Culinary and medicinal herbs as feed additives, effect on performance, serum biochemical parameters and microbial population of broiler chickens

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Abstract. Study was carried out to evaluate the effect of culinary and medicinal herbs as feed additive on the performance of broiler chickens. One day old (N=280) unsexed broiler chicks were divided into seven treatments. For each treatment was allocated 40 chicks in four replicates of ten individuals. Single starter and finisher basal diet (BD) was formulated. Seven treatments were: (C) BD control, (C1) BD + *Cuminum cyminum* 1%, (C2) *C. cyminum* 0.5%, (F1) BD + *Foeniculum* vulgare 1%, (F2) BD + *F. vulgare* 0.5%, (Z1) BD + *Achillea wilhelmsii* 1%, and (Z2) BD + *A. wilhelmsii* 0.5%. The treatment groups supplemented with herbal additive revealed improved weight gain, average daily gain and feed conversion (P<0.05). Treatments supplemented with cumin showed better growth performance, followed by *F. vulgare* and *A. wilhelmsii* (P<0.05). Supplementation of herbal additives had effect on ileum bacterial enumeration (P<0.05). Herbal feed additives showed its activity as antibacterial as well as prebiotic and supported the lactic acid bacteria (P<0.05). In conclusion the present study revealed that supplementation of herbs as feed additives positively affected growth parameters in more than single criterion and could be used as growth promoters.
Key words: Herbal additives, intestinal flora, growth performance, growth promoter.

Introduction. It is thought that herbs can be used as alternative to antibiotic growth promoting feed additive. The long term use of antibacterial products as growth promoter at sub therapeutic dose resulted in antibiotic resistant microbial strains in poultry, therefore is considered threatening for humans and ban has imposed over the use of these products as growth promoter (Aarestrup et al 2001; Jang 2011) and in consequences there have been reports indicating in increased disease out breaks (Casewel et al 2003).

Herbs are long used to treat humans and animals and a huge proportion of the masses still depends and uses these natural remedies (Pan et al 2014). These natural compounds carry out different type of activities. Generally important activities both under in-vitro and in-vivo conditions has been reported are antibacterial, antifungal, antioxidant, immune modulators, enzyme secretions stimuli, antispasmodic and gut environment modifiers (Bhatt 2015).

The cumin (*Cuminum cyminum* L) vernacular name, Zeera is an aromatic spice and cultivated in many countries. Cumin seeds contain several phytobiochemicals known to have antibacterial, antioxidant, carminative and anti-flatulent properties. The active components in the cumin increase the motility of the gastro-intestinal tract, increased enzyme secretions. There are many reports attributing the effective use of cumin as growth promoter feed additive in broiler (Al-Kassi 2010; Shabaan 2012; Rafiee et al 2014).

The fennel (*Foeniculum vulgare* Mill) vernacular name Saunf, is an ancient seasonal herb. The herb was well-known to the ancient Egyptians, Romans, Indians, and

Chinese. It is a cultivated plant and also grows wild found worldwide. All parts of the plant are aromatic and can be used in many ways. Fennel has also been tested as feed additive and reported to cause the increase in the carcass yield, improved feed efficiency, and serum biochemical parameters (Mohammad & Abbas 2009).

Some chemical (coumarin derivative) constituents from fennel have been identified as active antimicrobial agent (Beleni et al 2015). The essential oil from the fruit and the seed and different type of extracts has been reported to exert antimicrobial and antifungal activity (Mohsenzadeh 2007; Pai et al 2010).

Another medicinal herb vernacular name Zawal/Bohe-Madran (*Achillea wilhelmsii* C. Koch) is a perennial herb and it prevails in uplands of Western Asia, Europe, Australia and New Zealand (Dokhani et al 2005). Traditionally it is used for the treatment of certain ailments in humans (Tareen et al 2010). The main compounds as reported by Alfatemi et al (2015) of the EO includes carvacrol (22.49%), dihydrocarvone (13.23%), linalool (12%), 1,8-cineol (11.42%), camphene (8.31%), thymol (5.28%), camphor (3.71%), pulegone (2.82%), α-pinene (2.2%), terpineol (2.11%), bornyl acetate (1.14%) and farganol (1.01%). *A. wilhelmsii* has a wide range of reported biological activities, including antioxidant (Alfatemi et al 2015), antihyperlipidemia (Asgary et al 2000), anti-tumoral (Csupor-Loffler et al 2009) and antispasmodic (Yaeesh et al 2006) properties. Different biochemical compounds like, monoterpenes, are found in essential oils and it has powerful antibacterial effects (Amjad et al 2011). Keeping in view study was carried out to evaluate the effect of culinary and medicinal herbs as feed additives on the performance of broiler chickens.

The objective of the study was to evaluate the effect of herbal additives and introduce new possible sources that could be used as growth promoter feed additives.

Material and Method

Experimental units management. A total of two hundred and eighty (n=280) day old mixed sex Hubbard broiler chicks were randomly divided in seven treatments groups (40 chicks per treatment) further divided into four replicates of ten chicks respectively in floor pans. Each replicate treated as experimental unit. Measured quantity of feed was administered in the morning and evening with slight modifications from Hubbard guide at high altitude. Water was provided *ad libitum*. Vaccination against Newcastle disease (intraocular/drinking water), infectious bronchitis and infectious bursal disease in drinking water was carried out.

Feed and experimental layout. Single basal diet (BD) starter (initial three weeks) and finisher (final three weeks) was formulated using indigenous ingredients. Ingredient and nutrient composition is given in Table 1. The seeds of cumin and fennel and aerial parts of herbs were ground and mixed thoroughly in the feed as per requirement and used during the experiment. The dietary treatments were: (C) BD control, (C1) BD + *C. cyminum* 1%, (C2) BD + *C. cyminum* 0.5%, (F1) BD + *F. vulgare* 1%, (F2) BD + *F. vulgare* 0.5%, (Z1) BD + *A. wilhelmsii* 1%, and (Z2) BD + *A. wilhelmsii* 0.5% respectively.

Weekly data of live body weight (LBW) and feed consumed (FC) per replicate was recorded and weekly weight gain (WG), average daily gain (ADG), feed efficiency (FE) was calculated. On day 42 at the end of the trial one broiler from each replicate (4 birds per treatment) was picked at random; weighed and slaughtered by severing jugular vein. Blood samples were collected from slaughtered broilers with and without heparin in tubes for hematological red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb) and mean corpuscular volume (MCV) and serum biochemical parameters total protein, albumin, globulin, alanine aminotransferase (ALT) and aspartate amino transferase (AST) were included. Weight of carcass and visceral organ were measured and their relative weight was calculated.

Table 1

Composition and calculated analysis of the starter and finisher diet

Ingredients	*Starter diet (%)*	*Finisher diet (%)*
Corn	52	58
Wheat bran	2.5	2.0
Soybean meal	18	19
Canola meal	5.0	4.0
Cotton seed meal	4.0	3.0
Peas	10	7.5
Corn gluten 60%	3.0	00
Oil	3.0	4.0
Lysine	0.2	0.2
Methionine	0.3	0.3
$CaCO_3$	1.8	1.8
Vitamin mineral premix	0.2	0.2
Total	100	100
	Calculated analysis	
Metabolizable energy Kcal/kg	2983.3	3103
Crude protein (%)	21	19.1
Lysine (%)	1.1	1.0
Methionine (%)	0.5	0.4
Calcium (%)	1.0	0.8
Phosphorus (%)	0.5	0.4

Vitamin mineral premix provides per kg of diet: vitamin A, 9,000 IU; D3, 2,000, IU; E, 18 IU; B1, 1.8 mg; B2, 6.6 mg; B3, 10 mg; B5, 30 mg; B6, 3.0 mg; B9, 1 mg; B12, 1.5 mg; K3, 2 mg; folic acid, 0.21 mg; nicotinic acid, 0.65 mg; biotin, 0.14 mg; choline chloride, 500 mg; Mn, 100 mg; Zn, 85 mg; Fe, 50 mg; Cu, 10 mg; I, 1 mg; Se, 0.2 mg.

Bacterial enumeration. Prior slaughter a 10 h diet was implemented. Immediately after slaughter intestine was removed and samples of the digesta from ileum (1 gram in 9 mL physiological saline) was collected in sterile falcon tubes for bacterial enumeration aseptically. Subsequently homogenate tenfold serially diluted up to 10^{-8} and cultured from 10^{-3} to 10^{-8} dilution for countable numbers. Brain heart infusion agar (LAB) plates were used for total aerobe bacterial counted, MacConkey agar (OXOID) for Coliform and MRS agar (OXOID) plate for lactic acid bacterial enumeration in duplicate were used at 37°C for 24 to 48 hours (Mountzouris et al 2007). The results are expressed as positive log_{10} colony forming units (log_{10} CFU) per gram.

Statistical analysis. For the comparison of means one way analysis of variance (ANOVA) technique was used using SPSS-16 software for windows. The difference between treatments was determined by Duncan’s Multiple Range test at 5% error and the results are presented as mean±standard error (Mean±SE).

Results and Discussion. The effect of treatment on the growth performance indices is presented in Table 2. The inclusion of culinary and medicinal herb to the broiler feed increased weight gain, daily weight gain and improved feed efficiency significantly ($P<0.05$). Highest daily weight gain was noted in cumin added treatment groups and rate of inclusion had no significant effect in all three additive groups ($P>0.05$). In this experiment feed was given at controlled rate and the results showed use of cumin slightly depressed feed intake. Similar trend observed in other additive groups. Highest feed intake observed in control ($P<0.05$) group followed by group Z1. Feed efficiency was significantly improved with the herbal additive ($P<0.05$). Treatment groups supplemented with cumin exhibited better performance in terms of feed utilization. There was no significant difference noted between treatments F1, 2 and Z1, 2 respectively ($P>0.05$).

Table 2
Effect of culinary and medicinal herbs on the performance of broiler chicken on day- 42

Treatments	*Live weight gain (kg/bird)*	*Feed consumed (kg)*	*Feed efficiency (feed kg/weight gain kg)*	*Average daily gain (g)*
C	1.96 ± 12.78^{c}	3.93 ± 29.51^{a}	2.00 ± 0.004^{a}	46.72 ± 0.3^{c}
C1	2.21 ± 16.3^{a}	3.84 ± 24.42^{b}	1.74 ± 0.012^{c}	52.55 ± 0.39^{a}
C2	2.21 ± 15.74^{a}	3.84 ± 36.21^{b}	1.73 ± 0.01^{c}	52.77 ± 0.37^{a}
F1	2.09 ± 12.8^{b}	3.88 ± 38.33^{ab}	1.85 ± 0.021^{b}	49.78 ± 0.30^{b}
F2	2.10 ± 13.96^{b}	3.90 ± 11.84^{ab}	1.85 ± 0.008^{b}	50.16 ± 0.33^{b}
Z1	2.09 ± 18.75^{b}	3.91 ± 21.88^{ab}	1.86 ± 0.021^{b}	49.97 ± 0.44^{b}
Z2	2.08 ± 11.38^{b}	3.90 ± 5.98^{ab}	1.87 ± 0.009^{b}	49.64 ± 0.27^{b}

Different superscript within same columns indicate significant difference ($P<0.05$).

The impact of medicinal and culinary feed additives on visceral organs characteristics is given in Table 3. Highest carcass yield (71.90) revealed in F2 treatment group, statistically significant differences were observed between groups ($P<0.05$). The relative weight of liver, gizzard and intestine was unaffected by the supplementation of additives ($P>0.05$). The additives increased abdominal fat deposition and highest values observed in F2 followed by Z2, least abdominal fat was present in the control group ($P<0.05$)

Table 3
Effect of culinary and medicinal herbs on relative organ weight of broiler chicken on day 42

Treatments	*Carcass*	*Liver*	*Gizzard*	*Heart*	*Intestine*	*Abdominal fat*
C	70.43 ± 0.34^{c}	2.21±0.058	2.72±0.02	0.65 ± 0.008^{a}	4.15±0.015	1.67 ± 0.08^{b}
C1	70.91 ± 0.25^{bc}	2.19±0.029	2.66±0.018	0.59 ± 0.0025^{b}	4.26±0.039	1.73 ± 0.025^{a}
C2	70.66 ± 0.25^{c}	2.10±0.048	2.67±0.029	0.59 ± 0.006^{b}	4.20±0.039	1.74 ± 0.058^{a}
F1	71.19 ± 0.50^{abc}	2.15±0.062	2.70±0.02	0.63 ± 0.007^{b}	4.22±0.074	1.70 ± 0.077^{a}
F2	71.90 ± 0.18^{a}	2.16±0.037	2.69±0.017	0.63 ± 0.016^{a}	4.24±0.061	1.76 ± 0.064^{a}
Z1	$71.68\pm0.14b^{c}$	2.14±0.065	2.69±0.016	0.63 ± 0.01^{a}	4.22±0.067	1.72 ± 0.09^{a}
Z2	71.02 ± 0.15^{abc}	2.16±0.046	2.71±0.005	0.65 ± 0.013^{a}	4.21±0.047	1.75 ± 0.03^{a}

Different superscript within same columns indicate significant difference ($P<0.05$).

Hematological parameters examined in the present study revealed no significant difference in RBC count ($P<0.05$). The WBC showed highest value in the control group and it differ significantly from other treatment groups ($P<0.05$), the effect of supplementation of additive to the feed had no effect on the WBC count. Significant differences in the mean corpuscular volume was seen among treatment groups ($P<0.05$). Highest Hb and MCV values were noted in C1 and C2, while lowest values was in the control group ($P<0.05$). Use of *F. vulgare* and *A. wilhelmsii* had no effect on MCV values (Table 4).

Table 4
Effect of culinary and medicinal herbs on hematological parameters of broiler chicken on day 42

Treatments	*RBC*	*WBC*	*Hb*	*MCV*
C	2.97±0.047	32.12±0.31a	9.29 ± 0.33^{b}	27.50 ± 0.28^{b}
C1	3.20±0.07	29.12±0.65b	10.75 ± 0.25^{a}	30.75 ± 0.47^{a}
C2	3.20±0.04	29.25±0.62b	10.60 ± 0.46^{a}	30.75 ± 0.75^{a}
F1	3.22±0.047	29.37±0.74b	10.37 ± 0.36^{ab}	29.25 ± 0.47^{ab}
F2	3.20±0.04	29.25±0.59b	10.42 ± 0.38^{ab}	28.25 ± 0.47^{b}
Z1	3.17±0.025	29.62±0.55b	10.51 ± 0.49^{B}	29.25 ± 0.75^{ab}
Z2	3.15±0.028	29.87±0.23b	10.50 ± 0.42^{ab}	28.50 ± 0.64^{b}
Total	3.14±0.026	29.80±0.26	10.35±0.15	29.17±0.29

Different superscript within same columns indicate significant difference ($P<0.05$). RBC (10^6); WBC (10^3); Hb mg/100mL; MCV %.

The effect of herbal additive on the serum biochemical parameters is summarized in Table 5. There was a significant differences in the total serum protein and albumin content of the treatment groups (P<0.05). Treatment groups C1, C2 and Z1, Z2 noted with highest values of total protein and albumin (P>0.05). ALT revealed insignificant (P>0.05) between treatments; while slight increased AST values noted in the control group as compare to other treatments (P<0.05).

Table 5

Effect of culinary and medicinal herbs on serum biochemical parameters of broiler chicken on day-42

Treatments	*Total Protein**	*Albumin**	*Globulin**	*ALT***	*AST ***
C	3.42 ± 0.047^{b}	1.37 ± 0.047^{b}	2.05 ± 0.095	26.32 ± 0.65	242.39 ± 3.23^{a}
C1	3.57 ± 0.085^{ab}	1.57 ± 0.047^{a}	2.00 ± 0.108	25.96 ± 1.43	230.57 ± 3.88^{b}
C2	3.57 ± 0.062^{ab}	1.60 ± 0.04^{a}	1.97 ± 0.075	25.87 ± 1.38	229.04 ± 4.24^{b}
F1	3.50 ± 0.091^{b}	1.50 ± 0.04^{ab}	2.00 ± 0.07	26.83 ± 1.62	233.44 ± 4.01^{ab}
F2	3.52 ± 0.047^{ab}	1.47 ± 0.047^{ab}	2.05 ± 0.064	26.55 ± 1.01	236.55 ± 2.51^{ab}
Z1	3.75 ± 0.028^{a}	1.57 ± 0.047^{a}	2.17 ± 0.062	25.76 ± 0.52	237.22 ± 2.61^{ab}
Z2	3.50 ± 0.108^{b}	1.50 ± 0.07^{ab}	2.00 ± 0.057	25.69 ± 1.09	235.92 ± 2.08^{ab}
Total	3.55 ± 0.030	1.51 ± 0.021	2.03 ± 0.028	26.14 ± 0.39	235.02 ± 1.36

Different superscript within same columns indicate significant difference (P<0.05).
* mg/100mL; ** IU/L.

The bacterial enumeration from the intestinal section (ileum) is given in Table 6. The total aerobe count was observed as ($7\log_{10}$) and highest count was in the control group followed by F (1,2) and Z2 (P<0.05). The coliform bacteria were enumerated at ($5\log_{10}$) and supplementation of the *C. cyminum* resulted in least number of coliform, while Highest CFUs were found in control group (P<0.05). The supplementation of additives supported lactic acid bacterial population proliferation and minimum CFUs were noted in control groups (P<0.05). The comparison between *C. cyminum* and *A. wilhelmsii* revealed no differences (P>0.05).

Table 6

Effect of culinary and medicinal herbs on the bacterial enumeration (CFU/g) on day-42

Treatments	*Aerobe*	*Coliform*	*Lactic acid bacteria*
C	8.25 ± 0.25^{a}	6.75 ± 0.25^{a}	4.50 ± 0.28^{c}
C1	6.25 ± 0.25^{d}	5.00 ± 0.40^{b}	6.75 ± 0.47^{a}
C2	6.50 ± 0.28^{cd}	5.25 ± 0.47^{b}	6.50 ± 0.50^{ab}
F1	7.50 ± 0.28^{ab}	6.00 ± 0.40^{ab}	5.25 ± 0.47^{bc}
F2	7.50 ± 0.28^{ab}	5.75 ± 0.25^{ab}	5.50 ± 0.50^{bc}
Z1	7.25 ± 0.25^{bc}	5.50 ± 0.28^{b}	6.50 ± 0.57^{ab}
Z2	7.50 ± 0.28^{ab}	6.00 ± 0.40^{ab}	6.25 ± 0.50^{ab}
Total	7.25 ± 0.15	5.75 ± 0.15	5.89 ± 1.06

*Different superscript in the same column indicate significant difference (P<0.05).

In present study the supplementation of culinary and medicinal herb used as additive exhibited better performance in terms of weight gain, feed efficiency and other important serum biochemical parameters. In the present study data suggests positive effects of all the herbal treatments. Ertas et al (2005), Toghyani et al (2010) and Goodarzi et al (2014) used different herbs as additives and reported increased performance parameters. Mohammad & Abbas (2009) used *F. vulgare* and reported significant enhanced weight gain. In another study Jeved et al (2009) used seven herbal extracts as supplement and suggested their usage as growth promoters could be a potential resource in the way to avoid usage antibacterial feed additives. However, there has been an argument that the condition in which the experiment is conducted plays a vital role; under germ free conditions additive and antibiotics fail to exhibit their growth promoting effects (Bedford 2000; Cross et al 2007; Hernandez et al 2004).

In the present study data regarding feed intake suggested a slight decrease in each treatment as compare to control. In earlier studies (Hernandez et al 2004; Windisch et al 2008) reported beneficial effects of herbal supplements and decreased feed consumption and better feed efficiency. This decline may be due to the presence of aromatic compounds. Earlier, Cabuk et al (2006) and Williams et al (2008) reported decreased feed intake while using fructo-oligo-saccharides (FOS) and blend of oils. However *F. vulgare* has been reported to contain appetite enhancement agents (Cabuk et al 2006).

The increased live weight gain of the treatments receiving supplement can be attributed to better nutrient utilization through proper digestion and their increased absorbance from the intestine. These differences among the treatments may be due to their stimulating effect on digestive secretion (Cabuk et al 2006; Franciosini et al 2016). Increased feed efficiency with the *F. vulgare*, *Origanum vulgare* and *Rosmarinus officinalis* has been reported by Goodarzi et al (2014) Mohammad & Abbas (2009), and Franciosini et al (2016).

The bacterial enumeration suggested that inclusion of herbal additive alter the gut micro flora characteristics. Supplementation of *C. cyminum* and *A. wilhelmsii* significantly reduced total aerobe and coliform count and enhanced the lactic acid bacteria. Franciosini et al (2016) reported that *R. officinalis* alone or in combination with *O. vulgare* increased lactic acid bacteria in the cecum. The mechanism behind could be direct bactericidal effect of active components in the *C. cyminum* and *A. wilhelmsii* or change in the gut environment decreasing the pH of the digesta, hence supported lactic acid bacteria and caused forced exclusion of other type of microbial population. Different strategies and possibilities and the role of lactic acid bacteria in competitive exclusion and decolonization of different bacteria have been argued by Mead (2000). Similarly Dofing & Gottschal (1997) suggested acidic environment increased the availability of nutrients by decolonization of pathogenic bacteria and increasing the availability of absorptive area of the intestine.

Conclusions. In the present study three herbs *C. cyminum*, *F. vulgare* and *A. wilhelmsii* was used as additive in broiler feed and they exhibited better performance of the subjects in terms of daily weight gain, feed efficiency and decreased ileal bacterial count as compare to control. In conclusion the growth performance of broiler can be enhanced due to the change of the microbial characteristics of the intestine resulting in better nutrient utilization. Hence on the basis of the present findings the tested culinary and medicinal herbs as feed additive could be an option to increase production performances in broiler chickens.

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