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Effect of different organic acids with probiotic supplementation on gut health of broiler chicken

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Abstract
The present research was conducted to study the effect of supplementation of acidifiers with probiotic on performance of broiler chicken. A total number of 300 birds were reared for a period of forty two days with dietary treatments; T_0 - control diet as per BIS (2007), T_1 - control + sodium diformate @ 0.2%, T_2 - control + sodium diformate @ 0.2 + probiotic @0.02%, T_3 - control + blends of acidifiers @0.2%, T_4 - control + blends of acidifiers @0.2% + probiotic @0.02%. Each treatment consist of sixty birds with four replicates containing fifteen birds per replicate. Gut parameter study showed significant decrease in ileal pH, *E. coli*, *Salmonella* and *Clostridia* count. However, there was increase in intestinal weight, length in all treatment groups as compared to control.

Keywords: Acidifiers, probiotic, gut parameters, *E-coli*, *Salmonella*

Introduction
Acidifiers are being considered as one of the viable option of the antibiotics as of late due to their antimicrobial activity against extensive variety of pathogenic microorganisms in light of their capacity to prompt a pH reduction in the gut and these can enhance nutrient utilization in poultry diets (Eidelsburger *et al*., 1992; Boling *et al*., 2000; Kil *et al*., 2011) [14, 9, 26]. Most organic acids having antimicrobial action have a pK value (characterized as the pH at which the acid is half dissociated) in the range of 3 to 5. These have been used either as single acid or combination of several acids (Wang *et al*., 2009) [43]. Utilization of organic acids and their salts in poultry has been permitted as safe by the European Union (Adil *et al*., 2010) [3]. Organic acids have growth-promoting properties (Fascina *et al*., 2012) [5] also its use could stimulate the natural immune response (Lohakare *et al*., 2005) [29]; Abbas *et al*., 2013) [1]. Organic acid supplementation significantly increased the villus width, height and area of GI tract (Kum *et al*., 2010; Rodriguez-Lecompte *et al*., 2012) [28, 36]. Probiotics are either single as well as blend of live microbial culture which elevate health benefits to the host (Fuller, 1992) [16]. Method of probiotics action includes competition with receptor sites in the intestinal tract, production of specific metabolites (short organic fatty acids, hydrogen peroxide, other metabolites possessing antimicrobial activity) and immune stimulation effect (Madsen *et al*., 2001; Sherman *et al*., 2009) [30, 37]. Microorganisms used as probiotics includes *Lactobacillus, Streptococcus, Enterococcus, Bacillus, Clostridium, Bifidobacterium* species and *E. coli* while yeast and fungus used as probiotics include *Saccharomyces cerevisiae* and *Aspergillus oryzae* (Fuller, 1999) [17]. Bacteria and yeasts have been included as spores or as living micro-organisms. *Saccharomyces* known to offer a good quality protein and B-complex vitamins. Due to immunomodulatory properties, yeast extract, the non-antibiotic functional product is suggested to be the potential non-antibiotic alternative for decreasing pathogenic bacteria in turkey production (Huff *et al*., 2010) [22]. At present yeast cell derivatives are gaining importance as zootechnical feed additives (Swiatkiewicz *et al*., 2014) [40]. Microencapsulation of probiotic can be used to enhance the viability during processing and also for the targeted delivery in gastrointestinal tract. The reason behind the use of probiotics has been primarily to establish normal intestinal flora with broad target of prevention or minimizing the disturbances caused by enteric pathogens (Dhama *et al*., 2008) [12] The strain of probiotic to be called as ideal should be resistant to acid, bile salts and digestive enzymes. Considering the wide scope for the research of combination of single or blends of acidifiers with probiotic to give optimum synergistic effect on performance of broiler chicken, the present study is planned.

Materials and Methods
The research was completed at Poultry Research Center, Post Graduate Institute of Veterinary and Animal Sciences, Akola (MAFSU Nagpur). The research was conducted on one day old

300 chicks of Cobb 430 strain for a span of 42 days from 22 January to 5 March 2018.A day old chicks were acquired from Amruta Hatcheries Pvt. Ltd. Amravati. These chicks were assigned to 5 dietary treatments, T_0 (control diet as per BIS, 2007) [8], T_1 (control plus sodium-diformate @ 0.2%), T_2 (control plus sodium diformate @ 0.2% plus probiotic @ 0.02%), T_3 (control plus blends of organic acid @ 0.2%), T_4 (control plus blends organic acid @ 0.2% plus probiotic @ 0.02%) with 60 birds in each group having 4 replicates of 15 birds each. Sodium diformate, mixes of various natural acids (Acidomix viz. buffered organic acids like Calcium Propionate, Sodium Formate ,Fumaric acid, Sorbic acid and Citric acid in equal quantity) and probiotic (encapsulated *Saccharomyces cerevisiae* @1 x 10^{10} CFU/g) were supported by Venkeys India Pvt. Ltd. Pune. The chemical analysis of different feed ingredients were carried out at Department of Animal Nutrition, PGIVAS, Akola. Based on chemical investigation, the diet was formulated for pre-starter, starter and finisher according to BIS 2007 [8] and shown in table 1. Standard managemental practices were followed during entire trail period. At the end of experiment, two experimental birds from each replicate (8 birds per treatment) were randomly selected and slaughtered to determine each of intestinal weight, ileum pH, intestinal length and microbial count. The carcasses of broilers were subsequently opened and the entire gastrointestinal tract was removed aseptically. Gut weight is determined after aseptical removal of intestine on digital weighing balance. To determine the pH, 10 g of intestinal content from ileum was collected aseptically in 90 ml sterilized physiological saline (1:10 dilution) (Al-Natour and Alshawabkeh, 2005) [6] and pH was measured by using digital pH meter.Gut length was measured with the help of measuring tape maintaining the aseptical conditions. Ceacal content specimens were taken aseptically and were transferred into sterile plastic bags and immediately transported in cold chain to the laboratory. One gram of each sample was diluted 1:9 (wt/vol) in sterile saline. All samples were subjected to 10 sequential dilutions 1:9 (vol/vol), and 0.1 mL of each sample was plated as duplicates by using spread plate method for *E coli*-EMB agar, *Salmonella-Salmonella shegell a*agar, *Clostridium*-nutrient agar .The samples were incubated for 22 ± 2 h at 37 ºC. Incubation procedure was conducted under aerobic (*E coli* and *Salmonella*) and anaerobic (*Clostridium*) condition in incubator. After incubation, typical colonies were counted. Results were expressed as $\log_{10}$ colony-forming units per g of ileal digesta ($\log_{10}$ CFU/g). Results for presence of each bacteria (*E. coli, Salmonella and Clostridia spp*.) were also checked. The data obtained was analyzed by utilizing Statistical Package for the Social Sciences (SPSS) Version 17.0. The differences between means were subjected to ANOVA by univariate analysis using General Linear Model. The Significant differences among treatment means were separated by using Duccan's Multiple Range test and considered as significant when P–value was less than 0.05.

Table 1: Composition of broiler ration

Ingredient	Pre-Starter					Starter					Finisher				
	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4	T_0	T_1	T_2	T_3	T_4
Maize	46.2	46.2	46.2	46.2	46.2	49	49	49	49	49	54	54	54	54	54
Soya (DOC)	43.5	43.5	43.5	43.5	43.5	40.6	40.6	40.6	40.6	40.6	35.1	35.1	35.1	35.1	35.1
Soya oil	5.57	5.57	5.57	5.57	5.57	6.3	6.3	6.3	6.3	6.3	6.92	6.92	6.92	6.92	6.92
L-Lysine	0.01	0.01	0.01	0.01	0.01	-	-	-	-	-	-	-	-	-	-
DL-Methionine	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
LSP	1.13	1.13	1.13	1.13	1.13	1.15	1.15	1.15	1.15	1.15	1.1	1.1	1.1	1.1	1.1
DCP	2.01	2.01	2.01	2.01	2.01	1.86	1.86	1.86	1.86	1.86	1.79	1.79	1.79	1.79	1.79
Trace-min mix	0.5	0.5	0.5	0.5	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vit mix	0.30	0.30	0.30	0.30	0.30	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30	0.30	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostat*	0.10	0.10	0.10	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder*	0.10	0.10	0.10	0.10	0.10	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium diformate*	-	0.2	0.2	-	-	-	0.2	0.2	-	-	-	0.2	0.2	-	-
Probiotic*	-	-	0.02	-	0.2	-	-	0.02	-	0.02	-	-	0.02	-	0.02
Acid Mixtures*	-	-	-	0.2	0.02	-	-	-	0.2	0.2	-	-	-	0.2	0.2
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CP (%)	23	23	23	23	23	22	22	22	22	22	20	20.1	20.1	20.1	20.1
ME (Kcal/kg)	3000	3000	3000	3000	3000	3100	3100	3100	3100	3100	3200	3200	3200	3200	3200

*****Over and above

Results and Discussion

The average value of Gut pH, Gut weight, Gut length and total bacterial count namely *E. coli*, *Salmonella*, *Clostridia* were determined at the end of experiment after sacrificing eight birds from each treatment (two birds from each replicate). The data was statically analyzed and the results are tabulated in Table 2.

Table 2: Gut parameters of different dietary treatment

Treatment Particular	T0	T1	T2	T3	T4	Pooled Mean
Ileal pH	$6.44^{b} \pm 0.25$	$6.36^{b} \pm 0.19$	$6.05^{ab} \pm 0.19$	$5.75^{a} \pm 0.12$	$5.56^{a} \pm 0.13$	6.03 ± 0.09
Intestinal Length	$160.91^{a} \pm 0.76$	$164.39^{ab} \pm 2.21$	$170.64^{b} \pm 5.03$	$175.11^{b} \pm 10.15$	$170.24^{b} \pm 0.5$	168.26 ± 7.06
Intestinal weight	$74.3^{a} \pm 2.79$	$78.86^{ab} \pm 2.91$	$80.75^{abc} \pm 7.27$	$82.45^{bc} \pm 7.99$	$86.5^{c} \pm 7.65$	80.57 ± 7.14
E. coli (*10^7 CFU/g)	$7.33^{b} \pm 0.24$	$7.04^{b} \pm 0.25$	$6.39^{b} \pm 0.46$	$6.77^{b} \pm 0.39$	$5.04^{a} \pm 0.36$	6.51 ± 0.2
Salmonella (*10^7 CFU/g)	$6.13^{c} \pm 0.59$	$5.29^{bc} \pm 0.18$	$4.4^{b} \pm 0.32$	$4.25^{ab} \pm 0.27$	$3.29^{a} \pm 0.22$	4.67 ± 0.21
Clostridia (*10^7 CFU/g)	$2.53^{c} \pm 0.22$	$2.35^{c} \pm 0.15$	$1.76^{b} \pm 0.06$	$1.34^{ab} \pm 0.21$	$1.03^{a} \pm 0.19$	1.8 ± 0.12

Treatments in column bearing common superscripts doesn't differ significantly (P<0.05)

It was revealed that values of ileal pH shows significant differences. The lowest pH was recorded in the treatment T_4 followed by T_3, T_2, T_1 and T_0 treatment groups. It was observed that ileal pH of all treatment group were found to lower as compared to control. These results obtained in present study are in agreement with AL-Tarazi and Alshawabkeh (2003) [7] who reported that lower levels of acids (0.5%) significantly ($P<0.05$) lowered the pH of the crop and cecal contents. Al-Natour and Alshawabkeh (2005) [6] also found reduced levels of pH of crop, small intestine, large intestine and caeca contents in all groups except 0.5% formic acid group. Similar results were observed by Thirumeignanam *et al.* (2006) [42], Brzoska *et al*. (2013) [10], Grashorn *et al.* (2013) [18], Ishfaq *et al.* (2015).However Huff *et al.* (1994) [23] found no consistent effects on pH of intestinal contents when supplemented with calcium propionate and propionic acid in the diet. Paul *et al.* (2007) [33] also reported that pH of different segments of gastrointestinal (GI) tract was unaffected by organic acid salt supplementation. Abdel-Fttah *et al.* (2008) [2] and Kral *et al.* (2011) [27] found non-significant differences in GI-tract segments.It was observed that values pertaining to intestinal length between the treatment groups were found to be significant. The highest value was recorded in treatment group T_3 (Diet containing mixtures of acidifiers) and lowest value observed in T_0 control group. Similar results were found to Adil *et al.* (2011) [4]. Also Rehman *et al.* (2016) [35] who performed a study to determine the influence of dietary acetic acid (AA) supplementation on gut parameters. Increased intestinal length were recorded in AA treated birds. The results observed in present study may be attributed to the fact that the acidifiers have direct stimulatory effect on the gastro-proliferation intestinal cell (Adil *et al*., 2011) [4]. It was revealed that, there were non-significant differences between the treatment groups for the intestinal weights (g).It was observed from table that the highest intestinal weight was in T_4 group whereas lowest intestinal weight was in control.In accordance with results obtained in present study Adil *et al.* (2011) [4] found significant increase in intestinal weight by supplementation of organic acid than control. Also Rehman *et al.* (2016) [35] worked on the influence of dietary acetic acid found higher intestinal weight. These results could be attributed to the fact that organic acids have direct stimulatory effect on the gastrointestinal cell proliferation as was reported by other workers that short chain fatty acids increase plasma glucagon-like peptide 2 (GLP-2) and ileal pro-glucagon mRNA, glucose transporter (GLUT2) expression and protein expression, which are all signals which can potentially mediate gut epithelial cell proliferation. (Tappenden *et al.*, 1998).Data pertaining to values of *E coli* count between the treatment groups were found to be significant .It was observed that Treatment group T_4 differed significantly lower than T_3, T_2 , T_1, T_0 .Whereas differences among the treatments T_3, T_2 , T_1, T_0 found to be non-significant. Similar results were obtained to Czerwinski *et al*. (2010) [11]. Total bacterial counts in cecal contents were slightly higher for birds fed the pea diets, but were not affected by OA or Pro supplements. The results indicate that the use of pea and probiotics in broiler feed may stimulate the cecal commensal microbiota (growth and/or activity) to some extent and hence prevent establishment of pathogenic and zoonotic enterobacteria in these segments of the gut. Hassan *et al.* (2010) [20, 21], Agboola *et al.* (2015) [15], Raga and Korany (2016) [34], Kazempour and Jahanian (2017) [25], Nosrati *et al.* (2017) [32], Youssef *et al.* (2017) [44] also reported similar results. However, Paul *et al.* (2007) [33] and Gul *et al.* (2014) [19] found non-significant differences for *E. coli* count among treatments and control.It was observed from the Table 2 that, there was significant differences for *Salmonella* count between the treatment groups. Treatment group T_4 differ significantly thanT_0 ,T_1, T_2. while treatment groups T_0, T_1, T_2, T_3 differed non-significantly. The lowest value was recorded in T_4 followed by T_3, T_2 ,T_1 and T_0 treatment group.The results of the present study are in accordance with Raga and Korany (2016) [34] who recorded significant decrease in the *salmonella* using potassium diformate and formic acid. Kazempour and Jahanian (2017) [25] also found similar results. There were significant differences for *Clostridia* count between the treatment groups (Table 2). Treatment group T_4 differ significantly than T_0 , T_1, T_2. while treatment groups T_2 and T_3 differed non-significantly. The lowest value was recorded in T_4 followed by T_3, T_2 ,T_1 and T_0 treatment group respectively .The results of the present study are in agreement with Hassanein and Soliman (2010) [20, 21] ,Raga and Korany (2016) [34]. The dissociation kinetics of organic acid salts such as KDF permits a proportion of FA to pass through the fore-gut intact and enter the small intestinal tract. So that, the KDF able to reduce *C. perfringens* and control necrotic enteritis in broiler flocks at (0.45%) (Mikkelsen *et al*., 2009) [31].

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