Dietary tributyrin intervention improves the carcass traits, organ indices, and blood biomarker profiles in broilers under the isocaloric diets administration

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ABSTRACT The objective of the current study was to investigate the effect of dietary tributyrin (**TB**) intervention on carcass traits, visceral and immune organ indices, and blood biomarker profiles in Arbor Acres (**AA**) broilers under the isocaloric diets administration. A total of 432-day-old healthy AA broiler chickens were assigned to 4 treatments, with 12 replicates per treatment and 9 birds per cage, for 42 d. The dietary treatments were a basal diet (control) and the basal diet supplemented with a TB product (Eucalorie) at doses of 0.50 g/kg (TB1), 1.0 g/kg (TB2), and 2.0 g/kg (TB3). The results showed that dietary TB treatment quadratically improved the average daily gain and average daily feed intake in the second (22−42 d) and overall (0−42 d) feeding periods ($P < 0.05$) while decreasing the feed conversion ratio in the second feeding period ($P < 0.05$). Dietary TB treatment improved the carcass traits, as evidenced by a higher eviscerated carcass rate and lower abdominal fat yield than those in the control group ($P < 0.05$). The breast meat yield rate was quadratically improved in response to dietary TB administration ($P < 0.05$). Dietary TB treatment improved the kidney, spleen, thymus, and bursa indices ($P < 0.05$) and reduced the lung indices compared with those in the control group ($P < 0.05$). In particular, the spleen and thymus indices were improved quadratically in response to dietary TB administration ($P < 0.05$). Dietary TB treatment improved the white and red blood cell counts, platelet count, hemoglobin and hematocrit at d 21, and platelet count at d 42 ($P < 0.05$), with those in the TB3 group being most affected. Dietary TB administration quadratically decreased the plasma content of uric acid at both d 21 and d 42 as well as that of creatine kinase at d 42 ($P < 0.05$), while it quadratically increased the plasma albumin/globulin ratio at both d 21 and d 42 ($P < 0.05$). Collectively, these results demonstrated that dietary TB intervention improved the growth performance, carcass traits, selected visceral and immune organ indices, and some blood biochemical markers under the isocaloric diets administration, which may facilitate better economic profit returns in poultry industry application.

Key words: Tributyrin, Carcass traits, Visceral and immune organ indices, Broiler

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INTRODUCTION

Antibiotics have long been used at subtherapeutic doses as growth promoters in commercial animal operations, as they effectively ameliorate animal health and improve animal performance and profit returns (Landers et al., 2012; Chattopadhyay, 2014). However, with growing public health concerns regarding the emergence of antibiotic resistance in livestock and dwindling antibiotic efficacy in humans (Vidovic and Vidovic, 2020; Ma et al., 2021), alternative products with not only antimicrobial properties and other value-added functions, such as anti-inflammation and antioxidization, but also safety to humans, animals, and the environment have been developed and applied in commercial feed operations. These products are mainly probiotics, prebiotics, organic acids, enzymes, phytogenic extracts, antimicrobial peptides, etc. (Gadde et al., 2017; Grant et al., 2018). However, with the increasing practice of antibiotic-free feeding regimens, the feed cost burden is inevitably increased (Crews, 2020). In this regard, antibiotic alternatives with energy- and/or amino

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acid-sparing potential may bring competitive profit returns to swine and poultry operations.

Of the available antibiotic alternatives, butyric acid and its derivative products are favorable choices, as they have been well demonstrated to play fundamental roles in modulating the intestinal structure, microbial composition, and immune response and attenuating intestinal inflammation and oxidative stress in broilers and pigs (Wang et al., 2019a,b; Sotira et al., 2020). For example, butyric acid and its derivative products could induce the immune response by upregulating the gene expression of intestinal host-defense peptides for the control of bacterial pathogens, particularly invasive pathogenic ones, in broiler chickens (Sunkara et al., 2011, 2012; Schulthess et al., 2019). With these results taken together, butyric acid proves to be a valid aid for gut health maintenance.

In scientific research, scientists are seriously concerned about the presence of butyrate in different parts of the gastrointestinal tract and its effects on digestive function, microbiota composition, and immune responses in broilers following administration of different butyrate sources (Moquet, 2018). The most commonly available butyrate products, sodium butyrate and tributyrin (**TB**) have demonstrated many beneficial yet different effects on the performance and wellbeing of broiler chickens (Bedford et al., 2017; Dhara et al., 2019; Lan et al., 2020a). Therefore, the poultry industry has long been entangled in choosing a favorable butyrate product because their marginal bio-beneficial effects on broilers and other animals are promising yet different.

Nonetheless, clinical and pharmacological research has demonstrated that TB shows advantages over sodium butyrate (Edelman et al., 2003). For example, with its longer metabolic half-life, a sufficient amount of ingested TB can be delivered to target tissues/organs for the initiation of its beneficial effects (Miyoshi et al., 2011). In particular, compared with sodium butyrate, TB efficiently induces the expression of host defense peptides under physiological conditions, demonstrating that its beneficial bio-efficiency is superior to sodium butyrate (Jiang et al., 2013). In addition, unlike butyric acid and sodium butyrate, butyrate glycerides have no offensive odor (Namkung et al., 2011), which facilitates their application in feed mill plants. Interestingly, butyrate not only is a preferred energy source over glucose and glutamine for colonic epithelial cells but also improves the apparent metabolizable energy (Kaczmarek et al., 2016), ileal energy digestibility coefficient (Liu et al., 2017), and apparent ileal digestibility of methionine (Moquet, 2018) in broilers, showing promising energy- and methionine-sparing potential. In this regard, the present study sought to investigate the changes in growth performance, carcass traits, selected visceral and immune organ indices, and blood biomarker profiles in response to dietary TB administration under isocaloric diets administration in Arbor Acres (**AA**) broilers.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

The animal trial protocol was approved by the Animal Care and Use Committee of the China Agricultural

Table 1. Ingredient and nutrient composition of the basal diets.

	Starter (1−21 d)				Finisher (22−42 d)			
Ingredients (%)	Control	TB1	TB2	TB3	Control	TB1	TB2	TB3
Corn	56.40	56.28	56.15	55.90	64.32	64.20	64.07	63.82
Soybean meal	32.67	32.67	32.67	32.67	25.46	25.46	25.46	25.46
Zein (Crude protein 90%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Soybean oil	1.39	1.39	1.39	1.39	1.15	1.15	1.15	1.15
Salt	0.30	0.30	0.30	0.30	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.12	0.12	0.12	0.12	0.17	0.17	0.17	0.17
DL-Methionine	0.18	0.18	0.18	0.18	0.10	0.10	0.10	0.10
Zeolite	1.15	1.22	1.30	1.45	1.15	1.22	1.30	1.45
Calcium phosphate	2.10	2.10	2.10	2.10	1.64	1.64	1.64	1.64
Limestone	1.14	1.14	1.14	1.14	1.20	1.20	1.20	1.20
Antioxidant	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
Choline chloride (50%)	0.30	0.30	0.30	0.30	0.20	0.20	0.20	0.20
Mineral premix [a]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix [b]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Tributyrin (Eucalorie®) [c]	0.00	0.05	0.10	0.20	0.00	0.05	0.10	0.20
Calculated composition								
ME (Mcal/kg)	2.87	2.87	2.87	2.87	2.93	2.93	2.93	2.93
Crude protein (%)	21.58	21.57	21.56	21.54	19.00	18.99	18.98	18.96
Ca (%)	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
Available phosphorus (%)	0.48	0.48	0.48	0.48	0.40	0.40	0.40	0.40
Lys (%)	1.14	1.14	1.14	1.14	1.00	1.00	1.00	1.00
Met (%)	0.51	0.51	0.51	0.51	0.40	0.51	0.51	0.51
Tributyrin (%, Analyzed)	0.000	0.044	0.089	0.190	0.000	0.042	0.087	0.176

TB, Tributyrin.

[a]Supplied per kilogram of diet: Zn ($ZnSO_4$), 75 mg; Fe ($FeSO_4$), 80 mg; Mn ($MnSO_4$), 100 mg; Cu ($CuSO_4$), 8 mg, I (CaI_2), 0.35 mg; Se (Na_2SeO_3), 0.25 mg.

[b]Supplied per kilogram of diet: Vitamin A, 18,750 IU; Vitamin D_3, 3,750 IU; Vitamin E, 28 IU; Vitamin K_3, 3.975 mg; Vitamin B_1, 3 mg; Vitamin B_2, 9 mg; Vitamin B_5, 18 mg; Vitamin B_{12}, 37.5 μg; Biotin, 150 μg; Folic acid, 1.875 mg; Nicotinic acid, 75 mg.

[c]Kindly contributed by Hubei Horwath Biotechnology Co., Ltd., Wuhan, Hubei, China.

University, with the ethical experimental statement NO.: AW13501202-2-1.

A total of 432 healthy AA broiler chickens (1-day-old) were selected for a 42-d feeding trial. The birds were sorted into four dietary treatments, with 12 replicates (cages) per treatment and 9 birds per cage. The dietary treatments were a basal diet (Control) and a basal diet supplemented with a TB product (Eucalorie) at doses of 0.50 g/kg (TB1), 1.0 g/kg (TB2), and 2.0 g/kg (TB3). Particularly, as outlined in the objectives, to achieve the isocaloric experimental diets, corn was replaced with the TB product based on equivalent metabolizable energy contents, and the formulas were rebalanced by adding zeolite to insure the sum of all the feed ingredients to 100%. Feed ingredients and feeding program are summarized in Table 1. The TB product (Eucalorie) contained 92% TB and was kindly contributed by Hubei Horwath Biotechnology Co., Ltd., Wuhan, Hubei, China. The metabolizable energy value of the Eucalorie product is 8 Mcal/kg for broilers (Hu et al., 2021).

Experimental Management

Immediately after receipt, the birds were allocated to cages following a completely randomized design. The cages were kept in an environmentally controlled room equipped with artificial illumination, ventilation, and heating. Consistent light was provided for 22 h, and temperature and humidity were adjusted according to the Arbor Acres broiler manual management guide. Feed and water were freely available. The birds were vaccinated against coccidiosis at 1 d of age, Newcastle disease at 7 and 20 d of age, and infectious bursal disease at 14 d of age. No antibiotics were used throughout the trial.

Growth Performance

Body weights were recorded on a cage basis prior to feeding in the morning on d 21 and 42, and the cumulative feed consumption per cage was recorded at the same time. The phase average daily body weight gain (**ADG**), average daily feed intake (**ADFI**), and feed efficiency (ratio of feed intake/body weight gain, **F/G**) were calculated.

Blood Sampling

On d 21 and 42, after weighing, 12 birds from each treatment (1 bird per cage, whose body weight was close to the cage average) were selected for blood sample collection, and the birds were returned to its original cage after blood sampling at 21 d. Four milliliters of blood were drawn from the wing veins, 2 mL of which was collected in test tubes (Greine Co Ltd, Monroe, NC) prefilled with EDTA as an anticoagulant and put on ice for later blood cell count analysis. Another 2 mL was collected into 5-mL vacutainer tubes prefilled with heparin (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and centrifuged at 3,000 g (Biofuge 22R centrifuge; Heraeus Instruments, Hanau, Germany) for 10 min at 4°C. The supernatant (plasma) was immediately collected and placed into test tubes and stored at −20°C until further biochemical analyses.

Carcass Traits, Visceral, and Immune Organ Indices

On d 42, after blood sample collection, 12 broilers per treatment (1 bird per cage, whose body weight was close to the cage average) were killed by exsanguination after applying carbon dioxide. The carcass weight was calculated after removing the feathers and blood, and the eviscerated weight was calculated after removing the head, feet, abdominal fat (fat surrounding the cloaca and gizzard), and all viscera except the lungs and kidneys. The dressed and eviscerated yields were expressed as percentages of the live body weight (**BW**). Breast and leg muscle and abdominal fat were excised and weighed for the calculation of muscle and abdominal fat yields, respectively, based on the eviscerated weight. After the carcass trait-related measurements were completed, the heart, liver, kidney, lung, spleen, thymus, and bursa of the same birds were separated and weighed for the measurement of organ indices. The organ indices were calculated as organ/body ratios.

Hematological Analysis

Selected hematological indicators, including red blood cells (**RBCs**), white blood cells (**WBCs**), hemoglobin (**HGB**), platelet count (**PLT**), and hematocrit (**HCT**), were measured with an automated blood cell counter and analyzer (Forcyte, Oxford Science, Las Vegas, NV) following corresponding guidelines.

Plasma Biochemical Analyses

The plasma concentrations of aspartate aminotransferase (**AST**), albumin (**ALB**), globulin (**GLB**), creatine kinase (**CK**), total cholesterol (**CHOL**), creatinine (**CREA**), glucose (**GLU**), total bilirubin (**TBIL**), total protein (**TP**), and uric acid (**UA**) were measured using an Automatic Biochemical Analyzer (Beckman, Miami, FL) with corresponding kits commercially available from Leadman Biochemistry Technology Company, Beijing, China.

Statistical Analysis

The data were subjected to a normality test before we conducted the treatment effect-related analysis. No abnormality was found within each treatment. The cage was used as the experimental unit and all data were analyzed with the SPSS program (version 17.0) using the mixed procedure. Orthogonal polynomial contrasts were used to study the linear and quadratic effects of dietary TB levels. Differences were considered significant at $P < 0.05$ and tendencies at $P < 0.10$.

Table 2. Effect of dietary tributyrin on growth performance in Arbor Acres broilers on a cage basis (n = 12).

Feeding phase	Item	Dietary treatment				P value	
		Control	TB1	TB2	TB3	Linear	Quadratic
0−21 d	ADG (g/d)	229.14 ± 12.95	235.62 ± 15.24	244.26 ± 17.15	248.49 ± 14.42	0.105	0.648
	ADFI (g/d)	364.14 ± 32.72	373.50 ± 25.50	379.53 ± 30.42	384.21 ± 26.81	0.057	0.926
	FCR	1.55 ± 0.04 [a]	1.54 ± 0.05 [a]	1.51 ± 0.04 [b]	1.51 ± 0.03 [b]	0.816	0.487
22−42 d	ADG (g/d)	636.75 ± 27.32 [b]	648.99 ± 22.17 [ab]	655.38 ± 16.28 [ab]	710.73 ± 24.57 [a]	0.071	0.024
	ADFI (g/d)	1,171.08 ± 65.11 [b]	1,191.69 ± 56.24 [b]	1,193.40 ± 75.55 [b]	1,278.81 ± 688.66 [a]	0.038	0.051
	FCR	1.80 ± 0.03 [a]	1.78 ± 0.02 [a]	1.76 ± 0.03 [b]	1.76 ± 0.02 [b]	0.052	0.049
0−42 d	ADG (g/d)	416.79 ± 42.67 [b]	429.66 ± 35.54 [ab]	438.21 ± 44.16 [ab]	470.16 ± 49.32 [a]	0.023	0.011
	ADFI (g/d)	749.79 ± 56.58 [b]	763.92 ± 71.45 [ab]	762.66 ± 78.87 [ab]	814.95 ± 64.45 [a]	0.097	0.052
	FCR	1.78 ± 0.02 [a]	1.74 ± 0.03 [ab]	1.70 ± 0.03 [b]	1.68 ± 0.02 [b]	0.365	0.300

Abbreviations: ADFI, Average daily feed intake; ADG, Average daily gain; FCR, Feed/gain ratio; TB, Tributyrin.

Control = basal diet; TB1 = basal diet supplemented with 0.5 g/kg TB-containing product; TB2 = basal diet supplemented with 1.0 g/kg TB-containing product; TB3 = basal diet supplemented with 2.0 g/kg TB-containing product.

[a,b]Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

RESULTS

Growth Performance

In the first feeding period (0−21 d), the FCR values in the TB2 and TB3 groups were lower than those in the other 2 groups ($P < 0.05$), although no significant differences in ADG and ADFI were observed among treatments ($P > 0.05$). The ADG, ADFI, and FCR in the second feeding period (22−42 d), and ADG and ADFI in the overall feeding period (0−42 d) were quadratically improved under dietary TB administration ($P < 0.05$). Notably, the ADG and ADFI were highest in the TB3 group ($P < 0.05$), while the FCR was lowest mainly in the TB2 and TB3 groups ($P < 0.05$) throughout the entire feeding period. The results are summarized in Table 2.

Carcass Traits

The eviscerated carcass rate was higher, and the abdominal fat yield was lower in the TB-fed groups than in the control group ($P < 0.05$), with the TB3 group being the most affected. There was a significant quadratic ($P = 0.047$) improvement in the breast meat yield rate in response to dietary TB administration. No significant differences were observed in dressing, breast muscle, or leg muscle yields among the treatments ($P > 0.05$). The results are summarized in Table 3.

Visceral and Immune Organ Indices

The kidney, spleen, thymus, and bursa indices were higher, while the lung indices were lower in the TB-fed groups than in the control group ($P < 0.05$), with the TB2 and TB3 groups being most affected (Table 4). In particular, the spleen and thymus indices were quadratically improved under dietary TB administration ($P < 0.05$).

Hematological Parameters

Dietary TB treatment significantly affected the selected hematological parameters, including WBCs, RBCs, HGB, HCT, and PLT at d 21 as well as PLT at d 42 ($P < 0.05$), with that in the TB3 group being most affected (Table 5). In particular, the HCT at d 21 and HGB, HCT, and PLT at d 42 quadratically improved in response to dietary TB administration ($P < 0.05$). In addition, the WBCs, HGB, and PLT at d 21 tended to quadratically improve following the dietary TB treatment ($P < 0.10$).

Plasma Biochemical Profiles

Dietary TB treatment significantly affected the plasma AST and UA levels at d 21 and the A/G ratio at both d 21 and d 42 ($P < 0.05$), with the TB3 group being most affected. Notably, the dietary TB treatment

Table 3. Effect of dietary tributyrin on carcass characteristics in Arbor Acres broilers at the age of 42 d.

Carcass trait	Dietary treatment				P value	
	Control	TB1	TB2	TB3	Linear	Quadratic
Dressing (%)	90.00 ± 1.22	90.91 ± 1.48	91.21 ± 1.14	91.26 ± 1.20	0.680	0.092
Eviscerated (%)	76.10 ± 3.04 [b]	77.74 ± 2.76 [a]	77.72 ± 3.21 [a]	77.79 ± 2.69 [a]	0.445	0.268
Breast meat (%)	19.47 ± 2.17	20.51 ± 2.24	21.23 ± 2.59	21.37 ± 1.31	0.216	0.047
Leg muscle (%)	13.43 ± 1.34	13.85 ± 1.83	13.97 ± 1.25	13.97 ± 1.27	0.342	0.055
Abdominal fat (%)	1.63 ± 0.33 [a]	1.34 ± 0.12 [b]	1.28 ± 0.12 [b, c]	1.18 ± 0.09 [c]	0.312	0.157

Abbreviation: TB, tributyrin.

Control = basal diet; TB1 = basal diet supplemented with 0.5 g/kg TB-containing product; TB2 = basal diet supplemented with 1.0 g/kg TB-containing product; TB3 = basal diet supplemented with 2.0 g/kg TB-containing product.

[a,b,c]Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

Table 4. Effect of dietary tributyrin on relative weight of selected visceral and immune organ in Arbor Acres broilers at the age of 42 d (g/100 g body weight).

Item	Organ	Dietary treatment				*P* value	
		Control	TB1	TB2	TB3	Linear	Quadratic
Visceral organ	Heart	0.45 ± 0.10	0.44 ± 0.04	0.43 ± 0.04	0.43 ± 0.05	0.871	0.904
	Liver	2.58 ± 0.24	2.52 ± 0.16	2.49 ± 0.10	2.43 ± 0.12	0.373	0.968
	Kidney	0.56 ± 0.06^{b}	0.59 ± 0.05^{ab}	0.60 ± 0.04^{ab}	0.64 ± 0.05^{a}	0.646	0.231
	Lung	0.65 ± 0.07^{a}	0.55 ± 0.04^{b}	0.54 ± 0.05^{b}	0.53 ± 0.06^{b}	0.265	0.128
Immune organ	Spleen	0.15 ± 0.02^{b}	0.17 ± 0.03^{ab}	0.19 ± 0.03^{a}	0.20 ± 0.02^{a}	0.767	0.031
	Thymus	0.32 ± 0.04^{c}	0.37 ± 0.02^{bc}	0.41 ± 0.05^{ab}	0.46 ± 0.05^{a}	0.060	0.019
	Bursa	0.07 ± 0.01^{b}	0.13 ± 0.01^{a}	0.13 ± 0.00^{a}	0.13 ± 0.02^{a}	0.542	0.167

Abbreviation: TB, tributyrin.

Control = basal diet; TB1 = basal diet supplemented with 0.5 g/kg TB-containing product; TB2 = basal diet supplemented with 1.0 g/kg TB-containing product; TB3 = basal diet supplemented with 2.0 g/kg TB-containing product.

^{a,b,c}Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

Table 5. Effect of dietary tributyrin on blood biomarkers in Arbor Acres broilers at the ages of 21 d and 42 d.

Age	Item	Dietary treatment				*P* value	
		Control	TB1	TB2	TB3	Linear	Quadratic
21 d	WBC (10^9/L)	128.05 ± 13.10^{b}	129.94 ± 10.21^{b}	138.11 ± 12.25^{a}	139.53 ± 11.43^{a}	0.145	0.087
	RBC (10^{12}/L)	2.20 ± 0.24^{bc}	2.33 ± 0.15^{b}	2.35 ± 0.32^{ab}	2.50 ± 0.16^{a}	0.153	0.151
	HGB (g/L)	102.60 ± 10.46^{b}	103.83 ± 9.25^{b}	110.13 ± 14.12^{a}	111.75 ± 12.23^{a}	0.119	0.079
	HCT (%)	27.47 ± 3.24^{c}	29.05 ± 2.66^{bc}	30.99 ± 4.12^{ab}	32.32 ± 3.45^{a}	0.067	0.020
	PLT (10^9/L)	7.00 ± 0.94^{b}	11.13 ± 1.12^{ab}	12.30 ± 1.56^{a}	15.10 ± 2.84^{a}	0.134	0.076
42 d	WBC (10^9/L)	128.29 ± 10.34	129.07 ± 6.86	131.83 ± 15.42	131.57 ± 13.54	0.248	0.101
	RBC (10^{12}/L)	2.14 ± 0.28	2.24 ± 0.32	2.25 ± 0.15	2.27 ± 0.24	0.826	0.350
	HGB (g/L)	124.18 ± 11.14	127.60 ± 10.35	129.45 ± 21.26	131.64 ± 13.30	0.094	0.025
	HCT (%)	30.35 ± 2.24	31.16 ± 3.11	31.57 ± 1.27	31.43 ± 2.25	0.425	0.022
	PLT (10^9/L)	32.73 ± 2.27^{b}	33.09 ± 3.03^{b}	34.75 ± 3.45^{ab}	40.00 ± 4.84^{a}	0.059	0.001

Abbreviations: HCT, hematocrit; HGB, hemoglobin; RBC, red blood cell; PLT, platelet; WBC, white blood cell.

Control = basal diet; TB1 = basal diet supplemented with 0.5 g/kg TB-containing product; TB2 = basal diet supplemented with 1.0 g/kg TB-containing product; TB3 = basal diet supplemented with 2.0 g/kg TB-containing product.

^{a,b,c}Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

quadratically decreased the plasma content of UA at both d 21 and d 42 as well as that of CK at d 42 ($P < 0.05$), while it quadratically increased the plasma A/G ratio at both d 21 and d 42 ($P < 0.05$). In addition, dietary TB administration tended to decrease the plasma GLB levels at both d 21 and d 42, as well as the CHOL level at d 21 ($P < 0.10$). The results are summarized in Table 6.

DISCUSSION

Chicken meat is widely consumed not only due to its favorable taste and nutritive values but also because it is free from religious and sociocultural restrictions (Henchion et al., 2014). Thus, the poultry industry is facing a sustainable, promising yet challenging future with the increase in worldwide demand for human-edible protein foods accompanying sustained population growth and rising incomes (Wu et al., 2014; Sanchez-Sabate & Sabaté, 2019). Such demand has attracted widespread effort to explore competitive strategies for the improvement of health and wellbeing in broilers for better performance. Interestingly, previous research demonstrated that directly adding glyceryl butyrate to the top of the control formula improved the growth performance and carcass yield in broiler chickens (Yin et al., 2016; Bedford et al., 2017), which has significant economic profit potential in industrial poultry operations. However, due to inevitable, sustained, and fierce commercial competitions, technical experts are eager to explore competitive strategies to obtain more profit in industrial poultry feed production. One encouraging approach is to add an optimal amount of butyrate to the feed formula without increasing the feed cost burden and compromising the birds' performance. Interestingly, the present study showed positive results that the ADG was quadratically improved in response to dietary TB intervention under the isocaloric and antibiotic-free feeding regime. This is of great interest to feed millers, as it may ensure birds gain greater marketing weight within a limited feeding period. Furthermore, dietary TB intervention also improved the eviscerated carcass and breast meat yield while reducing the abdominal fat yield of broilers in the current study, partially due to it improving the oxidation of lipids and fatty acids and increasing the net available energy for protein synthesis (Yin et al., 2016). This is of economic significance for the slaughtering companies, given that more broiler meats could be gained under their regular slaughtering and processing capacities. In addition, several pioneer studies demonstrated that a favorable weight gain and feed/gain ratio could be achieved when birds were fed a diet with reduced crude protein and/or metabolizable energy contents and supplemented with butyrate products (Bortoluzzi et al., 2017; Petrilla et al., 2018; Mátis et al., 2019).

Table 6. Effect of dietary tributyrin on plasma biochemical profiles in Arbor Acres broilers at the age of 21 and 42 d.

		Dietary treatment				*P* value	
Age	Item	Control	TB1	TB2	TB3	Linear	Quadratic
21 d	AST (U/L)	319.64 ± 29.14 [a]	317.55 ± 20.57 [a]	315.92 ± 15.25 [a]	276.83 ± 28.36 [b]	0.421	0.154
	ALB (g/L)	13.25 ± 1.32	13.92 ± 1.29	13.92 ± 1.93	14.00 ± 1.84	0.251	0.115
	GLB (g/L)	13.17 ± 1.13	13.17 ± 1.17	13.02 ± 1.32	12.17 ± 1.21	0.328	0.084
	A/G	1.01 ± 0.14 [b]	1.06 ± 0.16 [ab]	1.07 ± 0.13 [ab]	1.15 ± 0.15 [a]	0.378	0.044
	CK (u/L)	5541.30 ± 510.34	5415.80 ± 455.18	5267.00 ± 286.74	4633.82 ± 433.28	0.353	0.128
	CHOL (mmol/L)	3.20 ± 0.24	2.99 ± 0.23	2.98 ± 0.32	2.72 ± 0.22	0.075	0.074
	CREA (μmol/L)	7.33 ± 0.75	6.67 ± 0.56	6.58 ± 0.68	6.58 ± 0.56	0.420	0.155
	GLU (mmol/L)	15.24 ± 1.25	15.12 ± 1.12	15.03 ± 1.03	14.60 ± 1.64	0.042	0.114
	TBIL (μmol/L)	9.33 ± 0.95	10.15± 1.51	10.73± 1.03	11.00 ± 1.16	0.102	0.155
	TP (g/L)	26.42 ± 2.26	27.09 ± 2.07	26.94 ± 2.46	26.17 ± 2.17	0.198	0.405
	UA (μmol/L)	609.67 ± 6.96 [a]	547.10 ± 5.14 [ab]	505.22 ± 5.25 [ab]	411.50 ± 4.51 [b]	0.037	0.035
42 d	AST (U/L)	342.00 ± 3.24	340.09 ± 3.49	334.00 ± 4.34	303.36 ± 3.36	0.344	0.405
	ALB (g/L)	13.77 ± 1.36	14.22 ± 1.24	14.51 ± 1.15	14.94 ± 1.44	0.071	0.304
	GLB (g/L)	22.6 ± 2.12	22.14 ± 2.24	20.64 ± 2.46	20.22 ± 2.36	0.107	0.064
	A/G	0.61 ± 0.05 [bc]	0.64 ± 0.04 [b]	0.70 ± 0.08[ab]	0.74 ± 0.48 [a]	0.932	0.034
	CK (u/L)	9,178.10 ± 911.08	8,487.25 ± 848.52	7,801.82 ± 718.02	7,549.82 ± 725.48	0.016	0.017
	CHOL (mmol/L)	3.43 ± 0.24	3.42 ± 0.42	3.39 ± 0.40	3.09 ± 0.30	0.142	0.136
	CREA (μmol/L)	9.73 ± 0.37	9.50 ± 0.64	9.50 ± 0.92	9.42 ± 0.92	0.256	0.112
	GLU (mmol/L)	11.57 ± 1.17	11.35 ± 1.31	11.27 ± 1.12	11.17 ± 1.27	0.126	0.108
	TBIL (μmol/L)	21.21 ± 2.12	21.54 ± 2.45	23.76 ± 2.36	24.94 ± 2.44	0.479	0.401
	TP (g/L)	36.37 ± 0.36	36.36 ± 4.15	35.15 ± 2.55	35.16 ± 3.15	0.072	0.203
	UA (μmol/L)	358.00 ± 34.85	336.20 ± 36.23	335.56 ± 36.56	322.11 ± 23.21	0.137	0.029

Abbreviation: A/G, albumin/globulin ratio; AST, aspartate aminotransferase; ALB, albumin; CK, creatine kinase; CHOL, total cholesterol; CREA, creatinine; GLB, globulin; GLU, glucose; TBIL, total bilirubin; TP, total protein; UA, uric acid.

Control = basal diet; TB1 = basal diet supplemented with 0.5 g/kg TB-containing product; TB2 = basal diet supplemented with 1.0 g/kg TB-containing product; TB3 = basal diet supplemented with 2.0 g/kg TB-containing product.

[a,b]Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

Collectively, these results clearly demonstrated the application of butyrate for cost burden reduction and better performance in poultry operations.

Organ indices are apparent parameters indicating dietary treatment effects on the developmental and functional status of animals. In particular, well-developed and mature visceral and immune organs are correlated with improved immune responses and growth performance in healthy animals (Sikandar et al., 2017a; Selim et al., 2021). It is widely accepted that primary lymphoid organs, such as the bursa, thymus, and spleen, are key players in the immune system, protecting birds from infectious pathogenic bacteria and viruses by producing immune cells involved in cellular and humoral immunity (Lan et al., 2020b; Selim et al., 2021). In the current study, the spleen, thymus, and bursa indices were increased following dietary TB administration, indicating that the immune system was improved to provide better infectious diseases prevention and control. Similar results were observed in broilers and Japanese quails with sodium butyrate administration (Sikandar et al., 2017b; Elnesr et al., 2019). Notably, this effect was even significant in subdeveloped and subhealthy animals. For example, dietary tributyrin intervention increased the spleen indices by 62% in intrauterine growth-restricted piglets compared with that in control pigs (Dong et al., 2016). In addition, previous research demonstrated that butyrate protected the kidney from infectious pathogen-induced hemolytic uremic syndrome and reduced the urea level in the blood stream of pigs (Xiong et al., 2016; Sotira et al., 2020), thus further confirming that butyrate intervention effectively restored and/or even improved the normal function of kidney. In this regard, the kidney indices were increased under dietary TB administration, showing that kidney function was improved under the present feeding regimen. In addition, as expected, a particularly novel observation was that dietary TB administration significantly reduced the lung indices in the current study. Unlike monogastric and ruminant animals, the broiler's lung is firm and fixed within the thoracic cavity; therefore, it cannot expand and has little space to alleviate pressure due to increased blood flow (Rothschild, 2019). However, tributyrin intervention could reduce the heart action, blood flow, and arterial blood pressure (Onyszkiewicz et al., 2019), and improve O_2 exchange and transport (Witt et al., 2000; Mairbäurl & Weber, 2012). As a synergetic result, dietary TB administration could help to secure normal lung function without compensatory growth to meet the oxygen needs of the body. Taken together, these results provide scientific evidence that dietary tributyrin intervention improved the growth, development and function of some visceral and immune organs in broilers. Of particular importance, an optimal tributyrin dose is critically required in feed formulas for industrial poultry operations.

Hematological components, such as WBCs, RBCs, HGB, HCT, and PLT, are valuable parameters for the indication of health status in humans and animals. HGB is of significant importance for the cellular metabolism process, as it transports oxygen from the lung to other body tissues and organs, and transports carbon dioxide back to the lungs for expulsion via the respiratory tract (Jolliff and Mahan, 2011; Perri et al., 2015). Clinically, a higher HGB content is positively linked to higher RBC counts and hematocrit values, which synergistically facilitate the oxygen-carrying capacity in blood circulation (Balcerek et al., 2020). Interestingly, butyrate derivative products, such as sodium butyrate and tributyrin, have been demonstrated to effectively induce γ-HGB synthesis in a wide range of animal species (Faller & Perrine, 1995; Weinberg et al., 2005), and the function of tributyrin is superior to that of sodium butyrate (Witt et al., 2000). Furthermore, in the current study, in line with these findings, dietary TB treatment statistically and/or numerically ameliorated the RBCs, HGB and HCT parameters at 21 and 42 d of age, showing an improved oxygen exchange and transport capacity in blood circulation of broilers. Similarly, dietary sodium butyrate treatment also improved the HGB and RBC parameters in broilers (El-Sawy et al., 2015). Furthermore, efficient HGB synthesis is positively associated with growth performance improvement and higher survival rates in animals (Kim et al., 2013). Therefore, these results provide logical scientific evidence for the improvement of growth performance and reduction of the lung indices in broilers treated with tributyrin. In addition, the WBCs and PLT counts also improved following dietary TB treatment. WBCs, which are also known as leukocytes, are derived from hematopoietic stem cells, which actively protect the body from infectious pathogens and foreign invaders (Mahdavi and Poor, 2021). Interestingly, previous research also observed an improvement in blood WBCs counts in butyrate-treated broilers (Abonyi et al., 2020). Chicken PLTs, which are also known as thrombocytes, are nucleated blood leukocytes and represent the most abundant WBCs in avian blood (Chang and Hamilton, 1979), and they are involved in the processes of inflammation, hemostasis, and the initiation of tissue and wound repair (George, 2000; St Paul et al., 2012). In particular, PLTs act as the primary effector cells in innate host defenses against bacterial infections in chickens (Ferdous et al., 2008). Overall, the increase in blood WBCs and PLT levels demonstrated an improvement in the immune response for better infectious disease prevention and control in broilers following dietary TB treatment under the current energy-sparing formulation regime.

Blood metabolites and enzymes are valuable predictors for evaluating metabolic conditions in humans and animals (Stringer et al., 2015; Azeredo et al., 2016). In the current study, the plasma AST and UA levels were significantly affected by dietary TB administration. Furthermore, the A/G ratio and UA and CK levels were quatratically changed in response to dietary TB treatment. AST is an important bioindicator of liver integrity and function, and in particular, its activity has been adopted to evaluate the severity of liver disease (Obidike, 2009; Yildirim et al., 2011). Clinically, an increase in blood AST levels demonstrates hepatocyte and liver dysfunction (Tessari et al., 2010). Interestingly, the plasma AST level was statistically and numerically decreased at 21 and 42 d of age following dietary TB administration, demonstrating that TB treatment improved liver function. Furthermore, as the liver plays a major role in controlling GLU metabolism, flux, and storage (Lan et al., 2020a), a numerical decrease in plasma GLU levels within the normal physiological range indirectly indicated improved liver function in broilers. In line with this promising observation, previous research also observed a reduction in blood AST levels in sodium butyrate-fed broilers (Deepa et al., 2017; Abonyi et al., 2020). UA is a major end-product of amino acid catabolism, and its blood level is controlled by the balance between its synthesis and excretion (Maiuolo et al., 2016; Star et al., 2021). Normally, a lower blood UA value is positively linked to higher amino acid turnover, protein synthesis, and nitrogen retention under given dietary protein levels in broilers (Kriseldi et al., 2018; Star et al., 2021). In agreement with these findings, dietary TB treatment significantly and numerically reduced the plasma UA level at 21 and 42 d of age, demonstrating improvement in protein synthesis and nitrogen retention, which was in accordance with the improved body weight gain in the current study.

Physiologically, blood TP levels are the sum of ALB and GLB levels. The ALB level and A/G ratio are important biomarkers indicating nutritional status, as they are rapidly increased after meals and always have low values under malnutrition (Fuhrman et al., 2004). Notably, higher values of ALB and the A/G ratio are positively associated with higher body weight gain in animals (Doornenbal et al., 1986; Elbers et al., 1992). In agreement with this, dietary TB treatment significantly improved the A/G value while numerically improving the plasma ALB level, which was in accordance with the improved ADG in broilers in the current study. Similarly, such results were also observed in TB-fed pigs (Sotira et al., 2020). CK is a central regulator of the maintenance of intracellular energy supplies (Tao et al., 2011), it is also a reliable diagnostic marker indicating the degree of stress that to which hosts are subjected by the environment, such as heat stress and transport stress (Tao et al., 2011; Xing et al., 2015). In the current study, the plasma CK activity quadratically decreased under TB treatment, with the most significant decrease in the TB3 group. We can therefore predict that dietary TB administration mitigated the occurrence of stress status, although further evidence is needed.

In the current study, there was a quadratically decreasing trend of plasma CHOL levels in response to dietary TB administration at 21 d of age, and similar observations were also found in sodium butyrate-treated birds (Elnesr et al., 2019; Abonyi et al., 2020). Notably, the decrease in blood CHOL levels was even significant at earlier ages in butyrate glyceride-fed broilers (Yin et al., 2016; Bedford et al., 2017). Lower blood cholesterol and triglyceride levels were associated with improved fatty acid oxidation and energy expenditure, as well as the modified fatty acid synthesis and lipoprotein metabolism of the liver, thus reducing the occurrence of diet-induced liver and abdominal fat deposition (Musa et al., 2006; Panda et al., 2009; Yin et al., 2016). Therefore, the decreased blood CHOL level was further confirmed the reduction in the abdominal fat ratio of broilers in the current study.

CONCLUSIONS

The present study demonstrated that 0.2% TB administration significantly improved growth performance, carcass traits, selected visceral, and immune organ indices and some valuable plasma biomarker profiles under an equal metabolizable energy content formula. These findings provide scientific proof of better economic profit potential in industrial poultry operations.

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Author contributions: QH and YL designed the study. QH, BL and GL conducted the animal trial, sampling and data collection. QH and FY analyzed the data. QH and FY drafted the manuscript. LD and YY reviewed the manuscript. All authors approved the final version of the manuscript.

DISCLOSURES

The authors have declared that no competing interests exist.

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