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Evaluation of the effect of supplementation of Ashwagandha (*Withania somnifera)* root powder in the broiler's ration on gut morphology and bacteriology: A review

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Abstract
The global for poultry production experienced leaps and bounces to accommodate rising demand over past few years. On the other hand, popular demand and scientific interest for organic poultry production, particularly feeding with medicinal botanicals, has increased considerably in recent years. Ashwagandha (*Withania somnifera*) is a well known herb possessing several health benefits. The alkaloids and steroidal lactones (withanolides) are the main constituent found in it. These are responsible for modulating the gut environment of broiler birds which further responsible for better feed digestion and utilization. With use of Ashwagandha in the diet of broiler birds, there is decrease in harmful microflora and increase in beneficial microflora in gut. A positive change was observed in morphometry of gut, which finally lead to better feed utilization and growth performance of broiler birds. The aim of this study was to review research currently being carried out on the Ashwagandha and phytogenics that have been shown to modulate the gut environment, microflora and morphology and special attention is given to the use of herbal plants as poultry feed supplement.

Keywords: Microflora, morphometry, intestine, Ashwagandha

Introduction
Poultry industry in India is the most dynamic and fastest growing sector. Amongst the various animal husbandry enterprises, poultry production has assumed number one position and attained industrial status in India. Role of meat in human nutrition is important, being an excellent source of high quality protein, vitamins and minerals. Worldwide consumption of poultry meat is growing up in developed as well as in developing countries. 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. All over the world nowadays there is growing concern among the consumers about the ban on the use of antibiotics in poultry products (Wakeman *et al.*, 2005) [30]. Using antibiotics at sub therapeutic lead to the development of drug-resistant bacteria due to antibiotic residues in animals and human (Cervantes, 2006) [6]. Consequently, researchers are now looking towards viable alternatives for disease prevention and performance enhancing supplements. Today, production of poultry meat, physicochemical and sensory properties of meat is becoming more and more important. To the consumer, appearance is the major criterion for purchase, selection and initial evaluation of meat quality. There has been a resurgence of interests for medicinal plants like herbal feed additives, plant extracts with growth, flavour, colour enhancing, antioxidant and antibacterial activities (Omojasola and Awe, 2004) [22]. In ethnoveterinary practice, various herbal and medicinal plants have been used to control diseases in poultry (Waihenya *et al.*, 2002) [29]. Thus herbs could be expected to serve as safer alternatives as growth promoters due to their suitability and preference, lower cost of production, reduced risk of toxicity, minimum health hazards and environment friendliness (Devegowda, 1996) [9]. There is greater concern regarding the use of medicinal plants in improving immunity and growth rate because of easy availability, low cost, good antimicrobial action, reduced disease risks and diversified functions (Lewis *et al.*, 2003; Charis, 2000) [18, 7]. In traditional medical system herbal medicines have been used for thousands of years and have great contribution to maintain human health (Rahman *et al.*, 2011) [24]. Herbs have several pharmacological properties like anti-inflammatory (Udupa *et al.*, 1994) [28], immune-modulant (Mushtaq and Durrani, 2007) [21] and anthelmentic activity (Iqbal

et al., 2006) [14]. Use of herbs as feed additive is a new attention drawing field that has attracted animal nutritionists throughout the world. The beneficial effects of phytobiotics in poultry may arise from the activation of feed intake and the secretion of digestive enzymes, immune stimulation, antibacterial, coccidiostatic, anthelmintic, antiviral or anti-inflammatory activity, or from antioxidant properties.

Withania somnifera is a popular Indian medicinal plant, which is well documented for curing human diseases. *W. somnifera* is also known as Ashwagandha, ginseng, and winter cherry. It has been an important herb in the ayurvedic and indigenous medical system for over 3000 years. Numerous studies indicated that *W. somnifera* possesses antioxidant, antitumor, anti-stress, anti-inflammatory, immuno-modulatory, hematopoetic, anti-ageing, anxiolytic, anti-depressive rejuvenating properties and also influences various neurotransmitter receptors in the central nervous system (Pattipati *et al*., 2003) [23]. The constituents of *W. somnifera* are the steroidal alkaloids and steroidal lactones which are withanolides (Elsakka *et al*., 1990; Mishra *et al*., 2000) [10, 20] with the main active chemical constituent Withaferin A which is a phytosteroid (Lavi, 1965) [17]. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII). Experimental animals treated with *W. sominfera* showed significant increase in hemoglobin concentration, red blood cell count, white blood cell count, platelets count and body weight as compared to control (Ziauddin *et al*., 1996) [31]. It has hypoglycemic, diuretic, Hypocholesterolemic and immune-modulatory properties (Andallu and Radhika, 2000; Das *et al*., 2001; Gautam *et al*., 2004; Mushtaq and Durrani, 2007) [3, 8, 12, 21]. Ashwagandha is reported to be general tonic, anti-stress, hepato protective, haematinic, growth promoter and antioxidant in human practice and anticoccidal agent in poultry practice (Das *et al*., 2001) [8]. The herb Ashwagandha (*Withania somnifera*) reportedly have antioxidative, antistress, anticoccidial, immunomodulatory, cardioprotective and antilipidemic effect besides playing vital role in lowering blood sugar, serum cholesterol and stress induced gastric indigestion and ulcers (Abou-Douh, 2002) [1].

The present review describes effect of Ashwagandha supplementation as feed additive on gut micro flora and intestinal morphometry of poultry birds and its relevance with the results of the conducted study.

Gut Bacteriology and Morphology

An experiment was conducted on 300 day old chicks divided into six treatments with five replicate each having ten birds per replicate. The control group (T_1) was fed with standard diet, while T_2 was fed with antibiotic. Remaining four groups i.e. T_3, T_4, T_5 and T_6 were fed Ashwagandha @ 0.25%, 0.50%, 0.75% and 1.0%. At the end of feeding trial (6 weeks), parameters like gut bacteriology and morohometry were studied. Mean values of *E. coli* (log cfu/g) ranged from 2.89 (T_6) to 4.75 (T_1) among different dietary treatments. There was a significant reduction in *E. coli* count in T_5 and T_6 groups as compared with the control group (T_1). Total *Lactobacilli* (log cfu/g) ranged from 3.28 (T_2) to 5.24 (T_5) and highest value was recorded in the T_5 (5.24) group supplemented with 0.75% Ashwagandha root powder. It was significantly ($P<0.05$) higher than antibiotic supplemented group (T_2) as well as from control (T_1). Other treatment groups T_3, T_4 and T_6 were also significantly ($P<0.05$) higher as compared to control group. On the basis of this study, it can be concluded that there is increase in beneficial (*Lactobacillus* spp) microorganisms in the gut while decrease in harmful (*E.coli*) one. Gut micro flora can have either positive or negative impact on broiler growth. Harmful microflora such as coliforms can lower nutrient utilization by the host cell via increasing gut thickness, increasing gut mucosa turnover rate and/or competing with the host for the feed nutrients (Apajalahti *et al.,* 2004) [4]. On the other hand, beneficial bacteria such as Lactobacillus spp can improve bird growth and health due to their capacity to inhibit pathogenic bacteria via different mechanisms (competitive exclusion, bacteriocin and acid production and stimulation of the immune system) (Rolfe *et al.,* 2000) [26]. The caecal microflora in the current study was improved due to inclusion of Ashwagandha root powder in the feed as manifested by decreased coliform and increased lactobacilli count. Lowered caecal coliform count could be attributed to the antibacterial properties of Ashwagandha or to increased nutrient digestibility and subsequently less undigested nutrients available for bacterial fermentation in ceacum (Attia *et al.,* 2017) [5]. Researchers found that use of antibiotics reduce the gut count of both pathogenic and non-pathogenic bacteria (Ferket et al., 2002) [11].

On analysis of morphometrical data of intestine, the 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 ($P<0.05$). Increased villi height can be considered as an indicator for increased surface area available for nutrient absorption (Amat *et al.,* 1996) [2]. Similarly, decreased crypt depth can be considered as an indicator for lowered production of immature enterocyte with subsequent less tissue turnover rate and less maintainance requirements for building new enterocytes (Savage *et al.,* 1997) [27]. Therefore, improved villus height or villus height-to-crypt depth ratio is usually associated with efficient nutrient absorption and better performance (Geyra *et al.,* 2001) [13].

These findings are in agreement with report documented by Rizwana *et al.* (2012)[25] revealed that extracts of *Withania somnifera* especially methanolic leaf extract were more potent against MRSA (methicillin-resistant *Staphylococcus aureus*) than standard antibiotic, vancomycin (30µg) used in the study. They concluded that chloroform, acetone, methanolic and ethanolic extracts of *Withania somnifera* might be exploited as natural drug for treatment of several infectious

diseases. Similarly, Kumari and Gupta (2015)[16] concluded that the aqueous root extract of *W. somnifera* holds an excellent potential as an antibacterial agent against *E. coli* O78 and ascertains the value of medicinal plants used in Ayurveda, which could help in the development of an alternative drug. Another study by Attia *et al.* (2017) [5] reported a positive ($P<0.05$) influence on cecal microflora count (decreased *coliforms* and increased lactobacilli count) by inclusion of the plant extract blend. However; intestinal histomorphological parameters like 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. This lowered caecal *coliform* count could be attributed to the antibacterial properties of the utilized plant extract components in the plant extract blend or to increased nutrient digestibility and subsequently less undigested nutrients available for bacterial fermentation in the ceacum. The morphometric analysis of study conducted by Mirakzehi *et al.* (2017)[19] 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). Kumar *et al.* (2018) [15] reported that birds supplemented with corn soyabean based diet supplemented with 100mg *Withania somnifera* extract/kg diet reduced E. coli counts significantly ($P<0.05$) than other groups.

Conclusion

To summarize, the results of this study may lead us to conclude that the addition of Ashwagandha root powder at the level of 0.75% and 1.0% of feed either alone or in combination with other herbs in the diet of the broilers increased the beneficial microflora and decreased harmful microflora found in gut of broilers. Supplementation of herb also increased villus height and villus height to crypt depth ratio resulting in better utilization of feed by the broiler birds. An improvement is observed in gut morphology and microflora content that further improved feed efficiency. Thus, inclusion of Ashwagandha root powder could enhance the overall growth, gut microflora and morphometry of broiler birds. Supplementation of Ashwagandha in basal diet could be commercially interesting as it may have the potential to be an alternative to antibiotic growth promoter for broiler chickens.

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