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Effect of *Withania somnifera* (Ashwagandha) root powder on morphometry of the intestinal mucosa in broiler chicken

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Abstract
This study was designed with the aim to determine the impact of *Withania somnifera* (Ashwagandha) root powder on morphometry of intestinal mucosa of broiler chicken. For this, one day old three-hundred commercial broiler chicks were randomly divided into six treatments with five replicates per treatment and each replicate has ten birds. Feeding trial was conducted for six weeks in two different growth phases i.e. starter (0-28d) and finisher (29-42d). The experimental diets were: basal diet without antibiotic as control (T_1), basal diet with antibiotic (T_2), 0.25% Ashwagandha root powder (T_3), 0.50% Ashwagandha root powder (T_4), 0.75% Ashwagandha root powder (T_5) and 1.0% Ashwagandha root powder (T_6). At end of the feeding trial, one bird from each replicate was selected, slaughtered and ileal sample were collected for morphometric analysis. Mean villus height of the birds under different dietary treatments ranged from 1106.53 µm to 1352.72 µm. A significant increment in villus height was observed in Ashwagandha supplemented groups. Mean values of villi width were decreased significantly ($P< 0.05$) in Ashwagandha supplemented groups as compared to control. Height: Depth of intestinal crypts under different dietary treatments ranged from 4.20 to 5.16 and significant effect was observed in 0.75% Ashwagandha root powder supplemented group. Based on the results, it can be concluded that supplementation with Ashwagandha root powder influence the morphometry of the intestinal mucosa of broiler chicken upto a significant level.

Keywords: Broilers, ashwagandha, intestinal morphometry, villus

Introduction
Poultry meat has significant role in Indian diet; favoured by socio-economic conditions like rising purchasing power and changing food habits of the people this sector is driven by increased domestic consumption. Poultry meat is an excellent source of high quality protein, vitamins and minerals and is not subjected to cultural and religious restrictions (Singh *et al*., 2016) [14]. The production of safer poultry products without any chemical and microbial residues is the order of the day. Economic balanced feeding with optimum production is the need of hour. It becomes necessary to have an efficient programme with maximum utilization of the nutrients from the available feedstuffs. Over a period of time, extensive efforts have been made to lower down the cost of production by lowering the expenses on feed. Feed additives are one of the important tools used for improving feed conversion ratio, growth rate and disease resistance. The range of feed additives used in animal production industry is very broad ranging from growth promoters to disease preventing agents. The mechanism by which the majority of herbal feed additives exert their antibacterial effect is by acting on the bacterial cell wall structure, denaturing and coagulating proteins. Herbal feed additives effects include improving nutrient utilization and stimulation of the immune system. The possible mechanisms of action of herb in the animal for growth promotion include changes in the intestinal microbiota, increased digestibility, nutrient absorption, enhanced nitrogen absorption, improvement of the immune response, morphological and histological modifications of the gastrointestinal tract and antioxidant activity. Herbs can also contribute by stimulating the endocrine system and intermediate nutrient metabolism.

Materials and Methods
The experiment was conducted at poultry unit of College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. A total of three hundred, day-old commercial broiler chicks were used for the experiment.

The birds were randomly allotted into six dietary treatments with five replicates per treatment and each replicate has ten birds. The birds were from the same hatch and were reared under uniform management condition upto six weeks of age. All the birds irrespective of their treatments were fed maize crumble for first three days of their age, followed by experimental ration prepared as per BIS (2007) [2]. The first group was kept as a control (T_1) and given the basal diet without antibiotic while second (T_2) basal diet with antibiotic, third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups were supplemented with Ashwagandha root powder @ 0.25, 0.5, 0.75 and 1%, respectively in the diet. At the end of the experiment, one bird from each replicate was slaughtered and ileal samples were collected for intestinal morphological examinations. Cross-sections were prepared measuring 1 mm to 2 mm thick from ileum and enclosed in tissue cassettes. The tissues were fixed in 10% neutral buffered formalin over 24 h for each intestinal segment, a 5µm section was placed onto a glass slide and stained with alcian blue and hematox ylin-eosin. Slides were viewed under Fluorescent microscope and visual measurements were taken of the villous length (µm distance from apex of villus to the junction of the villous and crypt), villous width and crypt depth (µm distance from the junction to the basement membrane of the epithelial cell at the bottom of the crypt).

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC), 235/CPCSEA dated 1-8-2000 in the Department of Animal Nutrition, LUVAS.

Experimental design

Completely Randomized Design (CRD) was used as experimental design at uniform and standard management practices.

Composition of diets

Basal ration was formulated as per BIS (2007) [2] to fulfill the metabolizable energy (ME) and crude protein requirements of birds. Level of crude protein in starter (0-4weeks) and finisher (4-6weeks) ratio was 22 percent and 20 percent, respectively. The respective ME content was 3000 and 3200 Kcal/kg. All feed ingredients, additives and supplements used in the experiment were procured in one lot before the start of the experiment. The ingredients, additives and supplements used in the diet formulation were maize, soybean meal, vegetable oil, fish meal, mineral mixture, vitamins, coccidiostat, lysine, DL- methionine and Ashwagandha root powder. The sources, composition and mixing rate of additives/supplements used in ration formulations are presented in Table 1.

Table 1: Ingredient composition of experimental diets during different phases of growth

Ingredient (kg /100 of feed)	0-4 wk	4-6 wk
Maize	58	60
Soybean meal	30	25
Fish meal	7	7
Vegetable oil	3	6
Mineral mixture	2	2
Feed additives (g/100 kg feed)		
Spectromix	10	10
Spectromix BE	20	20
Veldot	50	50
Choline chloride	50	50
Lysine	50	50
DL-methionine	150	150

Composition, sources and rate of mixing of feed additives/supplements

1. **Spectromix:** Powder (Ranbaxy Animal Health, New Delhi). Each g. contained Vitamin A- 82,500 IU, Vit D_3-12000 IU, Vit B_2-50 mg and Vit.K-10 mg. mixing rate: 10 g/100 kg of feed.
2. **Spectromix BE:** Powder (Ranbaxy Animal Health, New Delhi). Each g contained Vit.B_1-8 mg, Vit.B_6-16 mg, Vit.B_{12}-80 mg, niacin-120mg, calcium pentothenate-80 mg, Vit.E-160 mg, Lysine hydrochloride-10 mg, DL-methionine-10 mg and calcium 260 mg. Mixing rate: 20g/100 kg of feed.
3. **Veldot:** Venkeys- Dinitro-O-Toluamide (Coccidiostat). Mixing rate: 50 g/100 kg of feed.
4. **Choline chloride:** Contain 60 percent choline. Mixing rate: 50 g/100 kg of feed.
5. **Lysine:** Contained 98% lysine. Mixing rate: 50 g/100 kg of feed.
6. **DL-methionine:** Contained 98% methionine. Mixing rate: 150 g/100 kg of feed.

Statistical analysis

The data were analyzed using general linear model procedure of statistical package for social sciences 20th version (SPSS) [15] and comparison of means tested using Duncan's multiple range test (DMRT) [3] and significance was considered at $P< 0.05$.

Results and Discussion

Data pertaining to gut morphology of the experimental birds under different dietary treatments are presented in Table 2 and Figure 1.

Table 2: Mean values of villi height, villi width, crypt depth and villi height: crypt depth of intestine of experimental birds under different dietary treatments

	Villi height (Micron)	Villi width (Micron)	Crypt depth (Micron)	Villi height: Crypt depth
T_1	1106.53^{a}±19.68	304.82^{b}±1.24	263.78±1.32	4.20^{a}±0.08
T_2	1112.77^{a}±3.00	297.02^{b}±4.35	263.50±2.01	4.24^{a}±0.03
T_3	1125.73^{a}±7.60	298.18^{b}±2.58	262.76±1.73	4.27^{a}±0.03
T_4	1256.80^{b}±9.18	253.60^{a}±4.07	262.62±0.81	4.79^{b}±0.03
T_5	1352.72^{c}±5.90	248.92^{a}±1.18	262.54±1.18	5.16^{c}±0.02
T_6	1330.34^{c}±10.89	251.34^{a}±3.12	262.50±1.39	5.06^{c}±0.02

Means bearing different superscripts in a column differ significantly ($P< 0.05$)

Villus height under different dietary treatments ranged from 1106.53 µm (T_1) to 1352.72 µm (T_5). Dietary supplementation of Ashwagandha root powder at the level of 0.50% (T_4), 0.75% (T_5) and 1.0% (T_6); significantly ($P< 0.05$) increased the villus height as compared to antibiotic supplemented (T_2) and control group (T_1). Mean values of villi width under different dietary treatments ranged from 248.92 µm (T_5) to 304.82 µm (T_1). There was a significant ($P< 0.05$) decrease in villi width in 0.50%, 0.75% and 1.0% Ashwagandha supplemented groups as compared to control. Under different dietary treatments crypt depth ranged from 262.50 µm (T_6) to 263.78 µm (T_1). Lowest crypt depth was obtained with 1.0% Ashwagandha supplemented group as compared to control group but the differences were non-significant among groups. Villi height: depth of intestinal crypts of the experimental birds ranged from 4.20 (T_1) to 5.16 (T_5) under different dietary treatments. Highest effect on villi height: depth ratio of intestinal crypts was recorded in 0.75% Ashwagandha supplemented group. This was significantly ($P< 0.05$) higher from the control group. The morphometric analysis results showed that the supplementation of Ashwagandha root powder increased villi height significantly ($P< 0.05$). The morphometric analysis result of the present study showed that the supplementation of antioxidants increased villus height, but decreased crypt depth in broilers and these results are in agreements with Park *et al.* (2016) [12] who concluded that the supplementation of antioxidants increased villus height but decreased crypt depth in broilers reared under chronic heat stress**.** Study conducted by Mirakzehi *et al.* (2017) [10] revealed that birds fed 75 mg/kg *Withania somnifera* and *Withania coagulans* with 0.5 µg/kg $1,25(OH)_2D_3$ had lowest values of villus width (100.76, and 102.03µm, respectively) while diet with only 150mg/kg *Withania somnifera* displayed the highest villlus width (160.07µm). Several relevant reports which favoured the findings of the present result; Xu *et al.* (2003) [16] reported the effect of dietary fructooligosaccharide (FOS) on digestive enzyme activities, intestinal microflora and morphology of male broilers. Addition of 4.0 g/kg FOS significantly increased ileal villus height, jejuna and ileal microvillus height, and villus-height to crypt-depth ratios at the jejunum and ileum and decreased crypt depth at jejunum and ileum. Higher villus height, low crypt depth and high villus height /crypt depth ratio are desirable parameters for better absorption of nutrients.

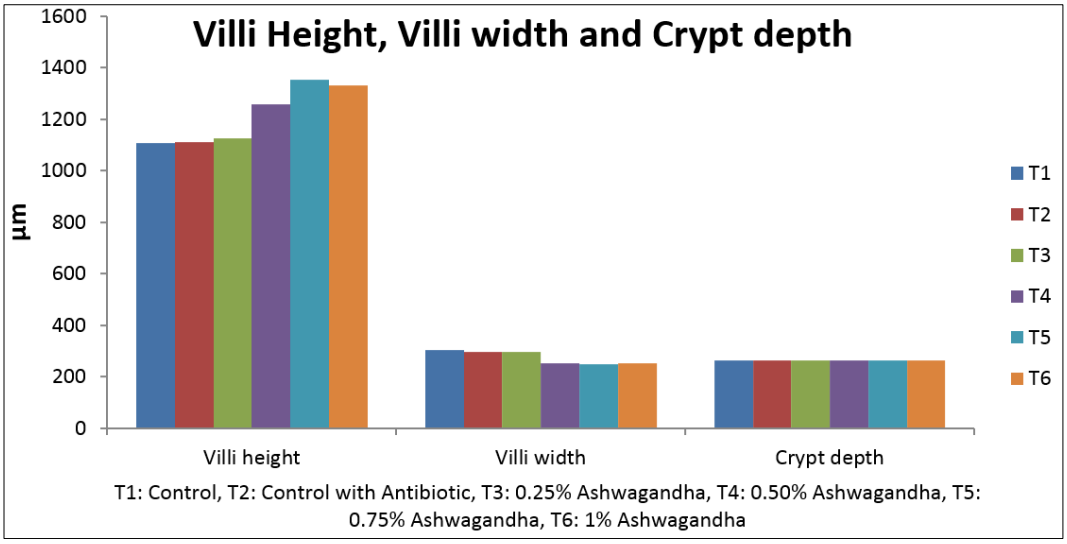

Fig 1: Villi height, villi width and crypt depth of intestine under different dietary treatments

Phenolic compounds administration may reduce gut inflammation, improve digestibility of nutrients and metabolism (Giannenas *et al*., 2010) [6]. The villus height: crypt depth ratio is an indicator of the likely digestive capacity of the small intestine and increase in this ratio corresponds to an increase in digestion and absorption (Montagne *et al*., 2003) [11]. Increased intestinal villi height and the ratio villi height to crypt depth is an indication of the vast area for nutrient absorption and higher absorption function (Sieo *et al*., 2005) [13]. Feng *et al.* (2007) [5] studied the effect of fermented soyabean meal (FBSM) on digestive enzyme activities and intestinal morphology in broilers. They also showed that there were no significant differences on enzymes activity between the treatments while increased villus height ($P< 0.05$) and decreased crypt depth ($P< 0.05$) of jejunum mucosa was observed in the whole growth stage of broilers fed with fermented soyabean meal. In contrast to our results, Attia *et al.* (2017) [1] reported that the villus height, crypt depth and villus height-to-crypt depth ratio of broiler's duodenum, jejunum and ileum were not significantly affected ($P< 0.05$) by dietary inclusion of the plant blend extract. Enhancement of intestinal activities of trypsin, lipase and amylase (Lee *et al*., 2004) [9] and improved gut morphological characteristics (Jamroz *et al*., 2003) [8] are the major mechanisms through which phytoadditives exert their beneficial effect on the nutrient digestibility. Phytogenic additives has antibacterial, antioxidant, antistress, gut microflora manipulation, immune enhancement properties and digestive enzymes stimulation could be the probable reasons for the positive effects exerted by them on the growth and health performance of animals (Durrani *et al*., 2007; Hashemi and Davoodi 2011) [4, 7].

Histological Demonstration of Intestinal Villi

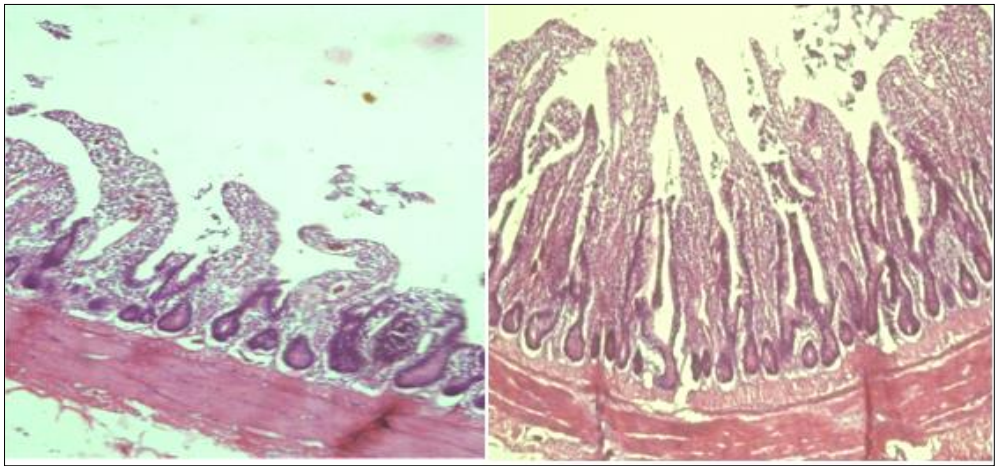

Fig 2: Gut Morphology (Control)

Fig 3: Gut Morphology (0.75% Ashwagandha root Powder)

Conclusion

Based upon the above study, it can be concluded that Ashwagandha root powder can be effectively supplemented as an alternative to antibiotics growth promoter in poultry ration and best results was obtained at 0.75% Ashwagandha root powder supplementation level (T_5) with regards to gut morphology. Production of short chain fatty acids by *Lactobacilli* results into effective migration of enterocytes along the tip of villi which enhanced the growth of intestinal villi. In present study, antioxidants present in amla may effectively scavenge the generated free radicals caused by stress, consequently resulting in improved ileal morphology. The villus: crypt depth ratio is an indicator of the likely digestive capacity of the small intestine. Increased villi height is an indicator of increased enzymatic digestion thereby providing more surface area for absorption of nutrients.

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