of fenugreek essential oil respectively. During the experimental period feed intake, body weight gain and feed conversion ratio were calculated. 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) respectively. For evaluation carcass traits 4 birds of the same weight in each group were slaughtered, separated and weighed. The obtained result showed that the highest feed intake related to the fenugreek essential oil and highest body weight was seen in the groups that the fed by fenugreek essential oil. Also there were significant differences between treatments about feed conversion ratio ( $p \leq 0.05$ ). The SRBC, ND and Influenza titer as immune system parameters was for fenugreek essential oil respectively. The carcass evaluation mentioned that the highest carcass percentage was for fenugreek oil and there were significant differences between groups about intestine and gizzard percentage. The results showed that different levels of fenugreek oil used in experimental broilers had significant effects on intestinal microbial population flora. We may conclude that fenugreek essential oil at the present levels could have better body performance, some carcass parameters, and immune system in Ross 308 broiler chicks.

**Keywords** broiler chicks; fenugreek essential oil; performance; immune system; intestinal microbial population.

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## 1 Introduction

Fenugreek is a well-known medicinal plant is cultivated in Iran and has some dietary protein for consumption by both human and animals. It is having properties of lowering blood sugar level, anthelmintic, antibacterial, anti-inflammatory, antipyretic, and antimicrobial. It contains minerals, Vitamin B, iron, phosphates, A and D vitamins, lecithin and choline that help to dissolve cholesterol and fatty substances (Abbas, 2010; Dixit et al., 2005). Fenugreek contains neurin, biotin, tri-methylamine which tends to stimulate the appetite by their action on the nervous system (Ahmadiani et al., 2001). Moderate level of anti proteolytic activity in fenugreek and it 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; Gacche et al., 2010). Dixit et al. (2005) and Elmahdi-Elbushra (2012) showed that fenugreek seeds powder improved broiler metabolism and (Gomez et al. 1998) 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. Weerasingha and Atapattu (2013) showed that dietary fenugreek linearly increased the relative length of the small intestine and the weight of the pancreas. Several researcher 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). The aim of present study was to determine the effect of using fenugreek oils on performance, immune response and intestinal microbial population on Ross 308 broiler chicks.

## 2 Material and Methods

### 2.1 Birds, diets and the management

A total 240 one day Ross 308 broiler chicks were divided and assigned into 4 groups and 5 replicates of 12 birds each. Fenugreek oil was purchased from Shahre-Kord vegetables market and was prepared and used as a feed additive. Broiler chicks were fed by basal diet as control and 3 levels (100, 200 and 250 ppm) of fenugreek oil respectively. During the experimental period feed intake, body weight gain and feed conversion ratio were calculated. The experimental groups received diet formulated according to NRC, 1994 (Table 1). Feed and water were given ad libitum.

### 2.2 Carcass yield and characteristics

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.

### 2.3 Immune parameter assay

To determine the sheep red blood cell 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 according to the method described by (Beard, 1989) with chicken red blood cells and four units of New castle disease and sheep red blood cell test 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. 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 colony forming unit (CFU) in 1 g of the sample described by (Downes and Ito, 2001).

Also illeocecal intestinal microbial population of *Escherichia coli* and lactobacillus bacteria colonies were investigated as described by (Dumonceaux et al., 2006).

**Table 1** Composition of the experimental diets for experimental chicks.

<b>Ingredients %</b>	<b>0-14 (days)</b>	<b>14-21 (days)</b>	<b>21-42 (days)</b>
Corn grain	54.8	59.4	65
Soybean meal	39.6	35.5	30
Oil	1.45	1.50	1.70
DCP	1.70	1.45	1.30
Oyster shells	1.05	0.95	0.86
Methionine -D-L	0.310	0.250	0.235
Lysine-L	0.22	0.10	0.10
Threonine-L	0.09	0.04	0.04
Nacl	0.30	0.30	0.30
Vitamin and Mineral Premix*	0.50	0.50	0.50
<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, 2000 IU vitamin D3, 30 Mg vitamin E, 1.5 µg vitamin B12, 2 Mg 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, 1Mg Folic acid 20 Mg Fe, 71 Mg Mn, 100 µg Se, 37 Mg Zn, 6 Mg Cu, 1.14 Mg I, 400 µg Cu.

## 2.4 Data 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).

## 3 Results and Discussion

Results showed that the average of feed intake was none significant in control and treated groups (Table 2). And the body weight and feed conversion ratio were significantly heavier ( $p \leq 0.05$ ) in treated compared to control instead. The results showed some positive effect of fenugreek oil supplementation on the body weight and pre-slaughter weigh ( $p \leq 0.05$ ). The effect of fenugreek oil in feed consumption can be explained on the basis of different perspectives that fenugreek oil as natural feed additives improved diet palatability and lead to higher feed intake, and 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 (Alloui et al., 2012).

**Table 2** The effects of experimental diets on Ross 308 broilers performance.

Treatments	FI (g.d)	BWG (g.d)	FCR	Pre-slaughter (g)
Control	94.5	47.5 <sup>a</sup>	1.98 <sup>b</sup>	2272.5 <sup>a</sup>
100 ppm Fenugreek	90.5	46.5 <sup>ab</sup>	1.97 <sup>b</sup>	2252.8 <sup>ab</sup>
200 ppm Fenugreek	91.2	41.5 <sup>b</sup>	2.25 <sup>a</sup>	2062.2 <sup>b</sup>
250 ppm Fenugreek	95.5	45.5 <sup>ab</sup>	2.10 <sup>ab</sup>	2242.5 <sup>ab</sup>
SEM	2.45	1.85	0.078	72.1

<sup>a, b</sup> Means within columns with different superscripts are different ( $p \leq 0.05$ ).

Supplementation of fenugreek oil improved significantly body weight and pre-slaughter weight. 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.

**Table 3** The effects of experimental diets on carcass characteristics.

Treatments	Intestine	Liver	Gizzard	Carcass
Control	5.55 <sup>ab</sup>	2.25	1.79 <sup>a</sup>	69.9 <sup>ab</sup>
100 ppm Fenugreek	6.02 <sup>a</sup>	2.55	1.52 <sup>ab</sup>	68.8 <sup>ab</sup>
200 ppm Fenugreek	5.64 <sup>ab</sup>	2.32	1.57 <sup>ab</sup>	66.5 <sup>b</sup>
300 ppm Fenugreek	5.25 <sup>b</sup>	2.35	1.48 <sup>b</sup>	70.7 <sup>a</sup>
SEM	0.30	0.28	0.085	1.41

<sup>a, b</sup> Means within columns with different superscripts are different ( $p \leq 0.05$ ).

There were no significant differences between liver weight percentages among treated and control group (Table 3). Weerasingha and Atapattu (2012) 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. Khan et al. (2011) reported that fenugreek seed extract had no impact on visceral organs (liver, heart, gizzard, and intestines) of broiler chicks. 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).

Fenugreek oil supplementation significantly changed influenza but not ND and SRBC titer and chicks were fed by fenugreek oil had the higher and significant values compared to the control (Table 4). 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). Abid et al. (2011) 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. Awad et al. (2015) results suggest that the fenugreek seed, 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. As (Abed et al., 2014) showed supplemented with 1% fenugreek recorded high anti-body titter against Newcastle disease virus and Gumboro disease virus.

**Table 4** The effects of experimental diets on some immune response.

Treatments	Influenza (Log <sup>2</sup> )	ND (Log <sup>2</sup> )	SRBC (Log <sup>2</sup> )
Control	4.15 <sup>b</sup>	5.75	6.60
100ppm Fenugreek	4.82 <sup>a</sup>	5.72	7.70
200ppm Fenugreek	4.85 <sup>a</sup>	5.80	8.21
300ppm Fenugreek	4.38 <sup>ab</sup>	5.71	8.41
SEM	0.20	0.15	0.60

<sup>a, b</sup> Means within columns with different superscripts are different ( $p \leq 0.05$ ).

**Table 5** The effects of experimental diets on intestinal microflora.

Treatments	<i>Escherichia coli</i> (CFU.g)	Lactobacillus (CFU.g)
Control	4.90 <sup>a</sup>	5.60 <sup>b</sup>
100 ppm Fenugreek	4.55 <sup>b</sup>	5.90 <sup>ab</sup>
200 ppm Fenugreek	4.15 <sup>c</sup>	6.21 <sup>a</sup>
250 ppm Fenugreek	4.05 <sup>c</sup>	6.32 <sup>a</sup>
SEM	0.10	0.12

<sup>a, b</sup> Means within columns with different superscripts are different ( $p \leq 0.05$ ).

Results showed that there were significant differences between treatments about intestinal micro-flora by using fenugreek oil (Table 5). Faghani et al. (2014) concluded that the use of turmeric extract enhanced useful microbial population in and decrease the *Escherichia coli* in broiler chicks. Additionally (Valiollahi et al., 2014) found that the inhibitory action of sumac on *Escherichia coli* population. Also Nasar-Abbas and Halkman (2004) reported that water extract of sumac at the rate of 0.1, 0.5, 1.0, 2.5 and 5% showed bacteriostatic and or bactericidal effects against gram positive and negative bacteria. In Zomorodian et al. (2011) study use of ajwain could inhibit *Escherichia coli* in intestinal tract. The findings of the present study are in agreement with Nayaka et al. (2013) who observed that inclusion of turmeric at 1g per kg diet lowered microbial colony in the ileal content of broiler chickens.

#### 4 Conclusion

In the current study some of beneficial acts toward using fenugreek oil on performance, some immune parameters and intestinal microbial population on broiler chicks were demonstrated. We may conclude it will be possible that the higher concentration of fenugreek oil may produce better result in term of weight of studied parameters in broilers chicks. This improvement may be due to the biological functions to improve

growth, digestibility and other activities that found in fenugreek. Although the beneficial effects on health and growth are believed to be mediated by effects on gastrointestinal microbiotic, the underlying mechanisms remain to be discovered. Also more research is needed on the other microbial and other biochemical parameters of fenugreek oil treated broilers and further studies are needed to more detail explanation.

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