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RESEARCH ARTICLE

EFFECT OF CERTAIN HERBS ON FECAL EXCRETION OF ANAEROBIC BACTERIA IN LAYER CHICKEN

***Kalaiselvi, G., Sakthi Priya, M., Puniyamurthy, N. and Sukumar, K.**

Centralized Research Laboratory, Veterinary College and Research Institute, Namakkal -637002

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ABSTRACT

A total of 225, 16 weeks old layer birds randomly divided into 4 groups with three replicates each. Birds were assigned to the basal diet (control). All birds employed in the experiment were fed according to applicable recommendations of the National Research Council Feed and water bacterial count assessed before and after completion of trial. Fresh purified herbal extracts of turmeric, fenugreek and ginger were subjected to sterility and in vitro antibacterial effect test. The herbal ingredients were incorporated to the basal diet at the level of 0.1%, 0.25% and 0.5% respectively. First week of feeding showed reduction of anaerobic bacterial count when compared with untreated control group. Second and third week of herbal feeding showed significance difference of anaerobic bacterial excretion in dropping materials compared to untreated control group. Simultaneously growth rate and feed conversion ratio also improved in the test groups when compared with untreated control group. Feeding of crude preparation of fenugreek, Ginger, turmeric along with basal diet at the level of 0.1%, 0.25% and 0.5% had significance influence on the reduction of excretion of fecal anaerobic bacteria and no changes in excretion of beneficial bacteria and feeding of herbal extract do not any negative impact on body weight and blood parameters.

INTRODUCTION

Poultry industry is an important profitable industry in India. The feed cost contributing 70% of the production cost and the economic loss due to enteric pathogens like Salmonella, *E.coli*, Coccidiosis also very high. A number of feed additives including antibiotics have been widely employed in the poultry industry for several decades. A manipulation of gut function and microbial habitat of domestic animal with feed additives has been recognized as an important tool for improving growth performance and feed efficiency (Jamroz *et al.* 2003). Recently, the concerns about possible antibiotic residues and antibiotic resistance have aroused great caution in the usage of antibiotics in the animal industry. The use of antibiotics as feed additives has accelerated and led to investigations of alternative feed additives in animal production. As one of the alternatives, herbal extracts are already being used as feed supplements to improve growth performance under intensive management systems (Lippens *et al.*2005). Plant extracts and spices as single compounds or as mixed preparations can play a role in supporting both performance and health status of the animal. Beneficial effects of herbal extracts or active substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral, antioxidant and antihelminthic actions. Isoprene derivatives, flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract (Manzanilla *et. al.* 2001). The stabilizing effect on intestinal microflora may be associated with intermediate nutrient metabolism. The pharmacological action of active plant substances or herbal extracts in humans is well known, but in animal nutrition the number of precise experiments is relatively low. Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases (Alloui *et.al* 2012). Garlic is thought to have various pharmacologic properties. For example, it has been found to lower serum and liver cholesterol, inhibit bacterial growth and inhibit viral growth and reduce oxidative stress in chicken (Qureshi *et al.*1983). Garlic as a natural feed additive, improves broiler growth and Feed Conversion Ratio (FCR), and decreased mortality rate. Aloe vera plant extract is widely used as a self prescribed agent against upper respiratory tract infections such as common cold and improves immunity. According to many *in vitro* studies, it is thought to stimulate macrophage activity and hence the immune system. In recent years, much effort has been made to identify the potential components in turmeric powder extract that could account for its *in vitro* immuno stimulatory effects (Lee *et al.* 2001). Some of these bioactives compounds include polysaccharides, cichoric acid and alkyamides, the principle active, its optimal

****Corresponding author: Kalaiselvi, G.,***
Centralized Research Laboratory, Veterinary College and Research Institute, Namakkal -637002.

dose level and its action in vitro, have not been well known. In older animals the effectiveness of plant extract supplementation was relatively low, but higher digestibility of nutrients and reduction of *E. coli* and Clostridium sp. in intestinal content were observed (Zaika *et al*.1988).

MATERIALS AND METHODS

A total of 240, 11 weeks old layer birds were selected from a local private farm of Namakkal. Birds were weighed and randomly housed in wood shavings covered floor pens (20 birds per pen). Continuous lighting was provided throughout the experiment. Sterile, well dried turmeric and fenugreek and ginger, were grinded finely and water solubility were assessed. The experimental diet was formulated according to the standards prescribed in Bureau of Indian Standard. The finely grinded herbal recipes sterility and antibacterial effect were assessed in *in vitro* condition. The herbal materials mixed with normal basal ration of birds at the level of 0.1%, 0.25%,0.5% . Feed and water bacterial count should be assessed before and after completion of experiment. Samples of fenugreek, garlic and black pepper used in the experimental feeds were analyzed. Phytoadditives, fenugreek, garlic and ginger combination are added as feed supplements and act as antibiotic alternatives.

Sample collection and analysis

Fecal sample collected all groups of birds in sterile manner. The randomly collected fecal samples are weighted and serially diluted in sterile distilled water.

next day10 fold serial dilution was made with distilled water. Agar media were prepared in sterile way for analyzing effect of herbs on controlling anaerobic bacteria and changes associated with beneficial bacteria (anaerobic agar, *Clostridium perfringens* isolation agar and Man-Rogosa – Sharp agar). The Pour plate technique is used to determine the number of microbes/ml of the fecal sample. This technique does not require any previously prepared plates. The agar was prepared, melted and sterilized and then allowed to cool. The sample was collected and serially diluted so as to get about 30 to 300 colonies /ml. When the agar was cooled to about 45° C, the samples were added to the agar and mixed thoroughly. Then this was poured into sterile Petri-plates and they were allowed to cool on a flat table top. Once the agar is cooled they get solidified and then the plates were inverted and kept in the incubator at 37° C for about 24 to 48 hours. The formation of the colonies were noted and counted. The colonies were examined microscopically by performing gram's staining. The separate organisms were observed and the morphological characteristics were observed. The result were found to be rod shaped, gram positive organism, thus confirming the presence of bacillus with terminal and subterminal spores. The micro organisms use carbohydrates in a different pattern depending on their enzyme complement. In fermentation, substrates such as carbohydrates and alcohols undergo anaerobic dissimilation and produce organic acids that may be accompanied by the production of gases such as hydrogen or carbon-dioxide. Therefore, all the anaerobic bacteria were subjected to glucose fermentation test in order to see the production of gas. *In vitro* analysis of antibacterial effect of herbal extract were performed by agar well diffusion method and disc diffusion

Table 1. Proximate composition of fenugreek, turmeric, ginger (% DM basis)

Nutrient	Turmeric	Ginger	Fenugreek	Garlic	Thulsi
DM%	90.1	89.1	93.1	93.9	89.2
Ash %	2.39	5.4	2.39	7.3	13.6
Fat %	2.9	4.0	7.5	1.3	3.6
Protein %	28.58	9.4	28.58	15.93	20.64
Fiber %	6.27	52.1	6.27	10.12	8.9
Carbohydrate %	11.1	18.1	6.4	-	39.58

Table 2. Effects of herbs in fecal excretion of anaerobic bacteria in experimental chicken

Days	Mean ± standard error			
	Control group	Group 1	Group 2	Group 3
7 days	$115.83^{a} \pm 3.55$	$64.50^{b} \pm 2.36$	$19.00^{c} \pm 1.64$	$4.83^{d} \pm 0.84$
14 days	$137.67^{a} \pm 5.79$	$43.33^{b} \pm 3.41$	$13.00^{c} \pm 1.04$	$2.00^{d} \pm 0.84$
21 days	$156^{a} \pm 2.40$	$41.33^{b} \pm 1.52$	$7.83^{c} \pm 0.84$	$0.83^{d} \pm 0.31$

Value given each cell is the mean of four observation. Means within the same row with different superscript differ significantly ($P<0.05$)

Table 3. Body weight gain of experimental chicken (average)

Particulars	0 day of treatment (gms)	21st day of treatment (gms)	Difference in weight gain (gms)	Feed intake In grams	Feed conversion ratio
Control group (Average)	750	1020	270	1070	3.96
Trail group 0.1% inclusion (Average)	745	1040	295	1078	3.65
Trail group 0.25% inclusion (Average)	755	1030	275	1075.5	3.91
Trial group 0.5% inclusion (Average)	735	1045	310	1061	3.42

The collected poultry dropping materials processed as per the method of Galey *et al.(*2000) 1 gram of fecal sample of trial groups mixed with 10 ml of distilled water and autoclaved at 121° C for 20 min and samples were centrifuged at 1500 rpm/10 mins. Supernatant were collected treated with equal quantity of ethanol for vegetative bacterial death and spores are allowed to germinate at strict anaerobic condition. Then

method. Simultaneously beneficial bacteria count were analyzed by spread plate method in MRS agar. Experimental chickens body weight were measured in 0 days, 7th day and 21st day and feed conversion ratio were calculated. Blood samples were collected by vine puncture from live birds and blood parameters were analyzed.

RESULTS AND DISCUSSION

Phytochemical analysis of herbal extract showed presence of saponin and tannin, flavonoids, Terpenoids, amino acids, carbohydrates, alkaloids carbohydrates, volatile acid, glycosides and absence of hydro soluble amines, phenols, cardiac glycosides and vitamin C. *In vitro* analysis of antibacterial activity of herbal extract which showed inhibition at the rate of 10% inclusion of herbal extract when compared with control culture. But the agar well diffusion and disk diffusion method of antibacterial activity analysis produced only small zone of inhibition (5 mm and 8 mm diameter zone) due to lack of diffusion of crude herbal extract in agar medium. The feed conversion ratio as well as body weight improved when compared with control group. Blood parameters like Hb, WBC, RBC, PCV counts did not showed any difference when compared with control group. Herbal feeding does not have negative impact on FCR and blood parameters. The microscopic examination of pure culture organisms morphological characteristics were observed. The strains were found to be rod shaped, gram positive organism with terminal and sub terminal spores. The anaerobic bacterial culture were subjected to glucose fermentation test in order to see the production of acid and gas. All the anaerobic pure culture isolated from experimental chicken fermented carbohydrate and produce acid and gas. Analysis of fecal lactobacillus spp. count by using MRS agar at the dilution rate of 10^{-5}, showed that there is no reduction of lactobacillus spp. by feeding of fenugreek, turmeric, garlic, thulsi and ginger at the level of 0.1%,0.25% and 0.5% respectively.

The cloacae fecal samples analysis of anaerobic bacteria, the first week of herbal feeding showed 50% reduction of anaerobic bacteria when compared with untreated control group. 2nd and 3rd week of herbal feeding showed significance reduction of anaerobic bacterial excretion in dropping materials compared with untreated control group. The mean value of anaerobic bacterial reduction for the groups I to IV were $115.83^{a} \pm 3.55$, $137.67^{a} \pm 5.79$, $156^{a} \pm 2.40$, $64.50^{b} \pm 2.36$, $43.33^{b} \pm 3.4$, $41.33^{b} \pm 1.52$, $19.00^{c} \pm 1.64$, $13.00^{c} \pm 1.04$, $7.83^{c} \pm 0.84$, $4.83^{d} \pm 0.84$, $2.00^{d} \pm 0.84$, $0.83^{d} \pm 0.31$ percent respectively. Significant difference noticed between the groups and within the groups. Phytochemical analysis of herbal extract showed presence of saponin and tannin, flavonoids, Terpenoids, amino acids, carbohydrates, alkaloids carbohydrates, volatile acid, glycosides and absence of hydrosoluble amines, phenols, cardiac glycosides, vitamin C. however Wang *et al*.(1998) described the herbs and plant extracts used as feed additives include many different bio active ingredients such as alkaloids, bitters, flavonoids, glycosides, mucilage, saponins and tannins and effects expected of herbs and plant extracts also varies. The herbal plant extract act as the appetite stimulant in intestinal microflora, stimulate the pancreatic secretion to increase endogenous enzyme activity and immune system. Many plant product and their constituents have a broad antimicrobial activity, anti oxidant and sedative activity.

In vitro analysis of antibacterial activity of herbal extracts which showed inhibition at the rate of 5-10% inclusion of herbal extract when compared with control culture but the agar well diffusion and disk diffusion method of antibacterial activity analysis produced only small zone of inhibition (5 mm and 8 mm diameter zone) due to lack of diffusion of crude herbal extract in agar medium similar to the finding of Sonali *et al*.(2016) but they used curry leaves, cloves, cardamom and they proved that curry leaves have maximum antibacterial activity against *E.coli,* whereas cloves proves to be most effective against the rhizopus however, in case of Aspergillus sp, cardamom exhibit maximum antifungal activity. Herbal feeding increase feed conversion ratio as well as body weight when compared with control group. Blood parameters like Hb, WBC, RBC, PCV counts do not had any difference when compare with control group herbal feeding does not have negative impact on FCR and blood parameters. However Jamroz *et al*. (2003), Geetha *et al.*(2011), Alloui *et.al*. (2012), Elkhair *et al*.(2014), Kirubakaran *et al.*(2016) studied the effect of herbal feeding like garlic block pepper, fenugreek, coriander seeds at the inclusion level of 0.1%,0.5% and 1% level and found herbal feeding increases weight gain, growth and performance and feed conversion ratio, immune response and morphological changes in the gastrointestinal tissue. However inclusion of more than 1% of nitrogen rich herbal material increases high amount of uric acid excretion leads to kidney damage. Antibiotic sensitivity test with pure anaerobic isolate from experimental birds were performed. The culture showed the sensitivity towards Cloxacillin, Cephotaxime and Sulphatrimethoprim and resistance to Erythromycin, Kenamycin. This study was agreement with finding of Nasir *et al.* (2005) and Barbosa *et al*. (2005).

Conclusion

In the short experimental study we concluded that feeding of crude preparation of fenugreek, Ginger, turmeric along with basal diet at the level of 0.1%, 0.25% and 0.5% had significance influence on the reduction of excretion of fecal anaerobic bacteria and no changes in excretion of beneficial bacteria and feeding of herbal extract do not any negative impact on body weight and blood parameters. We found that herbal extract had similar antimicrobial action like antibiotic and using herbal extracts as feed additives means we can avoid antibiotic resistance problem in poultry industry and microbial contamination of environment and meat and egg. In this study we found that by using herbal feed additive we produce quality antibiotic residues free and microbial contamination free meat and eggs but further in depth detailed study is needed.

REFERENCES

Alloui, N., S. Ben Aksa and M. N. Alloui 2012. Utilization of fenugreek(Trigonella foenumgraecum) as growth promoter for broiler chicken. *J.World Poult. Res.* 2 (2) : 55-58

Barosa, T.M., C.R Serra., R.M.La Ragione., M.J Woodward, and A.O.Henriques, 2005. Screening for bacillus isolate in the broiler gastrointestinal tract. *Appl.Environ.Microbiol*., 71: 968-978

Bolukbasi, S.C. and M.K. Erhan. 2007. Effect of dietary thyme (Thymus vulgaris)_on laying_hens performance and E.coli concentration in faeces. *Int.J.Nat.Eng.Sci.* 1(2) :55-58

Bruneton, J. 1995. Pharmacognosy, Phytochemistry, Medicinal Plants (Translator): Hatton, C. K., Lavoisier Publishers, Paris.

Collington, G. K., D. S. Park, and D. G.Armstrong, 1990. The Influence of inclusion of Both an Antibiotic and a Probiotic in the Diet on the Development of Digestive Enzyme Activity in the Pig. *Br. J. Nutr.,* 64 : 59–70.

Elkhair R.A., H.A Ahmad, and S. Selim 2014) Effect of block pepper (piper nigrum). Turmeric powder (Curcuma longa) and coriander seeds (Coriandrum sativum) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chicken. *Asian Aust. J. Anim. Sci*., 27(6) : 847-854

Emadi M and H.Kermanshahi 2007a. Effect of turmeric rhizome powder on immunity responses of broiler chicken. *Madwell Journal of Animal and Veterinary advances,* 6 (7):833-836.

Fry, J., R. I.Carr, and T. D. G. Lee, 1998. Echinacea stimulation of mouse macrophages. *FASEB J.* (Abstract), 12 (5) : A299.

Galey, F.D., R.Terra, R.Walker, J.Adaska, M.A.Etchebarne, , B.Puschner, E. Fischer, R.H. Whitlock, T.Rocke, D.Willoughby, E. Tor, 2000. Type C. botulism in dairy cattle from feed contaminated with a dead cat. *J.Vet. Diagn. Invest.,* 12: 204–209.

Geetha M., K.R. Suneel, A.M. Krupanidhi K.S.Muralikrishna, A.P.Avin and P.Prashanth 2011. Effect of fenugreek on total body and organ weight. A study in mice. *Pharm. Online,* 3 :747-752.

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