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Full Length Research Paper

The effect of butyric acid glycerides on performance and some bone parameters of broiler chickens

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A concern about enhancing the natural defense mechanisms of animals and reducing the massive use of antibiotics led to the banning of studies in this field. So, this research was done to investigate the effect of butyric acid glycerides and salinomycin sodium on the performance of the broiler chickens (strain Ross 308). A total of 800 chickens were reared for 42 days. A 3 factor statistical design was conducted with 4 replicates, and each factor contained 2 levels (25 broilers in each pen). The factors were butyric acid glycerides (0 and 0.3% of diet), salinomycin sodium - an anticoccidial drug (0 and 0.5% of diet) - and litter moisture (normal litter with average moisture of 35% and wet litter with average moisture of 75%). Data were collected and analyzed by SAS with GLM procedure. The results showed that butyric acid glycerides had no significant effect on feed intake. Weight gain and feed conversion ratio were not significantly affected by the mentioned factors. The effect of the treatments on the number of Eimeria oocytes excreta in the second and fourth week of breeding and feed intake were significant ($p<0.05$). Diet acidification with butyric acid glycerides caused an increase in ash, calcium and phosphorus of the chicken tibia, but this increase was not significant ($p>0.05$). Considering the result of this experiment, the use of butyric acid glycerides and salinomycin sodium in the aforementioned levels had no positive effect on the performance of broiler chickens ($p>0.05$).

Key words: Butyric acid glycerides, salinomycin sodium, ross, performance and broilers.

INTRODUCTION

In the past, antibiotics have been included in animal feed at sub-therapeutic levels, acting as growth promoters (Dibner and Richards, 2005). Worldwide concern about development of antimicrobial resistance and transference of antibiotic resistance genes from animal to human microbiota led to the placement of a ban on the use of antibiotics as growth promoters (Mathur and Singh, 2005; Salyers et al., 2004). There is the need to look for viable alternatives that could enhance the natural defense mechanisms of animals and reduce the massive use of antibiotics (Verstegen and Williams, 2002). A way is to use specific feed additives or dietary raw materials to favorably affect animal performance and welfare, particularly through the modulation of the gut microbiota which plays a critical role in maintaining host health (Tuohy et al., 2005). A balanced gut microbiota constitutes an efficient barrier against pathogen colonization, produces metabolic substrates such as vitamins and short-chain fatty acids, and then stimulates the immune system in a non-inflammatory manner. Using new feed additives (for example, enzymes, organic acids, probiotics, prebiotics and herbal extracts), towards host-protecting functions to support animal health, is a topical issue in animal breeding and it creates fascinating possibilities. Use of organic acids is very appropriate because of the ease of use, accessibility, reinfection improbability, positive effect on broiler performance, lack of bacterial resistance, providing proper balance of intestinal flora and prevention of feed nutrient destruction (Waldroup and Kanis, 1995). Organic acids mechanism

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of action is totally different from antibiotics. Organic acids are lipophilic in their unsegregated form and can easily pass through the bacterial cell membrane. An organic acid is segregated inside the bacterial cell and cause pH reduction in the cytoplasm which consequently cause enzyme activity and material transfer disorders. Bacterium tries to send H^+ ions out of the bacterial cell to protect homeostasis, which is an endergonic activity. Organic acids reduce accessible energy for other bacterial activities through this way. $Rcoo^-$ ions can also have negative effects on DNA and bacterial cell division. So organic acids can act as bactericide combinations and cause bacteria death (Chaveerach et al., 2008; Dibner and Buttin, 2002; Griggs and Jacob, 2005; Partanen and Mroz, 1999). Among short-chain fatty acids, butyric acid has been specially noticed. The liquid form of butyric acid is given to the bird mainly in combination with water, while the powder form is given with their diet. By using methods such as mineral carriers, esterification with glycerol and also encap-sulation, organic acids are protected from being absorbed in the upper parts of the digestive system. A study by Bolton and Dewar (1965) showed that 60% of butyric acid was absorbed only in crop and less than 1% of this acid reached the lower parts of the small intestine. So, butyric acid glycerides were used in this experiment in order to prevent quick absorption in upper parts of the digestive system. Various beneficial experiments have shown that organic acids were used to control disease causing bacteria such as Salmonella, Campylobacter and *E. coli* (Chaveerach et al., 2008; Van Immerseel et al., 2005), but only a few researches have been done to study the effect of butyric acid on other microorganisms of the digestive system.

This research was conducted to study the effect of butyric acid glycerides on the performance of some bone traits of the broiler chickens and the microbial population of the digestive system, especially Eimeria Protozoan. Different factors such as litter moisture and existence or absence of anti-coccidial drug (salinomycin sodium), were included in the experimental design in order to measure the anti-microbial power of butyric acid.

MATERIALS AND METHODS

Birds and diets

In this research, a completely randomized design was selected with factorial method. So, 3 factors were selected and the level number of each factor was 2. A total of 800 male broiler chickens (Ross 308) were obtained from a local breeding farm. Experimental factors were butyric acid glycerides (0 and 0.3% of the diet), salinomycin sodium - anticoccidail substance (0 and 0.5% of the diet) and litter moisture (normal litter with average moisture of 35% and wet litter with average moisture of 75%). Upon arrival, chickens were wing-banded, weighed and randomly allocated to 8 treatment groups of 100 birds each. Each group was further divided into 4 replicates of 25 birds. All replicates were housed in 32 separate wire-suspended cages equipped with plastic sides, and the bottoms covered with clean wood shavings. Light was continuously provided for the duration of the experiment. The temperature in the cages was 32°C on arrival of the chickens. From day 8 of the experiment, the temperature was gradually decreased by 2°C every day, until it reached 20°C by day 14. However, feed and water were available *ad libitum*.

UFFDA program was used for diets formulation, based on the National Research Council recommended table (National Research Council, 1994). However, mash diets were used in this experiment. In order to compare the effect of butyric acid glycerides with salinomycin sodium, this anti-coccidial drug was added to the experimental diets with the amount of 0.5 kg/ton, during the grower and finisher stages. Before the experiment, chemical analyses of experimental diets were determined according to the methods of AOAC (Association of Official Analytical Chemists, 1990). The ingredients and the composition of the experimental diets are presented in Table 1.

Butyric acid and salinomysin sodium were added to the basal diet by substitution at the expense of corn. The starter diet was fed until day 10, the grower diet was fed from day 11 to 28, and the finisher diet was fed from day 29 to 42.

Traits and data collection

Data were collected as per the number of coccidia oocytes in the excreta, feed intake, weight gain and feed conversion ratio, as well as the amount of mineral storage in chicken tibia (ash, calcium and phosphorus).

In order to determine the number of Eimeria oocytes, 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. Excreta collection was done in the evening and the samples were stored overnight in a refrigerator. The oocytes of each cage were counted the next day and the numbers were expressed per g of excreta. For oocyte 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 in 1 L of water). Then, the suspension was put into the McMaster chamber using a micropipette and the number of oocytes was counted (Peek and Landman, 2003).

Body weights were measured on days 10, 28 and 42. Feed intakes were determined per week for every cage and were expressed as g/bird/day. The feed conversion ratio was calculated as feed intake per cage divided by weight gain of birds in the cage. At the end of the experimental period (42 days of age), one broiler chicken from each replicate was randomly selected. Live weights of birds were recorded after a 12-h-hunger period. The selected birds were subjected to feed withdrawal overnight, permitting gut clearance, after which they were killed via neck cutting.

To study the effect of butyric acid glycerides on digestibility and absorption of minerals in the diet, measurement and comparison of the amount of mineral storage (ash, calcium and phosphorus) of the tibia were done for treatments 1 and 3 on days 14 and 35 of the breeding. After the chickens were suffocated by CO_2, the left tibia were removed from the body, packed in nylon bags, indexed and transferred to a cool mortuary (4°C) for storage. To determine ash, calcium and phosphorus contents, the bones were then transferred to a lab where they were boiled in water and dried in an oven for 24 h following flesh and cartilage removal. The products were then

Table 1. Composition of experimental diets.

Ingredient and analysis	Starter	Grower	Finisher
ingredient			
Corn	56.11	61.6	67.31
Soybean meal (44% CP)	34.71	27.94	21.91
Poultry wastage powder	2	3	4
Oil	1.27	1.26	1.42
DL-Methionine	0.34	0.28	0.22
L-Lysine HCL	0.26	0.24	0.2
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
Salt	0.23	0.23	0.24
Sodium bicarbonate	0.17	0.16	0.15
Formycine gold	0.1	0.1	0.1
Oyster shell	0.04	-	0.01
Avilamycin	0.01	0.01	0.01
Salinomycin sodium	-	0.05	0.05
Calculated analysis (%)			
Metabolizable energy (kcal/kg)	2850	3000	3100
Crude protein	21.1945	19.2978	17.5038
Calcium	0.9892	0.9363	0.8168
Total phosphorus	0.7200	0.7033	0.6219
Available phosphorus	0.4711	0.4681	0.4036
Sodium	0.1600	0.1600	0.1600
Potassium	0.8707	0.7559	0.6540
Chlorine	0.2300	0.2300	0.2300
Crude fat	4.2316	4.6872	5.2963
Crude fiber	3.1363	2.8974	2.6905
Linoleic acid	2.2180	2.3255	2.5233
Arginine	1.3660	1.2094	1.0667
Lysine	1.3472	1.1808	1.0091
Methionine + cystine	1.0080	0.9045	0.7967
Methionine	0.6593	0.5781	0.4933
Threonine	0.7967	0.7234	0.6556
Tryptophan	0.2483	0.2173	0.1891

[1]Content per 2.5 kg: Vitamin A, 9,000,000 IU; vitamin D, 2,000,000 IU; vitamin E,18,000 IU; vitamin K, 2,000 mg; vitamin B_1, 1.800 mg; vitamin B_2, 6.600 mg; vitamin B_3, 10.000 mg; vitamin B_5, 30.000 mg; vitamin B_6, 30.000 mg; vitamin B_9, 1.000 mg; vitamin B_{12}, 15 mg; vitamin H_2, 100 mg; choline chloride, 500,000 mg and antioxidant, 3000 mg; [2]Content per 2.5 kg: manganese, 100.000 mg; iron, 50,000 mg; zinc, 100,000 mg; copper, 10,000 mg; iodine, 1.000 mg; selenium, 200 ; mg; cobalt, 100 mg.

placed in Soxhlet apparatus for 16 h for fat extraction, after which they were transferred to dry oven and electric furnace for 8 h treatment in order to obtain ash. The ash was then weighed in order to determine the ash percentage of the bones. It was then used to determine the calcium and phosphorus contents using the standard methods recommended by the Association of Official Analytical

Table 2. Main and interactive effects of experimental factors on the number of Eimeria oocytes per g of excreta in different weeks of breeding.

Parameter	Week of breeding				
	2	3	4	5	6
Treatment (Interaction effect)					
1 ($A_1B_1C_1$)	$52.75^{ab} \pm 8.3$	$550^{a} \pm 73.21$	14625 ± 634^{b}	$95025^{a} \pm 4548$	$30350^{a} \pm 607$
2 ($A_1B_1C_2$)	$150^{a} \pm 9.1$	$4850^{a} \pm 39.1$	2407750 ± 3251^{a}	$11150^{a} \pm 850$	$26725^{a} \pm 396$
3 ($A_2B_1C_1$)	$0^{b} \pm 0.0$	$850^{a} \pm 50$	$44300^{b} \pm 4920$	$56900^{a} \pm 9411$	$14850^{a} \pm 933$
4 ($A_2B_1C_2$)	$75^{ab} \pm 5.7$	$450^{a} \pm 18.3$	$12600^{b} \pm 2400$	$10630^{a} \pm 741$	$10650^{a} \pm 767$
5 ($A_1B_2C_1$)	$100^{ab} \pm 8.6$	$400^{a} \pm 25.8$	$300^{b} \pm 21.4$	$36325^{a} \pm 670$	$63350^{a} \pm 297$
6 ($A_1B_2C_2$)	$25^{ab} \pm 2.3$	$350^{a} \pm 26.7$	$4050^{b} \pm 810$	$90825^{a} \pm 6895$	$28375^{a} \pm 2410$
7 ($A_2B_2C_1$)	$50^{ab} \pm 7.73$	$300^{a} \pm 41.42$	$25^{b} \pm 5.0$	$19650^{a} \pm 175$	$52850^{a} \pm 4024$
8 ($A_2B_2C_2$)	$50^{ab} \pm 4.36$	$350^{a} \pm 26.4$	$125^{b} \pm 25.4$	$49825^{a} \pm 812$	$24750^{a} \pm 185$
Significant	**	n.s	**	n.s	n.s
Factors (main effects)					
Butyric acid glycerides (A)*					
A_1	$81.94^{a} \pm 7.5$	$1538^{a} \pm 67.7$	$64938^{a} \pm 6836$	$58275^{a} \pm 2844$	$37200^{a} \pm 739$
A_2	$43.75^{a} \pm 5.6$	$488^{a} \pm 68.22$	$14263^{a} \pm 3091$	$58169^{a} \pm 3154$	$25775^{a} \pm 799$
Significant	n.s	n.s	n.s	n.s	n.s
Salinomycin sodium (B)					
B_1	$69.44^{a} \pm 10.9$	$1675^{a} \pm 40.2$	$78075^{a} \pm 6582$	$67288^{a} \pm 4015$	$20644^{a} \pm 304$
B_2	$56.25^{a} \pm 2.7$	$350^{a} \pm 21$	$1125^{a} \pm 402.3$	$49156^{a} \pm 3912$	$25775^{a} \pm 279$
Significant	n.s	n.s	n.s	n.s	n.s
Litter moisture (C)					
C_1	$50.69^{a} \pm 2.5$	$525^{a} \pm 44.49$	$14813^{a} \pm 3069$	$51975^{a} \pm 5571$	$40350^{a} \pm 511$
C_2	$75^{a} \pm 10$	$1500^{a} \pm 40.2$	$64388^{a} \pm 1685$	$64469^{a} \pm 5921$	$22625^{a} \pm 235$
Significant	n.s	n.s	n.s	n.s	n.s

* A_1 and A_2 were supplemented with 0 and 0.3% butyric acid glycerides, B_1 and B_2 were supplemented with 0 and 0.5% salinomycin sodium, and C_1 and C_2 were supplemented with normal litter with an average moisture of 35% and wet litter with an average moisture of 75%, respectively; ^{a,b} means within columns with different superscripts differ significantly at $P < 0.05$.

Chemists (1990).

Statistical analysis

Analysis of variance was performed by applying 3-way ANOVA procedure of the SAS (2004). Comparison of the mean test was done by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Data analysis showed that in the second and fourth week of breeding, experimental treatments had significant effect on the number of Eimeria oocytes per g of excreta ($p<0.05$) (Table 2). Treatment 2, which was found with wet litter and without butyric acid glycerides and salinomycin sodium, caused most of the infections by Eimeria Protozoan. This treatment had a significant difference from the control in the fourth week of the experiment. Litter moisture can increase infection and provide better environmental conditions for coccidia oocyte growth. In the second and fourth week of breeding, the fewest number of oocytes was observed in treatment 3 containing butyric acid glycerides and in treatment 7 containing salinomycin sodium and butyric acid glycerides, respectively. In other weeks of breeding, experimental treatments had no significant effect on this parameter ($p>0.05$). 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 butyric acid and salinomysin preparation has the potential to lower the severity and 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. In the different weeks of breeding, experimental factors such as butyric acid

Table 3. Main and interactive effects of experimental factors on feed intake, weight gain and feed conversion ratio.

Parameter	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Treatments (Interaction effect)			
1 ($A_1B_1C_1$)	5151.99^{a} ± 180.2	1971.18^{a} ± 86.2	2.616^{a} ± 0.12
2 ($A_1B_1C_2$)	4931.85^{b} ± 27.7	2003.43^{a} ± 95.9	2.465^{a} ± 0.11
3 ($A_2B_1C_1$)	5020.81^{ab} ± 88.6	1996.04^{a} ± 196.1	2.532^{a} ± 0.20
4 ($A_2B_1C_2$)	4935.62^{ab} ± 233.6	1927.33^{a} ± 111.5	2.577^{a} ± 0.19
5 ($A_1B_2C_1$)	4903.86^{b} ± 83.6	2043.83^{a} ± 73.3	2.402^{a} ± 0.10
6 ($A_1B_2C_2$)	4968.97^{ab} ± 123	2016.50^{a} ± 119.6	2.471^{a} ± 0.15
7 ($A_2B_2C_1$)	4882.79^{b} ± 76.8	2020.48^{a} ± 119.6	2.424^{a} ± 0.16
8 ($A_2B_2C_2$)	4977.02^{ab} ± 95	1998.13^{a} ± 99.5	2.494^{a} ± 0.10
Significant	**	n.s	n.s
Factors (main effects)			
Butyric acid glycerides (A)*			
A_1	4989.17^{a} ± 145.1	2008.74^{a} ± 94.2	2.489^{a} ± 0.13
A_2	4958.56^{a} ± 134.6	1985.05^{ab} ± 127.7	2.507^{a} ± 0.17
Significant	n.s	n.s	n.s
Salinomycin sodium (B)			
B_1	5014.57^{a} ± 164.3	1974.50^{a} ± 120.2	2.548^{a} ± 0.16
B_2	4933.16^{a} ± 95.7	2019.74^{a} ± 99.7	2.448^{a} ± 0.12
Significant	n.s	n.s	n.s
Litter moisture (C)			
C_1	4989.86^{a} ± 151.4	2007.89^{a} ± 117.8	2.495^{a} ± 0.17
C_2	4957.86^{a} ± 127.3	1986.35^{a} ± 106.5	2.502^{a} ± 0.13
Significant	n.s	n.s	n.s

* A_1 and A_2 were supplemented with 0 and 0.3% butyric acid glycerides, B_1 and B_2 were supplemented with 0 and 0.5% salinomycin sodium, and C_1 and C_2 were supplemented with normal litter with an average moisture of 35% and wet litter with an average moisture of 75%, respectively; ^{a,b} means within columns with different superscripts differ significantly at $P < 0.05$; n.s, not significant.

glycerides, salinomycin sodium and litter moisture had no significant effect on this parameter ($p>0.05$). Some researchers showed that using organic acids caused a significant reduction in the microbial balance of the digestive system and consequently improved the bird's performance (Van Immerseel et al., 2005), which did not agree with the result of this experiment. This difference can be because of these reasons: The tested strains in those researches such as Salmonella and Campylobacter were non-resistant against the acids, and the effect of organic acids on organic acid-resistant strains such as Eimeria was not studied in any of them. On the other hand, since each organic acid has its own anti-microbial power, using other acids or a mixture of acids with synergetic effect could cause different results.

Also, Eimeria was studied, but since each organic acid has its own anti-microbial power, using other acids or a mixture of acids with synergetic effect could cause different results. In addition, higher levels of butyric acid glycerides may be needed to destroy excreta Eimeria oocytes. In a study by Conway et al. (2002), it was reported that salinomycin sodium had no significant effect on the amount of infection by Eimeria Protozoan. These researchers showed that salimycin in comparison with diclazuril and roxarsone has less power to control the Eimeria oocytes. Increasing the resistance of Eimeria oocytes against ionospheres can also be one of the reasons for the insignificant reduction of oocytes in response to adding salinimycin sodium to the diet. This result agree with the findings of Ali et al., (2002) and Goncagul et al. (2004).

The effect of experimental treatments on the amount of feed intake was significant ($p<0.05$) (Table 3), in that the highest and lowest values for feed intake were observed

Table 4. Main and interactive effects of experimental factors on the amount of mineral storage in chicken tibia (ash, calcium and phosphorus).

Treatment*	Ash (%)		Calcium (%)		Phosphorus (%)	
	14 days	35 days	14 days	35 days	14 days	35 days
1 ($A_1B_1C_1$)	$36.37^a \pm 4.1$	$46.89^a \pm 2.1$	$12.66^a \pm 1.8$	$15.09^a \pm 2.4$	$12.23^a \pm 1.3$	$13.40^a \pm 0.6$
3 ($A_2B_1C_1$)	$38.51^a \pm 3.1$	$45.85^a \pm 2.5$	$12.72^a \pm 1.1$	$16.44^a \pm 1.09$	$13.25^a \pm 0.8$	$13.66^a \pm 0.5$
Significant	n.s	n.s	n.s	n.s	n.s	n.s

*Treatment $A_1B_1C_1$, without butyric acid glycerides and salinomycin sodium in a normal litter (control); $A_2B_1C_1$ supplemented with 0.3% butyric acid glycerides, without salinomycin sodium in a normal litter. ^{a,b} means within columns with different superscripts differ significantly at $P < 0.05$.

in the control and treatment 7, respectively. This reduction, in comparison with the control group was significant ($p<0.05$). Treatment 7 was supplemented with butyric acid glycerides and salinomycin sodium in a normal litter. Feed intake in treatment 5 (with salinomycin sodium) and treatment 7 (with salinomycin sodium and butyric acid glycerides) had a significant reduction in comparison with the control. The used level of salinomycin sodium could cause feed intake reduction, and this effect could increase by adding butyric acid glycerides to the diet containing salinomycin sodium. According to the results, the main effects of the experimental factors; butyric acid glycerides, salinomycin sodium and litter moisture were not significant on feed intake ($p>0.05$) although the numeric value of feed intake reduced by increasing these factors. This result is in agreement with the findings of Pinchasov and Jensen (1989). These researchers reported that butyric acid in contrast with propionate has no significant effect on broiler's performance and feed intake.

According to Table 3, different treatments had no significant effect on weight gain ($p>0.05$). The main effects of butyric acid glycerides and litter moisture on weight gain were not significant ($p>0.05$). This observation is in agreement with the findings of Leeson et al. (2005). Using salinomycin sodium with the amount of 5% in diet caused an improvement in weight gain, but this improvement was not significant ($p>0.05$), and was also reported elsewhere (Ali et al., 2002). The experimental treatments and factors had no significant effect on feed conversion ratio ($p>0.05$) (Table 3), although salinomycin sodium factor caused improvement in feed conversion ratio.

Using butyric acid glycerides had no significant effect on the percentage of tibia ash, calcium and phosphorus at 14 and 35 days of age ($p>0.05$) (Table 4). However, on these two dates, consumption of this feed additive and consequently diet acidification, caused an insignificant increase in the values of the mentioned parameters. A study by Boling-Frankenbach et al. (2001) showed that using citric acid caused a significant increase in tibia ash and calcium, but the result of this study's experiment did not agree with the findings of Boling-Frankenbach et al. (2001). This can be due to different acidity of butyric and citric acids. In addition, unprotected organic acids were lipophilic and were mainly absorbed in crop, but the organic acid used in this experiment was butyric acid glycerides and was mainly released in the lower parts of the digestive system which has fewer absorption sites.

In conclusion, considering the existing condition in this experiment and values of the parameters, butyric acid glycerides and salinomycin sodium used in the mentioned levels had no significant positive effect on the performance of broiler chickens.

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