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EFFECT OF SUPPLEMENTATION OF DIFFERENT COMBINATIONS OF ORGANIC ACIDS AS REPLACER OF GROWTH PROMOTING ANTIBIOTIC IN DUCK

A.K. Paul[1], S.K. Mukhopadhayay[1]*, D. Niyogi[2], and S. Ganguly[3]

[1]Department of Veterinary Pathology, Faculty of Veterinary & Animal Sciences,West Bengal University of Animal and Fishery Sciences, Kolkata - 700 037, WB, INDIA

[2]Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, Narendra Deva University of Agriculture and Technology, Faizabad - 224 229, UP, INDIA

[3]AICRP-PHT (ICAR), Department of Fish Processing Technology, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 094, WB, INDIA

ABSTRACT

A series of experiments have been conducted in the present study to evaluate the various aspects and effects of different combination of organic acids viz., formic acid and propionic acid as a replacer of growth promoter antibiotic(s) in ducks. The ducks were divided into five equal groups with one as Control. Studies on body weight gain revealed that after 48 weeks body weight gain was higher in treated groups as compared to the control Group C. Bacteriological studies of different portions of small intestine revealed that total coliform count and Clostridium perfringens count (log10 CFU/g) was significantly (P<0.05) reduced in the small intestine of the birds in treated groups as compared to Group C. Salmonella sp. was not found in any group. No significant results of Lactobacillus count (log10 CFU/g) were noticed in the intestinal digesta of the ducks in treated groups. Study on villus height of different potions of small intestine (i.e. duodenum, jejunum and ileum) revealed significantly higher villus height in treated groups as compared to Group C.

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***Corresponding author:** Email: skmpath@gmail.com; Tel: +91 9433036799

[I] INTRODUCTION

The use of organic acids in poultry nutrition can be an efficacious tool to replace antibiotic growth promoters. Organic acids are not antibiotics, but if used correctly along with nutritional, managerial and bio-security measures, they can be a powerful tool in maintaining the health of the gastro-intestinal tract of poultry, thus improving their zoo-technical performances [1]. Organic acids in proper dosage as prebiotic supplements in poultry feed act as potential and promising cause for body weight gain and growth promotion without having any deleterious and/ or residual effects, unlike the use of hazardous antibiotics growth promoters. The mode of action of organic acids on bacteria is that non-dissociated (non-ionized, more lipophilic) organic acids can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria [2].

The present investigation was conducted to study the effect of different combinations of organic acids on poultry intestine considering the parameters of body weight gain, pH of different parts of gastrointestinal tract, bacterial count of intestine, changes in villi height of different parts of small intestine and changes in crypt depth of different parts of small intestine respectively.

[II] MATERIALS AND METHODS

2.1. Experimental design

Sixty (60 No.) day-old Khaki Campbell ducks were reared in a poultry house. After attaining 24 weeks of age ducks were weighed randomly and distributed into five groups comprising of 12 birds each. One group was maintained as control and other 4 groups were maintained as organic acid treated groups as described in Table-1.

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Table. 1: Distribution of experimental groups of ducks

S.No.	Group	Number of ducks	Diet
1	C (control)	12	Diet without acidifier(s) and antibiotic(s)
2	T1	12	Diet with antibiotic BMD @ 50gm/100 Kg of feed
3	T2	12	Diet with ammonium formate @ 0.3% level
4	T3	12	Diet with calcium propionate @ 0.3% level
5	T4	12	Diet with ammonium formate (0.15%) + calcium propionate (0.15%)

*Supplied per Kg diet: Vitamin A: 8000 IU, Vitamin D3: 1200 IU, Vitamin E: 24 IU, Vitamin K: 1.5 IU, Thiamin: 1 mg, Riboflavin: 6 mg, Niacin: 60 mg, Pantothenic acid:10 mg, Pyrioloxime: 2.5 mg, Cobalamine: 20 mg, Biotin: 0.15 mg, Folic acid: 100 mg, Choline chloride: 800 mg, selenium: 150 µg.

2.2. Fortification of diet with OAS and AGP

The OAS (organic acid supplemention) and AGP (animal growth promoter) were added to the basal diet by substituting at the expense of maize .Ammonium formate (Molecular weight: 63.06, Minimum assay: 92%) and calcium propionate (Molecular weight: 188.22, Minimum assay: 97%) were used as a source of formic acid salt and propionic acid salt respectively in the diet. Final concentration of OAS in each of the respective treatments was 0.3% of the diet. Bacitracin methyl disalicylate was used as a source of AGP. The control diet was not fortified with either OAS or AGP.

2.3. pH value of feeds

10 samples were taken from each of the diets assigned for different treatment groups to determine their pH. 10 gm of each sample mixed with 90 ml of deionized water and pH was determined by pH meter.

2.4. Microbial counts in feed

1g each of 10 feed samples was taken in sterile test tubes and diluted to 10 ml with 0.1% peptone water (autoclaved at 15 lbs for 15 min) and mixed thoroughly with cyclo-mixer. 0.1 ml volume (0.5 ml for *Clostridium perfringens*) of the supernatant was spread with a sterile loop on the surface of specific culture media- Mc Conkey agar for total coliforms, EMB agar for *E. coli*, xylose lysine deoxycholate agar for *Salmonella*, *perfringens*, selective agar for *C. perfringens*, lactobacillus isolation selective agar for *Lactobacillus sp*. and potato dextrose agar media for *Aspergillus sp*. All the inoculated media except for *C. perfringens* were incubated aerobically at 37oC for 48 h and the confirmed colonies were enumerated after Gram's staining. The results which were expressed as log10 CFU/g of feed and were calculated as follows:

Total viable number of microbes = Mean plate count x dilution factor x 10 (The values were multiplied by 10 as the appropriate solution was of 10 ml)

2.5. Body weight

Body weight of each of the birds was recorded with the digital weighing balance on day '0' and subsequently at 2 weeks intervals up to 8 weeks (starter phase), 3 weeks intervals up to 48 weeks of age (layer phase) in the morning before the birds had any access to feed and water.

2.6. Study on gastrointestinal tract

The study on gastrointestinal tract was conducted at the end of 48 weeks age of the birds.

2.7. pH of digesta

Following evisceration the intact gastrointestinal tract was removed, segmented and mixed thoroughly in 15 ml deionized water. pH was determined by pH meter.

2.8. Microbial counts in digesta

Digesta present in the gastrointestinal tract was considered for bacteriological study on selective media for enumeration of *Salmonella*, *Lactobacillus* and *C. perfringens*. The results which were expressed as log10 CFU (colony forming unit) per gm of digesta, were calculated as follows:

Total viable number of microbes = mean plate count x dilution factor x 10 (The values were multiplied by 10 as the appropriate solution was of 10 ml.)

2.9. Determination of villus length and crypt depth in the small intestine

The investigation on the villus heights and crypt depths of different portions of small intestine was performed by histopathological studies and measurements were made microscopically. The results were expressed in micrometer (µm).

2.10. Statistical analysis

For the parameters one way Analysis of variance (ANOVA) technique was used for studying the effect of organic acids. Levels of significance were calculated by Duncan Test [3] whenever any effect was found significant using F-statistic either 1% or 5% level of significance. The analysis was done by SPSS software.

[III] RESULTS AND DISCUSSION

3.1. Effect on body weight

The data are presented in **Table-2** which depicts the live weight of the ducks in different phases of egg laying cycle. There was significant (P<0.001) difference in live weight of the birds fed FP diet with those fed propionate or formate supplemented feed.

Table. 2: Effect of organic acids and antibiotic on live body weight (g) of ducks

Age of bird (in weeks)	Live body weight (gm)						
	Group C	Group T1	Group T2	Group T3	Group T4	SEM	P-level
24	1671.4	1684.97	1702.63	1709.23	1716.13	6.54	P>0.05
28	1683.72d	1731.48c	1725.45c	1762.37b	1792.30a	0.91	P<0.001
32	1688.69e	1738.23c	1729.65d	1767.98b	1798.22a	1.29	P<0.001
36	1695.85e	1747.17c	1738.02d	1775.48b	1805.30a	1.24	P<0.001
40	1699.32e	1750.33c	1741.32d	1778.40b	1808.74a	1.10	P<0.001
44	1704.44e	1755.54c	1745.98d	1783.58b	1814.24a	1.02	P<0.001
48	1707.62e	1758.44c	1749.28d	1786.28b	1816.68a	0.87	P<0.001

Values bearing different superscripts in a column differed significantly, SEM: Standard error mean. Each value is an average of ten observations.

3.2. pH of digesta

The data related to pH of digesta in the ducks at 48 weeks of age are detailed in **Table– 3**. No significant changes in digesta pH in any part of the gastrointestinal tract except in the proventriculus were observed in the birds fed BMD or organic acids compared to those of control group. The pH of the digesta in the proventriculus of the birds fed formate (pH 2.71), propionate (pH 2.83) and FP (pH 2.99) supplemented diets were significantly (P<0.05) lower than that of the birds fed non-treated diet (pH 3.25).

Table. 3: Effect of organic acids and antibiotic on pH of digesta in ducks at 48 weeks

pH of different portion of small intestine	Group C	Group T1	Group T2	Group T3	Group T4	SEM	P-level
Crop	4.64	4.67	4.46	4.71	4.53	0.067	P>0.05
Proventriculus	3.25b	3.22b	2.71a	2.83a	2.99ab	0.051	P<0.05
Gizzard	3.43	3.39	3.32	3.43	3.33	0.079	P>0.05
Duodenum	5.60	5.46	5.36	5.47	5.43	0.082	P>0.05
Ileum	7.23	6.83	6.69	6.60	6.61	0.063	P>0.05
Cecum	7.23	6.70	6.72	6.71	6.67	0.095	P>0.05

Values bearing different superscripts in a column differed significantly, SEM: Standard error mean, Each value is an average of ten observations.

3.3. Bacteriology of small intestine

The bacterial counts of different portions of small intestine of the ducks at 48 weeks of age are shown in **Table-4**. Salmonella spp. was not detected in the intestine of the birds of any treatment group. It was found that total coliform count (log10 CFU/g) was significantly (P<0.05) reduced in the small intestine of the birds fed FP (3.64), propionate (3.77), formate (3.89) or BMD (4.23) supplemented diet compared to the birds fed non-supplemented control (4.42) diet.

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Table. 4: Effect of organic acids and antibiotic on total bacterial count (log_{10} CFU/g) of different portions of small intestine of ducks at 48 weeks age

Total count (log10 CFU/g) of bacterial type	Group C	Group T1	Group T2	Group T3	Group T4	SEM	P-level
Total coliform	4.42c	4.23bc	3.89abc	3.77ab	3.64a	0.078	P<0.05
E. coli	3.58b	2.88a	2.92a	2.61a	2.58a	0.052	P<0.01
C. perfringens	3.38b	2.83a	2.93a	2.68a	2.77a	0.057	P<0.05
Lactobacillus spp.	6.49	6.64	6.76	6.83	6.74	0.078	P>0.05

Values bearing different superscripts in a column differed significantly, SEM: Standard error mean, Each value is an average of ten observations.

The lowest (P<0.05) *C. perfringens* count (log10 CFU/g) was noticed in the small intestine of the birds fed propionate (2.68) supplemented diet followed by FP (2.77), BMD (2.83), and formate (2.93) groups compared to the birds of control group (3.38). No significant results of *Lactobacillus* count (log10 CFU/g) was noticed in the intestinal digesta of the ducks of treatment group.

3.4. Villus length

The results on villus length in the small intestine of the ducks at 48 weeks of age are presented in **Table-5**. Villus length of all the birds fed diets supplemented with either BMD or organic acids was significantly (P<0.001) higher than that of the birds fed control diet (966.50).

It was observed that in the birds fed with propionate (804.56), FP (803.45) or BMD (781.32) supplemented diet, villus length (µm) in the ileum was significantly (P<0.001) higher than that of the birds of control group (767.42). However, there was no significant difference in villus length (µm) in the ileum between the birds fed diets supplemented with propionate (804.56) and (803.45).

Table. 5: Effect of organic acids and antibiotic on villus height of different portions of small intestine of ducks at 48 weeks

Villus height (µm) of different portion of small intestine	Group C	Group T1	Group T2	Group T3	Group T4	SEM	P-level
Duodenum	1324.05d	1395.28b	1371.83c	1455.36a	1467.23a	1.053	P<0.001
Jejunum	966.50d	1037.84c	1017.68b	1094.34b	1103.01a	0.709	P<0.001
Ileum	767.42d	781.32b	774.69c	804.56a	803.45a	0.517	P<0.001

Values bearing different superscripts in a column differed significantly, SEM: Standard error mean, Each value is an average of ten observations.

3.5. Crypt depth

The results on crypt depth in the small intestine of the ducks at 48 weeks of age are presented in **Table-6**. Crypt depth (µm) in duodenum of the birds fed FP (302.30), propionate (302.29), BMD (309.46) and formate (319.94) supplemented diets was significantly (P<0.001) lower than that of the birds fed control feed (345.72). No significant variation was observed between the birds of FP, propionate and BMD groups as well as BMD and formate groups. In jejunum, lowest depth (µm) of crypt was measured in the birds fed FP supplemented diet (263.94) which was followed by the birds fed propionate (271.24), BMD (280.69), formate (287.63) and control (311.59) diets. Crypt depth of all the birds fed diets treated with organic acid and antibiotic was significantly (P<0.001) variable with that of the birds fed control diet.

The observations of variations in pH of digesta of organic acid treated groups of ducks in the present study can be correlated with the previous reports. Thomlinson and Lawrence [4] measured a lower gastric pH when 1% lactic acid was added to drinking water and offered to gastric cannulated piglets. Waldroup et al [5] found a reduction of cecal pH in relation to addition of formic acid-propionic acid blend in the diet of broiler chickens. The dietary mannan oligosaccharide (MOS) and *Saccharomyces cerevisiae* (SC) supplementation did not affect body weight and body weight gain, but MOS supplementation increased daily feed intake and feed conversion ration (FCR) of turkey toms. Carcass and part weights and yields, meat color and composition were not influenced by the dietary MOS and SC supplementation. There is a possibility that the level of MOS and SC generally recommended in finishing turkey diets by the companies could be too low to be effective on carcasses, cut yields and meat composition of turkeys [6]. The oral administration of yeast cell wall preparation improved innate immune responses in the chicken (broiler) [7].

Byrd et al. [8] found that treatment with lactic or formic acid caused significant (P<0.05) reduction in crop pH of broiler birds. Thirumeignanam et al. [9] reported reduced pH in the gut of broiler birds fed organic acids, but the values were within the physiological pH range. They also noticed reduced bacterial load and increased lactobacilli in crop, gizzard, duodenum and cecum.

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Table. 6: Effects of organic acids and antibiotic on crypt depth of small intestine of ducks at 48 weeks of age

Crypt depth (µm) in different portion of small intestine	Group C	Group T1	Group T2	Group T3	Group T4	SEM	P-level
Duodenum	345.72a	309.46c	319.94b	302.29d	302.30d	0.38	P<0.001
Jejunum	311.59a	280.69c	287.63b	271.24d	263.94e	0.44	P<0.001
Ileum	307.82a	279.50c	283.51b	269.77d	261.52e	0.30	P<0.001

Values bearing different superscripts in a column differed significantly, SEM: Standard error mean, each value is an average of ten observations

[VI] CONCLUSION

Based on the findings of the present study, it can be concluded that combination of organic acid in appropriate dose schedule may be used in duck feed as a replacer of growth promoter antibiotics, as their indiscriminate and injudicious use may cause some pathomorphological alteration in intestinal epithelium.

CONFLICT OF INTERESTS

None

FINANCIAL DISCLOSURE

Nil

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