International Journal of Research in Engineering, Science and Management
Volume-2, Issue-1, January-2019
www.ijresm.com | ISSN (Online): 2581-5792

Aloe Vera and Probiotic (*Lactobacillus Acidophilus*) on Immune Status and Histomorphological Changes in Broiler Birds

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***Abstract*: Two hundred one-day old broiler chicks were randomly distributed into five treatments groups with five replicates (n=5) per group. Each replicate contained 8 chicks five dietary groups consisted of: 1) basal diet of as per BIS standard, 2007 without any supplementation (control); 2) basal diet with BMD (Bacitracin methylene disalicylate) 0.5g/kg of feed (AB); 3) basal diet with 0.5% Aloe vera powder (ALV); 4) basal diet with Lactobacillus acidophilus (LAB) and 5) basal diet with 0.5%Aloe vera powder plus *Lactobacillus acidophilus* (ALVLB) . Antibody titre against Newcastle disease virus was not affected by the dietary treatment at day 28 and day 35. Intestinal morphology including villus length, crypt depth and their ratio was not generally affected by any dietary treatments compared with control. Aloe vera powder (0.5%) and Lactobacillus acidophilus can be used as an alternative to antibiotic growth promoters.**

***Keywords*: Aloe vera, Probiotic, Lactobacillus acidophilus, immune status, histomorphological, broiler birds**

1. Introduction

Poultry industry is one of the fastest growing segments of the agricultural sector today. The availability of feed is one of the major constraints for poultry industry. High levels of production and efficient feed conversion are the need of the modern poultry industry, which to a certain extent could be achieved by the use of specific feed additives. Antibiotic feed additive as growth promoters have long been supplemented to poultry feed to improve the general performance and prevent some specific intestinal pathology [1]. However, due to emergence of microbial resistance to antibiotics the European Commission (EC) decided to phase out, and ultimately ban, the marketing and use of antibiotics as growth promoters in feed since 2006. Among the alternatives phytobiotics and probiotics have the potential to improve the production performance of poultry.

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance, feed conversion efficiency, weight gain and reduce mortality or a live microbial feed that is beneficial to health [2]. Aloe vera (*Aloe barbadensis*) is a well-known medicinal herb and used for commercial and therapeutic properties in many parts of the world. Aloe vera gel contains compounds with proven antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, anti-diabetic, immunomodulatory, and wound healing properties [3]. Studies have reported that there is increased in the *Lactobacillus* count in the broilers by supplementing diets with Aloe vera (Lin *et al.*, 2005) due to its prebiotic properties [4].

2. Materials and methods

A. Location & Climate

This experiment was conducted at Instructional Livestock Farm Complex, Department of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, Selesih, Aizawl, Mizoram during the early summer months (Feb-April) during which environmental temperature was in the range of 18 ^{o}C to 25 ^{o}C. Permission for using the animals for the experiment was duly taken from Institutional Animal Ethics committee (IAEC) constituted as per the Article No. 13 of the CPCSEA rules laid down by Government of India.

B. Experimental birds, experiment design & diet

Two hundred one-day old broiler chicks were randomly distributed into five treatments groups with five replicates (n=5) per group. Each replicate contained 8 chicks five dietary groups consisted of: 1) basal diet of as per BIS standard, 2007 without any supplementation (control); 2) basal diet with BMD (Bacitracin methylene disalicylate) 0.5g/kg of feed (AB); 3) basal diet with 0.5% Aloe vera powder (ALV); 4) basal diet with Lactobacillus acidophilus (LAB) and 5) basal diet with 0.5%Aloevera powder plus Lactobacillus acidophilus (ALVLB). The chicks were reared group wise under the similar management condition and health care. The birds were vaccinated against Ranikhet and Infectious Bursal Disease at the 7th and 14th day respectively. The chickens were house in floor pen on fresh rice husk and saw dust. Plastic wire nest up was used to separate the different pens. Three types of standard broiler diets have been prepared i.e. broiler pre-starter (1-7 days of age), broiler starter (8-21 days of age) and broiler finisher (22-42 days of age) as per BIS (2007) specification.

C. Procurement of aloevera powder

The natural pure Aloevera powder was purchased from local market.

D. Preparation of probiotic culture (Lactobacillus acidophilus)

The probiotic product (Lactobacillus acidophilus) was procured from National Dairy Research Institute, Karnal, India. From the stock culture, a loop full of Lactobacillus acidophilus was transferred aseptically to glycerol solution for maintaining the micro-organism culture by incubating for 24 hrs. at 370C.The bacterial culture was kept in deep freeze. Then by using MRS agar the bacterial colony was grown, which was suspended in PBS solution and concentration was checked in Mac-Ferlend. So, Minimum concentration of Lactobacillus acidophilus was maintained 106/ g of feed. Aloe vera powder and culture of *L.acidophilus* were added to basal diet and mixed thoroughly to obtain different treatment diets.

E. Immune status

Vaccination against Newcastle disease (NDV) was done on 4th day and 20th day. Antibody titer will be measured by indirect ELISA using commercial kit following the manufacturer's instructions on 28th day and 35th day aged groups. The plates will be read by ELISA plate reader at required wavelength suggested by the manufacturer.

F. Histo-morphological study of small intestine

At day 42, four chickens from each dietary treatment were slaughtered by cervical disarticulation for measurement of the height and width of the intestinal villus and crypt length. The small intestine will be removed and 2 to 3 cm sections of duodenum, jejunum (between the entry of bile duct and Mackel's diverticulum) and ileum will be removed, rinsed with PBS and cross sectional lengths of 1 cm were fixed in 10% buffered formaldehyde (pH 7.2), followed by embedding in paraffin wax. The slides with the tissue sections were then stained with Delafield's Hematoxyline and Eosin, and mounted on distrene plasticizer xylene (DPX) as per the protocol described [5]. All the measurements will be made using an ocular micrometer (under a microscope fitted with a stage micrometer).

G. Statistical analysis

The data was analyzed by one way analysis of variance (ANOVA) using SPSS (1997) by completely randomized design. The test was employed for identifying the significant differences amongst the different treatments probability values less than 0.05 is considered to be statistically significant and the values P<0.01 was declared as trend.

3. Results and discussion

A. Immune status

Statistical analysis revealed non-significant difference (P>0.05) in antibody titre against Newcastle disease virus among the different groups on different day of collection. The lymphoid organ weights have been depicted in table 4.12. Statistical analysis revealed no significance difference (P>0.05) in weight of lymphoid organs of different treatment groups in experimental birds. Antibody titre against the common poultry diseases Newcastle Disease, Infectious Bronchitis and Infectious Bursal Disease was increased by the use of probiotic product Primalac [6]. It is also reported that bioactive peptide release by the lactic acid bacteria during fermentation could contribute to immunomodulation effect [7] and interaction between host cells and pathogen may lead to modulation of + cell mediated or B cell mediated immune response [8]. Acemannan stimulates immunity through potentiation of lymphocyte response to alloantigen with activation of nitric oxide production by macrophages and cytokines such as IL-1, 6, IFN and TNF. Weight of lymphoid organ viz. liver, spleen & bursa was not affected due to ALV & LAB supplementation individually or in combination. Similar findings were reported by [9] did not observe a significant difference in weight of lymphoid organs, but reported a weight gain in spleen and bursa. Such relative increase in the weight of lymphoid organs as a result of adding Aloe vera to feed or drinking water suggests immune (humoral and cellular) system readiness against antigens.

The mean ±SE of villi length were ranged from 973.30±20.80 (group-1) to 1359.3±98.20 (Group-2); Villi width ranged from 97.81±7.09 (Group-4) to 126.16±19.05 (Group-3); Crypt length ranged from 109.98±7.77 (Group-4) to 143.02±11.66 (Group-2); Crypt width ranged from 36.80±2.34 (Group-1) to 44.09±2.86(Group-4) ; Villi: Crypt length ranged

Table 1
Immune status in broiler chicken

Attributes	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	P value
d 28	2.33±0.33	2.66±0.33	3.33±0.33	3.33±0.33	3.33±0.33	0.17^{NS}
d 35	3.33±0.33	3.33±0.33	3.66±0.33	4.33±0.33	3.66±0.33	0.27^{NS}
lymphoid organ weight						
Spleen	1.91±0.08	1.86±0.03	1.92±0.07	1.86±0.02	1.84±0.03	0.782^{NS}
Spleen %	0.106±0.005	0.09±0.006	0.101±0.007	0.09±0.004	0.09±0.006	0.789^{NS}
Liver	31.66±1.66	31.66±1.66	33.33±8.81	41.66±6.00	30.00±5.77	0.609^{NS}
Liver %	1.76±0.08	1.65±0.04	1.72±0.38	2.16±0.25	1.53±0.21	0.41^{NS}
Bursa	0.99±0.12	0.86±0.02	1.28±0.11	1.03±0.02	1.09±0.05	0.052^{NS}
Bursa %	0.05±0.008	0.046±0.003	0.066±0.003	0.053±0.003	0.056±0.006	0.23^{NS}

NS=Non significant, AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*

Table 2
Intestinal morphology in broiler chickens

Attributes	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	P value
Villi length	973.30±20.80	1239.4±48.60	1157.2±101.99	1172.0± 138.80	1225.0±167.31	0.162^{NS}
Villi width	105.74±7.05	122.23±10.89	126.16±19.05	97.81±7.09	103.60±5.15	0.319^{NS}
Crypt Depth	116.60±9.84	124.53±11.66	112.22±2.03	109.98±7.77	119.68±3.83	0.104^{NS}
Villi:Crypt	8.58±0.70	10.80±1.82	10.32±1.00	10.72±1.10	10.14±1.11	0.233^{NS}

NS=Non significant, AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*

from 8.28±0.59 (Group-3) to 10.72±1.10 (Group-4) respectively. Statistically there was no significant difference (P>0.05) among the various experimental birds for Villi length, Villi width, Crypt depth, Villi: Crypt. The villi length is highest for the entire treatment group compared to control. Our findings are similar to [10] who found Villus height in probiotic (*B. coagulans* ATCC 7050) treated birds was greater than in birds treated with an AGP (Zinc–Bacitracin) when measured at 6 weeks age. [11] Found that the Probiotic (PRO) group had the greatest villus height (P < .05) while no significant difference was found between the PRO-WHP (whey protein) group and the WHP group in terms of villus height (P > 0.05). Small intestinal morphology including villus height, crypt depth & their ratio is an important indication of the gut health and proper functioning of intestine. Diet is one of the main factors which alter the intestinal morphology. Increased villus height and villus height/ crypt depth ratio are direct associated with increased epithelial turn over and activated cell [12].

4. Conclusion

Considering the overall performance of broiler birds in terms of feed intake, body weight gain, feed conversion efficiency and digestibility of nutrients it can be concluded that, Aloe vera and *Lactobaciluus acidophillus* have the potential to be used as alternative to antibiotic growth promoters.

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