EFFECT OF SUPPLEMENTING *MORINGA OLEIFERA* LEAF EXTRACT AND CLOVE BUD OIL TO THE DIET ON MICROFLORA POPULATION AND INTESTINAL MORPHOLOGY OF BROILER BIRDS

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Abstract

The present experiment was carried out to assess the effect of *Moringa oleifera* leaf extract and clove (*Syzygium aromaticum*) bud oil on the intestinal gut morphology and microbial status of broiler birds. Two hundred broiler chicks (day old) were randomly distributed into five treatment groups. Dietary supplemented groups were comprised of Group-1 (control); Group- 2 (basal diet with Bacitracin methylene disalicylate @0.5 g/kg of feed); Group-3 (basal diet with *Moringa oleifera* extract @ 0.55 g/kg of feed); Group- 4 (basal diet supplemented with clove bud oil @ 0.6 g/kg of feed) and Group- 5 (diet supplemented with *Moringa oleifera* extract and clove bud oil combination at same dose rate respectively). Result revealed that *Salmonella* spp. and *E. coli* counts in caecum were found similar ($P>0.05$) across different treatment groups but *Lactobacillus* spp. count tended ($P=0.10$) to be higher for group-3, group-4 and group-5 as compared to control and antibiotic fed group. Intestinal histomorphological studies showed significant difference ($P<0.05$) in villi height to crypt depth ratio (VH/CD) of duodenum of experimental birds in group-3 in comparison to other groups. VH/CD ratio of jejunum and ileum were tended to be higher ($P=0.07$ and $P=0.06$ respectively) in antibiotic, *Moringa* plant extract and clove oil supplemented group than control. The finding might have resulted from a controlled damage to the enterocytes, thus reducing the nutrient requirement for their turnover in *M. oleifera* and clove bud oil supplemented groups. It can be concluded that *Moringa oleifera* leaf extract and clove bud oil have the potential to be used as an alternative to antibiotic growth promoter to improve the health status of broiler birds.

Key words: Broiler, Intestinal morphology, Microflora, *Moringa oleifera*, *Syzygium aromaticum* oil

INTRODUCTION

Poultry industry has been growing at faster rate in India. Antibiotic growth promoters (AGP) have been used as feed additive in poultry industry to enhance gut health and to control sub-clinical diseases (David *et al.*, 2012). Antibiotics have generated resistant strains by transferring resistance to other species and made serious problem in public health and livestock. Since 1st January 2006, use

of in-feed antibiotics as growth promoters was banned by European countries (Catala-Gregori *et al.*, 2008). Therefore, several researchers had searched for alternatives to AGP such as, organic acids, phytobiotics, bacteriophages, probiotics and prebiotic (Yan *et al.*, 2012). Phytobiotics (essential oil, herbs, spices, and various plant extracts) are among those potential alternative to antibiotic growth promoters, which have received expansion

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as potential alternative to AGP. *M. oleifera* leaves are traditionally used widely for its antimicrobial properties (Suarez *et al.*, 2005) and its pharmacological effects (Mehta *et al.*, 2003). Clove found to inhibit some gut microbes and acts as an antiseptic and digestion stimulant, and has powerful antifungal and antimicrobial activity, anti-inflammatory, anti-carcinogenic and antioxidant effects (Ehrlich *et al.*, 1995; Mitsch *et al.*, 2004; Najafi and Torki, 2010). Eugenol, an active principle present in clove oil has antimicrobial (Ehrlich *et al.*, 1995), and anti-inflammatory (Feng and Lipton, 1987). Briozzo *et al.* (1989) revealed that clove oil with its active compound eugenol, destroy *Clostridium perfringens* and other bacteria. Hence, the present study was carried out to investigate the effect of *M. oleifera* and clove bud oil supplementation on microbial status and intestinal morphology of broiler birds.

MATERIALS AND METHODS

Two hundred (n=200) day old chicks (Vencobb) were distributed randomly into five treatment groups with five replicates (n=5) per group. Each replicate comprised of 8 chicks and five dietary groups consisted of: Group-1 (control) birds were fed basal diet; Group-2 (AB) were fed basal diet with Bacitracin methylene disalicylate @0.5 g/kg of feed; Group-3 (MPE) were fed basal diet with *Moringa oleifera* crude extract @ 0.55 g/kg of feed; Group-4 (CBO) had received the basal diet supplemented with clove bud oil @0.6g/kg of feed and birds in Group-5 (MPCBO) had received the basal diet supplemented with *Moringa oleifera* crude extract and clove bud oil combination at same dose rate respectively. The experimental house, feeder and watering trough were properly cleaned and poultry house was disinfected by white washing the floor and walls. All the birds were vaccinated against to Newcastle disease and Infectious Bursal disease. The chickens were housed in floor pen on fresh rice husk

and saw dust. Three types of standard diet of broiler were prepared i.e. broiler pre-starter (1-7 days of age), starter (8-21 days of age) and finisher (22-42 days of age) as per BIS (2007) specification. *Moringa oleifera* leaves were processed and the acetonic extract for the plants were prepared following the standard procedure. Level of inclusion of plant extracts and clove oil were considered based on minimum inhibition concentration (MIC). Antimicrobial activity of the *M. oleifera* extracts was done by disc diffusion method (CLSI, 2008). Clove bud oil was purchased from Allin exporters, New Delhi, India.

Microbial status in caecum of broiler birds:

The pre-caecal contents of the intestine were collected aseptically from the birds after slaughter on 42nd day of the trial. The pre-caecal contents were stored at 4°C and bacteriological enumeration was performed on the same day. Serial dilution of 1 g of the pre-caecal content was done (10-fold) with sterile phosphate buffer saline (PBS). Each dilution (10 µL) was poured on Nutrient agar (HiMedia, India), MRS agar (HiMedia, India), SS agar (HiMedia, Mumbai, India) and EMB agar (HiMedia, Mumbai, India), for finding the count of total bacteria, *Lactobacillus* spp., *Salmonella* spp. and *E.coli* respectively. The plates were incubated aerobically at 37°C for 24 h. The colonies of bacteria were enumerated in a colony counter (hand held digital colony counter, LA663, Himedia Lab. Pvt. Ltd., Mumbai, India) and the numbers were expressed as Log₁₀ colony forming units (CFU) per gram of sample.

Histo-morphological study of small intestine:

Five chickens from each dietary treatment were slaughtered on 42nd day by cervical disarticulation for the measurement of height of the intestinal villus and crypt depth. About 2 to 3 cm sections of duodenum, jejunum and ileum were removed and washed with PBS. Cross section (1 cm) was fixed in 10% buffered

formaldehyde (pH 7.2) for about 1-2 weeks, followed by paraffin wax embedding. With Delafield's Hematoxyline and Eosin, the tissue slides were stained, and mounted on distrene plasticiser xylene based on protocol described by Incharoen *et al.* (2009). All measurements were taken with Advanced Olympus trinocular research microscope.

Statistical analysis: The data were analyzed by one-way ANOVA using SPSS software in a completely randomized design as per the procedure described by Snedecor and Cochran (1994). Probability values $P \leq 0.05$ were declared as significant and the values of $0.05 > P \geq 0.10$ were declared as trend.

RESULTS

Microbial status in caecum of broiler birds:

There were no significance difference ($P > 0.05$) in the counts of total bacteria, *Salmonella* spp., *E.coli* spp. and *Lactobacillus* spp. of experimental birds among different treatment groups (Table 1). However, the counts of *Lactobacillus* spp. was tended to be higher ($P = 0.10$) in Moringa plant extract and clove oil supplemented groups than antibiotic and control group.

Histo-morphological of small intestine:

Morphometric measurements obtained for small intestines of experimental broiler chickens (duodenum, jejunum and ileum) are presented in Table 2. The villi height, crypt depth, VH/CD ratio

Table 1. Caecal bacterial count ($\log_{10}$ CFU/g) of experimental broiler birds fed diet supplemented with *Moringa oleifera* extract and clove bud oil

Bacteria	Control	AB	MPE	CBO	MPCBO	SEM	P value
Total bacteria	6.05	5.79	6.25	5.97	6.08	0.113	0.81
<i>Lactobacillus</i> spp.	5.38	5.86	6.01	6.01	6.03	0.09	0.10
<i>Salmonella</i> spp.	5.61	4.98	4.96	5.48	4.96	0.11	0.17
<i>E. coli</i>	5.35	5.10	4.81	4.46	4.84	0.12	0.22

AB- Antibiotic; MPE- Moringa plant extract; CBO- Clove bud oil; MPCBO- Moringa plant extract and clove bud oil

Table 2. Effect of supplementation of *Moringa oleifera* extract and clove bud oil on intestinal morphology in broiler chickens

Attributes	Control	AB	MPE	CBO	MPCBO	SEM	P value
Duodenum							
Villus height(μ m)	2169.5	2245.1	2238.8	2245.4	2237.6	16.68	0.29
Crypt depth(μ m)	207.76	212.88	176.17	196.36	201.49	4.87	0.12
Villus height/ crypt depth	10.47 ^a	10.55 ^a	12.77 ^b	11.46 ^a	11.18 ^a	0.27	0.028*
Jejunum							
Villus height(μ m)	1361.5	1408.8	1433.7	1396.6	1411.2	10.11	0.24
Crypt depth(μ m)	171.74	158.87	161.37	172.54	158.67	2.44	0.15
Villus height/ crypt depth	7.93	8.89	8.89	8.11	8.90	0.15	0.07
Ileum							
Villus height(μ m)	1015.5	1074.7	1079.8	1067.1	1062.7	11.21	0.41
Crypt depth(μ m)	174.19	163.90	165.33	163.14	166.08	1.59	0.17
Villus height/ crypt depth	5.83	6.55	6.53	6.54	6.40	0.09	0.06

AB- Antibiotic; MPE- Moringa plant extract; CBO- Clove bud oil; MPCBO- Moringa plant extract and clove bud oil; ^{a,b} means with in a row with different superscripts are significantly different.;*($p < 0.05$)

of duodenum, jejunum and ileum of experimental broiler birds did not differ significantly ($P>0.05$) among different treatment groups. Intestinal histomorphological studies showed significant difference ($P<0.05$) in villi height to crypt depth ratio of duodenum of experimental birds in MPE group in comparison to other groups. Villi height to crypt depth ratio of jejunum and ileum were tended to be higher ($P=0.07$ and $P=0.06$ respectively) in antibiotic, Moringa plant extract and clove oil supplemented group than control.

DISCUSSION

Microbial status: Gut microbes have significant effects on host nutrition and health. Gut microbiome benefit the host by supplying nutrients and modulate development of digestive and immune system (Pan and Yu, 2014). *M. oleifera* leaf extracts has a potential role as an antibacterial and antioxidant (Sreelatha and Padma, 2009). Eugenol, a principal constituents of clove bud oil had good antibacterial activity against *Listeria monocytogenes* and *Campylobacter jejuni* but had medium antibacterial activity against Enterobacteria (Brenes and Roura, 2010). In the present experiment, *Salmonella* spp. and *E. coli* counts were not found significantly different ($P>0.05$) between groups. *Lactobacillus* spp. count was tended to be higher in supplemented groups. In agreement with the present study, Abd El-Ghany and Eraky, (2019) revealed that there was no significant ($P>0.05$) difference in total bacterial and Coliform count in intestine between control and *M. oleifera* supplemented group. Agostini *et al.* (2012) reported that *Lactobacillus* count were increased due to supplementation of clove bud oil in the diet of broilers, which could be due to positive selection of eugenol towards lactic acid bacteria. On contrary to our findings, Chowdhury *et al.* (2018) reported that *Clostridium* spp. counts reduced ($P=0.029$) in the antibiotic growth promoter group compared with the clove bud oil supplemented group and control and *Lactobacilli* spp. count was not

affected ($P=0.39$) by any essential oil supplementation. Similarly, Gandomani *et al.* (2014) reported that incorporation of clove bud powder and fish oil either alone or in combination significantly ($p<0.05$) reduced the ileal *Escherichia coli* and *Salmonella* spp. counts.

Small intestinal morphology: The intestinal morphological characteristics in poultry are vital for nutrient utilization and indicate the sound physiology of the host. Longer villi are useful for better absorption of the nutrients due to more surface area and also indicators of improved gut health (Xu *et al.*, 2003). In this experiment, VH/CD ratio of duodenum of experimental birds in MPE group was significantly higher compared to other groups. Villi height to crypt depth ratio of jejunum and ileum were tended to be higher ($P=0.07$ and $P=0.06$ respectively) in antibiotic, Moringa plant extract and clove oil supplemented group than control. These findings may have resulted from a controlled damage to the enterocytes, thus reducing the nutrient requirement for their turnover in *M. oleifera* and clove bud oil supplemented groups. This finding is in agreement with Khan *et al.* (2017) who observed that inclusion of *M. oleifera* leaf powder at 1.2% increased significantly ($P<0.05$) VD/CD ratio in ileum compared to control. On contrary, Acosta *et al.* (2018) reported that at 42 days, the height of villi of the duodenum of the control group obtained the lowest values with respect to *M. oleifera* supplemented groups. Chowdhury *et al.* (2018) revealed that the villi height in jejunum, duodenum and ileum was higher ($P=0.04$) in the cinnamon bark oil supplemented group than control, clove bud oil and ajwain oil groups, while no significant difference was found between cinnamon oil and antibiotic growth promoter in broiler chicken. Kavoi *et al.* (2016) observed that no significant differences VH/CD ratio were noted between control and *M. oleifera* leaf meal supplemented birds. Gandomani *et al.* (2014) observed that feeding clove bud, at 2% level, increased in villus height, VH/CD ratio,

the number of lamina propria lymphatic follicles but decreased in crypt depth and goblet cells.

Considering the microbial status and intestinal morphology of the experimental broiler birds, it can be concluded that *Moringa oleifera* leaf extract

and clove bud oil have the potential to be used as an alternative to antibiotic growth promoter to improve the health status of broiler birds.

Conflict of interest: There is no conflict of interest.

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