Original Research

Effect of Supplementation of Organic Acids and Their Combination as an Alternative to Antibiotic Growth Promoter on Performance, Gut Health, Immune Status and Economics of Broiler Production

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Rec. Date:	Aug 12, 2018 07:46
Accept Date:	Nov 14, 2018 01:03
DOI	10.5455/ijlr.20180812074616

Abstract

An experiment was conducted on 360 Vencobb^{400} day old straight run chicks which were weighed and distributed randomly into six treatment groups viz. A, B, C, D, E and F with four replicates of 15 chicks. The treatment group A was served feed without organic acid and BMD. Treatment B had Bacitracin Methylene Disalicylate (BMD).The treatment groups C, D and E were supplemented with sodium citrate, ammonium formate, calcium propionate @ 1g/kg feed, respectively. Treatment F had combination of sodium citrate, ammonium formate and calcium propionate each @ 1 g/kg feed. The body weight, feed consumption and feed conversion ratio were significantly affected (P <0.01) by organic acids and their combination. The pH of crop (P<0.01) and ileum (P<0.05) differed significantly. The serum antibody titre of IBD at 21st day had significant (P<0.01) difference. The net profit per kg was higher in organic acid groups. It is concluded that ammonium formate, sodium citrate, calcium propionate and their combination are beneficial for growth performance, gut health, immune status, profitability and may be used as an alternative to BMD.

Key words: Broiler Performance, Economics, Gut Health, Immune Status, Organic Acid

How to cite: Ahmed, A., Dhumal, M., Nikam, M., Nandedkar, P., & Ingle, V. (2019). Effect of Supplementation of Organic Acids and Their Combination as an Alternative to Antibiotic Growth Promoter on Performance, Gut Health, Immune Status and Economics of Broiler Production. International Journal of Livestock Research, 9(2), 154-165. doi: 10.5455/ijlr.20180812074616

Introduction

Antibiotic growth promoters (AGP) have been beneficial for bird's health and productivity, but it is a double-edged sword (Miles *et al*., 2006). The rapid spread of drug-resistant pathogens and the public concerns over possible antibiotic residual problems as well as global concern over emergence of antibiotic-

related environmental pollutants led to their ban by the European Commission (Haq *et al*., 2017). In view of this the researchers have to explore the utility of other non-therapeutic alternatives like organic acids, probiotics, prebiotics, herbs and essential oils as feed additives in poultry production (Khan *et al*., 2016). The organic acids penetrate the cell wall of pathogenic microbes, disrupt the normal cell functioning thereby causing death of microbes (Afsharmanesh and Pourreza, 2005; Mroz, 2005). Beside this, the organic acids do not form residue in meat or environment, preventing microbial resistance. In recent years, there has been increasing attention on the blending type (mixtures) of organic acids based on the assumption that synergistically positive effects of individual organic acid may exist (Kil *et al*., 2011). Hence, the experiment was designed to assess effect of organic acids and their combination on performance, gut health, immune status and economics as alternative to AGP.

Materials and Methods

Experimental Site

The experiment was carried out at Department of Poultry Science, COVAS, Parbhani, affiliated to MAFSU, Nagpur, India. The trial was conducted from of 18th April to 29th May 2018 which had the heat stress index ranging from 100.56^{o}F to 105.51^{o}F.

Formulation of Experimental Ration

The quantity of feed ingredient required in the present experiment were purchased from local market. Rations were prepared as per BIS (2007) at Feed Mixing Plant Department of Poultry Science, COVAS, MAFSU, Parbhani (Table 1). The pre-starter ration was offered for first seven days of age, starter ration was offered from 8th day to 20thday and finisher ration was offered thereafter up to 42nd day of age. The *iso-caloric and iso-nitrogenous* feeding practices were followed. Organic acids (sodium citrate, ammonium formate and calcium propionate) and AGP (Bacitracin methylene disalicylate) were purchased from local market.

Experimental Birds and Data Collection

The experiment was approved by Institutional Animal Ethics Committee vide resolution no. IAEC/29/18 dated 11/05/2018. The experiment was carried out on 360 Vencobb^{400} day old straight run chicks for a period of 42 days. The chicks were weighed and distributed randomly into six treatment groups viz. A, B, C, D, E and F with four replicates of 15 chicks each. Experimental design used for housing of broilers is presented in Table 2. The standard feeding, watering, floor space and vaccination schedule were followed.

 DOI 10.5455/ijlr.20180812074616

Table 1: Percent ingredient and nutrient composition of (basal diet) pre-starter, starter and finisher rations with supplementation of organic acids

Feed Ingredients	Pre-Starter	Starter	Finisher
Maize	55.45	55.2	60.2
Vegetable oil	1.6	3	4
Soya-bean meal	40.15	39	33
Di-calcium phosphate	1.5	1.5	1.5
Limestone powder	1	1	1
Salt	0.3	0.3	0.3
Total	100	100	100
Mineral mixture (g/100 kgs)	300	300	300
Vitamin mixture	150	150	150
Methionine	180	190	160
Lysine	170	130	100
Choline chloride	60	60	60
Coccidiostat	60	60	60
*Sodium citrate, Ammonium formate, Calcium propionate and BMD			
Nutrient Composition Calculated			
Crude protein (%)	23.05	22.12	20.26
Metabolizable energy (Kcal/kg)	3015.32	3101.82	3213.32
Estimated Nutrient Composition			
Dry matter %	93.52	93.72	92.91
Crude Protein %	24.33	22.36	20.43
Total Phosphorus %	0.69	0.65	0.66
Calcium %	1.22	1.08	1.07

**Added in the diet as per the dose rate mentioned in experimental design*

Table 2: Experimental design for housing of broiler with organic acids

Treatment Group	Treatment Group Details	No. of Birds/Pen/ Replication	No. of Replicates	Total No. of Birds
A	Basal Diet	15	4	60
B	Basal Diet+ BMD* @ 30 mg/kg. diet	15	4	60
C	Basal diet + sodium citrate@ 1 g/ kg. diet	15	4	60
D	Basal diet + Ammonium formate@ 1g/ kg. diet	15	4	60
E	Basal diet + Calcium propionate @ 1g/ kg. diet	15	4	60
F	Basal diet + sodium citrate, Ammonium formate and calcium propionate each @ 1 g/ kg. diet	15	4	60
	Total	6	24	360

**Bacitracin Methylene Disalicylate*

Performance Parameters

The weekly live body weight, feed consumption, FCR, mortality and economics of broiler production were recorded replicate-wise.

Gut Health

pH of Different Parts of the Gastrointestinal Tract (GIT)

One bird per replicate was sacrificed on the 42nd day of experiment. 1 g of gut content from crop, proventriculus, gizzard, duodenum jejunum and ileum were collected aseptically in 9 ml sterilized physiological saline (1: 10 dilution) and pH was measured using digital pH meter.

DOI 10.5455/ijlr.20180812074616

Immune Status

Antibody Titre against RD (Ranikhet Disease)

Serum sample from one bird per replicate was collected on the 7th and 14th day post R.D. vaccination. Antibody titre against RD (Ranikhet disease) was estimated in the laboratory of Poultry Diagnostic and Research Centre of Venkateshwara Hatcheries Pvt. Ltd., Pune.

Antibody Titre against Infectious Bursal Disease (IBD)

Serum sample from one bird per replicate was collected on the 7th and 14th day post I.B.D. vaccination. An indirect ELISA for IBD antibody test kit developed by PDRC (Poultry Diagnostic and Research Centre, Loni Kalbhor, Pune) was used. ELISA antibody titre against IBD (Infecitious Bursal Disease) was estimated at the laboratory of Poultry Diagnostic and Research Centre, Pune.

Statistical Analysis

Data, thus collected were subjected to statistical analysis by using Randomized Block Design by Snedecor, and Cochran, (2002). The treatment means were compared by critical differences (CD) and analysis of variance.

Results and Discussion

Weekly Body Weight

The results from present study revealed significant ($P < 0.01$) influence on weight for different treatment groups. The organic acids (ammonium formate, sodium citrate, calcium propionate and their combinations) were found to be superior to Bacitracin methylene disalicylate (BMD) in enhancing the live weight of experimental birds. It may be related to the reduction in pH in the gastrointestinal tract (GIT) and selective promotion of beneficial bacteria in the gut. The reduced pH is condensive for the growth of favorable bacteria, simultaneously hampering the growth of pathogenic bacteria which grow at a relatively higher pH. The organic acids may affect the integrity of microbial cell membrane or cell macromolecule or interfere with nutrient transport and energy metabolism in the pathogenic bacteria causing the bactericidal effect. The inhibition of intestinal pathogenic bacteria leads to the reduced metabolic needs, thereby increasing the availability of nutrients to the host. This also could decrease the level of toxic bacterial metabolites, causing an improvement in the protein and energy digestibility, thus improving the live weight of the birds (Adil *et al.*, 2011). It indicates the growth promoting activity of organic acids and their combination. They may act as alternative to antibiotic growth promoter. These results were supported by the findings of Samanta *et al*. (2009), Ghazalah *et al*. (2011), Hedayati *et al*. (2013), Sohail *et al*. (2015), Sultan *et al*. (2015), Tanzin *et al*. (2015), Bagal *et al*. (2016), Lakshmi *et al*. (2016), Ragaa *et al*. (2016), Rout *et al*. (2016), Kanagaraju *et al*. (2017) and Ramigani *et al*. (2017). In contrast to present study, Paul

 DOI 10.5455/ijlr.20180812074616

et al. (2007), Aghazadeh and Tahayazdi (2012) and Naveenkumar *et al.* (2017) reported non-significant influence on body weight by organic acids.

Feed Consumption

Table 3 and 4 revealed significant (P<0.01) influence on feed consumption. These findings are in close agreement with the results of Haque *et al*. (2010), Hudha *et al*. (2010) and Tanzin *et al*. (2015).

Table 3: Live bodyweight (g), feed consumption (g), FCR and mortality at different age groups supplemented with organic acids

	A (Control diet)	B (30 mg BMD/kg)	C (1g sodium citrate/kg)	D (1g amm. formate/kg)	E (1g cal. propionate/kg)	F (1g of C,D &E each/kg)
Live Body Weight (g) [CD at 1% Significant Level = 5.332 g.]						
I	96.57	98.18	98.48	99.32	98.62	97.98
II	279.65	282.65	287.32	292.4	286.5	283.3
III	488.92	500.82	541.78	554.38	541.1	525.67
IV	887.15	914.68	948.24	957.9	947.6	949.3
V	1380.5	1417.24	1464.3	1489.74	1460.07	1451.25
VI	2020.25	2082.81	2209.85	2245.02	2205.6	2195.92
Overall Mean	858.83^{a}	882.73^{b}	924.99^{d}	939.79^{e}	923.24^{d}	917.23^{c}
Feed Consumption (g) [CD at 1% level = 9.387 g]						
I	58.97	59.8	59.18	59.18	59.13	59.55
II	256.72	257.27	260.83	261.98	256.6	255.13
III	313.82	322.05	365.66	374.23	364.32	349.75
IV	652.42	667.74	656.74	646.85	656.58	679.87
V	979.65	982.47	997.93	1006.58	990.4	984.06
VI	1264.83	1299.04	1445.39	1448.98	1433.05	1436.76
Overall Mean	587.73^{a}	598.65^{b}	630.89^{c}	632.96^{c}	626.96^{c}	627.52^{c}
FCR [CD at 1% level = 0.009]						
I	1.03	1.02	1	0.99	1	1.01
II	1.4	1.39	1.38	1.36	1.37	1.38
III	1.5	1.48	1.44	1.43	1.13	1.44
IV	1.64	1.61	1.62	1.6	1.62	1.6
V	1.99	1.96	1.93	1.89	1.93	1.96
VI	1.98	1.95	1.94	1.92	1.92	1.93
Overall Mean	**1.59^{e}**	**1.56^{d}**	**1.551^{b}**	**1.53^{a}**	**1.54^{b}**	**1.55^{bc}**
Mortality (%)						
Overall	**8.33**		**5**	**5**	**5**	**6.66**

Means bearing different superscript differ significantly row-wise

It may be attributed to the antimicrobial ability of organic acids. It enhances the digestibility of nutrients by improving the structure of intestinal flora (Tazoe *et al.*, 2008). In contrast to present findings, Adil *et al*. (2011) and Ramigani *et al*. (2017) reported decreased feed intake and justified it with decreased palatability of diet.

Feed Conversion Ratio

The FCR revealed significant (P< 0.01) differences (Table 3 and 4) in different treatment groups. An improvement in FCR may be due to increased feed intake by improving palatability of feed, increased

DOI 10.5455/ijlr.20180812074616

permeability of mucosal cells of intestine followed by increased rate of utilization of nutrients resulting in increased body weight gain. Similar findings were observed by Chowdhury *et al*. (2009), Panda (2009), Adil *et al*. (2010), Sohail *et al*. (2015), Bagal *et al*. (2016), Lakshmi *et al*. (2016) and Ramigani *et al*. (2017). In contrast to present findings, Rout *et al*. (2016) and Naveenkumar *et al*. (2017**)** recorded non-significant effect.

Mortality

There were no significant differences for mortality among different organic supplemented groups. These findings were in accordance with Hedayati *et al*. (2013), Lakshmi *et al*. (2013), Sultan *et al*. (2015), Kanagaraju *et al*. (2017) and Naveenkumar *et al*. (2017). They concluded that organic acid supplemented groups had mortality within normal range. It might be due to suppression of pathogenic bacteria by organic acids which led to healthy condition of birds throughout the experiment period. Similarly, Al-Natour *et al*. (2005) recorded a significant reduction in mortality in formic acid treatment group. Acikgoz *et al*. (2011) recorded numerically low mortality in organic acid treatment groups.

Table 4: ANOVA for body weight (g), feed consumption (g), FCR and at different age groups supplemented with organic acids

Sources		Body Weight		Feed Consumption		Feed Conversion Ratio	
	DF	MS	F	MS	F	MS	F
Treatments	5	22427.85	26.96**	8961.34	8.08**	0.01	14.68**
Replicate	3	81.67	0.09	141.46	0.12	0	0.044
Week	5	14600000	17575.32**	5988541.64	5403.70**	3.08	4625.17**
Error	130	831.61		1108.22		0.001	

*** Significant at 1% level.*

Gut Health

pH of Different Parts of Gastrointestinal Tract

Significantly (P<0.01) decreased pH was recorded for crop (Table 5 & 6). It concludes that organic acids inhibit pathogen bacteria growth in the feed, at the opening of digestive tract thereby improving the performance of birds. Similarly, the pH of ileum differed significantly (P<0.05) for organic acid groups. The pH of provenrticulus, gizzard, duodenum and jejunum was numerically decreased but non-significant. The pH level in specific areas of the GIT is a factor which established a specific microbial population and also affected the digestibility and absorption of most nutrients. Most pathogens grow at a pH close to 7. The beneficial microorganisms live in an acidic media (5.8-6.2) in which they compete with pathogens. Hence, organic acids can be used as an alternative to antibiotic, increasing birds' performance (Ghazalah *et al*. (2011).

Table 5: pH of different parts of gastrointestinal tract of broilers supplemented with organic acids

	A (Control diet)	B (30 mg BMD/kg)	C (1g sodium citrate/kg)	D (1g amm. formate/kg)	E (1g cal. propionate/ kg)	F (1g C,D,E each/kg)	C. D
Crop	4.71^{ab}	4.91^{abc}	4.28^{a}	4.12^{a}	4.24^{a}	4.53^{ab}	0.32
Proventriculus	4.38	4.29	4.32	3.81	3.63	3.63	N.S
Gizzard	3.79	4.33	3.94	3.53	3.53	3.39	N,S
Deodenum	5.69	5.55	5.36	5.38	5.3	5.59	N.S
Jejunum	5.76	5.89	5.51	5.73	5.61	6.1	N.S
Ileum	6.25^{a}	6.55^{ab}	5.70^{a}	5.82^{a}	5.93^{a}	6.85^{abc}	0.7

Means bearing different superscript differ significantly row-wise

Apart from antimicrobial and pH reducing effect, organic acids may have beneficial effect on the intestinal mucosa of broilers. As nutrient absorption in gut occurs from the intestinal mucosa, it may improve the nutrient utilization and consequently growth performance (Pelicano *et al*. 2005). These findings are supported by Ghazalah *et al*. (2011) and Ragaa *et al*. (2016), who observed that pH reduction was significantly greater in the upper part of GIT as compared to the lower part of the GIT. In contrast to the present findings, Salgado-Transito *et al*. (2011) and Marin-Flamand *et al*. (2014) reported non-significant differences in pH of GIT.

Table 6: ANOVA for pH values in different parts of the gastrointestinal tract of broiler supplemented with organic acids

	Crop				Ileum			
Source	**DF**	**SS**	**MS**	**F ratio**	**DF**	**SS**	**MS**	**F ratio**
Treatment	5	1.85	0.37	7.03**	5	4.06	0.81	3.36*
Replicate	3	0.03	0.01	0.21	3	0.4	0.13	0.554
Error	15	0.79	0.05		15	3.62	0.24	

***Significant at 1% level, * Significant at 5% level*

Immune Status

RD Titre

The differences between the overall antibody titre against Ranikhet disease (RD) at 14th and 21st day of age were non-significant (Table 7), however, it was numerically higher in sodium citrate and ammonium formate group. The findings in the present experimentation were in close agreement with the findings of Das *et al*. (2011), Marin-Flamand *et al*. (2014) and Deepa *et al.* (2017) who recorded non-significant differences in antibody titre against RD. In contrast, Lakshmi *et al*. (2013), Sohail *et al*. (2015) and Fascina *et* al. (2017) recorded significant increase in antibody titre against RD for organic acid supplemented groups.

DOI 10.5455/ijlr.20180812074616

Table 7: Antibody titre against Ranikhet disease at 14th day and 21st day and Infectious Bursal disease at 21stand 28th day of age of broiler supplemented with organic acids

Treatments	Ranikhet Disease (RD) Titre				Infectious Bursal Disease (IBD) Titre			
	14th day		21st day		21st day		28th day	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	8	4.61	7	3.41	560.25^{ab}	115.54	323.75	105.36
B	36	30.89	6.5	3.2	296.75^{a}	41.81	138.5	19.09
C	54	27.59	5	1	420.75^{a}	100.39	287.25	74.63
D	40	8	11.5	6.94	598.75^{ab}	118.53	365	88.77
E	12	2.3	7	3	910.50^{abc}	53.68	457	32.51
F	14	2	8	2.82	783.50^{ab}	34.66	399.25	25.3
CD	N.S		N.S		254.38		N.S	
CV%	127.11		102.22		28.55		40.55	

Means bearing different superscript differ significantly column-wise

IBD Titre

Significant (P<0.01) influence on antibody titre of IBD at 21st day was observed for calcium propionate (Table 7 & 8). These findings were in accordance with Ghasemi *et al*. (2014), Manafi *et al*. (2016) and Sohail *et al*. (2015) who found significant effect on antibody titer IBD. It indicates that broilers were healthy with higher immune status to fight against infectious diseases and enteric pathogens (Chowdhury *et al*., 2009). Similarly, calcium propionate and the combination of organic acids reported improved immune status of broilers. It indicates organic acids are capable of modifying gut microflora communities and therefore, might support improvement in immunity (Emami *et al.*, 2013). However, differences at 28th day for IBD titre were non-significant. It was in accordance with Lohakare *et al*. (2005).

Table 8: ANOVA of antibody titre against IBD at 21st day of age for dietary organic acids

Sources	DF	SS	MSS	F
Treatments	5	1022441	204488.2	7.08**
Errors	18	519721	28873.38	
Total	23	1542162		

***Significant at 1% level*

Economics of Broiler Production

Increased profitability per kg live weight (Table 9) was observed which is in agreement with findings of Haque *et al*. (2010), Tanzin *et al*. (2015) and Kamel and Mohamed (2016). It indicates more bioavailability of nutrients resulting in enhanced growth rate, superior FCR and ultimately more returns in the form of profit. Hence, organic acids could replace AGP (BMD) with no residual effect and optimum profitability of broiler production. In contrast, Chowdhury *et al*. (2009) and Rout *et al*. (2016) inferred a non-significant difference in profit between various treatments.

 DOI 10.5455/ijlr.20180812074616

Table 9: Economics of broiler production supplemented with different organic acids

S. No.	Particulars	A (Control diet)	B (30 mg BMD/kg)	C (1g sodium citrate/kg)	D (1g amm. formate/kg)	E (1g cal. propionate/kg)	F (1g C,D,E each/kg)
1	Chick cost (Rs)	35	35	35	35	35	35
2	Feed consumption (g)	3526.4	3588.39	3785.37	3797.81	3760.08	3765.12
3	Feed cost /bird (Rs.)	99.85	101.61	108.47	109.04	108.01	110.98
4	Misc. cost (Rs)	7	7	7	7	7	7
5	Production cost (Rs)	141.85	143.61	150.47	151.04	150.01	152.98
6	Av. Live wt. (g)	2020.25	2082.81	2209.85	2245.02	2205.6	2195.92
7	Sale receipt @ 83/kg wt	167.66	172.8	183.34	186.33	183.01	182.18
8	Net profit/ bird	25.81	29.19	32.87	35.29	33	29.2
9	**Net profit /kg (Rs)**	**12.77**	**14.02**	**14.88**	**15.71**	**14.96**	**13.3**

Conclusion

It is concluded that ammonium formate, sodium citrate, calcium propionate and their combination through feed were found to be beneficial for growth performance, gut health, immune status and economics. Hence, organic acids may be efficiently used as alternative to AGP (BMD).

Acknowledgement

The authors are highly grateful to Dr. N. M. Markandeya, Associate Dean, COVAS, MAFSU, Parbhani for providing necessary facilities and for his help at various stages of the experiment. The authors are also thankful to PDRC, Pune for providing serum estimation facilities.

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