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# Supplementation of Propionic Acid (PA), Butyric Acid (BA) or Antibiotic (AB) in Diets and their Influence on Broiler Performance, Carcass Parameters and Immune Response

**K. Vijaya Lakshmi[1], G. Shyam Sunder[2]**

[1]Department of Poultry Science, College of Veterinary Science, Rajendranagar, Hyderabad-30 and [2]Project Directorate on Poultry, Rajendranagar, Hyderabad-30

**Abstract:** ***The effectiveness of organic acids, Propionic acid (PA) and Butyric acid (BA) at two levels (0.2 and 0.3%) in replacing antibiotic (AB-Virginiamycin 11mg/kg) from chick diets was evaluated in a feeding trial with 270 broiler chicks (Vencobb) by recording their performance, meat yield and immune response up to 42 d of age. The experimental diets included two organic acids, PA and BA at two levels (0.2 and 0.3%) each in a factorial pattern, and compared with the antibiotic and control diets. Each diet was offered ad libitum to 9 replicates of 5 birds per replicate in battery brooders. The results indicated that organic acids, PA and BA at both levels of inclusion (0.2% and 0.3%) in diets enhanced ($p \leq 0.01$) broiler body weight by 8-10% over the AB and control diets (2154g vs 1960g) at 42d of age. Both organic acids significantly ($p \leq 0.01$) improved FCR (1.68- 1.91), tibia weight (5.75-5.31g), tibia length (7.96-6.16 cm), breast muscle yield (13.68-14.77%), and reduced leg scores (1.04-1.50) and abdominal fat (7.5%) compared to AB and control diets. However, there was no statistical difference between PA and BA for all the effects recorded. Organic acids supplemented at 0.2% in diets was as effective as or better than 0.3% level suggesting that the former level was adequate in broiler diets. In effect, the study suggested that organic acids, PA and BA supplemented at 0.2% was effective in replacing Virginiamycin (11mg/kg) from broiler diets with the advantage of better performance up to 42 d of age.***

**Keywords:** Organic acids, antibiotic, broiler performance, immune response

## 1. Introduction

Broiler chickens are under constant threat from pathogens like *Escherichia coli*, *Salmonella*, *Clostridium sps. etc.*, from the day of hatch to the marketable age (0-42 d). Conventionally, the practice has been to include sub-therapeutic dosage of antibiotics in feed as additives to achieve the twin objectives of protection from specific intestinal pathogens, and as growth promoter (Dibner and Richards, 2005). Organic acids as feed acidifiers have been found to suppress the growth of bacteria, particularly those of *Escherichia coli*, *Salmonella* and *Clostridium* species. The short chain fatty acids (C1-C7) are associated with antimicrobial activity. They are either simple mono-carboxylic acids (formic, acetic, propionic and butyric acids) or carboxylic acids with hydroxyl group (lactic, malic, tartaric and citric acids). In the undissociated state, organic acids penetrate the semi-permeable membrane of bacterial cell wall, enter the cytoplasm and decrease the internal pH of bacteria affecting the enzymes (e.g. decarboxylases and catalases) of bacteria, inhibiting glycolysis, preventing active transport and interfering with signal transduction. Hence, the supplementation of organic acids in feed in lieu of sub-therapeutic dosage of antibiotics is being seriously considered, particularly in the context of reservations in using antibiotic as a feed additive. Therefore, the present study attempted to include Propionic (PA) and Butyric (BA) acids in broiler diets at low levels (0.2% and 0.3%) to replace Virginiamycin (11mg/kg) from feed.

## 2. Materials and Methods

### Stock, husbandry and experimental diets

Day-old commercial male broiler chicks (270) of Vencobb brand were used in this trial. They were individually wing banded, weighed and randomly distributed to 6 dietary groups with 9 replicates of 5 chicks each, and housed in stainless steel battery brooders in open sided house. Each battery brooder had three tiers with two cells per tier (60 cm x 75 cm x 45 cm - for 5 chicks). Incandescent lights were used to brood chicks at 34 $\pm$ 1$^0$C up to 7 days of age, and the temperature was gradually reduced to 26 $\pm$ 1$^0$C by 21 d of age after which, no supportive heat was provided. They were vaccinated against Marek's disease, Newcastle's disease and Infectious Bursal disease, as per the standard vaccination schedule.

A basal diet was formulated with maize and soybean meal (Table 1) to which Propionic acid (PA) or Butyric acid (BA) was added at two levels, 0.2 and 0.3% in a factorial pattern and compared with the antibiotic (AB-Virginiamycin 11mg/kg) added diet and a control with no supplementation of PA, BA or AB. In all, there were 6 experimental diets, which were offered individually at *ad libitum* to each of the dietary groups.

### Response criteria

The relative efficiency of two organic acids, PA and BA, besides AB and control diets was evaluated in broiler chicken from 0-42 d of age, based on their response over

different parameters related to performance, carcass yields, bone measures and immune competence. Broiler chickens were weighed individually and the feed intake was recorded replicate-wise to calculate the feed consumption per unit weight gain (FCR). Mortality was recorded throughout experimental period for calculating the percent livability up to 42 d of age. Further, the hock joint scores of all birds were recorded at the end of the experiment following the score card described by Watson et al., (1970).The degree of leg abnormality was measured by examining the hock joints. The birds that appeared normal were scored 1; slight swelling of tibia metatarsal joint as 2; marked swelling of the joint as 3; swelling of the joint with symptoms of slipped achilles tendon as 4 and swelling with marked degree of slipped tendon as 5.

On the 43$^{rd}$ day, 6 birds from each dietary group were randomly selected, starved over night, weighed and sacrificed by cervical dislocation for measuring the eviscerated meat yield; breast meat, abdominal fat, spleen and bursal weights as percentage of pre-slaughter weight.

**Immune response**

The immune competence was evaluated based on humoral and cell-mediated immune response. Sheep RBC, a non-pathogenic antigen was used for evaluating humoral immune competence of the chicken. Sheep blood was collected by jugular puncture in EDTA tubes and washed thrice with 0.85% saline solution. The broiler chicks (6 no) from each dietary group were injected 0.1 ml of 0.5% SRBC suspension into brachial vein on the 36$^{th}$ d and the blood samples were collected after 5 d post-inoculation on the 41$^{st}$ d. Subsequently, the microhaemagglutination activity of serum was estimated following the procedure of Wegmann and Smithies (1966). Antibody titres were expressed as log2 of the reciprocal of the highest dilution in which agglutination occurred (Brugh, 1978).

The cell mediated immune response was assessed by conducting the cutaneous basophilic hypersensitivity test *in-vivo* by using PHA-P (Phytohaemagglutinin – plant origin). Broiler chicken (6 no) from each test groups were selected on the 36$^{th}$ d and the toe thickness between the 2$^{nd}$ and 3$^{rd}$ inter-digital space of both feet, left and right was measured by micrometer. Immediately after measurements, 100 µg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS) and 0.1 ml of PBS alone, were respectively injected into right and left foot (acted as control). The web swelling of both feet was measured after 24 hours of initial injection. The response was determined by subtracting the skin thickness of first measurement from the second and the values of left foot (control) from the right foot (Corrier and Deloach, 1990).

On the 43$^{rd}$ d, broiler chicken were sacrificed for meat traits from which the bursa and spleen were collected, weighed individually and expressed as the percent pre-slaughter weight.

**Statistical analysis**: The experiment was that of completely randomized design with 6 dietary treatments. Since, two organic acids were tested at similar levels of inclusion, two-way ANOVA was conducted on the organic acids and the same was compared with AB and control groups using the General Linear Models Procedure of SAS (1991). The means were compared by Duncan's Multiple Rang Test (Duncan 1955). A level of ($P< 0.05$) was used as the criterion for statistical significance.

## 3. Results and Discussion

**Growth, feed intake, FCR and livability in broilers**

The nutrient composition of control diet for pre-starter broilers was in line with the formulated levels (protein-22%; Ca - 0.90%; P- 0.45% and ME-2950 kcal/kg) of nutrients. Experimental diets supplemented with PA, BA or AB also contained similar composition as that of control (Table 1). The diets formulated for pre-starter, starter and finisher broilers also confined to the nutrient composition that was formulated. The diet supplemented with PA and BA produced significantly ($P< 0.01$) higher body weight (2138-2154 g) compared to AB and control (1995 and 1960g, respectively). The two organic acids and their levels of inclusion produced similar growth pattern. The average feed consumption per unit weight gain (FCR) was significantly better in groups fed organic acids at both levels of inclusion, followed by AB and control diets (Table 2). The interaction also revealed the advantage of FCR due to organic acids at either level of inclusion, followed by AB and control diets. Though not significant, the average percent livability was higher (93.1 to 98.7%) in birds fed with organic acids compared to AB (92.5%) and control (90.7%) group of birds during the experiment. The interaction also revealed the advantage of reducing the mortality due to organic acids at both levels of inclusion compared to AB and control group of birds.

In the early period of growth, the caeca of broilers are dominated by *Enterobacteriaceae* and *Enterococci*, whose number decreased with age (Vander *et al*., 2000). They compete for nutrients with the host and reduce nutrient utilization (Parks *et al*., 2001). It was also observed that young chicks have a low quantity of volatile fatty acids in gut and caeca (Vander *et al*., 2000), which provided favorable environment for multiplication of pathogenic microorganisms with negative effect on young broilers. Probably, in our experiment supplementation of PA or BA in broiler diets improved the concentration of volatile fatty acids, and restricted the growth of microbes facilitating utilization of dietary nutrients more efficiently for higher growth and FCR. These results were in conformity with those of Abdel-Fattah *et al.* (2008) who suggested that organic acids caused a reduction of bacteria in crop resulting in improved body weight. The organic acids might have also supported reduction in gastric pH to increase pepsin activity (Afsharmanesh and Pourreza, 2005) and peptide production, which triggered hormonal release (gastrin and cholecystokinin) for enhanced digestion and absorption of protein (Hersey, 1987) and minerals (Kishi *et al*., 1999).

**Eviscerated meat yield, breast meat and abdominal fat**

The percent eviscerated meat yield was not altered by the organic acids at both levels of inclusion, AB and control

diets, but the yield of breast meat was significantly (P<0.01) higher with diets having PA and BA at 0.2 and 0.3% levels compared to AB and control diets (Table 3). It appeared that organic acids enhanced protein accretion at both levels more than other treatments. Incidentally, the percent abdominal fat was significantly (P<0.01) less in broilers fed diets supplemented with PA or BA followed by AB compared to the control diet. The interaction between organic acids and their levels of supplementation showed that both acids, PA and BA at 0.2% produced higher breast meat weight, though both levels exhibited similar effect.

In the present study, organic acids were associated with growth stimulation and improvement in nutrient digestibility, but the same was not converted into higher carcass yield (Table 3). Similar observations were also made by Izat *et al* (1990b), Skinner *et al.* (1991) and Zhang *et al* (2005) for organic acids. However, a significant increase in breast meat and reduction in abdominal fat was recorded with organic acids. Concomitant to the findings of the present study, Izat *et al.* (1990) reported significant reduction in abdominal fat content in male broiler chickens by dietary supplementation of Propionic acid. This reduction in abdominal fat might be due to elevated serum $T_3$ concentration as observed by Abdel-Fattah *et al.* (2008). It was hypothesized by Fushimi *et al.* (2001) that acidification of diets might stimulate glycogenesis by increasing the influx of glucose 6-phosphate into glycogen synthesis pathway through the inhibition of glycolysis due to an increase in citrate concentration. Our results on interaction between organic acids and their levels indicated that both acids, PA and BA at 0.2% produced more breast meat and reduced abdominal fat which could be due to the reasons referred herein. Therefore, lower concentration of organic acids can be used under practical conditions.

**Bone measures**

The average tibia weight and tibia length was significantly (P<0.01) higher, and the leg scores lower in the groups fed PA, BA and AB diets compared to the control diet (Table 2 and 3). No difference (P<0.01) was observed in tibia weight, length and leg scores between the two organic acids and AB diets. However, the interaction between acids and their levels showed higher tibia weight and length (P<0.01) with optimum leg scores AB in diets. The interaction between acids and their levels showed enhanced response on tibia weight and tibia length when both organic acids were used at 0.2% levels. Sacakli *et al.* (2006) observed that addition of short chain organic acids to the quail diet improved utilization of dietary phosphorus and increased total ash in tibia bones. Organic acids improved the length of intestinal villus in broilers (Senkoylu *et al.* 2007), which perhaps stimulated higher mineral absorption. The improvement in Ca and P bio-availability was due to decreased pH in the upper part of the intestine, which was perhaps responsible for higher tibia weight, length and low leg scores. The results of our study corroborate with those referred above, in particular for tibia weight, length and leg scores. The interaction between acids and their levels indicated that the bone parameters were optimized by using organic acids at 0.2% level, and higher inclusion had little improvement in bone parameters.

**Immune response**

The antibody titers against sheep red blood cell (SRBC) antigen were significantly (P<0.01) higher in broilers raised on diets containing PA and BA followed by AB and control diets (Table 4). AB diets were intermediate between organic acids and control. The cell mediated response recorded as cutaneous basophil hypersensitivity was higher (P<0.01) in PA, BA and AB supplementation compared to control, but there was no difference among the supplemental groups. The concentration of organic acid at 0.2% was similar to or more effective than 0.3% for antibody titers, CBH response and weight of spleen and bursa. The organic acid at 0.3% was better (P<0.05) than AB and control diets for antibody titers and bursa weight. The interaction between organic acids and their levels revealed that BA at 0.2% maintained significantly (P<0.05) higher antibody titers, CBH response and weight of lymphoid organs than other dietary combinations.

The evidence of immune response attributable to dietary acidification could be on account for their inhibitory effects against the pathogenic microorganisms throughout the GI tract. The inclusion of organic acids in diets increased the percent gamma globulin and provided better protection against diseases (Rahmani and Speer, 2005). In the studies undertaken by **Grimmer (1986)** the serum globulin level was considered as an indicator of immune response and source of antibody production. It was suggested that high globulin level and, low albumin and globulin ratio signified better immune response and disease resistance. Although in our study, the serum albumin and globulin was not evaluated, high antibody titers to SRBC and better response to PHA-P due to the inclusion of organic acids in diets was perhaps related to higher albumin and globulin levels in serum. Few other studies have also indicated immunological advances due to relative increase of spleen and bursa weights (Abdel-Fattah *et al.* 2008). It is well known that spleen and bursa are considered as a part of immune system (Sturkie, 1986), which was responsible for producing cells that protected the birds from invasive microorganism. Our findings corroborated with these findings, because the lymphoid organs being heavier in birds fed PA and BA produced higher immune response than AB and control groups. Further, the supplemental level of organic acids at 0.2% was adequate for sustaining optimum immune response in broilers up to 42 d of age.

In conclusion , broiler diets supplemented with PA or BA at 0.2% level could effectively replace AB and improve weight gain (> 8-10%), FCR, breast meat yield, tibia weight, tibia length, immune response and reduced abdominal fat in broiler chicken at 42 d of age.

## 4. Acknowledgments

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**Table 1:** Ingredient composition of basal diet (g/kg)

| Ingredient | Pre-starter (0-10d) | Starter (11-21d) | Finisher (22-42d) |
|---|---|---|---|
| Maize | 560.600 | 569.370 | 574.009 |
| Soya bean meal | 376.840 | 354.350 | 335.894 |
| Common salt | 4.500 | 4.500 | 4.200 |
| Di-cal Phosphate | 19.020 | 19.200 | 17.684 |
| Shell grit powder | 7.230 | 7.340 | 8.616 |
| DL methionine | 2.370 | 2.090 | 1.993 |
| L lysine HCl | 0.560 | 0.060 | 0.000 |
| AB2D3K* | 0.150 | 0.150 | 0.150 |
| B complex * | 0.150 | 0.150 | 0.150 |
| Choline Chloride | 0.600 | 0.600 | 0.600 |
| Toxin binder | 2.000 | 2.000 | 2.000 |
| Trace minerals** | 1.200 | 1.200 | 1.200 |
| Anti-oxidant | 0.200 | 0.200 | 0.200 |
| Coccidiostat | 0.500 | 0.500 | 0.500 |
| Vegetable oil | 23.780 | 37.950 | 52.354 |
| **Nutrient composition** | | | |
| [1]ME (kcal/kg) | 2950 | 3050 | 3150 |
| [2]Crude protein (%) | 22 | 21 | 20.2 |
| [2]Lysine (%) | 1.2600 | 1.15 | 1.09 |
| [2]Methionine (%) | 0.56 | 0.52 | 0.50 |
| [1]Calcium (%) | 0.90 | 0.90 | 0.90 |
| [1]Avail. P (%) | 0.45 | 0.45 | 0.42 |

* Vitamin premix provided per kg diet: Vitamin A, 12375 IU; vitamin D3, 1800 IU; vitamin E 6 mg; vitamin K 1.5 mg; riboflavin 7.5 mg; vitamin B1 0.6 mg; B6 1.2 mg; B12 6 mcg; Niacin 9 mg; Calcium pantothenate 6 mg.
** Trace mineral provided per kg diet: Mn 60 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1mg and Selenium 0.1mg.
[1] calculated nutrients
[2] Estimated nutrients

**Table 2:** Influence of PA and BA inclusion in diets at different concentrations on the performance of broiler chicken at 42 d of age and its comparison with AB and control diets

| *Org acids and levels* | *B. wt (g)* | *FCR* | *Livability (%)* | *Leg Score* |
|---|---|---|---|---|
| PA 0.2% | $2165^{a}$ | $1.68^{c}$ | 98.7 | $1.00^{b}$ |
| PA 0.3% | $2142^{a}$ | $1.68^{c}$ | 93.7 | $1.08^{b}$ |
| BA 0.2% | $2141^{a}$ | $1.64^{c}$ | 95.0 | $1.00^{b}$ |
| BA 0.3% | $2135^{a}$ | $1.69^{c}$ | 93.1 | $1.08^{b}$ |
| AB | $1995^{b}$ | $1.83^{b}$ | 92.5 | $1.25^{ab}$ |
| Control | $1960^{b}$ | $1.91^{a}$ | 90.7 | $1.50^{a}$ |
| Source of variation | [2]Significance of treatment effect | | | |
| Org acids x levels | ** | ** | NS | ** |
| Organic acids | ** | ** | NS | ** |
| Levels | ** | ** | NS | ** |

Means within a column having different superscripts are statistically different ($p \leq$ **0.01 or *0.05)
PA- Propionic acid: BA – Butyric acid: AB-Antibiotic

**Table 3:** Influence of PA and BA at different levels of inclusion in diets on the eviscerated meat yield, breast meat, abdominal fat (%), tibia weight (g) and tibia length (cm) of broiler chicken at 42 days of age and its comparison with AB and control diets

| Org acids and levels | Eviscerated yield | Breast meat yield | Abdominal fat | Tibia weight | Tibia length |
|---|---|---|---|---|---|
| PA 0.2% | 73.08 | $14.96^{a}$ | $1.09^{bc}$ | $5.84^{a}$ | $8.44^{a}$ |
| PA 0.3% | 73.17 | $14.64^{ab}$ | $1.13^{bc}$ | $5.65^{b}$ | $7.48^{c}$ |
| BA 0.2% | 72.67 | $14.91^{a}$ | $1.08^{bc}$ | $5.89^{a}$ | $7.99^{b}$ |
| BA 0.3% | 72.73 | $14.39^{b}$ | $1.06^{c}$ | $5.61^{b}$ | $7.53^{c}$ |
| AB | 72.91 | $13.87^{c}$ | $1.24^{b}$ | $5.67^{b}$ | $7.74^{bc}$ |
| Control | 73.04 | $13.68^{c}$ | $1.60^{a}$ | $5.31^{c}$ | $6.16^{d}$ |
| Source of variation | | | | | |
| Org acids x levels | NS | ** | ** | ** | ** |
| Organic acids | NS | ** | ** | ** | ** |
| Levels | NS | ** | ** | ** | ** |

Means within a column having different superscripts are statistically different ($p \leq$ **0.01 or *0.05)
PA- Propionic acid: BA – Butyric acid: AB-Antibiotic

**Table 4:** Effect of organic acids and antibiotic inclusion in diets on the humoral immune response (antibody titers), cell mediated response and weight of lymphoid organs in broiler chickens at 42 d of age

| Org acids and levels | SRBC ($log_2$) | CBH | Spleen | Bursa |
|---|---|---|---|---|
| PA 0.2% | $6.21^{b}$ | $232^{a}$ | $0.170^{ab}$ | $0.175^{a}$ |
| PA 0.3% | $6.08^{bc}$ | $217^{b}$ | $0.159^{c}$ | $0.166^{a}$ |
| BA 0.2% | $6.38^{a}$ | $232^{a}$ | $0.173^{a}$ | $0.172^{a}$ |
| BA 0.3% | $6.03^{c}$ | $224^{ab}$ | $0.164^{bc}$ | $0.143^{b}$ |
| AB | $5.57^{d}$ | $222^{b}$ | $0.157^{c}$ | $0.141^{b}$ |
| Control | $5.35^{e}$ | $207^{c}$ | $0.143^{d}$ | $0.127^{c}$ |
| Source of variation | | | | |
| Org acids x levels | ** | ** | ** | ** |
| Organic acids | ** | ** | ** | ** |
| Levels | ** | ** | ** | ** |

Means within a column having different superscripts are statistically different ($p \leq$ **0.01 or *0.05)
PA- Propionic acid: BA – Butyric acid: AB-Antibiotic
SRBC: sheep red blood cell; CBH: Cutaneous Basophil hyper-sensitivity

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