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Full Length Research Paper

The effect of butyric acid glycerides on serum lipids and carcass analysis of broiler chickens

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A worldwide concern about the development of antimicrobial resistance led to the banning of the use of antibiotics as growth promoters. This research was carried out to investigate the effect of butyric acid glycerides and salinomycin sodium on serum lipids and carcass characteristics in Ross 308 broiler chickens. 800 broiler chickens were raised for 42 days. The experiment was conducted in a 2 × 2 × 2 factorial design with 4 replicates of 25 broilers each. The experimental factors were butyric acid glycerides in two levels (0 and 0.3% of the diet), salinomycin sodium- an anticoccidial drug in two levels (0 and 0.5% of the diet) and litter moisture in two levels (normal litter with average moisture of 35% and wet litter with average moisture of 75%). The level serum cholesterol decreased at 14 and 35 days of age, but this reduction was only significant at 35 days of age ($p<0.05$). The use of butyric acid glycerides in diet caused a significant increase in blood triglycerides at 14 days of age ($p<0.05$). The effect of the experimental treatments on carcass percentage, thigh, abdominal fat, pregastric and gizzard, pancreas and intestine were significant ($p<0.05$). Using butyric acid glycerides caused a significant increase in carcass percentage and a significant reduction in intestine weight ($p<0.05$). Salinomycin sodium caused a significant increase in carcass percentage and a significant reduction in the percentage of pregastric and gizzard, and also pancreas ($p<0.05$).

Key words: Butyric acid glycerides, salinomycin sodium, Ross, carcass, broiler chickens.

INTRODUCTION

The first goal of animal production is the delivery of safe foods for human consumption, taking the welfare of the animal and protection of the environment into account. In the past, antibiotics have been included in animal feed at sub-therapeutic levels, as growth promoters (Dibner and Richards, 2005). However, worldwide concern about development of antimicrobial resistance and transference of antibiotic resistance genes from animal to human microbiota (Mathur and Singh, 2005; Salyers et al., 2004) led to banning the use of antibiotics as growth promoters. 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). One of such ways 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 (example vitamins and short-chain fatty acids) and stimulates the immune system in a non-inflammatory manner. Using new feed additives, such as enzymes, organic acids, probiotics, prebiotics and herbal extracts for host-protecting functions to support animal health, is a topical issue in animal breeding and creates fascinating possibilities. Using 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

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intestinal flora and prevention of feed nutrient destruction (Waldroup and Kanis, 1995). Organic acids mechanism of action is totally different from antibiotics. Organic acids are lipophilic in their unsegregated form and can easily pass through the bacterial cell membrane. Organic acid is segregated inside bacterial cell and cause pH reduction in cytoplasm, which consequently cause enzyme activity and material transfer disorders. Bacterium sends H^+ ions out of the bacterial cell to protect homeostasis, which is an undergone 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 the short-chain fatty acids, butyric acid has been specially noticed. This product is available in 2 forms of powder and liquid. The liquid form is given to the bird mainly in combination with the water and the powder form is given with their diet. By using methods such as mineral carriers, estrification with glycerol and also encapsulation, organic acids are protected from being absorbed in upper parts of digestive system. A study (Bolton and Dewar, 1965) showed that 60% of butyric acid is absorbed only in crop and less than 1% of this acid reaches the lower parts of the small intestine.

Butyric acid glycerides were therefore 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 are used to control disease causing bacteria such as *Salmonella*, *Campylobacter* and *Escherichia coli* (Chaveerach et al., 2008; van Immerseel et al., 2005), but, only a few researchers have been done to study the effect of butyric acid on other microorganisms of digestive system.

The objective of this study was to evaluate the effect of butyric acid glycerides on serum lipid and carcass traits in broilers.

MATERIALS AND METHODS

Birds and dietary treatments

This experiment was conducted in a completely randomized design with 2 × 2 × 2 factorial arrangements of treatments. 800 day-old male broiler chickens (Ross 308) were obtained from a local breeding farm. 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 each. All replicates were housed in 32 separate wire-suspended cages equipped with plastic sides, and bottoms were 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. Feed and water were available *ad libitum*.

Experimental factors were butyric acid glycerides in two levels (0 and 0.3% of the diet), salinomycin sodium- anti-coccidail substance in two levels (0 and 0.5% of the diet) and litter moisture in two levels (normal litter with average moisture of 35% and wet litter with average moisture of 75%). UFFDA program was used for diets formulation, based on National Research Council recommended table (NRC, 1994). 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 (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 substituting at the expense of corn. The starter diet was fed until day 10. The grower diet was fed from days 11 to 28. The finisher diet was fed from days 29 to 42.

Traits data

Data were collected on the percentage of carcass, breast, thigh, abdominal fat, pregastric and gizzard, pancreas, liver and gall bladder, and intestine; as well as the amount of lipid parameters in blood serum (triglycerides and cholesterol).

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, and were then killed via neck cutting. After slaughter, percentage of body weight and percentage of body components weight were measured. After separating heat, feather, organs, viscera and abdominal fat pad (including fat surrounding gizzard, bursa of fabricius, cloaca and adjacent muscles), carcass weight was measured, and carcass percentage was expressed as a ratio of live weight. The percentage of breast and thigh were calculated as a ratio of the carcass weight. The percentage of abdominal fat, pregastric and gizzard, pancreas, liver and gall bladder and also intestine were calculated as a ratio of the live weight.

Statistical analysis

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

RESULTS AND DISCUSSION

According to Table 2, the effect of experimental treatments on the carcass percentage mean was significant ($p<0.05$). The lowest and highest values for carcass percentage were observed in treatments 2 and 7, respectively; but their differences with the control were not significant ($p>0.05$). The percentage of carcass in treatment 2 was significantly lower than its value in treatments 3, 4, 5, 6, 7 and 8 ($p<0.05$). The reason for carcass percentage reduction in treatment 2 can be lack of butyric acid glycerids and salinomycin sodium in diet and also highest litter moisture. Treatment 7 with both additives and normal litter had the highest carcass percentage among all treatments. The study of the

Table 1. Composition of experimental diets.

Ingredient and analysis	Starter	Grower	Finisher
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.

experimental factors in Table 2 showed that using butyric acid glycerides and salinomycin sodium can significantly increase carcass percentage ($p<0.05$). This increase can be due to the effect of these two additives on the bird's health and consequently performance improvement. This observation agreed with the findings of Antongiovanni et al. (2007). Increasing litter moisture cause insignificant reduction in carcass percentage ($p>0.05$).

Main and interactive effects of the experimental factors were not significant on breast percentage mean ($p>0.05$) (Table 2) which was also reported elsewhere (Antongiovanni et al., 2007; Garcia et al., 2007). The percentage of muscle tissue such as breast is mainly influenced by the bird genotype and is less influenced by its nutrition. However, with serious shortage in the diet, the bird's genetic capacity cannot be shown.

The effect of the experimental treatments was significant on thigh percentage mean ($p<0.05$) (Table 2). The highest and lowest values for thigh percentage were observed in treatments 4 and 3, respectively; but the mean difference between these two treatments and the control was not significant. The main effects of the

Table 2. Main and interactive effects of experimental factors on the percentage of carcass, breast, thigh and abdominal fat.

Treatment (Interaction effect)	Carcass (%)	Breast (%)	Thigh (%)	Abdominal fat (%)
1 ($A_1B_1C_1$)	$71.016^{ab} \pm 1.12$	$30.483^{a} \pm 0.82$	$27.380^{ab} \pm 0.64$	$1.1040^{b} \pm 0.37$
2 ($A_1B_1C_2$)	$69.528^{b} \pm 0.78$	$31.011^{a} \pm 1.25$	$27.503^{ab} \pm 0.42$	$1.3865^{ab} \pm 0.14$
3 ($A_2B_1C_1$)	$71.272^{a} \pm 1.03$	$31.192^{a} \pm 0.91$	$26.904^{b} \pm 1.10$	$1.2943^{ab} \pm 0.25$
4 ($A_2B_1C_2$)	$71.325^{a} \pm 0.77$	$30.073^{a} \pm 0.74$	$29.234^{a} \pm 1.12$	$1.0675^{b} \pm 0.31$
5 ($A_1B_2C_1$)	$71.767^{a} \pm 0.66$	$31.294^{a} \pm 1.25$	$28.307^{ab} \pm 0.47$	$1.6995^{ab} \pm 0.38$
6 ($A_1B_2C_2$)	$71.304^{a} \pm 1.20$	$30.945^{a} \pm 1.36$	$28.269^{ab} \pm 1.01$	$1.0860^{b} \pm 0.19$
7 ($A_2B_2C_1$)	$72.686^{a} \pm 1.10$	$31.182^{a} \pm 0.93$	$28.375^{ab} \pm 1.40$	$1.0275^{b} \pm 0.38$
8 ($A_2B_2C_2$)	$71.595^{a} \pm 1.15$	$31.020^{a} \pm 1.16$	$27.629^{ab} \pm 0.80$	$1.9275^{a} \pm 0.40$
Significant	**	n.s	**	**
Factor (Main effect)				
Butyric acid glycerides (A)*				
A_1	$70.904^{b} \pm 1.21$	$30.933^{a} \pm 1.10$	$27.865^{a} \pm 0.96$	$1.319^{a} \pm 0.37$
A_2	$71.716^{a} \pm 1.11$	$30.867^{a} \pm 0.97$	$28.035^{a} \pm 1.21$	$1.329^{a} \pm 0.49$
Significant	**	n.s	n.s	n.s
Salinomycin sodium (B)				
B_1	$70.78^{b} \pm 1.17$	$30.690^{a} \pm 0.93$	$27.755^{a} \pm 1.18$	$1.2131^{a} \pm 0.31$
B_2	$71.838^{a} \pm 1.16$	$31.110^{a} \pm 1.07$	$28.145^{a} \pm 1.21$	$1.3351^{a} \pm 0.46$
Significant	**	n.s	n.s	n.s
Litter moisture (C)				
C_1	$71.658^{a} \pm 1.13$	$31.038^{a} \pm 0.95$	$27.742^{a} \pm 1.20$	$1.2813^{a} \pm 0.39$
C_2	$70.935^{a} \pm 1.21$	$30.762^{a} \pm 1.11$	$28.159^{a} \pm 1.22$	$1.3469^{a} \pm 0.45$
Significant	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 with 0 and 0.5% salinomycin sodium, and also C_1 and C_2 were normal litter with average moisture of 35% and wet litter with average moisture of 75%, respectively.
[a,b] Means within columns with different superscripts differ significantly at $P < 0.05$.

experimental factors on thigh percentage were not significant ($p>0.05$). These results agreed with the findings of Antongiovanni et al. (2007).

According to Table 2, the highest and lowest values of abdominal fat percentage were related to treatments 8 and 7, respectively. Increasing abdominal fat percentage in treatment 8 in comparison with the control was significant ($p<0.05$), but other treatments had no significant difference with the control ($p>0.05$). The main effects of experimental factors on this parameter were not significant ($p>0.05$), which also agreed with the findings of Antongiovanni et al. (2007).

According to Table 3, the highest percentage of gizzard and pregastic weight was observed in treatment 2 and the lowest value was observed in treatment 7, which was significantly different from the control group ($p<0.05$). The means of other treatments were between the means of these two treatments. Salinomycin sodium significantly decreased the weight of this organ ($p<0.05$). But other factors had no significant effect on this parameter ($p>0.05$).

The effect of the experimental treatments on the percentage of pancreas weight was significant ($p<0.05$) (Table 3). The highest and lowest values were observed in the control and treatment 5, respectively; so a significant difference was observed between these two treatments. Salinomycin sodium significantly decreased the percentage of pancreas weight ($p<0.05$). This result can be due to the effect of this antibiotic on the reduction of intestinal bacteria and consequently harmful substances which are secreted by them. On the other hand the catabolism of secreted enzymes in pancreas which is done by bacteria, decreased by reduction in bacteria population. Butyric acid glycerides and litter moisture had no significant effect on this parameter ($p>0.05$).

According to Table 3, the experimental treatments and factors had no significant effect on the mean of liver and gall bladder percentage ($p>0.05$), which agreed with the findings of Antongiovanni et al. (2007). Considering liver detoxification role, this result showed that the metabolism of butyric acid glycerides and salinomycin sodium had no significant effect on liver.

Table 3. Main and interactive effects of experimental factors on the percentage of pregastric and gizzard, pancreas, liver and gall bladder and also intestine.

Treatment (Interaction effect)	Pregastric and gizzard (%)	Pancreas (%)	Liver and gall bladder (%)	Intestine (%)
1 ($A_1B_1C_1$)	3.0855^{ab} ± 0.46	0.4150^{a} ± 0.07	2.5735^{a} ± 0.29	5.5660^{a} ± 0.51
2 ($A_1B_1C_2$)	3.3513^{a} ± 0.31	0.3500^{ab} ± 0.05	2.8120^{a} ± 0.35	5.3840^{a} ± 0.21
3 ($A_2B_1C_1$)	3.0673^{a} ± 0.31	0.3900^{a} ± 0.07	2.4913^{a} ± 0.28	5.0868^{a} ± 0.45
4 ($A_2B_1C_2$)	2.9825^{abc} ± 0.22	0.3525^{ab} ± 0.06	2.5703^{a} ± 0.18	5.5878^{a} ± 0.80
5 ($A_1B_2C_1$)	2.7290^{abc} ± 0.24	0.2650^{b} ± 0.10	2.8808^{a} ± 0.68	5.0595^{a} ± 0.18
6 ($A_1B_2C_2$)	2.6873^{bc} ± 0.12	0.3300^{ab} ± 0.06	2.6240^{a} ± 0.08	4.7698^{a} ± 0.72
7 ($A_2B_2C_1$)	2.5643^{c} ± 0.24	0.3300^{ab} ± 0.02	2.8565^{a} ± 0.49	4.5713^{a} ± 0.42
8 ($A_2B_2C_2$)	3.2195^{a} ± 0.16	0.342^{ab} ± 0.03	2.4653^{a} ± 0.18	5.1038^{a} ± 0.49
Significant	**	**	n.s	**
Factors (Main effects)				
Butyric acid glycerides (A)*				
A_1	2.9633^{a} ± 0.39	0.3400^{a} ± 0.08	2.7226^{a} ± 0.39	5.1948^{a} ± 0.52
A_2	2.9584^{a} ± 0.33	0.3537^{a} ± 0.05	2.5958^{a} ± 0.32	5.0874^{a} ± 0.65
Significant	n.s	n.s	n.s	**
Salinomycin sodium (B)				
B_1	3.1216^{a} ± 0.33	0.3768^{a} ± 0.06	2.6118^{a} ± 0.28	5.4061^{a} ± 0.56
B_2	2.8000^{b} ± 0.31	0.3168^{b} ± 0.06	2.7066^{a} ± 0.43	5.8761^{a} ± 0.49
Significant	**	**	n.s	n.s
Litter moisture (C)				
C_1	2.8615^{a} ± 0.37	0.3500^{a} ± 0.08	2.7005^{a} ± 0.45	5.0709^{b} ± 0.52
C_2	3.0601^{a} ± 0.32	0.3437^{a} ± 0.05	2.6179^{a} ± 0.24	5.2113^{a} ± 0.60
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 with 0 and 0.5% salinomycin sodium, and also C_1 and C_2 were normal litter with average moisture of 35% and wet litter with average moisture of 75%, respectively.
[a,b] Means within columns with different superscripts differ significantly at $P<0.05$.

The effect of experimental treatments on the percentage of intestine weight was significant ($p<0.05$) (Table 3). The highest and lowest values were observed in treatments 4 and 7, respectively. The difference between treatment 7 and control was also significant. Butyric acid glycerides factor caused a significant reduction in the percentage of intestine weight ($p<0.05$). This result can be due to the effect of this organic acid on the population of intestinal bacteria, which consequently caused a reduction in intestine wall thickness and weight of this organ. Also, it can be noted that by changing the size and form of the intestinal villi, this organic acid could change the digestive organ weight. Studies (Antongiovanni et al., 2007; Leeson et al., 2005) showed that using organic acid could change the intestinal tissue. These researchers reported that using organic acids shortens the intestinal villi in ileum and jejunum, increases microvilli in jejunum and also deepens the intestinal crypts. Salinomycin sodium had no significant effect on this parameter ($p>0.05$).

According to Table 4, the effect of butyric acid glycerides on the amount of blood cholesterol was not significant on day 14 of age ($p>0.05$), however, using butyric acid caused a numeric reduction in blood cholesterol at this age. By using butyric acid, the amount of blood cholesterol was significantly reduced in comparison with the control at day 35 of age ($p<0.05$). The amount of blood triglycerides significantly increased in comparison with the control group at day 14 of age ($p<0.05$), but no significant difference was observed at day 35 of age ($p>0.05$). A study by Hara et al. (1999) showed that short-chain fatty acids such as butyric and propionic acids reduced cholesterol and triglyceride synthesis in rats. The results of this experiment revealed that butyric acid in chicken broiler diet reduces liver and intestinal synthesis of cholesterol. However, this reduction was only significant

Table 4. Main and interactive effects of experimental factors on the amount of lipid parameters in blood serum (triglycerides and cholesterol) (mg/dl).

Treatment*	Cholesterol		Triglyceride	
	14 day	35 day	14 day	35 day
1 ($A_1B_1C_1$)	$150.75^a \pm 5.1$	$157^a \pm 8.3$	$36^a \pm 6.5$	$66.5^a \pm 13.17$
3 ($A_2B_1C_1$)	$138.75^a \pm 6.2$	$130.75^b \pm 7.11$	$69.25^b \pm 9.91$	$52.5^a \pm 15.02$
Significant	n.s	**	n.s	n.s

*Treatment $A_1B_1C_1$ without butyric acid glycerides and salinomycin sodium in a normal litter (control) and treatment $A_2B_1C_1$ 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 $P<0.05$.

at 35 days of age ($p<0.05$).

In conclusion, considering the existing condition in this experiment and values of parameters, butyric acid glycerides and salinomycin sodium used in the earlier mentioned levels had significant effect on the carcass traits of broiler chickens.

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