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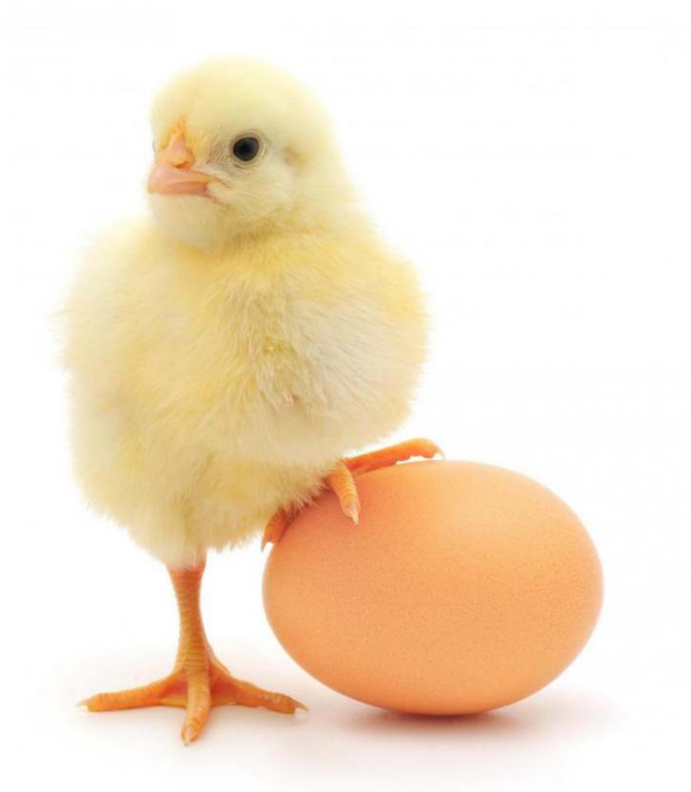

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Research Article

Comparison of Tributyrin and Coated Sodium Butyrate as Sources of Butyric Acid for Improvement of Growth Performance in Ross 308 Broilers

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Abstract

Background and Objective: Butyric acid in the form of fat-coated sodium or calcium butyrate has been widely used in commercial poultry production to improve body weight gain and reduce feed conversion. However, a quality coating process reduces the butyrate content and adds additional cost. Tributyrin, a glyceride containing three butyrate molecules attached to a glycerol backbone, is an alternative source of dietary butyrate which does not require a coating and can pass through the upper gastrointestinal tract to release butyric acid in the small intestine after cleavage by pancreatic lipase. The aim of this study was to compare the effects of two sources, coated sodium butyrate and tributyrin, on broiler growth performance. **Methodology:** In this study, two treatment diets were formulated at an iso-butyric level with either tributyrin or fat-coated sodium butyrate and compared to a control diet for body weight gain and feed conversion ratio over 35 days in Ross 308 broilers. **Results:** Both treatment diets showed significant improvements in body weight gain during the grower and finisher phases of the trial, with tributyrin having a numerical though not significant advantage over the sodium butyrate group. Feed conversion ratio was also significantly improved in both treatment groups during the grower phase of the trial and for the entire trial. No significant differences were seen between the tributyrin and sodium butyrate groups. **Conclusion:** The trial suggests that tributyrin is as effective as a fat-coated sodium salt for improvement of broiler performance and may confer advantages over traditional protected butyric acid salts.

Key words: Tributyrin, gut health, organic acid, feed additive, coated sodium butyrate

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Recent changes in modern animal production have primarily focused on a reduction or elimination in the use of antibiotics in feed and thus has led to an increase in the use of feed additives such as probiotics, prebiotics, enzymes, essential oils and organic acids. Among these, organic acids, specifically the short-chain fatty acids, have been a popular feed additive for the past two decades for their positive effect on gut health by creating a favorable environment for beneficial bacteria, improving digestibility and immunity and reducing inflammation of the gastrointestinal tract[1]. Of the short-chain fatty acids butyric acid (C4) has been extensively studied and used in animal agriculture for several decades, being first used in calves to stimulate early rumen development before finding use in swine and poultry[2-4]. Use of butyrates as a feed additive in poultry has been extensively studied and shown to improve body weight gain and feed conversion, reduce mortality and lessen the impact of gut-related disease[5-8]. A number of possible mechanisms of actions have been proposed including anti-bacterial activity, stimulation of villi development, upregulation of host defense peptide production and downregulation of the inflammatory response[9-11]. Due to butyric acid's offensive odor and rapid absorption in the upper gastrointestinal tract, the most common method of applying butyric acid in feed has been through the use of salts of butyric acid in a protected form[12]. This protection is often a fat matrix coating or encapsulation. A quality protection method is required to ensure that the butyrate is not released until it reaches the small intestine. This protection necessitates a reduction in the concentration of butyrate and adds a complex and costly production step. Tributyrin, a glyceride of butyric acid containing three butyrate molecules esterified to a glycerol backbone, has been shown to be an effective source of butyric acid for use in animal diets[12-15]. Tributyrin is a naturally stable molecule, able to pass through the upper gastrointestinal tract until it is cleaved by lipase in the small intestine, releasing butyric acid[16]. Since tributyrin is stable and non-volatile at room and pelleting temperatures and no coating is needed for protection, allowing for a higher butyric acid content. To date there are no published studies directly comparing tributyrin and coated salts of butyric acid for the improvement of performance in broilers, therefore, the purpose of this study was to compare tributyrin and fat-coated sodium butyrate for the improvement of broiler growth performance under field-simulated research conditions.

MATERIALS AND METHODS

Experimental animals and trial conditions: At the start of the trial, 720 DOH Ross 308 broiler males were randomly assigned to one of three groups of 240 birds each. Each group was further separated into 12 floor pens with 20 birds per pen. Throughout the trial all birds were provided feed and water *ad libitum*. The trial period was from day 0-35.

Temperature and ventilation were maintained by mechanical ventilation and gas-fired heaters. Carbon dioxide levels were tested weekly and carbon monoxide levels were monitored using a permanent alarm. An industry standard lighting program was used. A light intensity of 40 lux was used at placement and stepped down over ten days to a target intensity of 20 lux.

At placement birds were put in to floor pens with fresh wood shavings as the bedding material. On day 7 of the trial, the bedding in each pen was spiked with used litter collected from a commercial broiler operation to mimic commercial poultry conditions and provide a low-level challenge while not causing clinical symptoms of disease. The used litter was acquired approximately 4 weeks prior to the start of the trial and analyzed for the presence of *Salmonella*, *Campylobacter*, Rotavirus and *Eimeria* before use. Spiking of the trial bedding was accomplished by spreading 1.5 kg of litter into each pen by hand.

Experimental diets and treatments: The base diet was formulated as standard wheat-barley-soy diets with three phases during the trial. A starter diet (mash) was fed from day 0-9, a grower diet (pellets) from day 9-25 and a finisher diet (pellets) from day 25-35. The crude protein levels in the starter, grower and finisher diets were 21.8, 20.6 and 19.1%, respectively. Pelleting of the grower and finisher diets was done at a temperature below 85°C.

There were three treatments group: T1, T2 and T3 (Table 1). The three treatment diets will be offered for each of the three phases of the study. Formulation of each diet is presented in Table 1. For each phase, there will be a control diet (T1), control diet plus Tributyrin (T2) and control diet plus fat-coated sodium butyrate (T3). Treatment diets were formulated at an iso-butyric level. The tributyrin product had a butyric acid content of 53% and the sodium butyrate product had a butyric acid content of 24%. Tributyrin and sodium butyrate used in the treatment diets were both in powdered form. After mixing and prior to the start of the trial, all treated feeds were analyzed for butyric acid content to ensure proper dosing.

Table 1: Dosage programs for Tributyrin and fat-coated Sodium Butyrate treatment groups. Treatments were administered at iso-butyric levels throughout the trial

Treatment group	Diet details	Target inclusion rate (kg Mt^{-1}) Phase 1	Phase 2	Phase 3
T1	Standard broiler ration, no treatment	0.0	0.00	0.00
T2	Standard broiler ration, treatment with tributyrin	0.5	0.25	0.25
T3	Standard broiler ration, treatment with sodium butyrate, coated	1.0	0.50	0.50

Measured performance parameters and statistical analysis: Individual pens were considered the experimental unit for this trial. Pen weights were recorded at placement, directly prior to feed changes on days 9 and 25 and at the conclusion of the trial. Feed intake was recorded throughout the trial by weighing all feed added to the feeders for each pen. At feed changes any uneaten feed was weighed and recorded for each pen. The feed conversion ratio for each pen was calculated using the pen weights and total feed consumption for each pen for each feed phase in the trial. All mortalities during the trial were also recorded and mortalities weights were used to correct the FCR values.

Calculations for performance metrics were done using Microsoft Excel (Microsoft; Redmond, WA USA). Statistical analysis of the calculated data was performed using GraphPad Prism 7.04 (GraphPad Software; La Jolla, CA USA). One-way ANOVA was performed for all performance parameters using individual pens as the experimental unit. Dunnet's Multiple Comparisons test was used to further separate the treatment means from that of the control group and significance was considered as a p-value of 0.05 or less.

RESULTS AND DISCUSSION

Means±standard errors for body weight gain and feed conversion ratio are shown in Table 2. At chick placement no significant or numerical differences in starting body weight were observed between groups, with an average chick starting weight of 0.039 kg. At the first weigh point on Day 9 there was still no statistical difference in body weight gain between treatments though the tributyrin group was numerically heavier (0.229 kg) than the control group (0.222 kg) (p = 0.085), while the sodium butyrate group gained 0.225 kg (p = 0.63). One possibility for the numerical discrepancy is that, while both treatments require pancreatic lipase to release butyrate from either the tributyrin molecule or the fat coating, newly hatched chicks do not have high levels of lipase activity and fat digestibility is not mature until 1-2 weeks[17]. Pancreatic lipases tend to have a high preference for short-chain triglycerides, specifically the terminal fatty acids, so it is possible that the limited lipase in young birds is better able to release the butyrate from tributyrin compared to a fat-coated salt of butyrate, which consist of longer fat chains[18]. On Day 25, both treatment groups had gained statistically more body weight than the control group with the tributyrin group weighing 0.058 kg more and the sodium butyrate group weighing 0.043 kg more with p-values of 0.008 and 0.0496, respectively. At Day 35, both treatment groups maintained statistically higher body weight gain. The tributyrin group had 0.116 kg more body weight gain compared to the control group at the end of the trial (p = 0.008) while the sodium butyrate group had 0.094 kg more body weight gain compared to the control (p = 0.036). As might be assumed from the p-values, the coefficient of variation was lowest (2.6%) for the tributyrin group, followed by the sodium butyrate group (3.6%) and control group (4.4%). The lower C.V. value for the tributyrin group could be linked to the previously discussed preferential cleavage of tributyrin in young birds, allowing a more uniform start to life and thus higher uniformity at processing, which is highly desired in commercial poultry production[19-20].

As with body weight gain, feed conversion ratio in the starter phase was not significantly affected by treatment at Day 9. During the grower phase from Day 9-25 both treatments had improved FCR compared to the control. The FCR of the tributyrin group was improved by 5 points compared to the control (p = 0.006) and the FCR of the sodium butyrate group was improved by 6 points compared to the control group (p = 0.0007) and there was no significant difference between the two treatments (p = 0.69). In the final phase from Day 25-35 both treatment groups maintained their early improvements in FCR with the tributyrin group having an FCR 4 points improvement over the control group and the sodium butyrate group having 5 points improvement compared to the control group though neither of the treatment groups were significantly different from the control group during the final feeding phase. Both treatment groups were found to significantly improve FCR over the entire lifetime. Tributyrin and sodium butyrate treatments resulted in lifetime improvements of 4 and 5 points of FCR with p-values of 0.0022 and 0.0005, respectively. No significant difference was seen between treatments for lifetime FCR.

No significant differences were seen between groups for mortality or litter score during the trial and both remained low throughout the experiment. Total mortality during the trial was 5%, indicating a low level of pathogen challenge.

Table 2: Mean±standard error for body weight and adjusted feed conversion for each treatment

	D1 BW	D9 BW	D25 BW	D35 BW	D1-9 Adj FCR	D9-25 Adj FCR	D25-35 Adj FCR	Overall Adj FCR
Control	0.039±0	0.262±0.002	1.510±0.017	2.511±0.032	1.093±0.008	1.403±0.017	1.756±0.021	1.515±0.011
Tributyrin	0.039±0	0.268±0.003	1.568±0.014	2.627±0.020	1.079±0.007	1.352±0.009	1.713±0.019	1.472±0.007
Sodium butyrate	0.039±0	0.265±0.002	1.554±0.007	2.606±0.027	1.105±0.009	1.339±0.005	1.703±0.023	1.465±0.008

Means and standard error were calculated using pens as the experimental unit. Means designated by *indicate statistical significance from the control using Dunnet's Multiple Comparison test ($p \leq 0.05$)

In this study, tributyrin and a fat-coated sodium salt of butyrate were compared as sources of butyrate for the improvement of performance in broilers under simulated commercial conditions. Both groups were found to significantly improve body weight gain at days 25 and 35, with the tributyrin group having a numerically higher, though not significantly different, body weight gain throughout the trial as compared to the sodium butyrate group. It is possible this numerical advantage of tributyrin could be due to the relatively increased availability of tributyrin butyrate in the early life stages of the chick, allowing for higher butyrate activity and improved early development[17-18]. Additionally, endogenous avian lipase is only capable of cleaving the ester bonds of tributyrin at the sn-1 and sn-3 positions, leaving a monobutyrin to be absorbed in the jejunum and duodenum[21]. Monobutyrin has been shown to play an important role in stimulating angiogenesis and tissue development and could contribute to early gut development in chicks[22]. Previous studies have shown that broiler chick performance during the first week of life is a strong indicator of lifelong performance, with 1 gram of day 7 body weight corresponding to 6 grams of body weight on day 37[19]. Treatment effects on feed conversion were relatively even between the tributyrin and sodium butyrate groups throughout the trial and both treatments were found to significantly reduce overall feed conversion compared to the control diet. No treatment effect was observed for any group concerning mortality and litter score, though the birds were kept at low stocking densities with very little challenge. It is suggested that a larger trial under fully commercial conditions be conducted for further investigation of these parameters.

CONCLUSION

In conclusion, tributyrin was found to be an effective source of butyrate for the improvement of body weight gain and feed conversion in broilers under simulated commercial conditions. The delivery of butyrate by glyceride esters may have further advantages over the traditional fat-coated salts of butyrate, especially during early chick development when lipase activity is not yet fully matured. Further investigation is needed to determine if there are any differences between the modes of action between glyceride esters and fat-coated salts of butyrate, though this trial suggests that tributyrin is as effective, if not more, as fat-coated sodium butyrate when fed at iso-butyric levels at improving broiler body weight gain and reducing feed conversion ratio.

SIGNIFICANCE STATEMENTS

This study discovers the equivalence of two forms of butyric acid for improving broiler growth performance that can be beneficial for increasing growth in antibiotic-free poultry. This study will help researchers to investigate the mechanisms of action for butyric acid in broilers. Thus, a new theory of how butyric acid affects the broiler gut may be arrived at.

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