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Research Article

Effect of phytobiotics (Licorice, Asparagus and Lemongrass) on the intestinal histomorphometry of broiler chicken

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Abstract

This experiment was designed to investigate the effect of dietary inclusion of phytogenic feed additives (licorice root powder, asparagus root powder, and lemongrass oil) on the intestinal histomorphometry of broiler chicken for 42 days. A total of 440 day-old commercial male broiler chicks (Vencobb 400) were randomly distributed into 11 dietary treatment groups with 8 replicates for each treatment group and 5 birds in each replicate. Treatment groups included T1 as control i.e., basal diet (BD) without any growth promoter, T2 – BD+antibiotic (Bacitracin Methylene Disalicylate @ 0.05%), T3, T4 and T5 –BD+licorice root powder (LRP) at 0.05%, 0.1% and 0.15% respectively, T6, T7 and T8 –BD+asparagus root powder (ARP) at 0.1%, 0.15% and 0.2% respectively, T9, T10 and T11 –BD+lemongrass oil (LGO) at 0.015%, 0.025% and 0.035% respectively. Significantly (P<0.05) improved duodenal, jejunal and ileal villus height (VH), VH: CD ratio and villus width (VW) were observed with the supplementation of various concentrations of LRP, ARP, LGO when compared to control. Whereas, crypt depth (CD) was significantly (P<0.05) increased with antibiotic and LGO at 0.015% followed by LRP at 0.15% in the duodenum, with LRP at 0.05% and LGO at 0.025% and 0.035% in the jejunum and with ARP at 0.1% and 0.2% and LGO at 0.015%, 0.025% and 0.035% in the ileum. Therefore, it can be concluded that supplementation of PFAs has better effect on the gut morphology, however, more study is needed to optimize dosage and determine long-term effects.

Keywords: Asparagus, Broilers, Intestinal histomorphometry, Lemongrass oil, Licorice

Highlights

- Inclusion of PFAs as an alternative to antibiotics in the broiler diet improved the gut morphology.
- Inclusion of PFAs at different concentrations improved VH, VH: CD and VW in the GIT.
- LGO at lower concentration and antibiotics increased the duodenal crypt depth.
- Jejunal crypt depth was increased with LGO at higher level and LRP at lower level.
- Various levels of ARP and LGO improved the ileal crypt depth.

INTRODUCTION

Due to the prohibition on the use of antibiotics, natural growth promoters like phytogenic feed additives (PFAs) are necessary for the balance of gut microflora, better immune system, protection from enteric pathogenic bacteria and the digestion and absorption of nutrients (Castanon, 2007; Sharma et al., 2020). Licorice is a medicinal herb with various biological components such as saponins, flavonoids and triterpenes. The principal constituent is glycyrrhizin which has anti-inflammatory, immunomodulatory, anti-oxidant, antimicrobial and growth-promoting effects (Alagawany et al., 2019). Asparagus is medicinally important because of the presence of steroidal saponins and sapogenins, particularly Shatavarin I-IV present in the root (Vadivelan et al., 2011), responsible for its antibacterial effect (Shah et al., 2014). Lemongrass is an aromatic medicinal grass and the most important constituent is citral (lemonal) with various pharmacological actions (antioxidant, anti-inflammatory, antibacterial, antibiotic, and antifungal) and growth promoter (Khalifah et al., 2021; Otu et al., 2022). Other phytochemical components are also present, which protect the cells from oxidative stress and improve

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growth and health of bird's digestive system (Hasan-Al-Sharif et al., 2023).

However, there is limited research done over their impact on intestinal histomorphometry of broilers. Hence, the main aim of this study was to find out the effect of these PFAs on intestinal histomorphometry of broilers.

MATERIALS AND METHODS

Four-hundred-and-forty-day-old commercial male broiler chicks (Vencobb 400) were obtained and randomly allocated to 11 dietary groups with 8 replicates for each treatment group and 5 birds in each replicate and were fed with corn-soyabean meal-based isonitrogenous and isocaloric diets as shown in (Table 1) (Bureau of Indian Standards [BIS], 2007). Diets are as follows: T1 - control i.e., basal diet (BD), T2 –BD+antibiotic (Bacitracin Methylene Disalicylate @ 0.05%), T3, T4 & T5 –BD+licorice root powder (LRP) at 0.05%, 0.1% & 0.15% respectively, T6, T7 & T8 –BD+asparagus root powder (ARP) at 0.1%, 0.15% & 0.2% respectively and T9, T10 & T11 – BD+lemongrass oil (LGO) at 0.015%, 0.025% & 0.035% respectively. Licorice and asparagus root powders were procured from Suguna foods, Coimbatore, India. Whereas lemongrass oil was procured from CitSpray Aroma Sciences, Maharashtra, India.

Intestinal histomorphometry: On 42nd day, from each treatment, six birds were slaughtered, and 2 cm long segment of duodenum, jejunum and ileum were collected and then washed with physiological saline solution and fixed in 10% neutral buffered formalin solution. The samples were processed for histomorphological examination in terms of measurement of parameters like villus height (VH), cryptal depth (CD), villus width (VW) and villus height: crypt depth ratio. Histological sections were examined under 4X of light microscopy (OLYMPUS microscope) with micrometry and photographic attachment. The images were analyzed using image analyzing software (MICAPS-Microview, version 3.7). Twenty intact crypt-villus units were randomly selected from each bird and their mean length was calculated (Gurram et al., 2022).

The data obtained were analyzed using the General Linear Model of Statistical Package for Social Sciences (SPSS, version 20) and comparison of means was done using Duncan's multiple range test (Duncan, 1955) and significance was considered at P<0.05. Data were subjected to statistical analysis under completely randomized design employing one-way analysis of variance (Snedecor & Cochran, 1989).

Table 1. Ingredient and nutrient composition of basal diet (%) fed to the commercial broilers from 0 to 42 days of age

Ingredient	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	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

RESULTS

Intestinal histomorphometry

Duodenal morphometry- Broilers supplemented with phytogenic feed additives showed significant (P<0.05) effect on villus height when compared to the control group (Table 2). However, LRP 0.05% and 0.15%, ARP 0.15%, 0.2% and LGO 0.025% showed significantly (P<0.001) higher duodenal villus height among different levels of LRP, ARP and LGO and control. Higher duodenal crypt depth (P<0.001) was observed with LGO 0.015%, LRP 0.15% and antibiotic supplementation when compared to other treatment groups. Supplementation of all the phytogenic feed additives, except LRP 0.1% and ARP 0.1% showed significantly (P<0.001) higher duodenal VH: CD ratio when compared to antibiotic and control. Among all treatment groups LGO 0.025% and 0.035% had higher VH: CD values (P<0.001). Duodenal villus width was higher (P<0.001) with antibiotic supplementation and lower with control, LRP 0.15%, ARP 0.1%, and 0.15% supplementation.

Jejunal morphometry- Supplementation of phytogenic feed additives showed significantly (P<0.001) higher jejunal

Table 2. Effect of licorice root powder, asparagus root powder and lemongrass oil on histomorphometry of duodenum in broiler chicken

Trt	Diets	Villus height (µm)	Crypt depth (µm)	Villus height: crypt depth ratio	Villus width (µm)
T1	**Control**	936.78^{f}	225.37^{b}	4.39^{de}	94.52^{e}
T2	**Antibiotic**	1165.20^{de}	286.02^{a}	4.10^{e}	139.91^{a}
T3	**LRP 0.05%**	1259.91^{cd}	238.34^{b}	5.32^{bc}	111.87^{bcd}
T4	**LRP 0.1%**	1114.90^{e}	243.86^{b}	4.67^{cde}	97.43^{de}
T5	**LRP 0.15%**	1272.28^{cd}	258.94^{ab}	4.96^{bcd}	93.62^{e}
T6	**ARP 0.1%**	1072.49^{ef}	245.80^{b}	4.39^{de}	96.73^{e}
T7	**ARP 0.15%**	1300.81^{cd}	245.05^{b}	5.35^{bc}	94.78^{e}
T8	**ARP 0.2%**	1323.64^{c}	243.74^{b}	5.48^{bc}	103.03^{cde}
T9	**LGO 0.015%**	1582.82^{b}	289.92^{a}	5.56^{b}	123.00^{b}
T10	**LGO 0.025%**	1744.57^{a}	253.79^{b}	6.92^{a}	113.20^{bc}
T11	**LGO 0.035%**	1567.05^{b}	226.43^{b}	7.00^{a}	106.02^{cde}
SEM		26.147	3.590	0.115	1.902
P-value		0.001	0.001	0.001	0.001

Values bearing different superscripts within a column are significantly (P<0.05) different.
LRP: licorice root powder, ARP: asparagus root powder, LGO: lemongrass oil

Table 3. Effect of licorice root powder, asparagus root powder and lemongrass oil on histomorphometry of jejunum in broiler chicken

Trt	Diets	Villus height (µm)	Crypt depth (µm)	Villus height: crypt depth ratio	Villus width (µm)
T1	**Control**	839.63^{ef}	234.30^{cd}	3.63^{c}	72.27^{e}
T2	**Antibiotic**	809.99^{f}	215.73^{d}	4.11^{bc}	94.61^{cd}
T3	**LRP 0.05%**	1077.80^{bc}	306.92^{a}	3.57^{c}	94.96^{cd}
T4	**LRP 0.1%**	1089.29^{bc}	240.02^{bcd}	4.61^{ab}	120.08^{ab}
T5	**LRP 0.15%**	1118.00^{bc}	228.52^{cd}	5.05^{a}	117.29^{ab}
T6	**ARP 0.1%**	900.92^{de}	252.28^{bcd}	3.70^{c}	89.69^{d}
T7	**ARP 0.15%**	1044.89^{c}	266.56^{abc}	3.95^{bc}	95.64^{cd}
T8	**ARP 0.2%**	956.13^{d}	258.33^{bcd}	3.86^{bc}	98.33^{cd}
T9	**LGO 0.015%**	1209.46^{a}	246.48^{bcd}	4.98^{a}	131.46^{a}
T10	**LGO 0.025%**	1135.95^{ab}	302.65^{a}	3.84^{bc}	108.88^{bc}
T11	**LGO 0.035%**	1075.17^{bc}	280.31^{ab}	3.86^{bc}	85.90^{de}
SEM		14.325	4.766	0.086	2.107
P-value		0.001	0.001	0.001	0.001

Values bearing different superscripts within a column are significantly (P<0.05) different.
LRP: licorice root powder, ARP: asparagus root powder, LGO: lemongrass oil

villus height when compared to control and antibiotic (Table 3), where higher value was observed with LGO 0.015% and 0.025% and lower was observed with ARP 0.1%. Higher crypt depth (P<0.001) was observed with all phytogenic feed additives except LRP 0.15%when compared to control and antibiotic. Among the PFAs higher (P<0.001) crypt depth was observed with LRP 0.05% (T3) and LGO 0.025% and 0.035% (T10 & T11). Whereas, higher VH:CD ratio was observed with LRP 0.1% and 0.15% (T4 & T5) and LGO 0.015% (T9) and lower VH:CD was observed with LRP 0.05% (T3), ARP 0.1% (T6) and control fed (T1) group (P<0.001). Birds fed with phytogenic feed additives (T3 to T10) and antibiotic (T2) showed significantly (P<0.001) higher villus width when compared to control (T1) and LGO 0.035% (T11). Compared to control group, LGO 0.035% (T11) is numerically higher. Among all the treatment groups higher villus width was observed in birds fed with LRP 0.1% and 0.15% (T4 & T5) and LGO 0.015% (T9) (P<0.001).

Ileal morphometry- Significantly higher (P<0.001) villus height was observed in birds fed with LRP 0.15% (T5) followed by T3, T4, T8, T9, T10, T11 and T6 whereas ARP 0.15% (T7), antibiotic @ 0.05% (T2) and control group showed lower (P<0.001) villus height (Table 4 and Fig. 1-11). Among all the dietary treatments, significantly (P<0.001) higher crypt depth was observed with ARP 0.1%, 0.2% (T6 & T8), LGO 0.015% and 0.025% (T9 & T10), whereas, lower values were observed in LRP 0.1% (T4) and control group (T1). Higher VH:CD ratio was observed with LRP 0.05%, 0.1% and 0.15% (T3, T4 & T5) and the lowest ratio was seen with ARP 0.1%, ARP 0.15% (T6 & T7) and control (T1) (P<0.001). Villus width of birds fed with LRP 0.1% and 0.15% (T4 & T5) and LGO 0.015% (T9) was significantly (P<0.001) higher, when compared to control (T1), antibiotic (T2), LRP 0.05% (T3) and ARP 0.15% (T7) and 0.2% (T8) groups which showed significantly (P<0.001) lower villus width; whereas, ARP 0.1% (T6), LGO 0.025% (T10) and 0.035% (T11) exhibited intermediate villus width values.



Fig. 1. Photomicrograph of the cross section of ileum from control group (T1) (H&E, 4X)

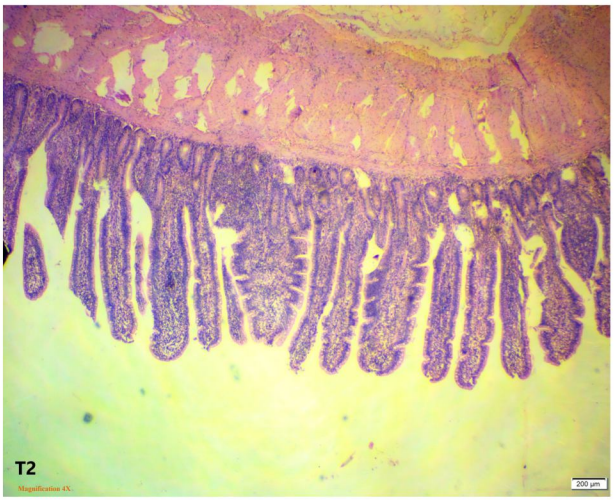


Fig. 2. Photomicrograph of the cross section of ileum from antibiotic group (T2) (H&E, 4X)

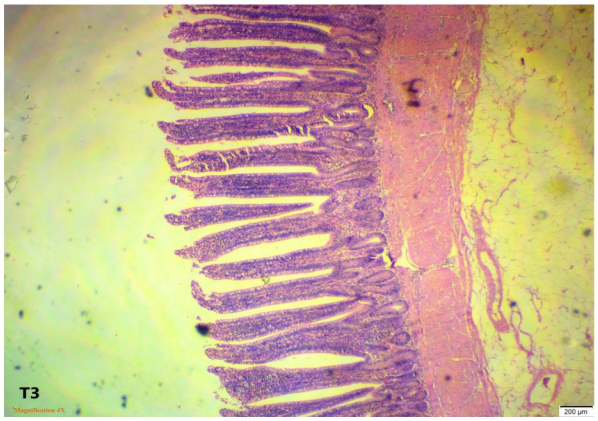


Fig. 3. Photomicrograph of the cross section of ileum from LRP 0.05% group (T3) (H&E, 4X)

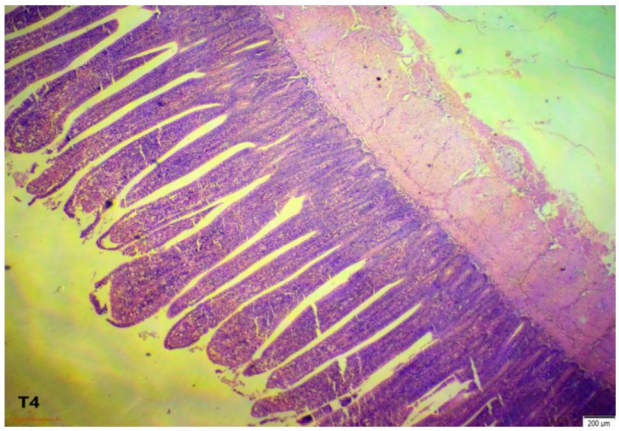


Fig. 4. Photomicrograph of the cross section of ileum from LRP 0.1% group (T4) (H&E, 4X)

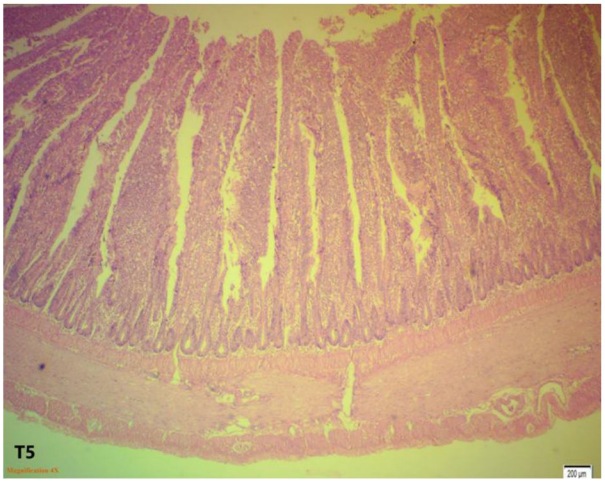


Fig. 5. Photomicrograph of the cross section of ileum from LRP 0.15% group (T5) (H&E, 4X)

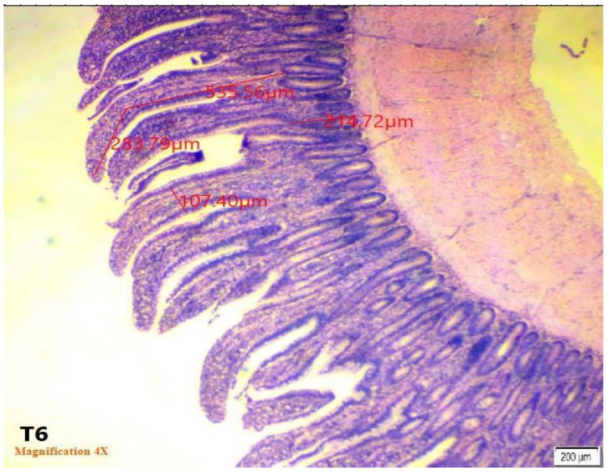


Fig. 6. Photomicrograph of the cross section of ileum from ARP 0.1% group (T6) (H&E, 4X)

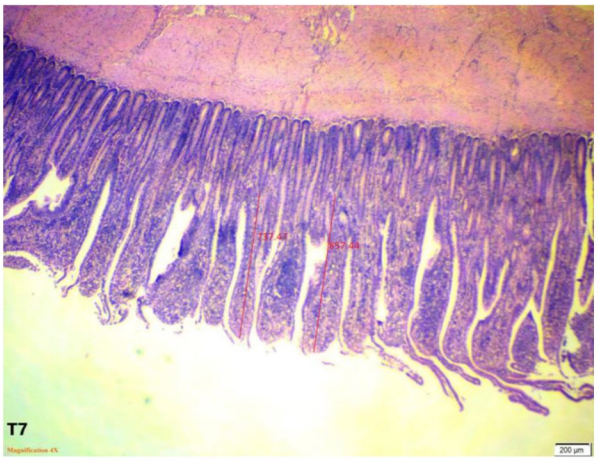


Fig. 7. Photomicrograph of the cross section of ileum from ARP 0.15% group (T7) (H&E, 4X)

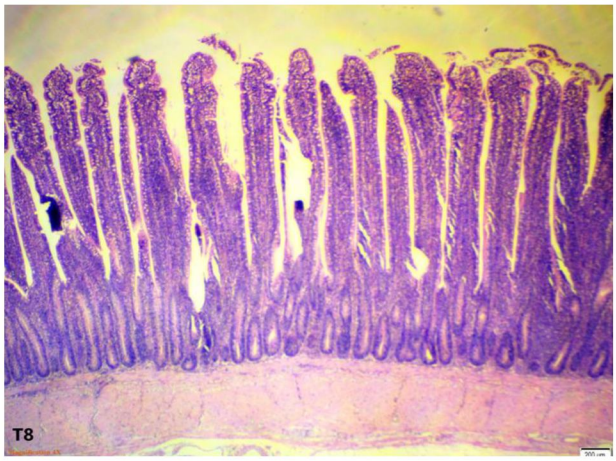


Fig. 8. Photomicrograph of the cross section of ileum from ARP 0.2% group (T8) (H&E, 4X)

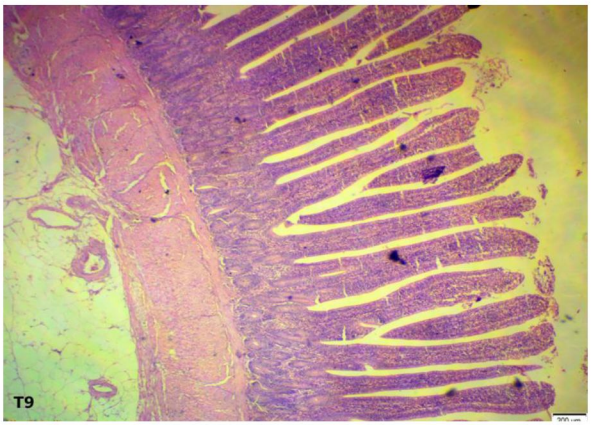


Fig. 9. Photomicrograph of the cross section of ileum from LGO 0.015% group (T9) (H&E, 4X)

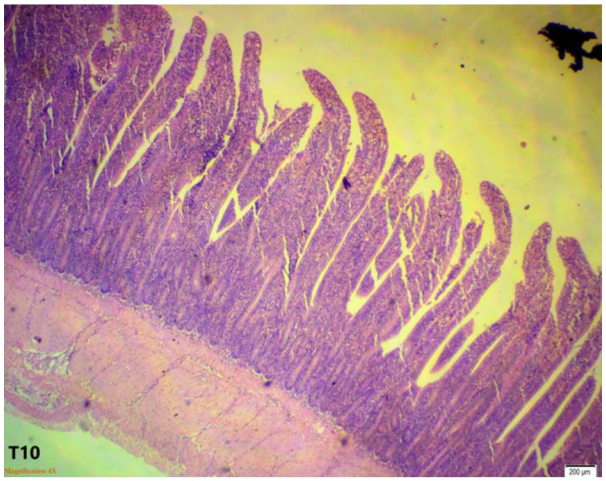


Fig. 10. Photomicrograph of the cross section of ileum from LGO 0.025% group (T10) (H&E, 4X)

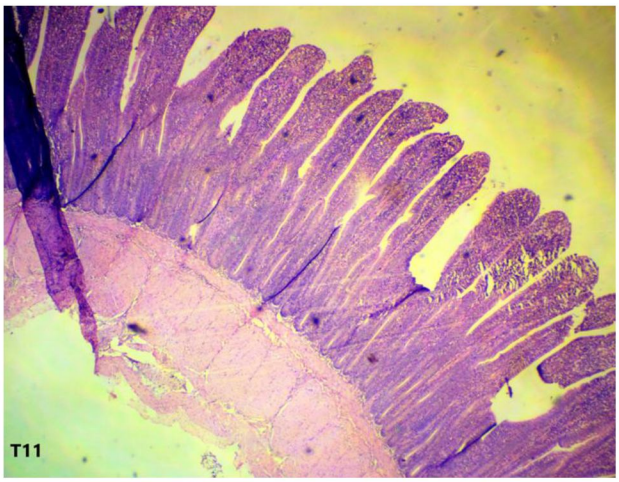


Fig. 11. Photomicrograph of the cross section of ileum from LGO 0.035% group (T11) (H&E, 4X)

DISCUSSION

Significant (P<0.05) improvement in the VH, CD, VH: CD ratio and VW was observed in the duodenum, jejunum and ileum with the supplementation of PFAs (LRP, ARP and LGO) when compared to the control group.

Similar results were reported by Abo-Samaha et al. (2022), who observed significant increase in the intestinal villi length, width, and villi length/crypt depth in broilers supplemented with licorice 0.4 gm/L. Gowthaman et al. (2024) also reported statistically (P≤0.05) higher VH and the ratio of the depth of the crypts in 0.3% LRP group than control group in Kadaknath chickens. Similarly, Nopparatmaitree et al. (2022) reported significantly increased duodenal and jejunal VH, jejunal and ileal CD and duodenal, jejunal, and ileal villus surface areas (P<0.01) with the supplementation of trimmed asparagus by products in broiler diets. El-Sahn et al. (2024) observed enhanced jejunal VH and CD values with supplementation of lemongrass extract in drinking water of broilers. Ujilestari et al. (2024) also reported that adding nanoemulsion of lemon grass essential oil increased the ratio of duodenal VH: CD (P<0.05) in 35-day-old broiler chickens. Inclusion of phytogenic feed additives (Digestarom®) in the broiler diet has significantly improved the VH and lowered CD of the small intestine (Murugesan et al., 2015). Supplementation of palmarosa oil derived from *Cymbopogon martini* in Japanese quails has increased the VH of duodenum, jejunum and ileum (Kurekci et al., 2021).

Further, inclusion of phytobiotics in poultry diets increases villus height with deeper crypt and enhances the digestive enzymes secretion such as proteases and amylases, to promote the growth and production performance of chickens along with enhanced absorptive cell growth in the gastrointestinal tract. Morphological parameters i.e., VH and CD are responsible for vigorous functioning of the intestine by facilitating larger surface area for better absorption of nutrients (Awad et al., 2009). Higher villus height/crypt depth ratio is linked with epithelial cell turn over while decreased ratio is associated with decreased intestinal bacterial population, along with reduced surface area for nutrients absorption (Laudadio et al., 2012).

Table 4. Effect of licorice root powder, asparagus root powder and lemongrass oil on histomorphometry of ileum in broiler chicken

Trt	Diets	Villus height (µm)	Crypt depth (µm)	Villus height: crypt depth ratio	Villus width (µm)
T1	**Control**	574.51^{e}	205.97^{d}	2.80^{c}	78.82^{d}
T2	**Antibiotic**	694.27^{d}	255.79^{bc}	2.86^{bc}	78.13^{d}
T3	**LRP 0.05%**	930.14^{b}	221.01^{cd}	4.29^{a}	75.73^{d}
T4	**LRP 0.1%**	946.23^{b}	205.42^{d}	4.73^{a}	140.56^{a}
T5	**LRP 0.15%**	1086.11^{a}	235.03^{cd}	4.65^{a}	140.59^{a}
T6	**ARP 0.1%**	834.20^{c}	304.76^{a}	2.73^{c}	98.87^{bc}
T7	**ARP 0.15%**	671.43^{d}	249.64^{c}	2.69^{c}	82.94^{cd}
T8	**ARP 0.2%**	998.97^{b}	316.33^{a}	3.28^{bc}	90.59^{cd}
T9	**LGO 0.015%**	983.26^{b}	334.35^{a}	3.02^{bc}	136.57^{a}
T10	**LGO 0.025%**	999.34^{b}	303.03^{a}	3.50^{b}	110.74^{b}
T11	**LGO 0.035%**	919.16^{b}	294.84^{ab}	3.17^{bc}	108.78^{b}
SEM	16.799	5.845	0.093		2.894
P-value		0.001	0.001	0.001	0.001

Values bearing different superscripts within a column are significantly (P<0.05) different.
LRP: licorice root powder, ARP: asparagus root powder, LGO: lemongrass oil

Improved VH, CD, VH: CD ratio and VW of duodenum, jejunum and ileum in this study might be due to the antibacterial property of licorice (Sharma et al., 2013), asparagus (Shah et al., 2014) and lemongrass (Wannissorn et al., 2005). By reducing the number of bacteria in the gut, less enterocytes destroyed and consequently less need to replace them with new ones. Newer cells have shorter VH and thus the ratio of VH: CD is low (Emami et al., 2012).

In contrast to the above results, Beski et al. (2019) observed no effect on the jejunal VH, CD and VH: CD with licorice extract in broilers. Ardiarini and Lase (2021) also reported no significant effect on the VH, VW and CD of duodenum, jejunum and ileum with herbals (turmeric, sambiloto leaves, soursop leaves, ginger, and lemongrass) supplementation in layer hens. Similarly, Thayalini et al. (2011) also reported no statistical differences in ileal VH and VH: CD in broilers fed with dried *Cymbopogon citratus* leaf and dried *Zingiber officinale* rhizome at 2% concentration each.

In conclusion, supplementation of LRP at 0.1% & 0.15%, ARP at 0.2% and LGO at 0.025% significantly (P<0.05) improved the duodenal, jejunal and ileal histomorphometry in terms of villus height, crypt depth, villus height to crypt depth ratio, and villus width when compared to control group in broiler chicken. However, further work has to be done to determine better levels and also regarding their mechanism of actions on intestinal health.

Conflict of interest: There is no conflict of interest among the authors.

Author's contribution: RP: Conception and design, investigation, data generation and preparation of the original draft; CPV: Conception and design, contribution to data analysis and critically revising the manuscript; VLK: Contribution to designing and critically revising the manuscript; ND: Contribution to designing and critically revising the manuscript; RMVLN: Contribution to designing and critically revising the manuscript.

Data availability statement: Data of research will be made available with the corresponding author on demand.

Ethical statement: The procedures in this study were approved by the Institutional Animal Ethical Committee, College of Veterinary Science, Rajendranagar, Hyderabad, Telan gana.

Use of artificial intelligence tools: No artificial intelligence tools were used.

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