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Full Length Research Paper

Investigation of oocyst count and performance affected by butyric acid and salinomysin in broiler

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Organic acids such as butyrate are considered potential alternatives to antibiotic growth promoters. The efficacy of butyric acid, salinomysin sodium and litter moisture on performance and number of oocysts in broiler chickens were tested in this study. The experiment was a factorial design, with/without butyric acid (0/5 and 0%), with/without salinomysin sodium (0/3 and 0%) and 2 levels of moisture (33 and 77%) of the litter of experimental pens. Eight hundred 1-day-old male broiler chickens (Ross 308) were purchased from a local hatchery. The birds randomly allocated to eight treatment groups of 100 birds each. Each group was further divided into 4 replicates of 25 birds each. The data obtained were analyzed by SAS (9.1) with a general linear models procedure. The obtained results showed that no significantly difference were observed in feed intake in starter and grower, weight gain in starter, grower and total periods, feed conversion ratio in starter, grower and total period and oocysts count in weeks 3, 5 and 6 ($p > 0.05$). Significantly difference were observed in feed intake in finisher, weight gain in finisher, feed conversion ratio in finisher and oocysts numbers in weeks 2 and 4 ($p < 0.05$). Base on this experiment results, the main effects of most parameters had no significant efficiency ($p > 0.05$). The interactive action between butyric acid, salinomysin sodium and litter moisture was significant in some parameters ($p < 0.05$). It is concluded that butyric acid and salinomysin sodium did not have clear positive effects on performance of broiler chickens.

Key words: Organic acid, oocyst, anticoccidial, performance, broiler chicken.

INTRODUCTION

The efficiency of a poultry digestion depends on the microorganisms which live naturally in its digestive tract. Dietary of certain feed additives are products which are incurporated into animal feed to create favorable conditions in the animal's intestine for the digestion of feed. Growth promoters have been used extensively in animal feeds and water all over world especially in the poultry and pig industries (Charles and Duke, 1978). Anti-biotics improve the production results of meat producing chicks, and the utilization of energy in particular was improved. However, the use of growth-promoting anti-biotics is being placed under more and more pressure as consumers increasingly fear that their use in feed rations of productive live stocks leads to the formation of resistance against bacteria which are pathogenic to humans (Langhout, 2000). Some probiotic microorganisms and organic acid are alternative to antibiotic to be used exclusively as a growth stimulant and for improvement of the feed conversion rate in farm animals (Esteive et al., 1997). Organic acids reduce production of toxic components by bacteria and a chance in the morphology of the intestinal wall, and it also reduces the colonization by pathogens on the intestinal wall, thus preventing damage to the epithelial cells (Langhout, 2000). In testing the survival of *Salmonella enterica* serovar typhimurium during exposure to short-chain fatty acids, Kwan and Ricke (1998) showed that butyrate and valerate have the greatest efficacy. Brons et al. (2002) credit butyrate derived from the fermentation of nonstarch polysaccharides with improved gastrointestinal health in

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human and reduced incidence of colon cancer.

Butyric acid therefore, appears to be both bactericidal and a stimulant of villi growth. As with any short-chain fatty acid, bactericidal activity of butyric acid is greatest when the acid is undissociated. Bolton and Dewar (1965) indicated that free butyrate quickly disappeared in the upper digestive tract, and whereas almost 60% of the feed source was intact in the crop, less than 1% was recovered from the upper small intestine. The efficacy of butyrate likely will be improved if it is protected from immediate absorption in the upper tract. The review of literature showed that some butyric acid glycerides are alternative to the treatment of broiler growth performance, thus to a certain feed additives can perform the role of antibiotics. The overall objectives of this study were to assess the effects of butyric acid glycerides and salinomysin sodium in broiler diet with litter moisture on performance and oocysts count.

MATERIAL AND METHODS

Animal and diet

The experiment was of a factorial design, with/without butyric acid (0 and 0/5%), with/without salinomysin sodium (0 and 0/3%) and 2 levels of moisture (33 and 77%) of the litter of experimental pens. Eight hundred 1-day-old male broiler chickens (Ross 308) were purchased from a local hatchery. On the day of arrival (Day 1), the birds were wing-banded, weighed, and randomly allocated to eight treatment groups of 100 birds each. Each group was further divided into 4 replicates of 25 birds each. All replicates were housed in 32 separate wire-suspended cages equipped with plastic sides, and bottoms covered with clean wood shavings. Continuous lighting was provided. The temperature in the cages was 32°C on arrival of the chickens, and from day 8 of the experiment, the temperature was decreased gradually by 2°C every day until it reached 20°C by day 14. Feed and water were available *ad libitum*.

Treatments include:

1. Diet without butyric acid and salinomysin sodium (with normal moisture litter).
2. Diet without butyric acid and salinomysin sodium (with high moisture litter).
3. Diet with butyric acid, but without salinomysin sodium (with normal moisture litter).
4. Diet with butyric acid, but without salinomysin sodium (with high moisture litter).
5. Diet without butyric acid, but with salinomysin sodium (with normal moisture litter).
6. Diet without butyric acid, but with salinomysin sodium (with high moisture litter).
7. Diet with butyric acid and salinomysin sodium (with normal moisture litter).
8. Diet with butyric acid and salinomysin sodium (with high moisture litter).

Butyric acid and salinomysin sodium were added to the basal diet by substituting at the expense of corn (Table 1). The starter diet was fed until day 10. The grower diet was fed from day 11 - 28. The finisher diet was fed from day 29 - 42. Body weights were measured on days 10, 28 and 42. Amounts of feed provided and leftovers were weighed per cage. Feed intakes were determined per week per cage and expressed as g/bird/day. Feed conversion ratio is calculated as feed intake per cage divided by weight gain of birds in the cage.

Oocyst counting

Fresh excreta samples were collected from the four corners and the middle of each cage on days 14, 21, 28, 35 and 42 of the experiment for oocyst counting. Excreta collection was done in the evening and the samples were stored overnight in a refrigerator. The oocysts of each cage were counted the next day and the numbers were expressed per gram of excreta.

For oocyst counting, a modified McMaster counting chamber technique of Hodgson (1970) was used. A 10% (w/v) feces suspension in a salt solution (151 g NaCl mixed into 1 L of water) was prepared. After shaking thoroughly, 1 ml of the suspension was mixed with 9 ml of a salt solution (311 g of NaCl mixed into 1 L of water). Then, the suspension was put into the McMaster chamber using a micropipette and the number of oocysts was counted (Peek and Landman, 2003).

Statistical analysis

The data obtained were analyzed by SAS (1990) with a General Linear Models procedure for ANOVA, for each experiment. Differences between means were analyzed with Duncan's multiple gaps test. The significant difference statements were based on the possibility $p < 0.05$, unless explained in another way.

The oocyst values were logarithmically transformed $[\log_{10} (X+1)]$ to create a normal distribution before being analyzed. When significant treatment effects were disclosed, differences between the eight treatments were evaluated by the post hoc multiple comparison least significant difference (LSD) test.

RESULTS

Performance results are presented in Table 2, 3 and 4. In starter period (1 to 10 days), there were no feed intake differences among treatment groups ($p > 0.05$), nor in weight gain and feed conversion ratio of broilers fed the different diets. As a result, no significant effects were observed with main effect of Butyric acid, Salinomysin, Litter moisture and interaction between them. From 11 - 28 days of age, there were no feed intake differences among treatment ($p > 0.05$), nor in weight gain and feed conversion ratio of broilers fed the different diets. No significant effects were observed with main effect of Butyric acid, Salinomysin, Litter moisture and interaction between them ($p > 0.05$). In the finisher period (29 - 42 days), there were significant differences in feed intake, weight gain and feed conversion ratio among treatment groups and the control group ($p < 0.05$). Highest feed intake, weight gain and feed conversion ratio were observed in the group that fed basal diet without Butyric acid and Salinomysin and had normal Litter moisture than other groups. In this period, individual effects of Butyric acid, Salinomysin and Litter moisture were not significant ($p > 0.05$). But the interactive effect of parameters was significant ($p < 0.05$).

In total experiments, significant difference was observed

Table 1. Ingredients and composition of the basal diet.

Ingredient	Starter	Grower	Finisher
Corn	56/11	61/6	67/31
Soybean meal	34/71	27/94	21/91
Poultry wastage powder	2	3	4
Oil	1/27	1/26	1/42
Dicalcium phosphate	1/56	1/48	1/31
Oyster shell	0/4	0/2	0/1
DL-Methionine	0/34	0/28	0/22
L-Lysine	0/26	0/24	0/2
Vitamin-mineral premix	0/5	0/5	0/5
Salt	0/23	0/23	0/24
Sodium bicarbonate	0/17	0/16	0/15
Calculated analyses			
Metabolizable energy (kcal/kg)	2850	3000	3100
Crude protein (%)	21/195	19/297	17/503
Calcium (%)	0/9892	0/9363	0/8168
Available phosphorus (%)	0/4711	0/4681	0/4036
Sodium (%)	0/1600	0/1600	0/1600
Lysine (%)	1/347	1/180	1/009
Methionine (%)	0/6593	0/5781	0/4933
Methionine + Cystine (%)	1/008	0/904	0/797

Table 2. Feed intake of broiler chickens fed diets containing butyric acid and salinomysin sodium with litter moisture.

Treatments	Starter	Grower	Finisher	Total
1	243 ± 15/18	2155 ± 73	2752^{a} ± 98/4	5151^{a} ± 180/2
2	245 ± 16/2	2098 ± 14/5	2588^{bc} ± 43/5	4931^{b} ± 27/7
3	231 ± 26/9	2144 ± 20/1	2645^{ab} ± 92/8	5020^{ab} ± 88/6
4	241 ± 16/2	2152 ± 63/1	2558^{bc} ± 63/6	4935^{ab} ± 233/6
5	246 ± 20/2	2105 ± 26/6	2551^{bc} ± 27/7	4903^{b} ±83/6
6	255 ± 13/3	2123 ± 40/5	2590^{bc} ± 72/8	4968^{ab} ±123
7	244 ± 21/7	2162 ± 14/9	2476^{c} ± 80/7	4882^{b} ± 76/8
8	240 ± 25/6	2115 ± 55	2621^{abc} ± 47/2	4977^{ab} ± 95
Main effects				
Butyric acid	n.s	n.s	n.s	n.s
Salinomysin	n.s	n.s	n.s	n.s
Litter moisture	n.s	n.s	n.s	n.s
Interaction effects	n.s	n.s	**	**

in feed intake between treatments ($p < 0.05$), and highest feed consumption related with group that had Butyric acid and Salinomysin and normal Litter moisture than other groups. Also no significant difference were observed in weight gain and feed conversion ratio between treatments ($p > 0.05$). No significant effects were observed with main effect of Butyric acid, Salinomysin, Litter moisture and interaction between them ($p > 0.05$).

As mentioned in Table 5, the results of Oocyt count showed that there were significantly differences in number of oocysts per gram of litter between treatments in weeks 2 and 4 ($p < 0.05$). In these weeks, individual effects of Butyric acid, Salinomysin and Litter moisture were not significant ($p > 0.05$). But interaction effect of

Table 3. Weight gain of broiler chickens fed diets containing butyric acid and salinomysin sodium with litter moisture.

Treatments	Starter	Grower	Finisher	Total
1	144/08 ± 10/26	914/41 ± 61/37	912/69[ab] ± 41/72	1971/18±86/2
2	144/57± 10/58	895/50 ± 74/39	963/35[ab] ± 39/65	2003/43±95/9
3	139/01 ± 22/83	934/13 ± 75/28	922/90[ab] ± 99/39	1996/04±196/1
4	141/42 ± 7/73	910/88 ± 91/81	875/04[b] ± 35/05	1927/33±111/5
5	146/79 ± 18/67	908/79 ± 40/43	988/25[a] ± 38/29	2043/83±73/3
6	150/81 ± 10/55	918/25 ± 96/54	947/44[ab] ± 52/78	2016/50±119/6
7	150/33 ± 12/41	950/73 ± 45/37	919/42[ab] ± 70/87	2020/48±119/6
8	145/63 ± 20/52	914/83 ± 42/32	937/67[ab] ± 67/88	1998/13±99/5
Main effects				
Butyric acid	n.s	n.s	n.s	n.s
Salinomysin	n.s	n.s	n.s	n.s
Litter moisture	n.s	n.s	n.s	n.s
Interaction effects	n.s	n.s	**	n.s

Table 4. Feed conversion ratio of broiler chickens fed diets containing butyric acid and salinomysin sodium with litter moisture.

Treatments	Starter	Grower	Finisher	Total
1	1/69 ± 0/05	2/34 ± 0/15	3/01[a] ± 0/16	2/61 ± 0/12
2	1/69 ± 0/04	2/35 ± 0/19	2/69 [ab] ± 0/14	2/46 ± 0/11
3	1/67 ± 0/09	2/30 ± 0/18	2/89 [ab] ± 0/20	2/53 ± 0/20
4	1/70 ± 0/04	2/38 ± 0/18	2/92 [ab] ± 0/20	2/57 ± 0/19
5	1/68 ± 0/07	2/31 ± 0/09	2/58 [b] ± 0/17	2/40 ± 0/10
6	1/69 ± 0/03	2/32 ±0/15	2/74 [ab] ± 0/19	2/47 ± 0/15
7	1/62 ± 0/02	2/27 ±0/11	2/70 [ab] ± 0/20	2/42 ± 0/16
8	1/65 ± 0/08	2/30 ±0/06	2/80 [ab] ± 0/20	2/49 ± 0/10
Main effects				
Butyric acid	n.s	n.s	n.s	n.s
Salinomysin	n.s	n.s	n.s	n.s
Litter moisture	n.s	n.s	n.s	n.s
Interaction effects	n.s.	n.s.	**	n.s.

factors was significant ($p < 0.05$). But in other weeks (3, 5 and 6) no significant difference were observed in oocysts count of treatments ($p > 0.05$). No significant effects were observed with main effect of Butyric acid, Salinomysin, Litter moisture and interaction between them ($p > 0.05$).

DISCUSSION

Although short-chain fatty acids such as acetate and propionate have been successfully used as water sanitizers, there is little information available on butyrate metabolism by poultry. Bolton and Dewar (1965) indicate that free butyric acid is absorbed very quickly in the upper digestive tract, and will likely be of limited use other than as a feed sanitizer. By inference, butyrate needs to be stabilized, and hence the testing of butyrate glycerides used in this study.

Pinchasov and Jensen (1989) reported that butyric acid, unlike other acids such as propionate, did not depress feed intake and do not have effect on the performance. In the current studies, adding up to 0.5% butyrate glycerides had no detrimental effect on feed intake, weight gain and feed conversion ratio. There is an indication from the present study of performance relative to birds fed Salinomysin. Sakata (1987) showed that infusion of Salinomysin into fistulated rats decreased feed intake and number of oocysts. Sharma et al. (1995)

Table 5. Oocyst count in feces of broiler chickens fed diets containing butyric acid and salinomysin sodium with litter moisture.

Treatments	Weeks				
	2	3	4	5	6
1	52/75 [ab] ± 8/30	550 ± 73/21	14625[b] ± 634	95025 ± 4548	30350 ± 607
2	150 [a] ± 9/1	4580 ± 39/1	2407750[a] ± 3251	11150 ± 850	26725 ± 396
3	0 [c]	850 ± 50	44300[b] ± 4920	56900 ± 9411	14850 ± 933
4	75 [ab] ± 5/7	450 ± 18/3	12600[b]± 2400	10630 ± 741	10650 ± 767
5	100 [ab] ± 8/6	400 ± 25/8	300[b]± 21/4	36325 ± 670	63350 ± 297
6	25 [ab] ± 2/3	350 ± 26/7	4050[b] ± 810	90825 ± 6895	28375 ± 2410
7	50 [ab] ± 7/73	300 ± 41/42	25[b] ± 5/0	19650 ± 175	52850 ± 4024
8	50 [ab] ± 4/36	350 ± 26/4	125[b] ± 25/4	49825 ± 812	24750 ± 185
Main effects					
Butyric acid	n.s	n.s	n.s	n.s	n.s.
Salinomysin	n.s	n.s	n.s	n.s	n.s.
Litter moisture	n.s	n.s	n.s	n.s	n.s.
Interaction effects	**	n.s.	**	n.s.	n.s.

suggested that the effect on oocysts growth may reflect changes in the litter moisture, which is known to be a major modulator of Coccidiosis Infection. Further studies are warranted on the effects of graded levels of butyrate and Salinomysin on oocysts count and performance of young broilers.

Van der et al. (2000) showed a correlation between the presence of undissociated butyrate (and acetate and propionate) and pathogen control in the ceca of young birds. Studies with cocci-vaccinated birds confirm the additional benefit of litter moisture condition in terms of growth rate after coccidiosis challenge, which is a common occurrence under commercial conditions. The interaction between Butyric acid, Salinomysin and Litter moisture reduction in oocysts production indicates that it might have anticoccidial activities. Thus, it can be concluded that the Butyric acid and Salinomysin preparation has the potential to lower the severity and the pressure of the infection and at the same time maintain the oocysts production, which is crucial for the re-infection and the maintenance of the immunity stimulated by the initial infection. Williams (1995) has reported that there is reciprocity between the immune status of chickens and their excretion of oocysts. In conclusion, the results of this experiment show that supplementation of the diet with Butyric acid and Salinomysin with normal moisture of litter may have negative effect on performance and affect oocysts count of litter.

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