



EFFECT OF RAW AND ENZYME-TREATED DANDELION LEAVES AND FENUGREEK SEED SUPPLEMENTED DIET ON GUT MICROFLORA OF BROILER CHICKEN

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ABSTRACT

Present study was aimed to assess the effects of diet supplemented with or without enzyme treated 0.5% dandelion (*Taraxacum officinale*) leaves (DL) or/and 1% fenugreek (*Trigonella foenum graecum*) seeds (FS), fed for 42 days on gut microflora of broiler chicken. Cobb chicks (273) were randomly distributed into 7 groups with 13 chicks in each replicate treatment. Birds in control were fed with only basal diet. The study revealed that caecal total viable and coliform counts were significantly ($p < 0.05$) low in birds fed with supplemented diets as compared to control with least population of 7.05 ± 0.03 and 4.07 ± 0.05 cfu g⁻¹, respectively, in chicken fed with enzyme treated 0.5% DL + 1% FS basal diet. The pH and moisture content of chicken litter showed no significant variation amongst the treatments. Both DL and FS showed beneficial health effects in terms of reducing the harmful gut bacteria.

Keywords: Antibacterial, broiler, dandelion, fenugreek, litter

INTRODUCTION

Phytogenics are defined as a group of natural growth promoters or non-antibiotic growth promoters derived from herbs, spices or other plants. In recent past, there has been an increasing trend in their use as feed additives in poultry. As against antibiotics, these products are safe, less toxic, residue free; hence ideal feed additives in animal production (Hashemi *et al.*, 2008). They primarily affect the ecosystem of gastrointestinal microbiota in a positive way through controlling potential pathogens (Hashemi and Davoodi, 2010). Dandelion (*Taraxacum officinale*) is a well known medicinal plant mostly found in Asia, Europe and North America (Malik *et al.*, 2011). Their roots primarily support digestion and liver function; and leaves serve as diuretic and digestive stimulant (Mir *et al.*, 2013). Yarnell and Abascal (2009) reported that dandelion leaves contain phenylpropanoids, triterpenoid, saponins and many other components that attribute to their anti-bacterial activity. Fenugreek (*Trigonella foenum graecum*) is grown mainly in India, Pakistan and China and contains lecithin and choline that are helpful in dissolving the cholesterol and fatty substances. It also possesses many other components like neurin, biotin, trimethylamine, etc. which tend to have a stimulatory effect on appetite by their action on the nervous system. Its seeds reportedly have many therapeutic effects such as anthelmintic, anti-inflammatory, hypoglycaemic and anti-microbial properties (Bash *et al.*, 2003). Based on the aforementioned properties, and availability of these two herbal plants in Kashmir, a research study was conducted to explore antimicrobial potential of dandelion leaves and fenugreek seeds as natural feed additives in the diets of broiler chicken.

MATERIALS AND METHODS

One day-old 'Cobb' broiler chicks (273) were used for the study in the year 2015. The chicks were reared in battery cages until 7 days age. During this period all the birds were provided with a pre-starter diet (23% crude protein and 2800 kcal kg⁻¹ metabolisable energy). Birds were reared in deep litter system; had free access to feed and water and were maintained on a constant 24 hr light schedule. On 8th day, the chicks were individually weighed, distributed into 7 groups with 13 chicks in each group, replicated 3 times, in a completely randomized design. The birds in control group were fed basal diet only as per the recommendations of BIS (1992). The other 6 groups were fed basal diet supplemented with either 0.5% dandelion leaves or 1% fenugreek seeds or a combination of 0.5% dandelion leaves and 1% fenugreek seeds with or without enzyme treatment. The enzyme used comprised of a cocktail product of cellulase 180000000, amylase 125000, xylanase 1800000, phytase 200000, protease 16000, lipase 40000 and pectinase 7000 IU g⁻¹ and added @ 50 g 100 kg⁻¹ feed. The plant products were procured from local market, dried and subjected to proximate analysis (Table 1) as per AOAC (2005). Dandelion leaves and fenugreek seeds in powder form were mixed thoroughly in aforesaid quantities to 1 kg feed in a premixer which was then mixed with the remainder of feed in a mechanical blender until a thorough and consistent mixture was obtained. All chicks were vaccinated against Ranikhet disease on 5th day with F1 strain vaccine and IBV-95 vaccine against infectious bursal disease on 16th day. Chicks were checked twice daily for mortality, if any. Birds were kept under similar management, environmental and hygienic conditions.

At the end of experiment (i.e. after 42 days) two birds per treatment-replicate were randomly selected and sacrificed by dislocation of atlantooccipital joint. The intestines were opened and ligated at both sides. The caecum contents were collected aseptically. Caecum samples (1 g from each bird) was transferred to sterile tubes; homogenized with sterile 0.9% normal saline solution (1:1) and then the resultant solutions mixed on vortex. Serial dilutions of samples were achieved upto 6th dilution. The 0.1 mL of each dilution was poured and uniformly spread on brain heart infusion (BHI) agar for caecal total viable count and on McConkey's agar for caecal coliform count. The plates were incubated at 37°C for 48 hr and bacterial colonies counted as per Quinn *et al.* (1992).

At the end of study period, litter samples from each pen were collected at five locations (four peripheral, equidistant from each pen corner and one central) and mixed thoroughly to obtain representative material of entire shed. For pH measurement, 5 g of each litter sample was suspended in 50 mL distilled water for 30 min., stirred for 5 min. and pH measured with the help of a pH meter. For moisture determination, 100 g litter sample from each treatment was oven-dried for 24 hr at 105°C in a hot air oven and then reweighed and moisture content calculated as per AOAC (2005). The data was statistically analyzed using analysis of variance and values expressed as mean $\pm$ standard error through general linear model procedure of SPSS (10.0) software. DMRT test was applied to assess significant difference between means at $p < 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

The results of proximate analysis of dandelion leaves (DL) and fenugreek seeds (FS) obtained in the present study were in close approximation with the results those of Khan *et al.* (2013) who reported that DL contains 15.3% crude protein (CP) and 14.5% crude fibre (CF). Kochhar *et al.* (2006) reported that fenugreek seeds have 11.7% moisture, 25.8% CP, 6.5% ether extract and 6.24% CF. Some variations in these values may be due to the species variation of the seed, its maturity level and nutrient composition of the soil.

The gut microflora revealed significant ($p < 0.05$) difference in caecal total viable and coliform bacterial counts in birds fed diets supplemented with either raw or enzyme treated dandelion leaves

or fenugreek seeds as compared to control (Table 2). However, there was no significant difference in groups supplemented with dandelion leave or fenugreek seeds or combination of both with or without enzyme. Lowest counts in caecal total viable (7.05 ± 0.03 cfu g⁻¹) and coliform (4.07 ± 0.05 cfu g⁻¹) bacteria were observed in birds fed an

Table 1: Proximate analysis of dandelion leaves and fenugreek seeds

Attribute	Dandelion leaves	Fenugreek seeds
Dry matter (%)	90.66	89.79
Moisture (%)	9.34	10.21
Crude protein (%)	11.4	25.2
Ether extract (%)	3.3	6.46
Crude fibre (%)	13.81	6.84

Table 2: Effect of raw and enzyme treated Dandelion leaves (DL) and Fenugreek seeds (FS) on gut microflora count (cfu/g) of broiler chicken and litter pH and moisture level

Parameter	Control	0.5% DL	1% FS	0.5% DL + 1% FS	Enzyme + 1% FS	Enzyme + 0.5% DL	Enzyme + 0.5% DL + 1% FS
Caecal total viable count	8.72±0.01 ^a	8.27±0.01 ^b	7.22±0.03 ^c	7.07±0.6 ^d	8.29±0.02 ^b	7.24±0.03 ^c	7.05±0.03 ^d
Caecal coliform count	5.77±0.04 ^a	5.17±0.07 ^b	4.19±0.02 ^c	4.08±0.02 ^d	5.16±0.01 ^b	4.18±0.05 ^c	4.07±0.05 ^d
pH	7.48±0.03	7.51±0.05	7.50±0.02	7.44±0.03	7.51±0.01	7.43±0.03	7.41±0.08
Moisture (%)	20.54±0.65	19.88±0.44	21.11±0.09	19.98±0.23	19.89±0.67	20.69±0.28	21.25±0.57

Means within the same row with different superscripts are significantly different ($p \leq 0.05$)

enzyme treated dandelion leaves and fenugreek seeds as compared to 8.72 ± 0.01 and 5.77 ± 0.04 cfu g⁻¹ observed in control birds. When individual effects of dandelion leaves and fenugreek seeds were compared, dandelion leaves showed better effect on gut microflora viz., 8.27 ± 0.11 and 5.17 ± 0.07 cfu g⁻¹ caecal total viable and coliform counts compared to 7.22 ± 0.03 and 4.19 ± 0.02 cfu g⁻¹, respectively, in birds fed diet supplemented with fenugreek seeds. Yan *et al.* (2011) have reported a significant reduction in faecal coliform count with dandelion extract powder @ 1g kg⁻¹ feed compared to the control group in pigs. Jassim *et al.* (2012) reported that dandelion extract has antibacterial property both against Gram positive and Gram negative bacteria, especially against *Staphylococcus aureus*, *Proteus mirabilis* and *Escherchia coli*. Yarnell and Abascal (2009) attributed the antibacterial activity of dandelion leaves to the presence of phenylpropanoids, triterpenoid, saponins and many other components in it. The antibacterial activity of fenugreek seeds has also been reported (Becker *et al.*, 2010, Akbari *et al.*, 2012). Fenugreek seeds significantly bind with the toxins produced by enterogenic *E. coli* (Becker *et al.*, 2010). Akbari *et al.* (2012) reported that the fenugreek seeds were significantly effective in inhibiting the growth of *E. coli*, *Shigella dysenteriae*, *Salmonella typhi* and *Pseudomonas* species which has been attributed to the presence of phenols and saponins in fenugreek. Thus both dandelion leaves and fenugreek seeds showed beneficial impact on caecal microflora of broiler chicken.

The pH of litter materials at the end of experimental period of 42 days varied in birds from 7.41 ± 0.08 to 7.51 ± 0.05 with no significant variations within treatments (Table 2). The pH in different treatment groups was within normal range and was in agreement with earlier workers (Wheeler *et al.*, 2008, Valéria *et al.*, 2011). The moisture content of litter material varied from 19.88 ± 0.44 and $21.25 \pm 0.57\%$ with no significant difference in various treatment groups. The litter moisture content in different treatments was within reported normal range (Benabdewelil and Ayach, 1996, Monira *et al.*, 2003). Litter quality is of utmost importance e.g., the litter material having too high moisture could increase the risk of pathogenic microbial growth. The results suggest that the supplementation of dandelion leaves and fenugreek seeds in diet decreased the caecal total viable and coliform bacterial count, particularly when used in combination. The strategy of feeding dandelion leaves and fenugreek alone or in combination may be useful in maintaining caecal microflora ecology for better performance.

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