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Evaluation of Alternatives to Antibiotic Feed Additives in Broiler Production

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Authors' Contribution
MR and NR designed and supervised the study, and executed experimental work. RBT helped in identification of herbs. MZM and A worked on bacterial enumeration of the samples. MMT statistically analyzed the data. MR wrote the manuscript.

Key words
Herbs, Additives, Broilers, Performance, Bacterial count.

ABSTRACT

The study was conducted to evaluate the effect of herbs as feed additive on performance of broiler alternative to antibiotics. One-day-old (N=320) broiler chicks were divided into eight treatment groups with four replicates of ten chicks. Six treatments were three herbs *i.e. Allium sativum*, *Cassia angustifolia* and *Artemisia scoparia* @ 0.5 and 1.0% respectively, control (basal diet), and a positive control (Oxyfeed® @ 2g/kg). In the study, the treatment groups, which were given diet supplemented with herb and antibiotic, exhibited improved weight gain, average daily gain, and feed conversion ($P<0.05$). No significant difference was observed in feed intake ($P>0.05$). The treatment groups supplemented with *Allium sativum* showed higher growth performance compared to *Cassia angustifolia* and *Artemisia scoparia* and control ($P<0.05$). Relative organ weights and relative carcass yield of treatment groups were not significantly different ($P>0.05$). The Relative length of the intestine of the treatment groups showed significant difference ($P<0.05$). Supplementation of herbal additives and antibiotic had a positive effect on bacterial enumeration of Ileum ($P<0.05$). The use of antibiotic caused a reduction in all three forms of microbial population. On the other hand, herbs added as feed additive acted as prebiotic and enhanced the lactic acid bacteria. In conclusion, supplementation of herbs as feed additive improved growth parameters and present results suggests herbs could be used as alternative to antibiotics growth promoting feed additives.

INTRODUCTION

Feed additives are substances that have the potential to enhance production performance without significantly altering the composition of feed. Broiler chicken requires high dietary energy and protein with balanced amino acid profile in the compound feed (Boling and Firman, 1998). Feed costs approximately about 70% of total production expenditure and nutrient lost in the feces either undigested or unabsorbed due to intestinal microbial population by parasitic action or occupation of receptors on the surface of intestinal epithelium could result in the economic loss (Lu and Walker, 2001). Therefore, dietary energy and protein in the feed and their utilization have significant effects on growth performance of broiler and overall production cost.

Growth enhancing effects of antibiotics were also explored soon after (ten years) their discovery in the mid-twentieth century. Initially animals were fed with dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues, which resulted in improved growth (Castanon, 2007). The mechanism, by which antibiotics act as growth promoters, operates through inhibiting pathogenic bacteria (Dibner and Richards, 2005; Niewold, 2007), decreasing competition between host and bacteria and thus making available nutrients for the host, otherwise consumed by bacteria for their propagation (Hardy *et al*., 2013). However, the concerns about the use of antibiotics as growth promoters were expressed within ten years of their use in the poultry feed industry (Mathew *et al*., 2007). Later, discovery of antibiotic resistant bacteria (Aarestrup *et al*., 2001) from different parts of the world resulted in a ban on the use of certain antibiotics as feed additive (FAO, 2003) and it resulted in increased disease outbreak in different countries (Casewell *et al*., 2003).

In order to find alternative to antibiotic feed additives to control diseases and increase production efficiency in poultry different types of additive alternative including probiotics, prebiotics, synbiotics, organic acids, enzymes and herbs (Cabuk *et al*., 2006; Dahiya *et al*., 2006; El-Latif *et al*., 2013) have been used, which exhibited encouraging results. Medicinal herbs and culinary spices have been used in different parts of the world for centuries to cure diseases in human and animals. Herbs are known to have a wide range of activities such as antibacterial, antiparasitic,

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antioxidant, antifungal, immune enhancer properties, feed intake stimulation, and enhanced endogenous enzyme secretions. Many of these effects are associated with different types of secondary metabolites like isoprene, flavonoids and tannins (Shin *et al*., 1995).

Garlic (*Allium sativum*) of the family Amaryllidaceae is used for both culinary purposes and medicine. It has major bioactive compounds g-glutamyl-S-allyl-L-cysteines and S-allyl-L-cysteine sulfoxides which have antibacterial (Andleeb *et al*., 2014) and immune enhancing effects (Amagase, 2006). The studies have also revealed that garlic consumption reduces risk for heart diseases and cancer. It is also reported that garlic consumption reduces cholesterol and hypertension (Dorhoi *et al*., 2006; Zeybek *et al*., 2007). Jir or Bootae (*Artemisia scoparia*) (Family Asteraceae) is an indigenous local medicinal herb contains monoterpenoids, sesquiterpenoids, β-pinene, capillin, limonene and murcene in the oil (Negahban *et al*., 2006; Singh *et al*., 2009) and its decoction or tea is used to treat indigestion, cold, and fever (Tareen *et al*., 2010) and have hepato-protective effects (Gilani and Janbaz, 1993). Sanna Makki (*Cassia angustifolia*) (Family Leguminosae) has different bioactive compounds such as galactomannan, epimelibiose, galactobiosylylmannose, mannobiose and galactobiose in water soluble fraction (Alam and Gupta, 1986). Its antipyretic, laxative, and diuretic effects are documented (Sultana *et al*., 2012). The purpose of this study was to find out the effect of indigenous culinary and medicinal herbs as feed additive on growth performance and enumeration of intestinal microflora.

MATERIALS AND METHODS

Management of the birds

A total of 320 one day-old Hubbard broiler mixed sex chicks were randomly divided into eight treatment groups (40 chicks for each treatment) with four replicates of ten chicks respectively, reared in littered floor pens. Standard managerial conditions were maintained with 24 h lighting. Controlled feeding program was selected and measured feed at morning and evening was offered to the birds with slight modification in accordance with Hubbard guide for altitudes. Water was provided *ad libitum*. Vaccination against Newcastle, Infectious Bronchitis, and Infectious Bursal disease was carried out. The experiment lasted for six weeks (42 days).

Feeding and treatments

Two-phase feeding regime was applied and for this purpose, a basal diet was formulated (Table I). The dietary treatments were *Allium sativum* 1.0% (T1), *Allium sativum* 0.5% (T2), *Cassia angustifolia* 1.0% (T3), *Cassia angustifolia* 0.5% (T4), *Artemisia scoparia* 1.0% (T5), *Artemisia scoparia* 0.5%, (T6) Control (Basal diet) (T7) and Positive control (Oxyfeed @ 2g/kg) (T8). The powdered herbs were initially mixed in small amount of feed and later in the total required feed thoroughly. The nutritive value of the herbs was considered as negligible.

Weekly data of body weight (BW) and feed intake (FI) per replicate were recorded and weight gain (WG) and feed conversion ratio (FCR) were calculated. At the end of the trial, one bird was randomly picked from each treatment of each replicate, weighed before being slaughtered by severing jugular vein.

Table I.- 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	18
Canola meal	5.0	4.0
Cotton seed meal	4.0	3.0
Peas	10	7.6
Corn gluten 60%	3.0	00
Oil	3.1	4.0
Lysine	0.2	0.2
Methionine	0.3	0.3
Vitamin mineral premix[a]	2.0	2.0
Total	100	100
Calculated analysis		
Metabolizable energy Kcal/kg	2992	3103
Crude protein (%)	21	19.1
Ether extract (%)	6.2	6.8
Crude fiber (%)	4.3	4.1
Lysine (%)	1.1	1.0
Methionine (%)	0.5	0.4
Calcium (%)	1.0	0.8
Phosphorus (%)	0.5	0.4

Vitamin mineral pre mix provides per kg of diet: vitamin A, 9000 IU; D3, 2000, IU; E, 18 IU; B1, 1.8 mg; B2, 6.6 mg B2,; B3, 10 mg; B5, 30 mg; B6, 3.0 mg; B9, 1 mg; B12, 1.5 mg; K3, 2 mg; H2, 0.01 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.

Immediately the intestine was removed and samples of the digesta from Ileum were collected in sterile falcon tubes for bacterial enumeration. Briefly, 1 g digesta was added to 9 ml physiological saline and vortexed to make homogeneous slurry. Subsequently, homogenate was serially diluted up to 10^{-8}. Total aerobe bacteria were counted on brain heart infusion agar (LAB) plates. Coliform bacterial enumeration was carried out by using

MacConkey agar (Oxoid) and lactic acid bacterial count was made using MRS agar (Oxoid) plates in duplicate for 24 to 48 h at 37°C. The results expressed log_{10} colony forming units (log_{10} CFU) per gram.

Statistical analysis

The data were statistically analyzed by using analysis of variance technique and, general linear model (GLM) procedure in SPSS-16 for windows and the results were presented as mean±standard error. In order to determine the difference between treatments, Duncan's Multiple Range (DMR) test was applied at 95% confidence interval.

RESULTS

At the start of the experiment, the chicks were randomly divided into the respective replicates and no difference (P>0.05) was observed in the weight of the chicks was observed and overall mean was 39.24g/chick. The dietary supplementation of the herbs as additive indicated a significant (P<0.05) effect on performance parameters compared to control (T7) (Table II). The highest weight gain was observed in T1, T2 and T8 (P>0.05), which was followed by T4, T3, T6 and T5 which was insignificant with each other (P>0.05). The supplementation of the *Allium sativum* @ 1.0 and 0.5% in the feed exhibited results comparable to antibiotic. There was no significant difference (P>0.05) in feed intake between treatment groups (Table II).

In the present study, treatment groups supplemented with additives, showed improved feed efficiency and average daily gain (Table II). The use of garlic at both (0.5 and 1%) levels exhibited better feed conversion and daily weight gain compared to the other herbal treatments (P<0.05). However, feed efficiency and daily gain of garlic treatment group was not significantly different from antibiotic treatment group (P>0.05). Similarly other herbal additive groups showed better feed efficiency and daily gain compare to control (P<0.05). The carcass and internal organ characteristics are shown in Table III.

Table II.- Weight gain (g/bird), feed consumed (g/bird), feed conversion ratio (g feed/g gain) and Average daily gain (g/bird) of broiler chicken on day 42 (Mean±SE).

Treatments	Weight gain (g/bird)	Feed consumed (g)	Feed conversion ratio (feed g/weight gain g)	Average daily gain (g)
T1	2195.8±18.86[a]	3903.5±35.68	1.778±.009[a]	52.28±0.241[a]
T2	2188.3±10.12[a]	3891.1±10.28	1.778±.019[a]	52.10±0.449[a]
T3	2128.7±16.11[b]	3883.0±11.17	1.824±.012[b]	50.68±0.383[b]
T4	2133.9±14.93[b]	3932.4±26.50	1.842±.008[c]	50.80±0.355[b]
T5	2117.9±10.74[b]	3911.8±26.41	1.847±.003[c]	50.42±0.255[b]
T6	2121.1±6.02[b]	3923.s6±15.00	1.849±.005[c]	50.50±0.143[b]
T7	1962.2±12.78[c]	3935.9±29.51	2.005±.004[d]	46.71±0.304[c]
T8	2192.1±11.88[a]	3954.8±14.56	1.804±.012[ab]	52.19±0.282[a]
Total	2130.0±13.34	3917.0±8.21	1.841±.012	50.71±0.317

*Different superscript within same columns indicate significant difference (P<0.05). T1, *Allium sativum* 1.0%; T2, *Allium sativum* 0.5%; T3, *Cassia angustifolia* 1.0%; T4, *Cassia angustifolia* 0.5%; T5, *Artemisia scoparia* 1.0%; T6, *Artemisia scoparia* 0.5%; T7, negative control (Basal diet) and T8, positive control (Oxyfeed @ 1g/kg).

Table III.- Effect of additive on relative organ weight of broiler chicken on day-42 (Mean±SE).

Treatments	Carcass	Liver	Gizzard	Heart	Spleen	Pancrease	Bursa	Abdominal fat
T1	72.17±0.20	2.12±0.03	2.65±0.03	0.61±0.008	0.14±0.007	0.20±0.009	0.10±0.005	1.73±0.08
T2	71.85±0.37	2.14±0.05	2.68±0.01	0.61±0.005	0.14±0.01	0.20±0.01	0.11±0.01	1.73±0.06
T3	71.68±0.34	2.18±0.05	2.70±0.02	0.61±0.01	0.15±0.007	0.23±0.01	0.11±0.006	1.73±0.08
T4	71.26±0.16	2.12±0.04	2.71±0.01	0.62±0.01	0.15±0.01	0.20±0.008	0.12±0.008	1.78±0.06
T5	71.45±0.13	2.18±0.04	2.70±0.01	0.62±0.01	0.14±0.005	0.12±0.01	0.11±0.005	1.78±0.06
T6	71.08±0.12	2.15±0.03	2.71±0.01	0.62±0.0009	0.14±0.01	0.23±0.01	0.12±0.008	1.74±0.05
T7	70.43±0.34	2.19±0.05	2.72±0.02	0.62±0.009	0.15±0.006	0.24±0.01	0.12±0.008	1.67±0.08
T8	71.66±0.56	2.16±0.04	2.69±0.05	0.63±0.01	0.14±0.007	0.52±0.33	0.12±0.005	1.76±0.04
Total	71.45±0.37	2.15±0.04	2.70±0.02	0.62±0.01	0.14±0.01	0.25±0.23	0.13±0.008	1.74±0.06

*Different superscript within same columns indicate significant difference (P<0.05). For abbreviations see Table II.

Table IV.- Effect of additives on the intestinal characteristics of broiler on 42 day (Mean±SE).

Treatments	Duodenum		Jejunum		Ileum		Intestine	
	cm	RL	cm	RL	cm	RL	cm	RL
T1	29.0±0.40	1.31±0.026^{bc}	70.00±0.41	3.17±0.014^{bc}	85.75±1.7	3.89±0.65^{bc}	184.75±0.75	8.08±0.07^{d}
T2	28.76±0.48	1.29±0.03^{c}	69.00±0.7	3.09±0.002^{c}	86.25±2.39	3.87±0.1^{c}	184.00±1.45	8.25±0.06^{cd}
T3	29.25±0.25	1.35±0.018^{bc}	69.50±0.28	3.21±0.03^{b}	87.25±1.25	4.03±0.09^{bc}	186.00±0.75	8.61±0.06^{bc}
T4	29.75±0.25	1.36±0.011^{b}	70.00±0.4	3.22±0.03^{b}	88.00±1.29	4.05±0.07^{bc}	187.75±0.85	8.64±0.05^{b}
T5	28.50±0.29	1.31±0.014^{bc}	69.5±0.28	3.20±0.027^{bc}	89.00±1.47	4.09±0.06^{bc}	187.00±0.85	8.60±0.04^{bc}
T6	29.75±0.25	1.37±0.007^{b}	70.25±0.25	3.25±0.013^{b}	89.00±1.15	4.12±0.05^{b}	189.00±0.75	8.75±0.03^{b}
T7	29.75±0.48	1.53±0.029^{a}	70.00±0.41	3.42±0.06^{a}	90.25±0.85	4.41±0.05^{a}	191.50±0.5	9.37±0.14^{a}
T8	29.50±0.29	1.32±0.002^{bc}	70.00±0.41	3.14±0.002^{bc}	89.75±0.62	4.03±0.07^{bc}	189.25±0.4	8.50±0.06^{bc}
Total	29.28±0.38	1.35±0.04	69.78±0.41	3.21±0.05	88.15±1.48	4.06±0.1	187.41±0.35	8.60±0.03

*Different superscript within same columns indicate significant difference (P<0.05). RL, relative length cm/100g body weight. For explanation of T1-T8, see Table II.

Table V.- Effect of additive on intestinal aerobe, coliform and lactic acid bacterial enumeration of broiler chicken on day-42 (log_{10} CFU/g digesta) (Mean±SE).

Treatments	Aerobe	Coliform	Lactic acid
T1	6.25±0.25^{c}	4.75±0.47^{b}	6.00±0.40
T2	7.00±0.40^{bc}	5.75±0.25^{ab}	5.75±0.25
T3	7.00±0.40^{bc}	4.75±0.47^{b}	5.75±0.47
T4	7.25±0.47^{ab}	5.75±0.47^{ab}	5.50±0.28
T5	7.25±0.47^{abc}	5.25±0.62^{b}	5.75±0.47
T6	7.50±0.28^{abc}	5.75±0.47^{ab}	5.75±0.25
T7	8.25±0.25^{a}	6.75±0.25^{ab}	5.25±0.47
T8	6.75±0.50^{bc}	5.00±0.40^{b}	4.75±0.25
Total	7.15±0.14	5.46±0.17	5.56±0.13

*Different superscript in the column indicate significant difference (P<0.05). For explanation of T1-T8, see Table III.

The relative weights of carcass and various organs were found to be insignificant between treatment groups (P>0.05). However, numerical differences were observed in the relative weight of the organs in the study. The prominent features of the intestinal segments observed in the study are given in Table IV. There was no significant difference (P>0.05) found between treatment groups in the length of duodenum, jejunum, ileum and intestine. But the relative length of the different segments and intestine of treatment groups revealed a significant difference (P<0.05). The highest relative length (9.37±0.28) of the intestine was observed in T7 and lowest (8.08±0.14) was in T1 (P<0.05). Supplementation of the additives to the broiler diets caused a reduction (numerical) in the length of the intestine.

The composition of bacterial enumeration of ileum at the end of the experiment on the day-42 is shown in Table V. Different treatments had a significant (P<0.05) effect on CFU count of aerobe, coliform, and lactic acid bacteria. The lowest aerobe CFU count was noted in T1 (6.25) and T8 (7.25), respectively. The highest count was recorded in T7 (8.25). The other herbs also exhibited a reduction in aerobe numerically compared to the control (P>0.05). Coliform bacterial CFU count was lowest in T1and T3, which were followed by T8. Lactic acid bacterial enumeration showed no significant difference (P>0.05) between treatments. Least (4.75±0.25) lactic acid bacterial count was observed in T8 followed by (5.25±0.47) in T7 and highest count (6.00±0.40) log_{10} CFU/g digesta was in T1, respectively.

DISCUSSION

In the present study, body weight gain and feed conversion, which were broiler performance indices, increased in additive supplemented groups compared to control. The effect of herbal additive and antibiotic additive was found to be insignificant (P>0.05). The use of antibiotic as growth promoter has previously been reported in addition to their drawback resulting in antimicrobial resistance (Aarestup *et al*., 2000). The beneficial effects of herbs in the present study are in line with studies of Elagib *et al*. (2013) and Lukanov *et al*. (2015), suggesting that supplementation of herbs to broiler diet increased performance indices. This increase in weight gain and feed conversion efficiency could be due to beneficial effects of the herbs in terms of change in gut environment, decreased microbial metabolites, competitive elimination of the pathogenic bacteria and their toxic metabolites from the intestinal tract (Chrubasik *et al*., 2005; Kabir, 2009; Ramiah *et al*., 2014).

In the present study, the control feeding system was opted to neglect the effect of height as the study was undertaken at about 1635meter above sea level. However, in other studies, even though *ad libitum* feed was offered, no significant difference was observed between control and additive supplemented groups in terms of feed intake (Choi *et al*., 2010). Supplementation of the antibiotic and herbs as additive resulted in better FCR. The improved feed conversion could be due to better digestion, increased absorption, of nutrients (Kabir, 2009). The reduced number of pathogens in the intestinal tract could also result in nutrient sparing effect, available for the host bird otherwise utilized by the bacteria or lost in the feces unabsorbed (Wenk, 2003).

The length of the intestine could be affected by the type of ingredients used in the feed (Wang *et al*., 2005**)** and wheat based diet has been reported to cause an increase in the length of intestine due to the presence of arabinoxylans (Annison and Choct, 1991). In the present study, difference in length of intestine might also be due to the changes in the digesta characteristics like viscosity.

The result of the present study indicated that it affected the microbial composition of the ileum. The use of antimicrobial as additive had the most profound effects on microbial population and caused the reduction in the all kinds of bacterial population. The results are in agreement with the study of Engberg *et al*. (2000) showing that antibiotic additive reduced intestinal microbial load. Similarly, there are reports suggesting phytogenic feed additives also act as antimicrobial agents under in-vivo conditions as synthetic antibiotics with different mode of action and beside exclusion of pathogenic bacteria they act as prebiotic (Jamroz *et al*., 2003; McReynolds *et al*., 2009) and support the enumeration of the lactic acid bacteria thought to be beneficial bacterial population (Wati *et al*., 2015). In general, the improved performance of the treatment groups supplemented with herbs used as feed additive are due to different bioactive ingredients present in herbs and exhibiting their activities in different ways.

CONCLUSION

In conclusion, the result of the present study revealed that culinary and medicinal herbs had multiple additional potentials compared to antibiotic feed additives and can be used as alternative to antibiotic feed additives. The inclusion level of herbs used in the present study was observed to be beneficial. Moreover, supplementation of feed with *A. sativum* in particular and *C. angustifolia* and *A. scoparia* to some extend had beneficial effects on performance parameters and gut microbial population.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Aarestrup, F.M., Bager, F. and Andersen, J.S., 2000. Association between the use of avilamycin for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers: epidemiological study and changes over time. *Microb. Drug Resist.*, **6**: 71-75. https://doi.org/10.1089/mdr.2000.6.71

Aarestrup, F.M., Seyfarth, A.M., Emborg, H.D., Pedersen, K., Hendriksen, R.S. and Bager, F., 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob. Agents Chemother.,* **45**: 2054-2059. https://doi.org/10.1128/AAC.45.7.2054-2059.2001

Alam, N. and Gupta, P.C., 1986. Structure of a water-soluble polysaccharide from the seeds of *Cassia angustifolia*. *Pl. Med.,* **4**: 308-310. https://doi.org/10.1055/s-2007-969161

Amagase, H., 2006. Clarifying the real bioactive constituents of garlic. *J. Nutr.*, **136**: 716S-725S.

Andleeb, S., Tahir, M., Khalid, M., Awan, U. A., Riaz, N. and Ali, S., 2014. Antibacterial and antioxidant activities of traditional herbs and honey against fish associated bacterial pathogens. *Pakistan J. Zool*, **46**: 933-940.

Annison, G. and Choct, M., 1991. Anti-nutritive activities of cereal non-starch polysaccharides in broiler diets and strategies minimizing their effects. *World Poult. Sci. J.,* **47**: 232-242. https://doi.org/10.1079/WPS19910019

Boling, S.D. and Firman, J.D., 1998. Digestible lysine requirement of female turkeys during the starter period. *Poult. Sci.,* **77**: 547-551. https://doi.org/10.1093/ps/77.4.547

Cabuk, M., Bozkurt, M., Alcicek, A., Akbas, Y. and Küçükyilmaz, K., 2006. Effect of an herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. *S. Afri. J. Anim. Sci.,* **36**: 135-141. https://doi.org/10.4314/sajas.v36i2.3996

Casewell, M., Friis, C., Marco, E., McMullin, P. and Phillips, I., 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J. Antimicrob. Chemother.,* **52**: 159-161. https://doi.org/10.1093/jac/dkg313

Castanon, J.I.R., 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult. Sci.,* **86**: 2466-2471. https://doi.org/10.3382/

ps.2007-00249

Choi, I.H., Park, W.Y. and Kim, Y.J., 2010. Effects of dietary garlic powder and α-tocopherol supplementation on performance, serum cholesterol level, and meat quality of chicken. *Poult. Sci*., **89**: 1724-1731. https://doi.org/10.3382/ps.2009-00052

Chrubasik, S., Pittler, M.H. and Roufogalis, B.D., 2005. Zingiberisrhizoma: a comprehensive review on the ginger effect and efficacy profiles. *Phytomedian,* **12**: 684-701. https://doi.org/10.1016/j.phymed.2004.07.009

Dahiya, N., Tewari, R. and Hoondal, G.S., 2006. Biotechnological aspects of chitinolytic enzymes: a review. *Appl. Microb. Biotech.,* **71**: 773-782. https://doi.org/10.1007/s00253-005-0183-7

Dibner, J.J. and Richards, J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. *Poult. Sci.,* **84**: 634-643. https://doi.org/10.1093/ps/84.4.634

Dorhoi, A., Dobrean, V., Zăhan, M. and Virag, P., 2006. Modulatory effects of several herbal extracts on avian peripheral blood cell immune responses. *Phytother. Res.,* **20**: 352-358. https://doi.org/10.1002/ptr.1859

Elagib, H.A., El-Amin, W.I.A. and Malik, H.E.E., 2013. Effect of dietary garlic (*Allium sativum*) supplementation as feed additive on broiler performance and blood profile. *J. Anim. Sci. Adv*., **3**: 58-64. https://doi.org/10.5455/jasa.20130219104029

El-Latif, A.S.A., Saleh, N.S., Allam, T.S. and Ghazy, E.W., 2013. The effects of rosemary (*Rosemarinus officinalis*) and garlic (*Allium sativum*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. *Br. J. Poult. Sci.,* **2**: 16-24.

Engberg, R.M., Hedemann, M.S., Leser, T.D. and Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broiler. *Poult. Sci*., **79**: 1311. https://doi.org/10.1093/ps/79.9.1311

FAO, 2003. *OIE/WHO Expert workshop on non-human antimicrobial usage and antimicrobial resistance*: First Workshop on Scientific Assessment, 1–5 December 2003, Geneva, World Health Organization.

Gilani, A.U.H. and Janbaz, K.H., 1993. Protective effect of Artemisia scoparia extract against acetaminophen-induced hepatotoxicity. *Gen. Pharmacol. Vascu. Syst*., **24**: 1455-1458. https://doi.org/10.1016/0306-3623(93)90434-Y

Hardy, H., Harris, J., Lyon, E., Beal, J. and Foey, A.D., 2013. Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immune pathology. *Nutrients*, **5**: 1869-1912. https://doi.org/10.3390/nu5061869

Jamroz, D., Orda, J., Kamel, C., Wiliczkiewicz, A., Wertelecki, T. and Skorupinska, J., 2003. The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. *J. Anim. Feed Sci.,* **12**: 583-596. https://doi.org/10.22358/jafs/67752/2003

Kabir, S.M., 2009. The role of probiotics in the poultry industry. *Int. J. mol. Sci*., **10**: 3531-3546. https://doi.org/10.3390/ijms10083531

Lu, L. and Walker, W.A., 2001. Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium. *Am. J. clin. Nutr.,* **73**: 1124S-1130S.

Lukanov, H., Genchev, A. and Ribarski, S., 2015. Effect of feed supplementation with garlic power on meat productivity and meat quality traits of classic ross 308 male hybrid chickens. *Trak. J. Sci.,* **13**: 67. https://doi.org/10.15547/tjs.2015.01.009

Mathew, A.G., Cissell, R. and Liamthong, S., 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Fd. Borne Path. Dis.,* **4**: 115-133. https://doi.org/10.1089/fpd.2006.0066

McReynolds, J., Waneck, C., Byrd, J., Genovese, K., Duke, S. and Nisbet, D., 2009. Efficacy of multistrain direct-fed microbial and phytogenetic products in reducing necrotic enteritis in commercial broilers. *Poult. Sci.,* **88**: 2075-2080. https://doi.org/10.3382/ps.2009-00106

Negahban, M., Moharramipour, S. and Sefidkon, F., 2006. Chemical composition and insecticidal activity of *Artemisia scoparia* essential oil against three coleopteran stored-product insects. *J. Asia-Pacific Ent.*, **9**: 381-388. https://doi.org/10.1016/S1226-8615(08)60276-9

Niewold, T.A., 2007. The non-antibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poult. Sci.,* **86**: 605-609. https://doi.org/10.1093/ps/86.4.605

Ramiah, S.K., Zulkifli, I., Rahim, N.A.A., Ebrahimi, M. and Meng, G.Y., 2014. Effects of two herbal extracts and virginiamycin supplementation on growth performance, intestinal microflora population and fatty acid composition in broiler chickens. *Asian-Aust. J. Anim. Sci.,* **27**: 375. https://doi.org/10.5713/ajas.2013.13030

Shin, L., Pelley, R.P. and Kripke, M.L., 1995. Use of Aloe vera yoghurt for acid production, viscosity, sensory properties and quality retention. *J. Invest. Derm*. **102**: 197-204.

Singh, H.P., Mittal, S., Kaur, S., Batish, D.R. and Kohli, R.K., 2009. Chemical composition and antioxidant activity of essential oil from residues of *Artemisia scoparia*. *Fd. Chem.,* **114**: 642-645. https://doi.org/10.1016/j.foodchem.2008.09.101

Sultana, S., Ahmad, M., Zafar, M., Khan, M.A. and Arshad, M., 2012. Authentication of herbal drug Senna (*Cassia angustifolia* Vahl.): A village pharmacy for Indo-Pak subcontinent. *Afri. J. Pharm. Pharmacol.,* **6**: 2299-2308. https://doi.org/10.5897/ajpp12.446

Tareen, R.B., Bibi, T., Khan, M.A., Ahmad, M. and Zafar, M., 2010. Indigenous knowledge of folk medicine by the women of Kalat and Khuzdar regions of Balochistan, Pakistan. *Pak. J. Bot.*, **42**: 1465-1485.

Wati, T., Ghosh, T.K., Syed, B. and Haldar, S., 2015. Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. *Anim. Nutr.,* **1**: 213-219. https://doi.org/10.1016/j.aninu.2015.08.003

Wang, Z.R., Qiao, S.Y., Lu, W.Q. and Li, D.F., 2005. Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poult. Sci.,* **84**: 875-881. https://doi.org/10.1093/ps/84.6.875

Wenk, C., 2003. Herbs and botanicals as feed additives in monogastric animals. *Asian Aust. J. Anim. Sci.,* **16**: 282-289. https://doi.org/10.5713/ajas.2003.282

Zeybek, U., Yaylim, I., Yilmaz, H., Ağaçhan, B., Ergen, A., Arikan, S., Bayrak, S. and Isbir, T., 2007. Methylene tetra hydro folate reductase C677T polymorphism in patients with gastric and colorectal cancer. *Cell Biochem. Funct.,* **25**: 419-422. https://doi.org/10.1002/cbf.1317