



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 353-356
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www.thepharmajournal.com
Received: 08-05-2023
Accepted: 16-06-2023

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Effect of dietary supplementation of phytogetic feed additives (Licorice root powder, asparagus root powder & lemongrass oil) in combination on the intestinal histomorphometry of commercial broiler Chicken

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Abstract

This study was aimed to determine the effect of phytogetic feed additives i.e., licorice root powder (LP), asparagus root powder (AP) and lemongrass oil (LGO) on the intestinal histomorphometry of broiler chickens. 300-day-old commercial male broilers (Vencobb 400) were randomly assigned to six treatments and housed for 42 days. Diets included T1, as control, T2 – 0.05% antibiotic (AB), T3 – 0.1% LP + 0.2% AP, T4 -0.1% LP + 0.025% LGO, T5 – 0.2% AP + 0.025% LGO and T6 – 0.1% LP + 0.2% AP + 0.025% LGO. Results revealed that significantly ($p<0.05$) higher duodenal villus height (VH) was noticed in LP+AP, villus width (VW) was higher in LP+AP and AP+LGO and VH/CD ratio with LP+AP and LP+LGO. Significantly ($p<0.05$) higher Jejunal VH was noticed with antibiotic, LP+AP and LP+AP+LGO and VW was higher with all PFA combinations, whereas, VH/CD ratio was higher with LP+AP and LP+AP+LGO groups compared to control groups. All dietary combinations showed significantly ($p<0.05$) higher ileal VH, VW and VH/CD ratio compared to control and antibiotic. However, no significant effect was observed in crypt depth (CD) of duodenum, jejunum and ileum. Therefore, it can be concluded that inclusion of licorice root powder (0.1%), asparagus root powder (0.2%) and lemongrass oil (0.025%) in combinations has positive effect on intestinal histomorphometry and can be included in broiler diets as a best replacement to antibiotic growth promoter.

Keywords: antibiotic, licorice, asparagus, lemongrass oil, broilers, intestinal histomorphometry

Introduction

For better production, growth rate and feed efficiency, the GIT plays an important role in broilers. The healthy gut is directly involved in the balance of gut microflora, better immune system, protection from enteric pathogenic bacteria and the digestion and absorption of nutrients. For which poultry industry has been using multiple agents such as medicinal herbs, prebiotics organic acids, enzymes, probiotics and synbiotics as feed additives alternative for antibiotics.

Phytobiotics are natural bioactive components or substances derived from plants such as, terpenoids, glycosides, alkaloids, and phenols [14]. Phytobiotics are further classified into herbs, non-woody and non-persistent plants, spices such as fruits, seeds, bark or roots, essential oils or extracts, and oleoresins [6]. Licorice (*Glycyrrhiza glabra*), Asparagus (*Asparagus racemosus*) and lemongrass (*Cymbopogon citratus*) are some of these phytoetics with beneficial effects. Licorice is a perennial herb belonging to Fabaceae family and the main primary active ingredient is glycyrrhizin, which improves growth rate and have other pharmacological activities like immunomodulation, antioxidative, antiviral and anti-inflammatory properties [2, 3, 11]. *Asparagus racemosus* commonly called as “Shatavari” and belongs to family Liliaceae, commonly found throughout India, Asia, Australia and Africa. The main active components of *Asparagus racemosus* contains four steroid saponins: Shatavarins I to IV, with Shatavarin I and Shatavarin IV being major glycoside present in the roots. Lemongrass is an aromatic perennial tropical plant belonging to the family Poaceae and was originally found growing wild in India. Main active compound in lemongrass essential oil is citral α and citral β along with others like geraniol, citronellal, nerol, etc. which might be reason for its various pharmacological activities [13].

Though the beneficial effects of the phytoetics on production performance of poultry was studied. There is limited research done over the impact of Licorice root powder, Asparagus

root powder and Lemongrass oil on intestinal histomorphometry of broilers. Hence, the main aim of this research trial was to find out combined effect of these phytogetic feed additives on intestinal histomorphometry of broilers.

Materials and Methods

300 day-old commercial male broiler chicks (Vencobb 400) were obtained and randomly distributed into six dietary groups and were fed with corn-soyabean meal based isonitrogenous and isocaloric diets as shown in [5] (Table 1). Diets are follows: T1 - control i.e., basal diet (BD), T2 – BD + antibiotic (BMD @ 0.05%), T3 – BD + LP 0.1% + AP 0.2%, T4 – BD + LP 0.1% + LGO 0.025%, T5 – BD + AP 0.2% + LGO 0.025% and T6 – BD + LP 0.1% + AP 0.2% + LGO 0.025%.

Table 1: Ingredients and nutrient composition of basal diet (%) (0 - 42 days).

| Ingredients | Pre-starter | Starter | Finisher |
|--|-------------|---------|----------|
| Maize | 51.94 | 52.86 | 57.05 |
| Oil | 3.70 | 5.10 | 6.00 |
| Soyabean meal | 40.10 | 37.80 | 32.70 |
| Stone grit | 1.31 | 1.31 | 1.31 |
| Dicalcium phosphate | 1.66 | 1.70 | 1.78 |
| Salt | 0.40 | 0.40 | 0.40 |
| DL-Methionine | 0.204 | 0.210 | 0.230 |
| L-Lysine HCl | 0.252 | 0.190 | 0.100 |
| Vitamin trace mineral mixture | 0.13 | 0.13 | 0.13 |
| Nutrient composition (calculated values) | | | |
| ME (kcal/kg) | 3002 | 3101.57 | 3200.70 |
| Crude protein (%) | 23.05 | 22.05 | 20.07 |
| Lysine (%) | 1.30 | 1.20 | 1.01 |
| Methionine (%) | 0.52 | 0.51 | 0.51 |
| Calcium (%) | 1.02 | 1.01 | 1.01 |
| Available phosphorous (%) | 0.45 | 0.45 | 0.45 |

Birds were slaughtered on day 42nd and 2-cm sections of duodenum, jejunum, and ileum were obtained from six birds in each group and examined under 4X of light microscopy (MICAPS-Micro view, version 3.7) for histomorphological examination of villus height (VH), cryptal depth (CD), villus width (VW) and villus height: crypt depth ratio (VH:CD).

General linear model of statistical package for social sciences (SPSS, version 20) was used to analyze the data and Duncan's multiple range test [7] was used to compare the means and significance was considered at $p < 0.05$. Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance [17].

Results and Discussion

Intestinal Histomorphometry

Duodenal Histomorphometry

Results showed that phytogetic feed additives at different combinations i.e., LP + AP, LP + LGO, AP + LGO and LP + AP + LGO and antibiotic showed higher ($p < 0.05$) duodenal VH (Table 2). Among all the dietary combinations, higher VH was observed with LP + AP followed by LP + LGO and lowest was observed with AP + LGO and LP + AP + LGO. No ($p > 0.05$) effect was seen in duodenal CD. Higher ($p < 0.05$) VH:CD ratio was observed in LP, AP and LGO at different combinations than control and antibiotic. Among these combinations, higher VH:CD ratio was observed with LP + AP and LP + LGO groups. VW was significantly ($p < 0.001$)

higher in LP + AP and AP + LGO followed by LP + LGO. Whereas, significantly ($p < 0.001$) lower villus width was observed in control, antibiotic and LP + AP + LGO.

Table 2: Effect of licorice root powder, asparagus root powder and lemongrass oil in combination on duodenal histomorphometry.

| Trt | Diets | Villus Height (µm) | Crypt Depth (µm) | Villus Height: Crypt Depth ratio | Villus Width (µm) |
|----------------|---------------|-----------------------|------------------|----------------------------------|----------------------|
| T ₁ | Control | 1551.22 ^d | 254.62 | 6.11 ^c | 141.47 ^c |
| T ₂ | Antibiotic | 1693.98 ^{cd} | 286.10 | 5.94 ^c | 153.84 ^c |
| T ₃ | LP + AP | 2096.96 ^a | 287.11 | 7.51 ^a | 212.50 ^a |
| T ₄ | LP + LGO | 1889.92 ^b | 259.84 | 7.46 ^a | 176.73 ^b |
| T ₅ | AP + LGO | 1641.27 ^{cd} | 273.34 | 6.24 ^{bc} | 194.71 ^{ab} |
| T ₆ | LP + AP + LGO | 1793.90 ^{bc} | 246.67 | 7.31 ^{ab} | 155.16 ^c |
| | SEM | 34.328 | 4.866 | 0.179 | 4.349 |
| | N | 6 | 6 | 6 | 6 |
| | P-value | 0.001 | 0.064 | 0.010 | 0.001 |

Values bearing different superscripts within a column are significantly ($p < 0.05$) different.

LP- licorice root powder @ 0.1%, AP- asparagus root powder @ 0.2%, LGO- lemongrass oil @ 0.025%.

Jejunal Histomorphometry

Jejunal VH was higher ($p < 0.05$) in LP + AP, LP + AP + LGO and antibiotic when compared to control, LP + LGO and AP + LGO. While no ($p > 0.05$) effect was observed in jejunal CD (Table 3). Higher ($p < 0.05$) VH:CD ratio was seen in all combinations of phytogetic feed additives and antibiotic when compared to control. Among all the combination groups, LP + AP and LP + AP + LGO showed significantly ($p < 0.05$) higher VH:CD ratio compared to other groups. VW was significantly ($p < 0.05$) higher in all different combinations of LP, AP and LGO.

Table 3: Effect of licorice root powder, asparagus root powder and lemongrass oil in combination on jejunal histomorphometry.

| Trt | Diets | Villus Height (µm) | Crypt Depth (µm) | Villus Height: Crypt Depth ratio | Villus Width (µm) |
|----------------|---------------|----------------------|------------------|----------------------------------|----------------------|
| T ₁ | Control | 1386.81 ^b | 193.30 | 7.20 ^c | 118.75 ^c |
| T ₂ | Antibiotic | 1686.23 ^a | 211.63 | 8.14 ^b | 126.19 ^{bc} |
| T ₃ | LP + AP | 1638.21 ^a | 188.83 | 9.20 ^{ab} | 165.37 ^a |
| T ₄ | LP + LGO | 1412.30 ^b | 191.26 | 7.91 ^b | 151.61 ^{ab} |
| T ₅ | AP + LGO | 1418.32 ^b | 196.06 | 7.55 ^{bc} | 154.88 ^{ab} |
| T ₆ | LP + AP + LGO | 1630.79 ^a | 175.81 | 9.31 ^a | 152.61 ^{ab} |
| | SEM | 30.364 | 5.707 | 0.235 | 4.718 |
| | N | 6 | 6 | 6 | 6 |
| | p-value | 0.002 | 0.649 | 0.037 | 0.023 |

Values bearing different superscripts within a column are significantly ($p < 0.05$) different.

LP- licorice root powder @ 0.1%, AP- asparagus root powder @ 0.2%, LGO- lemongrass oil @ 0.025%.

Ileal Histomorphometry

Results showed significantly ($p < 0.001$) higher VH in LP + AP and LP + LGO followed by AP + LGO and LP + AP + LGO when compared to antibiotic and control (Table 4). VH:CD ratio was higher in LP + LGO followed by other combinations compared to antibiotic and control ($p < 0.001$). VW of birds supplemented with LP + AP, LP + LGO and AP + LGO was significantly higher ($p < 0.05$) followed by LP + AP + LGO and lower in control and antibiotic. No difference was seen in ileal CD ($p > 0.05$).

Table 4: Effect of licorice root powder, asparagus root powder and lemongrass oil in combination on ileal histomorphometry.

| Trt | Diets | Villus Height (µm) | Crypt Depth (µm) | Villus Height: Crypt Depth ratio | Villus Width (µm) |
|----------------|---------------|----------------------|------------------|----------------------------------|----------------------|
| T ₁ | Control | 1006.79 ^d | 196.47 | 5.29 ^d | 113.26 ^c |
| T ₂ | Antibiotic | 1117.85 ^c | 212.17 | 5.45 ^{cd} | 120.29 ^c |
| T ₃ | LP + AP | 1575.24 ^a | 207.39 | 7.73 ^b | 151.30 ^{ab} |
| T ₄ | LP + LGO | 1529.44 ^a | 172.67 | 9.27 ^a | 150.82 ^{ab} |
| T ₅ | AP + LGO | 1309.08 ^b | 192.48 | 6.81 ^{bc} | 156.73 ^a |
| T ₆ | LP + AP + LGO | 1331.86 ^b | 200.89 | 7.24 ^b | 127.28 ^{bc} |
| | SEM | 30.146 | 5.373 | 0.263 | 4.074 |
| | N | 6 | 6 | 6 | 6 |
| | P-value | 0.001 | 0.357 | 0.001 | 0.002 |

Values bearing different superscripts within a column are significantly ($p < 0.05$) different.

LP- licorice root powder @ 0.1%, AP- asparagus root powder @ 0.2%, LGO- lemongrass oil @ 0.025%.

Similar results were reported in broilers fed with a combination of phytobiotics and organic acids and reported significantly improved villus height and ratio of villus height/crypt depth in ileum, jejunum, and duodenum [10]. Supplementation of plant extract blend (PEB) in broiler diets improved the height of villi and the villi length to crypt depth in duodenum, jejunum and ileum [9]. Similarly, results were observed with significantly increased jejunal and ileal villus height and lower jejunal crypt depth with no effect on duodenal and ileal crypt depth with phytochemical feed additives (Digestarom®) inclusion in broiler diets [12]. Another study stated that villus height and width of small intestine was increased significantly in broiler diets containing a combination of carvacrol + cinnamaldehyde + capsaicin and essential oil of thyme and anise [11].

Active components of licorice, asparagus and lemongrass essential oil can reduce various intestinal infections due to its high activity to resist pathogenic bacteria [15, 16, 19]. In addition, they can promote enterocyte maturation, increase villus height, decrease crypt depth, and increase their absorption efficiency [20].

In contrary, no significant effect was observed on the villus height (VH), VH: CD ratio and villus width (µm) of duodenum, jejunum and ileum with various phytochemical feed additives supplementation in the broiler diets [4, 8, 18].

Conclusion

The present study has confirmed that inclusion of licorice root powder (0.1%), asparagus root powder (0.2%) and lemongrass oil (0.025%) in combinations has positive effect on intestinal histomorphometry of broilers and hence can be included in their diets as a best replacement to antibiotic growth promoter.

Acknowledgements

We are grateful for the support given by the Department of poultry science, Rajendranagar, Hyderabad and Directorate of Poultry Research, Hyderabad.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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