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Performance, Safety and Tissue Residue Study for Coated Sodium Butyrate Added to Broiler Feed

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Abstract: The aim of this study was to evaluate whether administration of feed containing 0.1, 0.5 or 1.0% fat-coated sodium butyrate (coated sodium butyrate) to one-day old broiler chicks for 49 consecutive days caused toxicity to the animals, or altered the fatty acid profile or butyric acid metabolite concentrations (ß-hydroxybutyrate, acetoacetate and acetone) of ingestible tissues compared to control animals. Treatment groups that consumed coated sodium butyrate exhibited low mortality and good general health. No statistical differences were found between groups for performance parameters, with the exception of the feed: gain ratio in the period D0-D21, which was improved by coated sodium butyrate at the 1.0% concentration. There was no effect of coated sodium butyrate on hematology, clinical chemistry, organ weight or organ histology of broiler chickens. The fatty acid profile of breast, liver, kidney or subcutaneous fat (with skin) tissues from chickens fed diets containing coated sodium butyrate did not differ from control chickens, with the exception of a statistically significant decrease in palmitoleic acid (C16:1) in liver tissue in the 0.5% and 1.0% groups. Small, dose-dependent, toxicologically insignificant increases in tissue levels of butyric acid and its metabolites occurred in edible tissues, which were expected based on the dose-dependent absorption of butyric acid into plasma. The results show that coated sodium butyrate may be safely used in poultry feed at up to 10,000 g/tonne feed (1.0%), from the day of hatching to 49 days of age.

Key words: Broiler, chicken, sodium butyrate, toxicity, residue

INTRODUCTION

Butyric acid is a short-chain aliphatic carboxylic acid that is found naturally in many plants, fruits, vegetables, beverages, dairy products and in the essential oils of a number of herbs and spices (Coleman *et al*., 1981; Opdyke, 1981; Burdock, 2010). Butyric acid is also produced in the gastrointestinal (GI) tract of mammals, resulting from the fermentation of dietary fiber or starch by resident bacteria (McNeil, 1984). In the GI tract, both butyric acid and sodium butyrate (the sodium salt of butyric acid) dissociate into n-butyrate and the corresponding cation (H^+ for butyric acid and Na^+ for sodium butyrate). Butyric acid is primarily absorbed by colonic epithelial cells (colonocytes), but may also be absorbed into the portal vein and transported to the liver, where it is metabolized to other substances that may be used for energy or eliminated (Guilloteau *et al*., 2010; den Besten *et al*., 2013).

In the United States, butyric acid is approved for use in animal feed as a synthetic flavoring substance and adjuvant (21 CFR §582.60). Because butyric acid as a free acid is volatile and corrosive, the formation of the corresponding salt stabilizes the molecule; therefore, the sodium salt of butyric acid (sodium butyrate) is the preferred form for addition to feed (Cortyl, 2014). Fat-coated sodium butyrate has a number of desirable properties for use in chicken feed. When uncoated butyrate is added to chicken feed, the majority is absorbed from the proximal digestive tract, prior to reaching the duodenum (Van den Borne *et al*., 2015), limiting its ability for systemic absorption and nourishment of colonocytes. Coating of butyrate with fat results in absorption of butyrate along the entire intestinal tract of the chicken (Van den Borne *et al*., 2015) and improves butyrate's ability to nourish colonocytes.

Fat-coated forms of sodium butyrate have been evaluated in several poultry feeding trials that measured effects on performance (Smulikowska *et al*., 2009; Czerwinski *et al*., 2012; Chamba *et al*., 2014; Levy *et al*., 2015); however, these trials did not evaluate parameters required to demonstrate safety for poultry and consumers of poultry meat. This study was undertaken to determine whether use of 0.1, 0.5 or 1.0% fat-coated sodium butyrate (coated sodium butyrate) in the feed of broiler chickens over their entire lifespan produces toxicity, or alters the fatty acid profile or butyric acid metabolite concentrations in tissues that may be consumed by humans.

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MATERIALS AND METHODS

Guidelines: The animals used in the study were reared and treated in compliance with Directive 2010/63/UE covering the protection of animals used for experimental or other scientific purposes, adopted by the Italian legislation in D.Lgs 4th March 2014, No. 26 and according to Recommendation 2007/526/CE. The study was in accordance with current EFSA FEEDAP technical guidance on the conduct of tolerance and efficacy studies in target animals and with VICH GL43 for Target Animal Safety (TAS) measurements.

Test substance: The test substance was sodium butyrate (30% by weight) coated with partially hydrogenated vegetable oil supplied by Nutriad Inc., Hampshire, IL (coated sodium butyrate).

Animals and diets: Five hundred forty one-day old male, ROSS 708 chicks were placed on the study. On the day of hatching (AVIZOO, Longiano, Italy), the chicks were vaccinated against Marek's disease, Newcastle disease and infectious bronchitis (H120) and treated with Hipracox to prevent coccidiosis. They were then shipped to the test site (CERZOO, Piacenza, Italy). Immediately after arrival, the chicks were examined by a designated veterinarian for animal welfare evaluation and weighed (35.8±0.29 g). According to standard procedures, all birds were vaccinated at CERZOO against Gumboro disease on Day 14 (with Nobilis Gumboro D78 from Intervet International BV, Wim de Koverstraat, 35, NL-5831 AN Boxmeer Holland) and Newcastle Disease vaccine on Day 16 (with IZOVAC Brescia produced by IZO S.r.l., Via San Zeno 99/A, 25124 Brescia Italy).

During the entire 49-day study period, the animals were fed using one feeder per pen. All diets were administered as meal feed. Test diets were prepared in the CERZOO feed mill using a vertical (0.15 tonne) mixer for the starter (Days 0-21) diet and a horizontal (0.60 tonne) mixer for the grower/finisher (Days 22-slaughter) diet. Diet were prepared by mixing the appropriate amount of test substance into a portion of the basal diet (approx. 5 kg feed), then mixing this premix into the entire batch of feed to produce the necessary target coated sodium butyrate concentration. An amount of each diet sufficient for each period was prepared immediately before use. Diets were formulated according to NRC (1994) recommendations. The composition and expected analytical characteristics of the basal feed are shown in Table 1 and 2, respectively. The diets were stored at room temperature under dry conditions in a room separated from the study house. Pens and feeds were coded with numbers that were not revealed to the study personnel during the study. Drinking water from an internal water system network was supplied *ad libitum*.

Study design: Healthy animals were randomly placed 15 animals/pen in order to stay within a maximum density of 0.13 m^2/animal according to the Recommendation 2007/526/CE of 18 June 2007 and Italian D.Lgs 26/2014 of March 4, 2014 for welfare of animals used for scientific purposes. Each pen served an experimental unit and there were nine pens per experimental group: T1 = control group fed basal feed; T2 = basal feed containing 1000 g/tonne (0.1%) coated sodium butyrate; T3 = basal feed containing 5000 g/tonne (0.5%) coated sodium butyrate and T4 = basal feed containing 10,000 g/tonne (1.0%) coated sodium butyrate. The corresponding concentrations of sodium butyrate were 0.03, 0.15 and 0.30%. The control and coated sodium butyrate treatments were assigned to each pen according to Fig. 1. The diets were fed for a minimum of 49 consecutive days and up to 4 h prior to slaughter (from D50 to D53).

Twice daily (morning and afternoon), animals were inspected for general health status and feed and water supply and the housing facilities were inspected for temperature and ventilation variations. The mean temperature and relative humidity throughout the study were 26.00±2.00°C and 78.49±0.88%, respectively. Dead or culled birds were removed and autopsied by the designated veterinarian to determine the possible cause of death/culling. The following details for chickens that died or were culled were noted: day of death, body weight, reason for culling and/or most probable cause of death and necropsy findings. During the study, the following endpoints were recorded: body weights (per pen) on Days 0, 21 and 49, feed intake per pen during study periods 0-21, 21-49 and 0-49 and average daily gain and feed/gain ratio for Days 0-21, 21-49 and 0-49.

On Day 49, during weighing, all birds were individually tagged with a numbered leg band. On Days 50-53, birds were fasted for four hours and one animal per pen was selected by random number generation (nine animals/group) for slaughter, blood sampling from the wing vein, necropsy, histopathological examination and tissue analysis of fatty acids, butyric acid and butyric acid metabolites (β-hydroxybutyrate, acetoacetate and acetone). Animals from each group were slaughtered on each day from Days 50-53 (mostly two/group, but sometimes one or three), for a total of nine animals/day. Blood samples for hematological analyses were placed in vacuum tubes (10 ml) containing sodium citrate [for activated partial thromboplastin time (APTT), prothromin time (PT) and fibrinogen only] or lithium heparin as an anticoagulant. Vacuum tubes (10 ml) without anticoagulant were used for biochemical analyses. Blood collected for biochemical analyses was centrifuged for 10 min. at 3500 g (4°C) to prepare serum. Blood and serum samples were shipped under refrigeration to La Fontana, Piacenza, Italy (an ISO 9001-2008 certified laboratory) for the following analyses:

Hematology (blood collected with anticoagulant): erythrocytes (total counts), leukocytes: (total and

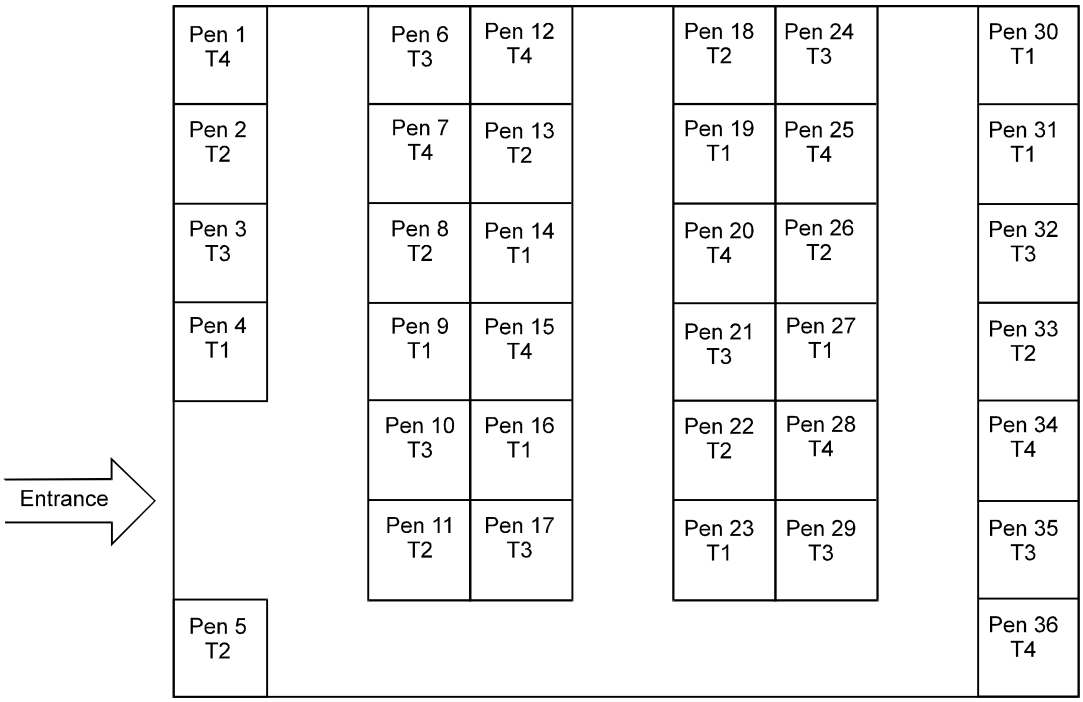

Fig. 1: Plan of the rooms and treatment allocation (T1, T2, T3 and T4 = control, 0.1, 0.5 and 1.0% coated sodium butyrate, respectively)

differential counts), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin, prothrombin time, platelet count, MPV (mean platelet volume), PCT (Plateletcrit, total platelet mass), PDW (Platelet Distribution Width), RDW (Red blood cell distribution width), Howell test, whole blood clotting time, APTT, PT and fibrinogen.

Biochemical analyses (serum): sodium, urea nitrogen, potassium, chloride, alanine aminotransferase (ALT), calcium, aspartate aminotransferase (AST), phosphate, lactate dehydrogenase (LDH), magnesium, gamma-glutamyl transferase (GGT), total protein, alkaline phosphatase (AP), albumin, creatine kinase (CK), globulin (including α1, α2, β and γ globulin), phosphate, total bile acids, glucose, cholesterol, alpha amylase, butyric acid, fibrinogen and acute phase protein (haptoglobin, creatinine).

The necropsy included a gross examination of the adrenal gland, bone marrow, brain, bursa of Fabricius, cecum, colon, crop, duodenum, epididymis, eyes, gall bladder, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, marrow smear, muscle, pancreas, parathyroid gland, pituitary gland, proventriculus, skin, spinal cord, spleen, stomach, testes, thymus, thyroid gland and ventriculus. The adrenal glands, brain, heart, kidneys, liver, pituitary gland, spleen, testes and thyroid were weighed. These organs, plus the aorta, breast muscle (no less than 30 g, from the outer to inner pectoral muscle layer, right side), femur (with articular surface), femur bone marrow, eyes (with retina and optic nerve), large intestine, larynx, air sacks, pancreas, sciatic nerve, thigh muscle, small intestine, spinal cord (cervical, mid-thoracic and lumbar segments including roots and dorsal root ganglia at the lumbar level), subcutaneous fat with skin in natural proportion (no less than 30 g from the sternum/dorsal region), tongue, trachea, ureter, cloaca, crop, bursa of Fabricius, ventriculus, proventriculus and all gross lesions were placed in 10% buffered formalin, Davidson's solution (eyes with retina and optic nerve only) or modified Davidson's solution (testes only), excepting the left kidney and left liver lobe. The fixed organs and tissues of the animals in the negative control and highest dose group were trimmed, embedded, sectioned, stained with haematoxylin and eosin (further stains if needed) and examined microscopically by a pathologist at AnaPath GmbH (Liestal, Switzerland). Histopathological findings were analyzed using the PathData System (version 6.2e, PDS Life Sciences, Basel, Switzerland).

The left liver lobe, left kidney and additional samples of breast muscle (no less than 30 g, from the outer to inner pectoral muscle layer, right side) and subcutaneous fat with skin in natural proportion (no less than 30 g from the sternum/dorsal region) were collected from the slaughtered animals and stored frozen (-80°C) until analysis for fatty acids, butyric acid and butyric acid

metabolites (β-hydroxybutyrate, acetoacetate and acetone) by La Fontana laboratory (Piacenza, Italy). Tissue samples were homogenized in water and extracted according to a two-step Bligh and Dyer method (Bligh and Dyer, 1959). Extracted fatty acids were analyzed using GC/MS. The identification of the peaks of each fatty acid was made by comparing the spectra with a fatty acid standard solution (FAME mix, C4:C22, NIST 2014 official, Agilent Technologies) analyzed under the same conditions. The butyric acid metabolites β-hydroxybutyrate and acetoacetate were analyzed using GC/MS according to the Yoon (Yoon, 2015) method. Acetone was analyzed with a gas chromatography with flame ionization detection method (GC-FID) with direct injection, using a capillary column according to the Pontes *et al.* (2009) method.

Homogeneity of the test item in feed was examined by collecting samples of each diet (D0-21 and D-22-49) from the top, middle and bottom of the feed mixer at the time of diet preparation and analyzing for sodium butyrate. The concentration of sodium butyrate and stability of the ingredient in feed were determined by collecting samples of each diet collected at diet preparation and weekly thereafter and analyzing for sodium butyrate. At each sampling time, the samples were immediately frozen and stored until analysis. Sodium butyrate was measured by CERZOO as butyric acid and extracted in 1% formic acid in n-hexane (using an Ultra-Turrax® (IKA, Staufen Germany) to homogenize the samples). The extract was centrifuged, diluted and then analyzed by gas chromatography coupled to mass spectrometry (GC/MS), with selected ion monitoring (SIM). Two ions (m/z+205 and 220) were used for both identification and quantification of sodium butyrate. Calibration was achieved using a certified reference standard. The validation procedure involved spiking a precise amount of untreated feed with a known amount of sodium butyrate standard (0, 200, 2000 or 4000 mg/kg feed), which bracketed the lowest (1000 g coated sodium butyrate/tonne feed, corresponding to 300 mg sodium butyrate/kg feed) and highest inclusions levels (10,000 g coated sodium butyrate/ton feed, corresponding to 3000 mg sodium butyrate/kg feed) of coated sodium butyrate. The spiked samples were extracted and analyzed according to the method used for study samples. The limit of detection of sodium butyrate in feed was 20 mg/kg feed.

Statistical analysis: Quantitative data were analyzed using the statistical software program SAS. The basic statistical model employed was analysis of Variance (ANOVA). The raw data were analyzed for outliers, which were found in performance parameters for each group. These data were not excluded from the statistical analysis because the animals in each pen were in good health and did not require removal from the trial and because the raw data of the animals was not an outlier in two subsequent measurements. Student's "t" and Tukey's tests were used to compare the means of each group. The criterion for significance was $p \leq 0.05$, with $0.05 < p \leq 0.10$ as a near-significant trend.

RESULTS

Feed analyses and environmental conditions: The analytical characteristics of the experimental diets are shown in Table 3. The analytical characteristics of the experimental diets were similar, within expected values for each treatment group and were not different from calculated values, with the exception of a slightly lower ash content for all diets used during the first experimental period. The sodium butyrate concentrations of the diets for both treatment periods were 89-93% of the theoretical concentrations of 300, 1500 and 3000 mg/kg for groups T2, T3 and T4, respectively. The results of recovery tests evidenced that the analytical method was suitable for the analysis of sodium butyrate in feed. Mean recovery of sodium butyrate in feed was 84%, with a Relative Standard Deviation (RSD) of approximately 19% for each concentration. Results of the homogeneity analyses showed 8-15% variability in sodium butyrate concentrations between top, middle and bottom positions of the mixer for first period diets and 2-11% variability for second period diets. There was no distinct pattern of increasing or decreasing concentrations of sodium butyrate with sampling site (top, middle or bottom of mixer) or concentration. In general, results of weekly test diet analyses showed similar degrees of variation as the samples analyzed for homogeneity. For each concentration, there was no distinct pattern of increasing or decreasing concentrations of sodium butyrate in feed over the course of the study. The variability in results of the sodium butyrate analyses in feed is similar to the RSD for the recovery analysis, suggesting that the variability in results of the concentration and homogeneity/stability in feed analyses are predominantly due to variations in recovery.

Health and performance: The health of the animals in the study was generally good. Feces were of normal consistency and mortality was low in all groups (Table 4). The post mortem examination did not show evidence of any known disease. Live weight, average daily gain and feed intake were similar for all groups. No statistical differences between groups were found for the feed: gain ratio except for the period D0-D21, which was improved in the T4 group *vs.* the untreated group (1.82 vs. 1.71, respectively; $p<0.05$). The feed:gain ratio of the T2 and T3 groups was numerically lower than control, but it did not reach statistical significance.

Results of the hematological and biochemical analyses are shown in Table 5 and 6, respectively. No differences

Table 1: Ingredients (% composition) used in preparation of the basal diets in the two experimental periods

Component	First experimental period (0-21 days)	Second experimental period (21 days-termination)
Corn meal (%)	52.00	59.25
Soybean meal (48% protein) (%)	40.00	33.00
Soybean oil (%)	2.25	2.40
Hydrogenated palm fat (%)	2.00	2.00
DL Methionine (95% purity) (%)	0.18	0.10
Calcium carbonate (%)	0.25	0.50
Dicalcium phosphate (%)	2.50	2.00
Salt (%)	0.30	0.30
Sodium bicarbonate (%)	0.27	0.20
Vitamins and minerals[1] (%)	0.25	0.25

[1]Vitamins and minerals premix (Zoodry B extra/1) was provided by Istituto delle Vitamine, Via G., di Vittorio, 20090 Segrate (MI), Italy. Each kg of vitamin and mineral premix contains (as on the label): vitamin A: 2.500.000 IU; vitamin D3: 600.000 IU; vitamin E: 15.000 IU; vitamin K: 1.200 mg; vitamin B1: 400 mg; vitamin B2: 1.600 mg; Pantothenic acid: 2.500 mg; vitamin B6: 1.200 mg; Biotin: 30 mg; Folic acid: 250 mg; vitamin C: 20.000 mg; vitamin PP: 8.000 mg; vitamin B12: 6 mg; Cu: 1.000 mg; Fe: 10.000 mg; Mn: 30.000 mg; Se: 40 mg; Zn: 15.000 mg; I: 200 mg; Co: 40 mg

Table 2: Calculated composition (%) of the basal diets in the two growing periods

Component	First experimental period (0-21 days)	Second experimental period (21-49 termination)
Dry matter (%)	89.79	89.93
Crude protein (%)	23.10	20.51
Crude fiber (%)	3.33	3.08
Crude fat (%)	7.18	7.48
Ash (%)	6.70	6.33
Total carbohydrates (%)	41.25	45.31
Lysine (%)	1.31	1.12
Methionine + cysteine (%)	0.93	0.78
Calcium (%)	1.00	0.91
Non-phytate phosphorus (%)	0.44	0.36
Sodium (%)	0.20	0.18
Metabolizable energy (kcal/kg)	3000	3166

Table 3: Analytical characteristics of the experimental diets (% as feed)

Parameter	T1 control	T2 coated sodium dutyrate (1000 g/tonne feed)	T3 coated sodium butyrate (5000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)
First experimental period (0-21 days)				
Sodium butyrate (mg/kg)[1]	ND	362±59	1406±144	3033±336
Dry matter (%)	89.56	89.45	89.56	89.65
Crude protein (%)	23.27	23.45	23.45	23.15
Crude fiber (%)	3.84	3.90	3.80	3.75
Crude fat (%)	6.67	6.25	6.32	6.45
Ash (%)	5.71	5.84	5.91	5.62
Starch (%)	36.41	36.70	36.84	36.12
Sugar (%)	3.42	3.45	3.52	3.61
Metabolizable energy (kcal/kg)	2862	2846	2857	2828
Second experimental period (21 days-termination)				
Sodium butyrate (mg/kg)[1]	ND	301±22	1484±183	3025±344
Dry matter (%)	89.90	89.85	89.76	89.70
Crude protein (%)	20.45	20.49	20.51	20.53
Crude fiber (%)	3.12	3.20	3.24	3.40
Crude fat (%)	7.34	7.39	7.42	7.50
Ash (%)	6.25	6.28	6.30	6.41
Starch (%)	43.45	43.48	43.50	43.48
Sugar (%)	3.70	3.68	3.70	3.67
Metabolizable energy (kcal/kg)	3093	3100	3104	3110

[1]Value is mean±standard deviation, adjusted for recovery. ND: Not detected

were found in any hematological variable examined. The concentration of butyric acid in plasma increased linearly with the level of inclusion of coated sodium butyrate in the diet, from 0.39 mg/dl in the control (T1) group to 0.76

mg/dl in the T2 group, 1.16 mg/dl in the T3 group and 1.53 mg/dl in the T4 group ($p<0.05$). Dose-dependent trends towards a decrease in urea ($p = 0.0955$) and increases in lactate dehydrogenase (LDH) ($p = 0.0598$) and aspartate aminotransferase (AST) ($p = 0.0740$) were also observed; however, none of the urea, LDH or AST values of treated animals were significantly different from control animals. There was no effect of sodium butyrate on organ/tissue histology (gross or microscopic) or organ weight (either absolute organ weight or when expressed as a percentage of body weight) (Table 7 and 8). All microscopic findings recorded in animals of the T1 and T4 groups were spontaneous background changes known to occur in ROSS 708 chickens maintained under laboratory conditions.

Fatty acid profile and butyric acid (and metabolites) content of edible tissues: No statistical differences were found between groups in the fatty acid profile of breast, liver, kidney or subcutaneous fat (with skin) tissue (Table 9-12), except for a significant decrease in palmitoleic acid (C16:1) in liver tissue in the T3 and T4 groups (Table 11). There was a trend ($p = 0.0738$) towards an increase in palmitoleic acid with increasing concentration of coated sodium butyrate in subcutaneous fat with skin tissue (Table 10) and a trend ($p = 0.0586$) towards a decrease in palmitoleic acid with increasing concentration of coated sodium butyrate in kidney tissue, which resulted in a trend ($p = 0.0945$) toward a decrease in monounsaturated fatty acids (MUFA) in the kidney (Table 12). The concentrations of butyric acid, β-hydroxybutyrate, acetoacetate and acetone in breast muscle increased with increasing levels of coated sodium butyrate ($p<0.05$) (Table 9). Levels of butyric acid and butyric acid metabolites in subcutaneous fat with skin, liver and kidney also increased in a dose-dependent manner ($p<0.05$).

DISCUSSION

Several studies have examined the effect of sodium butyrate (either coated or uncoated) on the performance of chickens (Smulikowska *et al*., 2009; Czerwinski *et al*., 2012; Chamba *et al*., 2014; Levy *et al*., 2015); however, none of the previous studies have demonstrated safe use of the ingredient in chickens. The results of this study show that the administration of coated sodium butyrate to male chicks over a growing phase of 49 days is safe at up to 10,000 g/tonne feed (1.0%), ten times higher than the recommended usage concentration of 1,000 g/tonne feed (0.1%). Mortality was low in all groups. Live weight, average daily gain, feed intake and fecal consistency were similar for all groups. The feed:gain ratio of the 1,000 g/tonne groups and 5,000 g/tonne groups was numerically lower than control and for the 10,0000 g/tonne group was statistically lower than control ($p = 0.05$, which is not considered an adverse effect. No statistical differences were found between groups for hematological and biochemical parameters, excepting a linear increase in plasma butyric acid concentration with the level of inclusion of coated sodium butyrate in the diets ($p = 0.0001$).

There was no effect of coated sodium butyrate on organ weight, organ histology or fatty acid profile of tissues and organs, with the exception of slight changes in palmitoleic acid, which tended to increase in a dose-dependent manner in subcutaneous fat with skin and decrease in liver and kidney. The results suggest a slight tendency for the concentration of palmitoleic acid to increase in fatty tissue after administration of coated sodium butyrate to chickens, rather than the liver or kidney. The reason for this finding is unclear. As palmitoleic acid is a relatively minor fatty acid in terms of concentration in poultry tissues, the overall balance of monounsaturated fatty acids, unsaturated fatty acids and ratio of saturated fatty acids to unsaturated fatty acids in any tissue measured was not significantly affected by inclusion of up to 10,000 g/tonne coated sodium butyrate in feed. In contrast to the results observed with coated sodium butyrate, chicks fed a diet containing 400 g/tonne microencapsulated sodium butyrate (30%) coated with specific vegetal fats for 42 days exhibited clear changes in the fatty acid composition of breast tissue, including decreased stearic (C18:0) and saturated fatty acids and increased arachidonic acid (C20:4) ($p\leq0.05$) (Zhang *et al*., 2011). The results suggest that the type of fat used to encapsulate sodium butyrate may have a significant impact on the fatty acid profile of chicken meat and that not all formulations of fat-coated sodium butyrate may be as ideal for use as the one used in the current study, which had virtually no effect on the fatty acid profile of breast, liver, kidney or subcutaneous fat (with skin) tissue.

In chickens, the majority of uncoated butyrate is absorbed from the proximal digestive tract, before the duodenum (Van den Borne *et al*., 2015). Coating with fat results in absorption of butyrate along the entire gastrointestinal (GI) tract of broilers (Van den Borne *et al*., 2015). Butyric acid is metabolized in the body to produce acetyl coenzyme A (CoA), β-hydroxybutyrate, acetoacetate and acetone (von Oettingen, 1960; Opdyke, 1981; Katz and Guest, 1994; Guilloteau *et al*., 2010). Because it was likely that butyric acid would be absorbed into the blood of chickens that were provided coated sodium butyrate in the feed, the design of the study included measurement of butyric acid in plasma and butyric acid (and metabolites) in edible tissues to determine whether the use of coated sodium butyrate in chicken feed could possibly increase concentrations of these substances to levels that could not be safely consumed by humans. The results of the study in fact show that concentrations of butyric acid and butyric acid metabolites increased significantly in breast tissue,

Table 4: Performance of chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Mortality (n)					
D0-D21	0	0	0	0	ND
D21-D49	3	1	1	2	ND
D0-D49	3	1	1	2	ND
D0-D49 (%)	2.22	0.74	0.74	1.48	ND
Live weight (g)					
D0	35.9±0.28	35.8±0.30	35.8±0.34	35.7±0.25	0.7850
D21	669.2±26.5	681.5±40.7	689.0±30.2	692.9±20.3	0.3766
D49	2237.7±69.3	2181.1±111.6	2233.9±75.8	2246.6±74.1	0.3594
Average daily gain (g)					
D0-D21	30.2±1.26	30.7±1.94	31.1±1.44	31.3±0.97	0.3749
D21-D49	56.1±1.64	53.6±3.09	55.3±2.20	55.5±2.25	0.1557
D0-D49	44.9±1.36	43.8±2.28	44.9±1.53	45.1±1.50	0.3414
Daily feed intake (g)					
D0-D21	54.8±1.56	53.6±1.53	54.2±1.20	53.5±0.84	0.1442
D21-D49	148.6±3.46	146.4±4.64	148.1±2.96	149.0±3.52	0.4899
D0-D49	108.2±2.05	106.6±3.11	107.8±1.98	108.0±1.99	0.4492
Feed: gain ratio					
D0-D21	1.82±0.11^{b}	1.75±0.07^{ab}	1.75±0.07^{ab}	1.71±0.05^{a}	0.0394
D21-D49	2.65±0.06	2.74±0.10	2.68±0.06	2.69±0.07	0.1394
D0-D49	2.41±0.05	2.44±0.08	2.40±0.04	2.40±0.05	0.4778

Data are presented as mean±standard deviation for all parameters except mortality. Different letters in the same row denote significant differences at $p \leq 0.05$, D: days, ND: Not determined

Table 5: Results of hematological analyses of chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
WBC (10^3/ml)	20.60±3.01	20.62±1.79	19.90±3.43	20.44±1.21	0.9223
RBC (10^6/ml)	2.48±0.09	2.47±0.22	2.55±0.18	2.54±0.17	0.6783
PCV (%)	29.58±1.49	29.58±2.90	30.18±2.09	30.64±1.63	0.6531
MCV (fl)	119.44±2.70	119.78±3.67	118.67±3.12	120.78±3.31	0.5795
MCH (pg)	51.42±3.77	50.89±3.25	50.33±1.42	50.53±2.99	0.8751
MCHC (g/dl)	43.14±3.13	42.61±3.41	42.52±1.75	41.91±2.35	0.8209
Hemoglobin (g/l)	12.78±1.16	12.54±0.64	12.83±0.75	12.84±0.75	0.8646
PT (sec.)	15.22±1.38	15.03±1.08	15.12±0.97	15.51±1.64	0.8736
Platelets (10^3/ml)	59.70±6.37	63.22±2.86	64.37±2.23	61.52±5.49	0.1706
APTT (sec.)	37.22±4.44	37.11±5.37	40.00±5.87	38.22±3.53	0.4782
WBCT (sec.)	337.00±67.8	339.44±91.4	303.11±46.1	324.22±83.3	0.7180
RDW (%)	18.96±1.24	18.22±2.32	18.26±1.75	18.57±3.53	0.9049
Neutrophils (%)	25.37±3.73	28.23±4.66	26.06±6.46	31.46±9.71	0.2149
Lymphocytes (%)	69.39±4.05	67.48±5.46	67.53±7.08	63.47±10.05	0.3488
Monocytes (%)	2.23±0.29	2.33±0.40	2.12±0.31	2.22±0.32	0.6150
Eosinophils (%)	0.93±0.55	0.77±0.24	0.90±0.40	1.13±0.81	0.5569
Basophils (%)	2.08±0.41	1.57±0.34	1.80±0.64	1.72±0.37	0.1336

Table 5: Continue

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Fibrinogen (mg/dl)	325.11±39.1	360.56±38.6	367.56±44.4	346.00±54.6	0.2115
MPV (fl)	9.07±0.55	9.17±0.56	8.79±0.36	8.99±0.49	0.4305
PCT (%)	0.11±0.19	0.12±0.16	0.11±0.16	0.11±0.18	0.9996
PDW (fl)	18.67±2.94	19.02±2.51	18.70±2.48	18.67±1.75	0.9879
Howell test (sec.)	115.22±12.6	125.33±8.5	119.22±14.0	115.56±14.6	0.3112

Data are presented as mean±standard deviation. APTT: activated partial thromboplastin time, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, MPV: M=mean platelet volume, PCT: plateletcrit, PCV: packed cell volume, PDW: platelet distribution width. PT: prothrombin time, RBC: red blood cells, RDW: red blood cell distribution width, WBC: white blood cells, WBCT: whole blood clotting time

Table 6: Results of biochemical analyses of chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Glucose (mg/dl)	231.22±27.07	224.78±14.58	225.89±21.83	218.78±22.16	0.6915
Urea (mg/dl)	7.41±2.56	5.30±2.35	4.76±2.01	5.60±2.14	0.0955
Butyric acid (mg/dl)	0.39±0.11^{a}	0.76±0.19^{b}	1.16±0.12^{c}	1.53±0.15^{d}	0.0001
Creatinine (mg/dl)	0.74±0.05	0.73±0.05	0.79±0.06	0.76±0.05	0.1712
Haptoglobin (mg/dl)	6.41±0.35	6.34±0.36	6.25±0.47	6.10±0.55	0.4716
Cholesterol (mg/dl)	149.33±29.40	136.67±8.67	136.00±13.48	136.00±10.76	0.3069
α Amylase (U/l)	756.22±109.4	693.22±136.7	695.44±63.7	668.11±89.4	0.3325
Total protein (g/dl)	20.89±3.44	20.11±2.42	21.44±2.83	21.55±2.01	0.6667
Albumin (g/dl)	9.21±1.67	8.31±1.66	8.55±1.54	9.00±1.68	0.6362
Globulin (g/dl)	11.44±1.88	11.37±1.52	12.43±1.60	12.42±1.64	0.3483
α 1 globulin (g/dl)	0.63±0.24	0.73±0.91	0.76±0.61	1.07±0.80	0.5574
α 2 globulin (g/dl)	2.43±0.50	2.21±0.61	2.30±0.54	2.51±0.45	0.6543
β globulin (g/dl)	3.56±1.38	3.58±0.80	3.78±1.21	3.73±1.31	0.9737
γ globulin (g/dl)	4.82±1.40	4.85±1.43	5.59±1.65	5.11±0.60	0.5874
Albumin/globulin	0.81±0.14	0.74±0.17	0.73±0.11	0.74±0.19	0.6555
Creatine kinase (U/l)	1780.4±575.0	2275.6±383.3	2216.7±712.5	2225.4±656.5	0.2742
AP (U/l)	1228.2±374.2	1097.2±406.1	853.6±446.1	877.6±403.3	0.1767
LDH (U/l)	1275.4±143.7	1275.8±299.4	1500.6±208.9	1502.7±264.9	0.0598
ALT (U/l)	12.89±3.89	10.78±4.29	10.22±2.22	9.00±1.87	0.1003
AST (U/l)	261.67±72.0	328.56±41.3	326.55±55.8	328.33±74.3	0.0740
GGT (U/l)	16.22±6.06	17.89±1.83	17.22±3.07	17.00±3.57	0.8443
Potassium (mEq/l)	6.53±1.09	6.03±1.08	6.28±0.80	6.42±1.07	0.7505
Chloride (mEq/l)	115.54±4.66	115.51±2.32	115.19±3.86	116.00±5.37	0.9820
Sodium (mEq/l)	148.33±8.61	144.67±6.56	148.00±8.06	149.78±6.70	0.5361
Calcium (mg/dl)	13.57±1.67	13.22±1.29	13.20±1.32	12.64±1.12	0.5560
Magnesium (mg/dl)	1.17±0.17	1.23±0.25	1.23±0.20	1.25±0.17	0.8306
Phosphate (mg/dl)	9.62±2.94	7.99±0.64	7.94±1.00	7.73±2.26	0.1634
Total bile acids (mmol/L)	17.29±3.39	17.59±5.10	17.83±4.10	17.76±4.66	0.9938

Data are presented as mean±standard deviation. Different letters in the same row denote significant differences at $p \leq 0.05$. ALT: alanine aminotransferase, AP: Alkaline phosphatase, AST: aspartate aminotransferase, GGT: gamma glutamyl transferase, LDH: Lactate dehydrogenase

Table 7: Necropsy results from chickens fed diets containing coated sodium butyrate

Organ or tissue	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Skin	0.67±0.50	0.56±0.53	0.67±0.50	0.56±0.53	0.9351
Brain	0.11±0.33	0.22±0.44	0.11±0.33	0.11±0.33	0.8884
Eyes	0.33±0.50	0.44±0.73	0.33±0.50	0.44±0.53	0.9516
Pituitary gland	0.56±0.53	0.44±0.53	0.67±0.50	0.56±0.53	0.8440
Thyroid gland	0.44±0.53	0.33±0.50	0.56±0.53	0.22±0.44	0.5356
Parathyroid gland	0.22±0.44	0.22±0.44	0.22±0.44	0.11±0.33	0.9224
Crop	0.22±0.44	0.22±0.44	0.11±0.33	0.33±0.50	0.7574
Heart	0.11±0.33	0.11±0.33	0.22±0.44	0.22±0.44	0.8661
Ventriculus	0.11±0.33	0.33±0.50	0.11±0.33	0.11±0.33	0.5238
Lung	0.67±0.50	0.78±0.44	0.44±0.53	0.67±0.50	0.5452
Spinal cord	0.11±0.33	0.00±0.00	0.11±0.33	0.00±0.00	0.5787
Stomach	0.67±0.50	0.56±0.53	0.56±0.53	0.78±0.44	0.7487
Gall bladder	0.78±0.44	0.44±0.53	0.67±0.50	0.78±0.44	0.4191
Lymph nodes	0.44±0.53	0.44±0.53	0.56±0.53	0.56±0.53	0.9395
Spleen	0.44±0.53	0.33±0.71	0.44±0.53	0.33±0.50	0.9516
Liver	0.22±0.44	0.11±0.33	0.22±0.44	0.33±0.50	0.7574
Pancreas	0.11±0.33	0.22±0.67	0.11±0.33	0.22±0.44	0.9145
Kidneys	0.33±0.50	0.22±0.44	0.33±0.50	0.33±0.50	0.9491
Adrenal gland	0.56±0.53	0.56±0.53	0.67±0.50	0.56±0.53	0.9580
Proventriculus	0.11±0.33	0.11±0.33	0.22±0.44	0.22±0.44	0.8661
Bone and marrow	0.11±0.33	0.56±0.73	0.33±0.50	0.33±0.50	0.3879
Marrow smear	0.56±0.53	0.67±0.50	0.67±0.50	0.33±0.50	0.4694
Duodenum	0.22±0.44	0.00±0.00	0.00±0.00	0.11±0.33	0.2808
Muscle	0.56±0.53	0.33±0.50	0.22±0.44	0.33±0.50	0.5452
Jejunum	0.33±0.50	0.44±0.53	0.44±0.53	0.22±0.44	0.7487
Ileum	0.89±0.33	0.78±0.44	0.56±0.53	0.44±0.53	0.1825
Colon	0.33±0.70	0.00±0.00	0.22±0.44	0.11±0.33	0.4425
Caecum	0.56±0.53	0.56±0.53	0.22±0.44	0.44±0.53	0.4694
Thymus	0.33±0.50	0.67±0.50	0.56±0.52	0.22±0.44	0.2272
Bursa of fabricius	0.22±0.44	0.67±0.50	0.56±0.53	0.44±0.53	0.2926
Testes	0.11±0.33	0.11±0.33	0.00±0.00	0.00±0.00	0.5787
Epididymis	0.11±0.33	0.11±0.33	0.00±0.00	0.00±0.00	0.5787

Data are presented as mean±standard deviation. Score: 0 = no alteration found, 1= slight alterations, 2 = alteration of medium intensity, 3 = serious alteration

Table 8: Organ weights of chickens fed diets containing coated sodium butyrate

Organ	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Brain					
(g)	3.12±0.27	3.23±0.23	3.12±0.18	3.04±0.50	0.6526
(% lw)	0.13±0.01	0.14±0.01	0.14±0.01	0.13±0.02	0.3105
Heart					
(g)	17.51±3.51	16.94±2.73	17.64±2.25	17.20±2.05	0.9457
(% lw)	0.75±0.13	0.75±0.12	0.77±0.08	0.75±0.06	0.9869
Liver[1]					
(g)	61.59±14.26	55.28±8.45	62.96±12.95	57.10±4.81	0.3972
(% lw)	2.65±0.57	2.45±0.30	2.73±0.48	2.50±0.18	0.4498
Spleen					
(g)	3.66±0.85	3.27±0.81	3.68±0.97	3.20±0.78	0.5125
(% lw)	0.16±0.03	0.15±0.04	0.16±0.04	0.14±0.03	0.5861
Left kidney					
(g)	10.02±1.53	9.87±1.43	9.90±1.85	9.89±1.36	0.9968
(% lw)	0.43±0.06	0.44±0.05	0.43±0.07	0.43±0.05	0.9900
Right kidney					
(g)	10.01±1.63	9.44±1.92	10.67±2.15	10.13±1.36	0.5462
(% lw)	0.43±0.06	0.42±0.08	0.46±0.08	0.44±0.05	0.5567
Left adrenal glands					
(g)	0.11±0.012	0.11±0.012	0.12±0.012	0.10±0.012	0.5769
(% lw)	0.005±0.002	0.005±0.002	0.005±0.001	0.004±0.001	0.5091
Right adrenal glands					
(g)	0.11±0.04	0.12±0.05	0.12±0.03	0.12±0.04	0.9516
(% lw)	0.005±0.002	0.005±0.002	0.005±0.001	0.005±0.002	0.9380
Testes					
(g)	2.20±0.23	2.24±0.15	2.26±0.17	2.38±0.15	0.1927
(% lw)	0.10±0.01	0.10±0.01	0.10±0.007	0.10±0.008	0.2660
Thyroid[2]					
(g)	2.00±0.70	1.97±1.19	1.71±0.67	1.87±0.57	0.8838
(% lw)	0.09±0.03	0.09±0.04	0.07±0.03	0.08±0.03	0.8696
Pituitary gland					
(g)	0.06±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.8214
(% lw)	0.002±0.000	0.002±0.000	0.002±0.000	0.002±0.000	0.8540

Data are presented as mean±standard deviation, lw: live weight of chicken. [1]including gallbladder, [2]including parathyroid

Table 9: Fatty acid analysis of breast tissue from chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Butyric acid and butyric acid metabolites (mg/g tissue)					
Butyric acid	0.009±0.007[a]	0.015±0.004[ab]	0.018±0.006[b]	0.030±0.003[c]	0.0001
β-hydroxybutyrate	0.008±0.005[a]	0.012±0.004[ab]	0.014±0.005[b]	0.023±0.004[c]	0.0001
Acetoacetate	0.002±0.001[a]	0.003±0.001[a]	0.004±0.002[ab]	0.005±0.001[b]	0.0005
Acetone	0.0002±0.0001[a]	0.0003±0.0001[a]	0.0004±0.0002[ab]	0.0005±0.0001[b]	0.0005
Fatty acid profile (% of total fatty acids)					
Myristic acid (C14:0)	0.46±0.05	0.46±0.06	0.43±0.07	0.41±0.05	0.2078
Palmitic acid (C16:0)	21.82±0.80	22.31±0.63	33.29±0.95	22.49±0.74	0.3379
Palmitoleic acid (C16:1)	2.74±0.80	2.71±0.57	2.67±0.89	2.26±0.79	0.5101
Stearic acid (C18:0)	9.35±0.66	9.06±0.44	9.17±0.57	9.06±0.34	0.6015
Oleic acid (C18:1n9)	31.06±0.69	30.67±0.80	30.47±0.71	30.80±0.89	0.4411
Linoleic acid (C18:2n6)	25.20±0.75	25.02±0.67	25.25±0.64	25.22±0.71	0.8965
Linolenic acid (C18:3n3)	2.51±0.09	2.52±0.16	2.56±0.16	2.58±0.14	0.6992
Arachidic acid (C20:0)	0.34±0.03	0.35±0.02	0.35±0.03	0.34±0.03	0.9695
Eicosadienoic acid (C20:2n6)	0.44±0.03	0.45±0.03	0.46±0.02	0.45±0.03	0.4894
Eicosatrienoic acid (C20:3n3)	0.34±0.03	0.35±0.02	0.36±0.02	0.36±0.02	0.3078
Arachidonic acid (C20:4n6)	2.49±0.15	2.41±0.13	2.46±0.10	2.41±0.13	0.5120
Docosapentaenoic acid (C22:5n3)	0.54±0.05	0.54±0.03	0.55±0.07	0.55±0.07	0.9100
Docosahexaenoic acid (C22:6n3)	0.75±0.11	0.74±0.17	0.75±0.10	0.77±0.18	0.9720
Others	1.95±1.57	2.42±1.37	2.24±1.46	2.30±0.81	0.8967
SFA	31.97±1.27	32.17±0.94	32.23±1.17	32.29±0.80	0.9235
MUFA	33.81±1.29	33.38±0.93	33.14±0.77	33.05±1.10	0.4355
PUFA	32.28±0.80	32.03±0.72	32.39±0.58	32.35±0.76	0.7234
USFA	66.08±1.65	65.41±1.37	65.53±0.83	65.40±0.83	0.6047
SFA/USFA	0.48±0.03	0.49±0.02	0.49±0.02	0.49±0.02	0.7750
n3 fatty acid	4.15±0.16	4.15±0.26	4.22±0.28	4.26±0.25	0.6955
n6 fatty acid	28.13±0.85	27.88±0.63	28.17±0.61	28.09±0.78	0.8418
n6/n3	6.80±0.40	6.75±0.46	6.71±0.52	6.61±0.47	0.8597

Data are presented as mean±standard deviation. Different letters in the same row denote significant differences at $p \leq 0.05$. MUFA = total monounsaturated fatty acids; Others: not identified fatty acid, which includes fatty acids measured in other tissues that are not in this Table, PUFA = total polyunsaturated fatty acids, SFA: total saturated fatty acids, USFA = total unsaturated fatty acids

Table 10: Fatty acid analysis of subcutaneous fat with skin from chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Butyric acid and butyric acid metabolites (mg/g tissue)					
Butyric acid	0.011±0.008[a]	0.017±0.004[ab]	0.023±0.008[b]	0.033±0.004[c]	0.0001
β-hydroxybutyrate	0.009±0.006[a]	0.013±0.003[ab]	0.018±0.006[b]	0.025±0.003[c]	0.0001
Acetoacetate	0.002±0.002[a]	0.004±0.001[ab]	0.005±0.002[b]	0.006±0.001[c]	0.0001
Acetone	0.0002±0.0002[a]	0.0004±0.0001[ab]	0.0005±0.0001[bc]	0.0006±0.0001[c]	0.0001
Fatty acid profile (% of total fatty acids)					
Myristic acid (C14:0)	0.99±0.30	0.87±0.39	0.84±0.39	0.79±0.38	0.6843
Palmitic acid (C16:0)	23.79±1.01	23.34±1.14	23.03±1.08	22.98±1.18	0.4032
Palmitoleic acid (C16:1)	3.00±0.85	3.31±1.00	3.87±0.94	4.03±0.82	0.0738

Table 10: Continue

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Stearic acid (C18:0)	6.94±0.44	6.80±0.57	6.88±0.72	7.08±0.86	0.8359
Oleic acid (C18:1n9)	34.12±0.82	34.10±1.01	33.33±1.13	33.67±1.45	0.3972
Linoleic acid (C18:2n6)	27.55±1.31	28.24±1.54	28.42±1.43	27.86±1.17	0.5437
α Linolenic acid (C18:3n3)	1.54±0.11	1.51±0.15	1.53±0.12	1.52±0.12	0.9333
γ Linolenic acid (C18:3n6)	0.43±0.08	0.42±0.10	0.42±0.05	0.43±0.12	0.9697
Eicosaenoic acid (C20:1n9)	0.60±0.08	0.61±0.08	0.61±0.07	0.61±0.10	0.9804
Others	1.04±0.49	0.80±0.55	1.09±0.57	1.01±0.58	0.6858
SFA	31.72±1.08	31.01±1.21	30.75±1.02	30.86±1.38	0.3137
MUFA	37.72±0.87	38.03±1.69	37.80±1.26	38.31±1.71	0.8103
PUFA	29.52±1.35	30.16±1.43	30.36±1.44	29.82±1.21	0.5734
USFA	67.23±0.84	68.18±1.37	68.16±1.30	68.12±1.37	0.3044
SFA/USFA	0.47±0.02	0.46±0.03	0.45±0.02	0.45±0.03	0.3051
n3 fatty acid	1.53±0.11	1.50±0.15	1.53±0.12	1.52±0.12	0.9333
n6 fatty acid	27.98±1.34	28.66±1.53	28.84±1.45	28.30±1.23	0.5714
n6/n3	18.27±1.56	19.34±2.96	18.98±1.81	18.70±1.78	0.7433

Data are presented as mean±standard deviation. Different letters in the same row denote significant differences at $p \leq 0.05$. MUFA = total monounsaturated fatty acids; Others: not identified fatty acid, which includes fatty acids measured in other tissues that are not in this Table, PUFA = total polyunsaturated fatty acids, SFA: total saturated fatty acids, USFA = total unsaturated fatty acids

Table 11: Fatty acid analysis of liver from chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne feed)	T3 coated sodium butyrate (5,000 g/tonne feed)	T4 coated sodium butyrate (10,000 g/tonne feed)	Treatment effect p =
Butyric acid and butyric acid metabolites (mg/g tissue)					
Butyric acid	0.024±0.014^{a}	0.036±0.007^{ab}	0.045±0.014^{b}	0.063±0.006^{c}	0.0001
β-hydroxybutyrate	0.020±0.010^{a}	0.028±0.005^{ab}	0.034±0.012^{b}	0.048±0.005^{c}	0.0001
Acetoacetate	0.004±0.003^{a}	0.008±0.002^{b}	0.009±0.003^{b}	0.012±0.001^{c}	0.0001
Acetone	0.0004±0.0003^{a}	0.0008±0.0002^{b}	0.0009±0.0003^{b}	0.0012±0.0001^{c}	0.0001
Fatty acid profile (% of total fatty acids)					
Myristic acid (C14:0)	1.43±0.60	1.41±0.52	1.43±0.66	1.44±0.53	0.9994
Palmitic acid (C16:0)	26.47±0.94	26.02±0.48	26.66±0.89	26.18±0.72	0.3181
Palmitoleic acid (C16:1)	4.23±1.20^{b}	4.24±1.09^{b}	2.94±0.60^{a}	3.00±0.45^{a}	0.0022
Stearic acid (C18:0)	17.43±0.93	17.48±0.75	17.79±1.06	17.74±0.62	0.7573
Oleic acid (C18:1n9)	32.51±0.95	31.85±1.15	32.79±1.04	32.12±1.16	0.2765
Linoleic acid (C18:2n6)	12.15±1.21	13.05±0.94	12.32±1.42	13.12±1.21	0.2290
Linolenic acid (C18:3n3)	0.17±0.06	0.16±0.05	0.16±0.04	0.17±0.05	0.9587
Eicosadienoic acid (C20:2n6)	0.68±0.20	0.66±0.18	0.68±0.13	0.69±0.18	0.9749
Arachidonic acid (C20:4n6)	2.71±0.76	2.78±0.44	2.72±0.43	2.79±0.74	0.9882
Docosapentaenoic acid (C22:5n3)	0.78±0.17	0.78±0.06	078±0.06	0.75±0.10	0.9675
Docosahexaenoic acid (C22:6n3)	0.43±0.03	0.41±0.03	0.41±0.03	0.43±0.03	0.9561
Others	1.02±0.37	1.16±0.42	1.31±0.40	1.55±0.67	0.1287
SFA	45.33±1.01	44.91±0.60	45.88±0.91	45.37±1.18	0.2128
MUFA	36.74±1.91	36.09±1.51	35.74±1.17	35.12±0.96	0.1317

Table 11: Continue

PUFA	16.91±1.15	17.84±1.20	17.07±1.41	17.97±0.62	0.1355
USFA	53.65±0.97	53.93±0.87	52.81±0.80	53.08±1.10	0.0657
SFA/USFA	0.84±0.03	0.83±0.02	0.87±0.03	0.85±0.04	0.1260
n3 fatty acid	1.38±0.27	1.36±0.16	1.35±0.21	1.36±0.21	0.9940
n6 fatty acid	3.38±0.84	3.43±0.55	3.41±0.54	3.49±0.89	0.9917
n6/n3	2.40±0.40	2.53±0.27	2.57±0.45	2.59±0.64	0.8117

Data are presented as mean±standard deviation. Different letters in the same row denote significant differences at $p \leq 0.05$. MUFA = total monounsaturated fatty acids, Others: not identified fatty acid, which includes fatty acids measured in other tissues that are not in this Table, PUFA = total polyunsaturated fatty acids, SFA: total saturated fatty acids, USFA = total unsaturated fatty acids

Table 12: Fatty acid analysis of kidney from chickens fed diets containing coated sodium butyrate

Parameter	T1 control	T2 coated sodium butyrate (1,000 g/tonne)	T3 coated sodium butyrate (5,000 g/tonne)	T4 coated sodium butyrate (10,000 g/tonne)	Treatment effect p =
Butyric acid and butyric acid metabolites (mg/g tissue)					
Butyric acid	0.011±0.011^{a}	0.020±0.007^{ab}	0.026±0.010^{b}	0.041±0.006^{c}	0.0001
ß-hydroxybutyrate	0.009±0.008^{a}	0.016±0.005^{ab}	0.019±0.008^{b}	0.032±0.005^{c}	0.0001
Acetoacetate	0.003±0.002^{a}	0.004±0.002^{ab}	0.005±0.002^{b}	0.008±0.001^{c}	0.0001
Acetone	0.0003±0.0003^{ab}	0.0005±0.0002^{b}	0.0005±0.0002^{b}	0.0008±0.0001^{c}	0.0001
Fatty acid profile (% of total fatty acids)					
Myristic acid (C14:0)	3.13±0.92	3.13±0.59	3.15±0.67	3.45±0.66	0.7406
Palmitic acid (C16:0)	24.99±0.72	24.90±0.68	25.13±0.62	24.76±0.69	0.7073
Palmitoleic acid (C16:1)	2.72±0.24	2.68±0.44	2.32±0.45	2.37±0.30	0.0586
Stearic acid (C18:0)	21.66±0.86	21.65±0.83	21.19±0.85	21.74±0.79	0.5032
Oleic acid (C18:1n9)	31.54±0.52	30.97±0.61	31.39±0.68	31.07±0.65	0.1934
Linoleic acid (C18:2n6)	14.79±0.72	15.14±0.58	15.37±0.69	14.99±0.64	0.3183
Others	1.20±1.24	1.54±1.18	1.45±0.92	1.62±0.88	0.8492
SFA	49.74±1.28	49.68±1.07	49.48±1.44	49.94±0.77	0.8667
MUFA	34.27±0.40	33.65±0.82	33.71±0.54	33.44±0.89	0.0945
PUFA	14.79±0.72	15.14±0.58	15.37±0.69	14.99±0.64	0.3183
USFA	49.06±0.71	48.79±0.39	49.07±0.86	48.44±0.58	0.1560
SFA/USFA	1.01±0.03	1.02±0.02	1.01±0.04	1.03±0.02	0.5080

Data are presented as mean±standard deviation. Different letters in the same row denote significant differences at $p \leq 0.05$. MUFA = total monounsaturated fatty acids, Others: not identified fatty acid, which includes fatty acids measured in other tissues that are not in this Table, PUFA = total polyunsaturated fatty acids, SFA: total saturated fatty acids, USFA = total unsaturated fatty acids

subcutaneous fat with skin, liver and kidney with increasing levels of inclusion of coated sodium butyrate. However, the increases were small (on the order of µg/g tissue variations) and not toxicologically relevant for humans, using levels of meat consumption provided by the CVM for the purpose of risk assessment (300 g/day muscle, 100 g/day liver, 50 g/day kidney or 50 g/day fat from animals) (Center for Veterinary Medicine of U.S. Food and Drug Administration, 2006). The levels of butyric acid in the tissues were similar to or lower than the levels in plasma, indicating a lack of bioaccumulation of butyric acid in tissues of chickens.

Conclusion: The results of the study show that coated sodium butyrate may be safely used in poultry feed at up to 10,000 g/tonne feed (1.0%), from the day of hatching to 49 days of age. Use of coated sodium butyrate had no adverse effects on poultry and did not affect the fatty acid profile of consumable tissues to any significant extent. Small, dose-dependent, toxicologically insignificant increases in tissue levels of butyric acid and its metabolites occurred in edible tissues, which were expected based on dose-dependent absorption of butyric acid into plasma.

Conflict of interest: All authors have a financial relationship with the sponsor of the studies and manuscript.

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