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Optimization of level of feeding herbal preparation in reducing faecal bacterial load in layer chicken

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Abstract
The present study was undertaken to optimize the level of feeding of the herbal preparation (*Ocimum sanctum, Zingiber officinale, Allium sativum, Trigonella foenum graceum* and *Curcuma longa*) in different doses @ 0.1percent, 0.25 percent and 0.5 percent in layer feed to reduce the faecal total bacterial count, faecal coliform count and screening for the presence of *E. coli.* The study was conducted in eleven weeks old Lohman breed growers at 11 weeks of age with 20 birds per group. Control group was fed only with the regular feed without any herbal preparation and the treatment groups I, II and III were fed with the herbal preparation in different levels as mentioned above in the regular feed. The experimental trial was conducted for twenty one days and the data were collected from a sample of six birds from each group. There was a significant reduction ($p<0.01$) in faecal total bacterial count and faecal coliform count in all the groups with the highest reduction nearing to one log in the group III when compared to the control. There was presence of *E. coli* in the contol and absence in the treatment groups. Thus, 0.5 percent level of the herbal preparation in the feed can be claimed as the optimum level of feeding in the layer chickens to combat enteric infections. However, further study is required to make concrete recommendation for commercial layer chicken productivity.

Keywords: Herbs, Faecal bacteria, Coliform count, *E. coli*

Introduction
Indian Poultry Industry is one of the fastest growing segments of the agricultural sector today in India and has become a means of improving the economy of the farming community due to its enhanced production performance. Apart from improving the livelihood, it also provides proteinaceous food. However mortality in layer type chicken plays a major role in determining the economic stability [1]

A number of factors have to be considered for maintaining the productivity of the birds, of which gut health is the major area of study in recent times [2].When gut health is affected, digestion and nutrient absorption is impaired as gut is an important and complex area involving nutrition, microbiology, immunology and physiology. Gut is composed of numerous community of microorganisms like bacteria, fungi, protozoa and viruses, but bacteria are the predominant microorganisms [3]. The colonization of bacteria in the gut is determined by its substrate preferences and the chemical composition of the contents [4]. Among the portions of the Gastro- Intestinal tract, there is diversity in the bacterial colonies inhabiting the gut from the proximal to the distal end with increased density in the distal part [5].

The bacterial diversity and the balance of microbes in the gut is influenced by many factors like periods of high challenge, feed, biosecurity, environment, brooding conditions [6] and bacterial and viral infections [7]. The most common viral infections which cause enteric diseases include rotavirus [8], coronavirus [9] adenovirus [10], astrovirus [7] and reovirus [11]. Among the bacterial infections, the common enteric diseases are necrotic enteritis, ulcerative enteritis, and spirochetosis and include other diseases such as salmonellosis, colibacillosis, mycobacteriosis, erysipelas and fowl cholera. These infections pose a serious threat to the livability of the flock as it impairs the feeding efficiency [12]. To combat these enteric infections and to improve the gut health, antibiotic growth promoters were widely used in poultry feed since last 50 years. But the risk associated with the development of resistant organisms made European Union to ban the use of antibiotics since January 2006 [13]. However the removal of antibiotics also led to performance problems [14] and search for an alternative began. Phytogenic feed additives in the present scenario are gaining importance to improve the growth performance in the animals [15, 16]. The herbs could be expected to serve as safer alternative as growth promoters [17] and hence the present study was proposed to evaluate the herbal preparation comprising of *Ocimum sanctum* (Tulsi), *Allium sativum* (Garlic), *Curcuma longa* (curcumin), *Trigonella foenum graceum* (Fenugreek) and

Zingiber officinale (ginger) in reducing the faecal bacterial count and to optimize the level of feeding at three different doses in layer chicken.

Materials and Methods

Experimental Site and Experimental Birds

The experimental trial was carried out at a private layer farm at pudhur, vaiyappamali, Thiruchengodu taluk of Namakkal district, Tamil Nadu, India with atotal of 80 grower birds Lohman breed @ 20 birds per group (Grower 11 weeks). The birds were reared in cage with controlled feeding and followed vaccination as per the schedule before the initiation of the experiment.

Experimental groups

The birds were allotted at random into four experimental groups and fed with the experimental diet for 21 days (11-14 weeks).

S. No	Group (n=20)	Treatment
1	Control	Regular feed without any herbal preparation
2	I	Regular feed with 0.1% herbal preparation
3	II	Regular feed with 0.25% herbal preparation
4	III	Regular feed with 0.5% herbal preparation

Collection and preparation of samples

The herbs Ocimum sanctum (Tulsi), Allium sativum (Garlic), Zingiber officinale (ginger), Trigonella foenum graceum (Fenugreek) and Curcuma longa (curcumin) were purchased fresh from market. Their rinds (ginger and garlic) were peeled off using knife, washed, shade dried and later ground to fine powder. 100 g of each herb was taken, blend and packed in an air tight plastic container.

Experimental diets

The feed formulation for grower is presented in the Table 1

Assessment of Faecal total bacterial count, Coliform count and Screening for *E. coli*

Collection of faeces

Faecal samples were collected aseptically from the experimental birds during the experimental period before initiation of the trial and at weekly intervals for three weeks after initiation of the trial. The samples were suspended immediately after collection in 9 mL of sterile normal saline and serially diluted from test tube 1 to test tube 7 and discarded 1 mL from test tube 7. The samples were subjected for estimation of faecal total bacterial count, faecal coliform count and screening for *E. coli* immediately after processing.

Assessment of faecal total bacterial count

Plate count agar medium was prepared by suspending 23.5 g in 1 litre of distilled water. This was brought to boil to dissolve completely and then sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 55ºC, it was poured into the the petri dish and checked for sterility by overnight incubation. Then the diluted faecal sample (100µL) was spread on the plates. The inoculated plate was then incubated at 37 ºC for 24 hours as per the standard method. The colonies on the plate were counted using a colony counter. The bacterial count was carried out at the Poultry Disease Diagnosis and Surveillance Laboratory, Namakkal.

Assessment of faecal coliform count

Mac Conkey agar medium was prepared by suspending 55.07g in 1 litre of distilled water. This was brought to boil to dissolve completely and then sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 55 ºC, it was poured into the petri dish and checked for sterility by overnight incubation. Then the diluted faecal sample (100µL) was spread on the plates. The inoculated plate was then incubated at 37 ºC for 24 hours as per the standard method. The colonies on the plate were counted using a colony counter. The coliform count was carried out at the Poultry Disease Diagnosis and Surveillance Laboratory, Namakkal.

Screening for the presence of *E. coli*

Eosin Methylene Blue (EMB) agar medium was prepared by suspending 36.0 g in 1 litre of distilled water. This was brought to boil to dissolve completely and then sterilized by autoclaving at 121ºC for 15 minutes. After cooling to about 55 ºC, it was poured into the petri dish and checked for sterility by overnight incubation. The next day, the freshly collected faecal sample *i.e* 1 gram of faeces from the experimental birds at random from each group in three replicates was suspended in 9 mL of sterile normal saline and serially diluted from test tube 1 to test tube 7 and discarded 1 ml from test tube 7. Form the last dilution a loopful of inoculum was streaked on the plate and then incubated at 37 ºC for 24 hours to screen for the presence of *E.coli* as per the standard method. The test was carried out at the Poultry Disease Diagnosis and Surveillance Laboratory, Namakkal.

Statistical analysis

The data collected on various parameters were subjected to Duncan's test as per the method suggested by Snedecor and Cochran [18].

Results and Discussion

The present work involving the herbal preparations to optimize its level of feeding in layer chicken at 11-14 weeks of age (growers) was carried out in field conditions to look for the phytobiotic approach in egg production, free from the issues of drug - residues and antimicrobial resistance. The parameters assessed were faecal total bacterial count, faecal coliform count and screening for *E. coli.*

Effect of the herbal preparations on assessment of the faecal total bacterial count

The effect of the herbal preparations on the faecal total bacterial count (Cfu/mL) is presented in the Table 2, Plate 1 and Figure 1.

The total faecal bacterial count (Cfu/mL) seven days, fourteen days and twenty one days post treatment was significantly ($p<0.01$) reduced in the treatment groups when compared to the control groups (0 day before initiation of the experiment and control group maintained after initiation of the experiment). The total faecal bacterial load was increased in the control which was fed only with the regular feed as against the treatment groups. Similarly there is significant ($p<0.01$) reduction between groups I, II and III. However one log reduction was noticed in the group III.

Effect of the herbal preparations on assessment of the faecal coliform count

The effect of the herbal preparations on the faecal coliform

count is presented in the Table 3, Plate 2 and Figure 2.
The total faecal coliform count (Cfu/mL) seven days, fourteen days and twenty one days post treatment was significantly ($p<0.01$) reduced in the treatment groups when compared to the control groups (0 day before initiation of the experiment and control group maintained after initiation of the experiment). The total faecal bacterial load was increased in the control which was fed only with the regular feed as against the treatment groups. Similarly there is significant ($p<0.01$) reduction between groups I, II and III. However one log reduction was noticed in the group III.

Screening for the presence of *E.coli*

The effect of the herbal preparations in reducing the *E.coli* is presented in the plate 3.
The control group showed the presence of metallic sheen in the Eosin methylene blue agar, whereas there was no metallic sheen appearance noticed in the treatment groups.
The reduction in the total faecal bacterial count and faecal coli from count in the herbal preparation fed groups in layer chicken may be attributed to the following findings. Gazuwa [19] reported that the flavonoids present in *Allim sativum exhibits* bacteriostatic and bacteriocidal effects on some strains of bacteria by inhibiting the reverse transcriptase and proteases activity in bacteria. Cardelle [20] stated that the garlic exerts a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria. Inhibiton observed in *E. coli* was more than 10 times greater than that seen in *Lactobacillus casei* for the same garlic extract dose. He concluded that the behavior may be due to a greater sensitivity of enterobacteria to allicin possibly because of the different composition and the increased permeability to allicin of their cell membrane. Sharma [21] reported that fructo-oligosaccharides present in the garlic found to selectively stimulate the growth and activity of beneficial bacteria (bifidobacteria and lactobacilli) in the colon, and thus act as a prebiotic and improve the host health.
Rivlin [22] reported that in laboratory animals, gingerols increase the motility of the gastrointestinal tract and have analgesic, sedative, antipyretic and antibacterial properties. Bharkava [23] reported that *Zingiber officinale* contain potent antimicrobial property against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*. Deshmuk [24] investigated the effect of turmeric against enteric pathogens. The study showed that *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Salmonella typhimurium* were very much sensitive to turmeric extract as compared to other tested pathogens. Ramya [25] suggested that the ethanolic extract of fenugreek extract of leaf is an important source for antibacterial components. Basu [26] proved that the fenugreek was found to strongly inhibit the growth of *Staphylococcus aureaus* and *Pseudomonus aeruginosa* in *in vitro* studies. The antimicrobial activity of *Ocimum sanctum* methanolic extract was evaluated against *Bacillus subtilis* and *E.coli* and reported that 5mg of the extract exhibited antibacterial activity against the test organisms with zone sizes 2.5 cm and 2.3 cm respectively [27].
Ashish [28] investigated the antimicrobial activity of the extracts quantitatively by measuring zone diameter of inhibition at three different concentrations of 50mg/l, 100 mg/l and 200 mg/l for methanol and aqueous extract. Erythromycin and Ciprofloxacin antibiotic were used as control for *E. coli*, *Proteus mirabilis* and *Staphylococcus aureus*. Both antibiotics showed no effect on the growth of *E. coli* and *P. mirabilis* whereas 17 and 19 mm zone diameter of inhibition was seen in *S. aureus* for Erythromycin and Ciprofloxacin respectively. Thus his study reported that *E. coli* and *Proteus mirablis* was resistant to both the extracts, with *Staphylococcus aureus* sensitive to both the extracts.
Thus the reduction in the total faecal bacterial count, coliform count and *E.coli* in the treated groups at 0.5% level of feeding of the herbal preparation (Group III) where there was one log reduction was also in accordance with the findings of Allinson [29] and Ghalib [30] who reported effects on pathogens and hence this 0.5% level of herbal preparation can be claimed as an optimum level for reducing the enteric pathogens and thereby improve the gut health. However further studies are needed to assess the effect of the herbs on production performance of layers and to assess its effect on broilers so as to recommend the optimum level of feeding in poultry.

Table 1: Regular feed composition for Growers (11-14 weeks)

Ingredients	Quantity (Kg)
Maize	600
Pellet SF(36%) protein	150
Soya bean meal	150
Deoiled rice bran	115
Calcite	15
Dicalcium Phosphate	12
Salt	4
Soda bicarbonate	1
DL methionine	1
Lysine	0.5
Choline chloride	1
Vitamins	500 gram
Traceminerals	1 kg
Phytase	100gram
Toxin binder	1 kg
Liver powder	500gram

Table 2: Effect of the herbal preparation (Mean±SE) on assessment of the faecal total bacterial count (Cfu/mL) in layer chicken n=6

Variables	Treatment (Mean ± SE)				
	Control		Group I	Group II	Group III
	Days				
	0 day before initiation of treatment (representative sample from all the groups)	After initiation of the treatment (only regular feed)			
7 days post treatment	$198.67^{a} \pm 4.31$	$216.33^{b} \pm 4.20$	$106.83^{c} \pm 3.06$	$88.67^{d} \pm 0.72$	$14.17^{e} \pm 0.80$
14 days post treatment	$198.67^{a} \pm 4.31$	$205.50^{a} \pm 2.20$	$100.17^{b} \pm 1.89$	$39.17^{c} \pm 1.68$	$11.50^{d} \pm 1.39$
21 days post treatment	$198.67^{a} \pm 4.31$	$243.33^{b} \pm 11.57$	$97.17^{c} \pm 1.61$	$31.50^{d} \pm 2.81$	$2.83^{e} \pm 0.54$

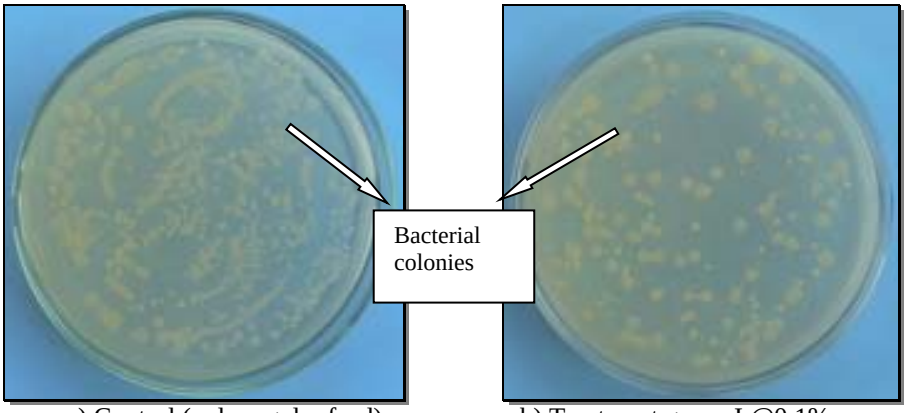
Mean values within the same row with different superscripts differ significantly ($p<0.01$)

Table 3: Effect of the herbal preparation (Mean ±SE) on assessment of the faecal coliform count (Cfu/mL) in layer chicken n=6

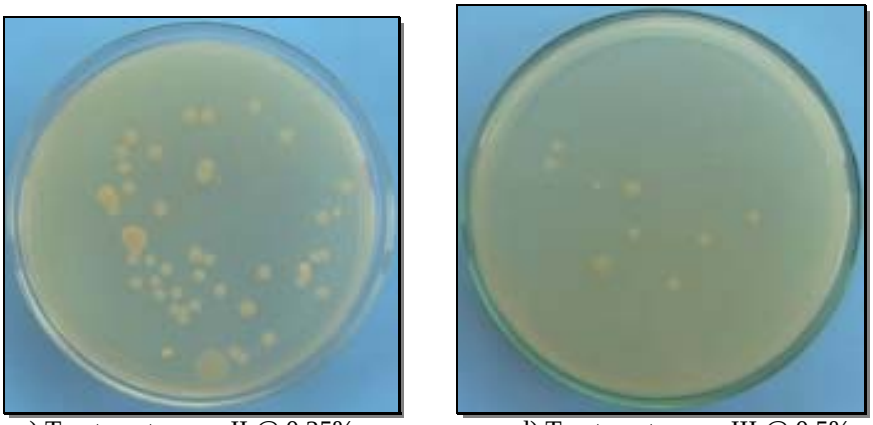
Variables	Treatment (Mean ±SE)				
	Control		Group I	Group II	Group III
	Days				
	0 day before initiation of treatment (representative sample from all the groups)	After initiation of the treatment (only regular feed)			
7 days post treatment	70.67 [a] ± 1.52	83.0 [b] ±3.38	29.33 [c] ±2.30	25.83[c]±3.65	11.50[d]±1.09
14 days post treatment	70.67 [a] ± 1.52	92.0 [b] ±1.21	30.50 [c] ±2.37	25.50 [d] ±1.67	5.83[e]±0.60
21 days post treatment	70.6 7 [a] ± 1.52	93 [b] ±1.84	11.0 [c] ±0.90	6.67 [d] ±0.67	1.33[e]±0.21

Mean values within the same row with different superscripts differ significantly (p<0.01)

Bacterial colonies on plate count agar

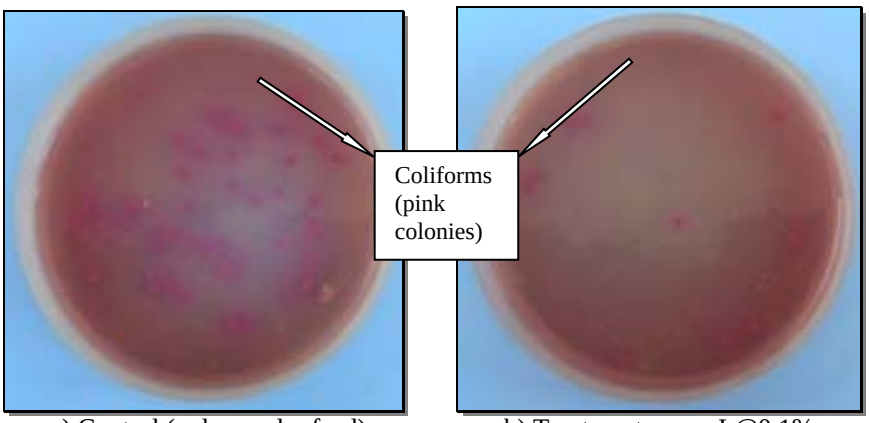


a) Control (only regular feed) b) Treatment group I @0.1%

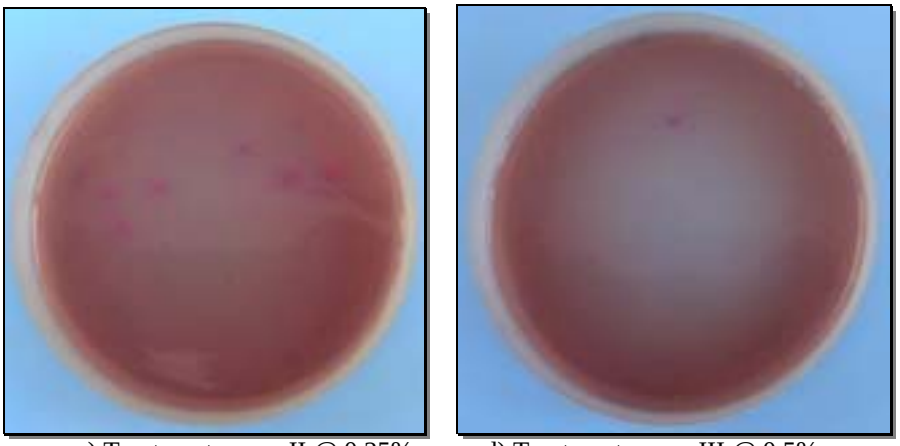


c) Treatment group II @ 0.25% d) Treatment group III @ 0.5%

Plate 1: Effect of the herbal preparation on assessment of the faecal total bacterial count (Cfu/mL) in layer chicken

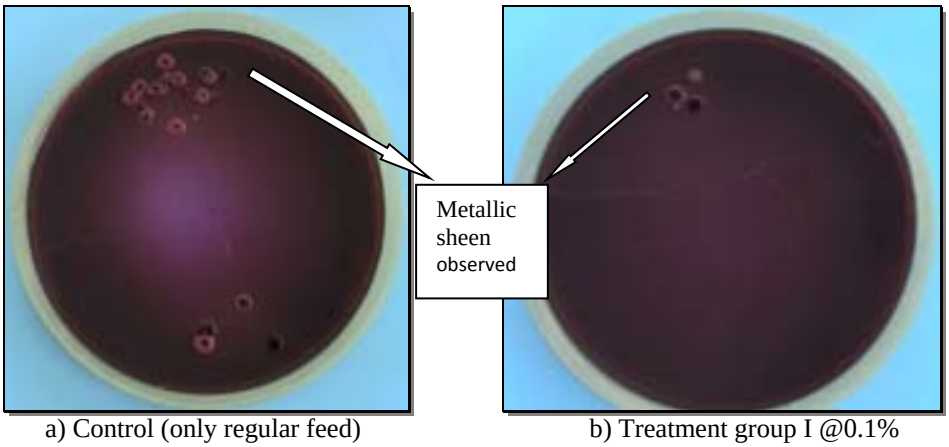


a) Control (only regular feed) b) Treatment group I @0.1%

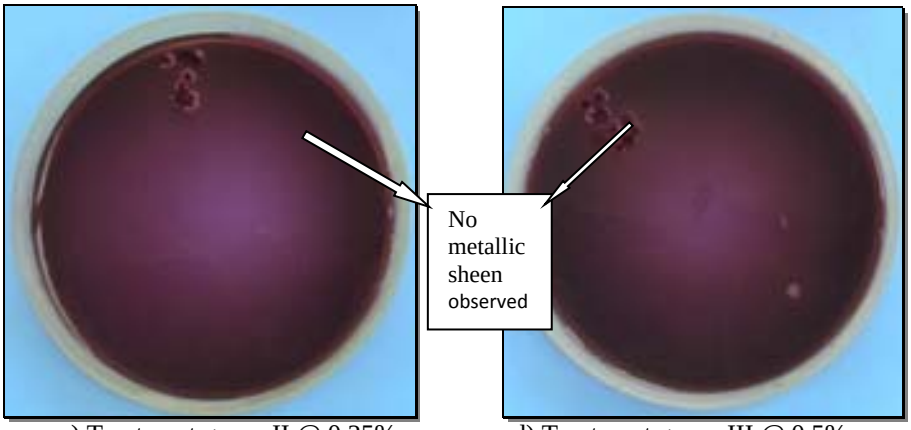


c) Treatment group II @ 0.25% d) Treatment group III @ 0.5%

Plate 2: Effect of the herbal preparation on assessment of the faecal coliform count (Cfu/mL) in layer chicken Coliform bacterial colonies on MacConkey agar



a) Control (only regular feed) b) Treatment group I @0.1%



c) Treatment group II @ 0.25% d) Treatment group III @ 0.5%

Plate 3: Screening for the presence of *E.coli* in layer chicken *E. coli* colonies on EMB agar

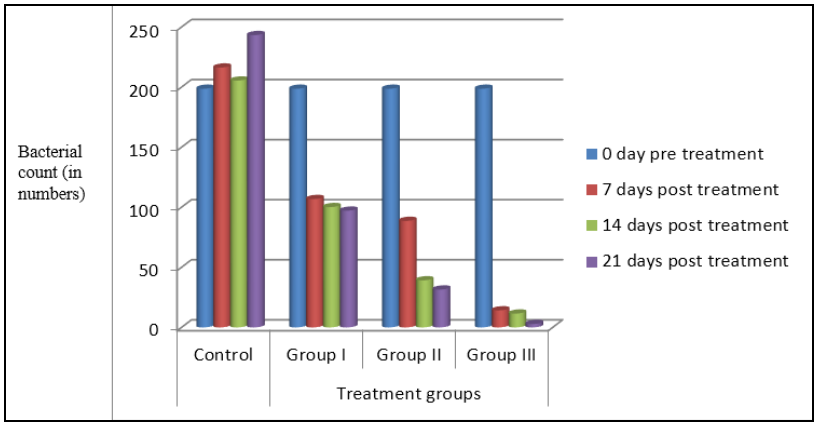


Fig 1: Effect of the herbal preparations on assessment of the faecal total bacterial count (Cfu/mL) in layer chicken

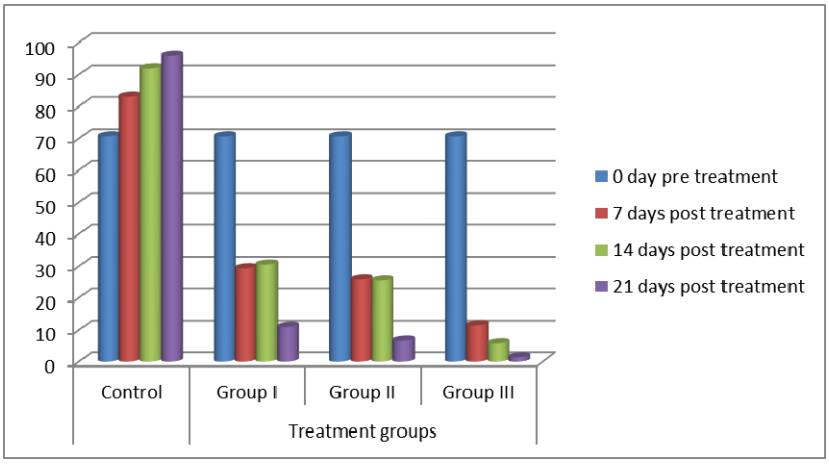


Fig 2: Effect of the herbal preparations on assessment of the faecal coliform count (Cfu/mL) in layer chicken

Conclusion

From the above findings, it can be concluded that by supplementation of the herbs *Ocimum sanctum* (Tulsi), *Allium sativum* (Garlic), *Curcuma longa* (curcumin), *Trigonella foenum graceum* (Fenugreek) and *Zingiber* officinale (ginger) at 0.5% level causes a definite reduction in the faecal total bacterial count and faecal coliform count and *E.coli.* However further studies are needed to assess the effect of the herbal preparations on production performance of layers

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Conflict of Interest: None declared

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