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Effects of Dietary *Clostridium butyricum* on Carcass Traits, Antioxidant Capacity, Meat Quality, and Fatty Acid Composition of Broilers

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Abstract: The demand for identifying substitutes for antioxidant feed additives in broiler production is increasing. The current study aimed to investigate the effects of *Clostridium butyricum* (*C. butyricum*) on carcass traits, antioxidant capacity, meat quality, and fatty acid composition of broiler chickens. A total of 330 one-day-old mixed-sex commercial Ross 308 broilers were randomly divided into five groups with six replicates per group and eleven broilers per replicate and reared for 39 days. The control (CON) group was fed a basal diet, the AM group was fed a basal diet containing 150 mg aureomycin/kg feed, and the CBL, CBM, and CBH groups were fed a basal diet containing 2×10^8, 4×10^8, and 8×10^8 colony-forming units (CFU) *C. butyricum*/kg feed. On day 21, compared to the AM group, serum total antioxidant capacity (T-AOC) level was enhanced in the CBH group and serum total superoxide dismutase (T-SOD) concentrations were increased in the CBM and CBH groups ($p < 0.05$). Dietary *C. butyricum* resulted in the liver T-AOC, T-SOD, and catalase (CAT) of broilers linearly increased at day 21 ($p < 0.05$). On day 39, supplementation with *C. butyricum* in broiler diets linearly increased concentrations of T-SOD ($p < 0.05$), CAT ($p < 0.001$), but linearly reduced MDA (malondialdehyde) contents ($p < 0.001$) in the liver. For the breast muscle, the redness for meat color increased in a linear manner and the shearing force decreased in a quadratic manner in response to *C. butyricum* inclusion ($p < 0.05$). The pH_{45min}, pH_{24h}, and the shearing force changed in a quadratic pattern ($p < 0.05$). The contents of total MUFA (monounsaturated fatty acid) and total PUFA (polyunsaturated fatty acid) were altered and quadratically responded to the doses of *C. butyricum* ($p < 0.05$). For the thigh muscle, the inclusion of *C. butyricum* in broiler diets showed the negative linear effects on the cooking loss and shearing force ($p < 0.001$). The total MUFA contents were changed linearly and quadratically ($p < 0.001$; $p < 0.05$), and the contents of total PUFA and the ratio of PUFA to SFA were quadratically responded to the doses of *C. butyricum* ($p < 0.05$). In brief, dietary *C. butyricum* could beneficially enhance liver antioxidant capacity, and improve meat quality and fatty acid composition in broilers.

Keywords: *Clostridium butyiricum*; broiler; carcass traits; antioxidant capacity; meat quality; fatty acid composition

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1. Introduction

Antibiotics have been used as a growth-promoting feed additive in livestock farming for many years. Although antibiotics provide advantages to animal production, the misuse and abuse of antibiotics have caused the evolution of bacteria and drug-resistant pathogens in poultry products, which directly or indirectly endangers human health and environmental safety [1]. Fortunately, many countries realized the severity of this safety problem and adopted "antibiotics ban" measures in the feed additives [2]. It is imperative to conduct intensified searches for alternative natural growth promoters to sustain growth, health, and

meat quality of chickens. On the other hand, the global demand for high-quality protein for a healthy life has been increasing during these years. Poultry meat, rich in protein and valuable nutrients, is the first most consumed and produced meat today globally [3]. According to Food and Agriculture Organization (FAO) statistics, global production of poultry meat accounted for 35 percent of meat production in 2019, and poultry meat showed the largest growth in absolute and relative terms since 2000 and was the most produced type of meat in 2019. Obviously, poultry meat has the potential to be a functional food because the substantial beneficial nutrients can be diverted from feed to poultry products. Therefore, the current work should be highlighted to improve nutritive value of poultry meat.

Probiotic feed additives have gained widespread interest worldwide in the poultry industry [4]. Probiotics have become more important alternatives as feed additives because they play a vital role in enhancing anti-bacterial, anti-inflammatory, and antioxidant effects, modulating the structure of host microflora and stimulating the digestive systems of animals [5–7]. In these probiotics, *Clostridium butyricum* (*C. butyricum*) is a direct-fed additive used widely in poultry production as it possesses positive properties, including promoting growth, antioxidant, regulating immunity, and improving meat quality [8–11]. Massive data show the potential application of *C. butyricum* to poultry feed. It qualifies unique advantages. Generally, *C. butyricum* can resist the adverse environment in the gastrointestinal tract due to its stress tolerance, then colonize the gut, and finally create a beneficial environment in vivo. For instance, some metabolites from *C. butyricum* enhance disease resistance and host innate immunity and coin a stable internal environment without suffering from oxidative stress [12,13].

Fatty acid (FA) profiles of meat have always been a focus of healthy food, in which the balance between saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) is one of the most attractive components. Poultry meat is a significant provider of essential polyunsaturated fatty acids (PUFAs), forcing it to oxidate more quickly [14]. However, those long-chain PUFAs are responsible for the color, texture, odor, and flavor of the meat, thus determining meat value in markets. As a result, measures must be adopted to impede the oxidation of PUFA. Recent studies have suggested that dietary supplementation of antioxidants is beneficial for preventing diseases and improving quality of life [15,16]. Previously, supplementation of *C. butyricum* has been reported to enrich meat with some functional FAs and increase the PUFA to SFA ratio in Peking ducks [17]. Moreover, *C. butyricum* can produce both butyrate and H_2, which have been proved to exert antioxidant properties by increasing the activity of antioxidative enzymes and decreasing reactive oxygen metabolites [18–20]. Dietary *C. butyricum* ameliorated antioxidant enzyme activity and lower malondialdehyde (MDA) content in weaned piglets [21]. Consequently, we speculated that *C. butyricum* may make an impact on resisting antioxidative stress in poultry production.

C. butyricum NF, isolated from cattle feces, has strong resistance to heat and simulated gastric and intestinal fluid (data not published) [22]. Our previous study showed that dietary *C. butyricum* NF improved hepatic antioxidant capability, meat quality and fatty acid composition, while decreased serum lipid and abdominal fat in Arbor Acres chicks [23]. Our findings could also verify its antioxidative properties that cell-free extracts of *C. butyricum* NF could scavenge some free radicals (data not published) [22]. Moreover, *C. butyricum* NF could ameliorate serum lipid in oxidative stress induced by corticosterone exposure of mice (data not published) [22]. However, little information is available on examining the response of *C. butyricum* NF on carcass traits and fatty acid composition in the meat of broiler chickens. We assumed that feeding *C. butyricum* NF might exert antioxidant properties in the broiler's body. Therefore, the present study aimed to evaluate the effects of probiotic *C. butyricum* NF on carcass traits, antioxidant capacity, meat quality and fatty acid composition of broiler chickens.

2. Materials and Methods

2.1. Bacterial Strains, Culture Conditions and Preparation

The *C. butyricum* used in this study is a strain originally isolated by our laboratory and was stored in the China General Microbiological Culture Collection Center (CGMCC). The collection number of *C. butyricum* is CGMCC 8187. The *C. butyricum* was cultured in Reinforced *Clostridium* Medium at 37 °C for 12 h, then inoculated at 4% and into a 50-liter vertical fermentation tank (GuJS-50, Zhenjiang Dongfang Bioengineering Technology Co., Ltd., Zhenjiang, China) and cultured for 24 h. The culture of the strain was spray dried directly with maltodextrin as the carrier. The final living bacteria count was 6.25×10^8 CFU per gram for *C. butyricum*.

2.2. Experimental Design, Animals and Housing

A total of 330 one-day-old, mixed-sex commercial Ross 308 broilers were procured from a commercial hatchery (Qilibao Chicken Farm, Hebi, Henan, China) and assigned into five treatment groups (66 per group) at random. Each group was subdivided into six replicates, 11 chicks per replicate. The assigned groups were as follows: broilers fed a basal diet (CON), broilers fed a basal diet containing 150 mg/kg aureomycin (AM); broilers fed a basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed (CBL); broilers fed a basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed (CBM); and broilers fed a basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed (CBH). The basal diets for the starter phase (days 1–21) and the finisher phase (days 22–39) complied with the recommendations of the National Research Council (NRC) (1994) for broiler chickens. The ingredients and nutrient composition of diets are shown in Table 1.

Table 1. Compositions and nutrient levels of basal diets for broilers (%).

Item (%)	Starter (d 1–21)	Finisher (d 22–39)
Ingredients		
Corn	54.70	56.70
High protein soybean meal [1]	34.70	25.90
$CaHPO_4$	1.50	1.20
Limestone	1.20	1.20
Soybean oil	0.90	3.50
wheat	5.00	7.50
Chicken bone meal [2]	0.00	2.00
Premix [3]	2.00	2.00
Total	100.00	100.00
Chemical composition analyzed		
ME, calculated, Mcal/kg	2.95	3.10
Dry matter	87.35	87.70
Crude protein	22.00	20.50
Calcium	0.90	0.87
Total phosphorus	0.61	0.57
Non-phytate phosphorus	0.45	0.45
Lysine	1.37	1.20
Methionine	0.67	0.60
Methionine + Cysteine	0.96	0.82
Threonine	0.95	0.84

[1] The protein content in the high protein soybean meal is 47.1%. [2] The protein content in the chicken bone meal is 51.3%.[3] Premix provided the following nutrients per kilogram of diet: Fe, 111 mg, Cu, 10 mg, Mn, 128 mg, Zn, 142 mg, NaCl, 3 g, L-lysine HCl, 2.4 g, DL- methionine 1.4 g, vitamin A, 14,000 IU, vitamin D3, 6000 IU, vitamin E, 70 mg, vitamin K3, 4 mg, vitamin B1, 7 mg, vitamin B2, 13 mg, vitamin B6, 13 mg, vitamin B12, 29 μg, choline, 1835 mg, folic acid, 3 mg, nicotinic acid, 93 mg, pantothenic acid, 27 mg.

The birds were fed in three-dimensional three-tier cages for 39 days. Throughout the entire period, the birds were fed ad libitum and had unlimited access to water. The ambient temperature was maintained at 33 °C for the first week, then decreased by 0.5 °C every day until the first 7 day, and then by 0.3 °C every day from the eighth day to the 30th day, until

it reached 22 °C. Chicks were exposed to continuous light for the first seven days and 22 h per day thereafter. The relative humidity in the bird's house was maintained at 60–70% in the first two weeks and 50% afterwards. All of the birds were vaccinated against infectious bronchitis (1 and 10 days of age) and Newcastle disease (10 days of age) (Nobilis® ND LaSota, Intervet International, Boxmeer, The Netherlands). The flocks were in good health, and no veterinary treatment measures were taken.

2.3. Sample Collection

At the end of the experiment, one bird from each replicate (6 replicates per treatment) with BW close to the average was selected at random, held without feed for 12 h, and weighed. Blood samples from the wing vein were individually obtained in a disposable vacuum blood collection tube and centrifuged at 845 × *g* for 15 min to obtain serum which was stored at −20 °C for further analysis. After blood collection, the selected broilers were exsanguinated by cutting the jugular vein. The dressing percentage, eviscerated percentage, breast and thigh muscle percentage, and abdominal fat percentage were calculated according to Ahmat [24].

2.4. Assay of Antioxidant Indices in Serum and Liver

Total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) activities, and malondialdehyde (MDA) content were measured by reagent kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China) according to the manufacturer's instructions.

2.5. Meat Colour, pH, Drip Loss, Cook Loss, and Shearing Force

Meat color was measured by a colorimeter (model WSC-S, Shanghai Shenguang Ltd., Shanghai, China) based on the CIELAB system (L* = Lightness; a* = Redness; b* = Yellowness) 45 min after slaughtering. A Testo 205 pH meter (Testo AG, Lenzkirch, Germany) equipped with an insertion electrode was used to test pH values at 45 min (pH_{45min}) and 24 h (pH_{24h}) postmortem. Twenty grams of breast and thigh muscle samples were stored in a refrigerator, and the drip loss was evaluated after 24 h of storage at 4 °C with a slight modification of the published method [17]. The drip loss at 24 h postmortem was expressed relative to the initial weight. Additionally, the cooking loss was performed with a slight modification of the published method by Cramer et al. [25]. Fifty grams of breast or thigh muscle sample were heated in water bath at 80 °C until the internal temperature reached 75 °C. The samples were then allowed to cool to an ambient temperature before being dried and weighed. The cooking loss was expressed as samples after cooking and cooling relative to the initial weight. Then, the cooked samples were held at 4 °C for 24 h after cooking as described above. Shear force was measured using a shear attachment on a texture analyzer (TMS-2000, Federal Trade Commission, American), of three strips (n × 1 × 1 cm^3) were cut from the middle of each cooked muscle by paralleling muscular fibers.

2.6. Fatty Acid Analysis

Fatty acid composition of breast and thigh muscles were determined by gas chromatography (6890, Agilent Technologies, Santa Clara, CA, USA). The total lipids were extracted following the chloroform-methanol procedure by Folch et al. [26]. Total lipid extracts were transmethylated into fatty acid methyl esters. Fatty acids were separated and identified using an HP 6890 gas chromatograph equipped with a DB-23 capillary column (0.25 mm × 60 m × 0.25 µm, JandW Scientific, Folsom, CA, USA). The oven temperature was 180 °C held for 10 min, up to 220 °C at 4 °C/min and held for 15 min, then increased to 250 °C at 3 °C/min held for 30 min. Helium was used as the carrier gas at the flow rate of 0.5 cm^3/min. The injector and detector temperatures were 250 °C and 280 °C, respectively. Nonadecanoic acid (C19:0, Fluka, Buchs, Switzerland) was used as the internal standard. The retention time of the fatty acids were compared to the international standard method, and concentrations were expressed as milligram per gram of muscle.

2.7. Statistical Analysis

The data were subjected to one-way ANOVA followed by F-test's factor significance and Tukey's multiple comparisons in IBM SPSS Statistics 20 statistical package (SPSS Inc., Chicago, IL, USA) as a completely randomized design with a pen (cage) as the unit. The results were expressed as means. The significance level was set at $p < 0.05$. The trend of *C. butyricum* doses at 2×10^8, 4×10^8, and 8×10^8 CFU/kg was analyzed using contrasts of linear and quadratic polynomial.

3. Results

3.1. Carcass Traits

Dietary *C. butyricum* has little effects on slaughter performance of broilers (Table 2). Dietary *C. butyricum* in the diet had no significant effect on dressing percentage, eviscerated percentage, breast muscle percentage, thigh muscle percentage and abdominal fat of broilers ($p > 0.05$).

Table 2. The slaughter performance of broilers feed with *C. butyricum*.

Item (%)	Treatment [1]					SEM [2]	*p*-Value	Response to CB	
	CON	AM	CBL	CBM	CBH			Linear	Quadratic
Dressing	91.77	90.32	90.66	90.18	90.78	0.003	0.510	0.286	0.239
Eviscerated	69.12	69.15	69.68	68.02	69.56	0.003	0.555	0.924	0.518
Thigh muscle	19.63	18.77	19.08	19.90	22.25	0.005	0.166	0.098	0.210
Breast muscle	28.18	29.07	30.52	30.21	30.64	0.004	0.284	0.062	0.244
Abdominal fat	2.36	1.97	1.86	1.84	1.96	0.001	0.358	0.202	0.151

Results are given as means, $n = 6$. [1] The treatments were as follows: CON, basal diet; AM: basal diet containing 150 mg/kg aureomycin; CBL, basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed; CBM, basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed; CBH, basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed. [2] SEM, standard error of the mean.

3.2. Antioxidant Indices Analysis

As shown in Table 3, the addition of *C. butyricum* to the feed improved the serum oxidant status in broilers. The addition of aureomycin to the feed caused a decrease in the serum T-AOC and T-SOD and an increase in the serum MDA of the birds at day 21 ($p < 0.05$). Moreover, the addition of aureomycin to the feed caused a decrease in the serum GSH-Px ($p = 0.070$) and an increase in the serum MDA ($p = 0.084$) of the birds at day 39. On day 21, compared with the AM group, the CBH group birds showed a higher T-AOC level ($p < 0.05$), and the CBM and CBH group birds had higher concentrations of T-SOD ($p < 0.05$). On day 39, the addition of *C. butyricum* to the feed linearly increased the serum GSH-Px ($p < 0.05$).

The addition of *C. butyricum* to the feed improved the liver oxidant status in broilers (Table 3). On day 21, increasing doses of *C. butyricum* led to a positive linear effect on the liver T-AOC, T-SOD, and CAT ($p < 0.05$). The addition of *C. butyricum* to the feed caused a decrease ($p = 0.086$) in the liver MDA in birds. Compared with the AM group birds, the CBH group birds had a higher content of liver CAT ($p < 0.05$). On day 39, inclusion of 2×10^8, 4×10^8, and 8×10^8 CFU/kg of *C. butyricum* in broiler diets linearly increased concentrations of T-SOD ($p < 0.05$) and CAT ($p < 0.001$), but linearly reduced MDA contents ($p < 0.001$) in the liver. Compared to the AM group, the CBH group birds showed a higher T-AOC level ($p < 0.05$).

Table 3. Effects of *C. butyricum* on serum and liver antioxidant capacity of broilers [1].

Item	Treatment [2]					SEM [3]	*p* Value	Response to CB	
	CON	AM	CBL	CBM	CBH			Linear	Quadratic
				Serum					
21 d									
T-AOC (U/mL)	5.73 ^{ab}	4.01 ^{b}	6.62 ^{ab}	6.33 ^{ab}	6.82 ^{a}	0.334	0.041	0.333	0.774
T-SOD (U/mL)	168.14 ^{a}	146.75 ^{b}	150.65 ^{ab}	166.40 ^{a}	165.07 ^{a}	2.856	0.033	0.806	0.185
CAT (U/mL)	4.74	4.47	4.66	5.32	5.45	0.152	0.163	0.030	0.697
GSH-Px (U/mL)	702.38	628.91	681.63	716.32	694.22	13.347	0.270	0.965	0.999
MDA (nmol/mL)	4.56 ^{b}	6.96 ^{a}	6.60 ^{ab}	4.69 ^{ab}	5.80 ^{ab}	0.299	0.019	0.519	0.461
39 d									
T-AOC (U/mL)	6.11	5.49	6.45	6.06	5.49	0.190	0.665	0.356	0.393
T-SOD (U/mL)	168.49	147.23	152.04	161.24	161.59	2.720	0.102	0.716	0.243
CAT (U/mL)	5.45	4.64	5.72	4.97	5.38	0.120	0.108	0.429	0.790
GSH-Px (U/mL)	653.40	619.39	682.99	735.37	733.33	13.810	0.070	0.021	0.552
MDA (nmol/mL)	4.53	6.91	5.60	5.47	5.73	0.238	0.084	0.155	0.455
				Liver					
21 d									
T-AOC (U/mL)	4.96	5.52	5.41	6.08	6.67	0.202	0.057	0.009	0.878
T-SOD (U/mL)	180.70 ^{b}	213.11 ^{ab}	195.70 ^{b}	205.19 ^{b}	262.15 ^{a}	7.548	0.003	0.001	0.152
CAT (U/mL)	3.16 ^{b}	3.04 ^{b}	3.39 ^{ab}	3.51 ^{ab}	3.84 ^{a}	0.722	0.001	0.001	0.666
GSH-Px (U/mL)	507.58	523.63	575.72	534.88	614.83	14.891	0.137	0.080	0.864
MDA (nmol/mL)	3.25	2.68	2.81	2.82	2.43	0.094	0.086	0.011	0.906
39 d									
T-AOC (U/mL)	5.87 ^{ab}	4.95 ^{b}	6.59 ^{ab}	6.71 ^{ab}	7.33 ^{a}	0.254	0.023	0.062	0.913
T-SOD (U/mL)	172.10 ^{b}	213.34 ^{ab}	184.82 ^{b}	203.97 ^{ab}	240.07 ^{a}	7.024	0.013	0.001	0.398
CAT (U/mL)	2.96 ^{b}	3.22 ^{b}	3.34 ^{ab}	3.97 ^{a}	4.05 ^{a}	0.108	0.001	<0.001	0.448
GSH-Px (U/mL)	599.51	620.25	635.75	637.50	692.94	18.572	0.621	0.189	0.838
MDA (nmol/mL)	4.39 ^{a}	3.49 ^{ab}	4.05 ^{a}	3.52 ^{ab}	2.86 ^{b}	0.151	0.008	<0.001	0.605

^{a,b} Means within a row with different letters differ ($p < 0.05$). Results are given as means, $n = 6$. [1] T-AOC, total antioxidant capacity. T-SOD, total superoxide dismutase. CAT, catalase. GSH-Px, glutathione peroxidase. MDA, malonaldehyde. [2] The treatments were as follows: CON, basal diet; AM: basal diet containing 150 mg/kg aureomycin; CBL, basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed; CBM, basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed; CBH, basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed.[3] SEM, standard error of the mean.

3.3. Meat Quality

There were significant differences ($p < 0.05$) in the meat quality in some indices by supplementing *C. butyricum* in the diet (Table 4). The lightness of the breast muscle showed an increasing but a limited trend in groups supplemented with *C. butyricum* as compared with the AM group. The inclusion of *C. butyricum* in broiler diets led to a linear effect on the redness for meat color in the breast muscle ($p < 0.05$). Supplementing *C. butyricum* in the diet had quadratic effects on pH_{45min} and pH_{24h} in the breast muscle ($p < 0.05$). Compared with the AM group, the pH_{45min} of the breast muscle in the CBL group was lower, and the drip loss was lower in all the *C. butyricum* supplemented groups ($p < 0.05$). Increasing the doses of *C. butyricum* had a quadratic effect on the shearing force in the breast muscle ($p < 0.05$).

The yellowness of thigh muscle in groups supplemented with *C. butyricum* showed an increasing but a limited trend when compared to the CON group. Compared with the AM group, the CBM group birds had a lower yellowness ($p < 0.05$) and a lower pH_{24h} in the thigh muscle ($p < 0.05$). The inclusion of *C. butyricum* in broiler diets showed negative linear effects on the cooking loss and shearing force in the thigh muscle ($p < 0.001$).

Table 4. Effects of *C. butyricum* on meat quality of broilers [1].

Item	Treatment [2]					SEM [3]	*p* Value	Response to CB	
	CON	AM	CBL	CBM	CBH			Linear	Quadratic
				Breast					
L*	55.66	51.34	54.29	54.81	56.93	0.662	0.083	0.519	0.248
a*	3.45 ^{b}	3.86 ^{ab}	3.83 ^{ab}	5.07 ^{a}	4.31 ^{ab}	0.180	0.038	0.027	0.120
b*	5.77	4.49	4.35	4.63	3.95	0.311	0.484	0.132	0.608
pH_{45min}	6.69 ^{ab}	6.90 ^{a}	6.31 ^{b}	6.55 ^{ab}	6.80 ^{ab}	0.066	0.032	0.373	0.034
pH_{24h}	5.62 ^{b}	5.81 ^{ab}	5.93 ^{a}	5.83 ^{ab}	5.71 ^{ab}	0.035	0.045	0.527	0.005
Drip loss (%)	1.74 ^{ab}	2.26 ^{a}	1.32 ^{b}	1.34 ^{b}	1.30 ^{b}	0.097	0.001	0.090	0.251
Cooking loss (%)	15.46	14.20	16.45	15.15	13.78	0.454	0.380	0.217	0.302
Shearing force (N)	30.67 ^{a}	23.27 ^{b}	24.89 ^{ab}	22.77 ^{b}	30.46 ^{a}	1.113	0.032	0.784	0.006
				Thigh					
L*	57.07	52.10	57.47	55.64	57.27	0.759	0.126	0.856	0.682
a*	6.33	6.40	5.26	6.34	6.35	0.151	0.064	0.371	0.063
b*	4.16 ^{b}	5.92 ^{a}	5.51 ^{ab}	4.06 ^{b}	5.04 ^{ab}	0.215	0.012	0.442	0.583
pH_{45min}	6.57	6.68	6.45	6.58	6.39	0.058	0.354	0.463	0.764
pH_{24h}	6.15 ^{ab}	6.25 ^{a}	6.05 ^{ab}	5.97 ^{b}	6.04 ^{ab}	0.030	0.024	0.369	0.582
Drip loss (%)	1.85	1.50	1.45	1.28	1.15	0.101	0.241	0.034	0.550
Cooking loss (%)	24.05 ^{a}	19.74 ^{b}	19.48 ^{b}	18.72 ^{b}	16.48 ^{b}	0.600	<0.001	<0.001	0.270
Shearing force (N)	27.80 ^{a}	13.70 ^{c}	25.78 ^{ab}	18.34 ^{c}	19.83 ^{bc}	1.147	<0.001	<0.001	0.305

^{a,b,c} Means within a row with different letters differ ($p < 0.05$). Results are given as means, $n = 6$. [1] L*, lightness. a*, redness. b*, yellowness. $pH_{45\ min}$: muscle pH value at 45 min postmortem; $pH_{24\ h}$: muscle pH value at 24 h postmortem. [2] The treatments were as follows: CON, basal diet; AM: basal diet containing 150 mg/kg aureomycin; CBL, basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed; CBM, basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed; CBH, basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed. [3] SEM, standard error of the mean.

3.4. Fatty Acid Composition

The addition of *C. butyricum* to the feed altered the fatty acid composition of breast muscle in broilers (Table 5). For monounsaturated fatty acid (MUFA) in the breast muscle, the contents of C16:1, C18:1n9c and total MUFA were quadratically responded to the doses of *C. butyricum* ($p < 0.05$). For polyunsaturated fatty acid (PUFA) in the breast muscle, the contents of linoleic acid (C18:2n6c, LNA), dihomo-gamma-linolenic acid (DGLA: C20:3n6), arachidonic acid (C20:4n6, ARA), and total PUFA were quadratically responded to the doses of *C. butyricum* ($p < 0.05$). Compared to the AM group, the CBM group birds showed a higher docosahexaenoic acid (C22:6n3, DHA) content ($p < 0.05$).

The addition of *C. butyricum* to the feed altered the fatty acid composition of the thigh muscle in the broilers (Table 6). For saturated fatty acid (SFA), dietary *C. butyricum* tended to linearly decrease the C23:0 content ($p = 0.052$). For MUFA in the thigh muscle, the contents of C18:1n9c ($p < 0.05$) and total MUFA ($p < 0.001$; $p < 0.05$) were changed linearly and quadratically, and the C20:1 ($p < 0.05$) content of broilers fed with *C. butyricum* was altered linearly. Compared with the AM group, the C14:1 content in the CBL and CBM groups was increased, the C16:1 content in the CBM and CBH groups was augmented, and the C20:1 content in the CBM group was increased ($p < 0.05$). For PUFA in the thigh muscle, the contents of DGLA and ARA were changed linearly and quadratically ($p < 0.05$), and the contents of total PUFA and the ratio of PUFA to SFA were altered quadratically of broilers fed with *C. butyricum* ($p < 0.05$). Compared with the AM group, the contents of LNA and total PUFA in the CBL and CBM groups were increased, and the PUFA to SFA ratio in the CBM group was increased ($p < 0.05$).

Table 5. Effects of *C. butyricum* on fatty acid composition (mg/g) in breast muscle of broilers [1].

Item	Treatment [2]					SEM [3]	*p* Value	Response to CB	
	CON	AM	CBL	CBM	CBH			Linear	Quadratic
C10:0	0.004	0.004	0.003	0.004	0.000	0.001	0.894	0.529	0.564
C12:0	0.012	0.014	0.010	0.017	0.004	0.002	0.335	0.435	0.286
C14:0	0.208	0.187	0.167	0.204	0.111	0.015	0.287	0.141	0.476
C14:1	0.023	0.030	0.027	0.039	0.014	0.003	0.059	0.572	0.033
C15:0	0.032	0.031	0.026	0.032	0.021	0.002	0.373	0.214	0.650
C16:0	9.424	8.907	8.207	9.535	6.130	0.577	0.350	0.184	0.430
C16:1	1.016 ^{ab}	1.234 ^{ab}	1.113 ^{ab}	1.755 ^{a}	0.656 ^{b}	0.125	0.048	0.678	0.030
C17:0	0.062	0.060	0.054	0.060	0.046	0.003	0.583	0.284	0.705
C18:0	4.973	4.780	4.776	4.722	3.931	0.211	0.629	0.218	0.592
C18:1n9c	10.629 ^{ab}	11.991 ^{ab}	11.126 ^{ab}	15.518 ^{a}	7.601 ^{b}	0.834	0.018	0.395	0.007
C18:2n6c	5.231 ^{b}	6.509 ^{ab}	6.998 ^{ab}	8.108 ^{a}	5.332 ^{b}	0.355	0.018	0.595	0.004
C18:3n3	0.227	0.187	0.166	0.208	0.107	0.018	0.294	0.119	0.640
C20:0	0.079	0.080	0.081	0.072	0.062	0.004	0.492	0.146	0.476
C20:1	0.165	0.201	0.152	0.184	0.132	0.011	0.387	0.585	0.469
C20:2	0.066	0.071	0.071	0.066	0.066	0.003	0.951	0.897	0.729
C20:3n3	0.038	0.041	0.037	0.041	0.045	0.004	0.972	0.593	0.793
C20:3n6	0.464 ^{b}	0.554 ^{ab}	0.530 ^{ab}	0.642 ^{a}	0.471 ^{b}	0.021	0.017	0.392	0.007
C20:4n6	2.182 ^{b}	2.313 ^{ab}	2.357 ^{ab}	2.893 ^{a}	2.375 ^{ab}	0.085	0.044	0.132	0.048
C20:5n3	0.083	0.094	0.088	0.085	0.076	0.003	0.494	0.478	0.366
C21:0	0.378	0.467	0.393	0.392	0.360	0.016	0.263	0.740	0.540
C22:0	0.081	0.076	0.076	0.075	0.064	0.003	0.391	0.113	0.691
C22:1n9	0.024	0.034	0.022	0.022	0.019	0.002	0.092	0.307	0.939
C22:2	0.018	0.021	0.021	0.016	0.008	0.002	0.383	0.202	0.341
C22:6n3	0.181 ^{ab}	0.159 ^{b}	0.169 ^{ab}	0.227 ^{a}	0.196 ^{ab}	0.008	0.030	0.143	0.509
C23:0	0.055	0.066	0.057	0.061	0.051	0.003	0.586	0.833	0.440
C24:0	0.032	0.023	0.030	0.030	0.022	0.002	0.371	0.176	0.548
C24:1	0.072	0.075	0.086	0.077	0.073	0.004	0.848	0.886	0.446
ΣSFA	15.340	14.694	13.879	15.204	10.802	0.816	0.422	0.190	0.466
ΣMUFA	11.930 ^{ab}	13.564 ^{ab}	12.526 ^{ab}	17.595 ^{a}	8.495 ^{b}	0.961	0.018	0.421	0.008
ΣPUFA	8.490 ^{b}	9.949 ^{ab}	10.430 ^{ab}	12.286 ^{a}	8.675 ^{b}	0.451	0.019	0.497	0.006
PUFA/SFA	0.586	0.687	0.775	0.811	0.803	0.033	0.124	0.042	0.158

^{a,b} Means within a row with different letters differ ($p < 0.05$). Results are given as means, $n = 3$. [1] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. [2] The treatments were as follows: CON, basal diet; AM: basal diet containing 150 mg/kg aureomycin; CBL, basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed; CBM, basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed; CBH, basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed. [3] SEM, standard error of the mean.

Table 6. Effects of *C. butyricum* on fatty acid composition (mg/g) in thigh muscle of broilers [1].

Item	Treatment [2]					SEM [3]	*p* Value	Response to CB	
	CON	AM	CBL	CBM	CBH			Linear	Quadratic
C10:0	0.015	0.009	0.008	0.013	0.015	0.001	0.522	0.862	0.140
C12:0	0.024	0.016	0.011	0.020	0.023	0.002	0.176	0.691	0.077
C14:0	0.442	0.256	0.373	0.382	0.472	0.027	0.091	0.682	0.163
C14:1	0.088 ^{a}	0.047 ^{b}	0.083 ^{a}	0.092 ^{a}	0.069 ^{ab}	0.005	0.010	0.206	0.274
C15:0	0.077	0.057	0.063	0.060	0.061	0.003	0.266	0.118	0.335
C16:0	20.261	16.157	20.276	19.652	19.442	0.549	0.064	0.533	0.918
C16:1	2.763 ^{a}	1.974 ^{b}	2.641 ^{ab}	3.033 ^{a}	2.843 ^{a}	0.113	0.007	0.383	0.832
C17:0	0.169	0.102	0.111	0.114	0.135	0.013	0.548	0.526	0.268
C18:0	9.666	8.406	8.437	8.323	8.574	0.253	0.471	0.148	0.157
C18:1n9c	15.702 ^{b}	18.182 ^{ab}	18.310 ^{ab}	21.393 ^{a}	20.403 ^{a}	0.599	0.003	0.001	0.034
C18:2n6c	17.450 ^{a}	11.611 ^{b}	18.471 ^{a}	19.205 ^{a}	16.400 ^{ab}	0.839	0.007	0.208	0.104
C18:3n3	0.558	0.418	0.429	0.575	0.483	0.026	0.181	0.721	0.716
C20:0	0.132	0.102	0.126	0.147	0.114	0.007	0.410	0.687	0.461

Table 6. *Cont.*

Item	Treatment [2]					SEM [3]	*p* Value	Response to CB	
	CON	AM	CBL	CBM	CBH			Linear	Quadratic
C20:1	0.374 [b]	0.382 [b]	0.411 [ab]	0.630 [a]	0.474 [ab]	0.032	0.033	0.032	0.064
C20:2	0.064	0.082	0.083	0.082	0.079	0.004	0.654	0.379	0.311
C20:3n3	0.041	0.052	0.059	0.061	0.056	0.003	0.099	0.089	0.066
C20:3n6	0.307 [b]	0.427 [ab]	0.464 [a]	0.439 [ab]	0.431 [ab]	0.019	0.038	0.035	0.028
C20:4n6	1.521 [b]	2.118 [ab]	2.607 [a]	2.789 [a]	2.418 [a]	0.135	0.003	0.008	0.004
C20:5n3	0.059	0.057	0.073	0.074	0.071	0.003	0.079	0.144	0.130
C21:0	0.345	0.411	0.385	0.381	0.416	0.011	0.268	0.055	0.903
C22:0	0.059	0.071	0.080	0.069	0.069	0.003	0.513	0.598	0.208
C22:1n9	0.029	0.026	0.031	0.033	0.032	0.006	0.719	0.528	0.749
C22:2	0.030	0.021	0.032	0.037	0.032	0.003	0.596	0.777	0.644
C22:6n3	0.122	0.142	0.160	0.168	0.160	0.006	0.142	0.074	0.121
C23:0	0.109 [a]	0.070 [b]	0.072 [b]	0.100 [a]	0.069 [b]	0.006	0.021	0.052	0.686
C24:0	0.028	0.028	0.032	0.029	0.028	0.002	0.965	0.918	0.607
C24:1	0.055	0.080	0.085	0.084	0.071	0.004	0.154	0.289	0.045
ΣSFA	31.329 [a]	25.687 [b]	29.975 [a]	29.290 [ab]	29.419 [ab]	0.595	0.010	0.112	0.384
ΣMUFA	19.011 [c]	20.692 [bc]	21.562 [bc]	25.265 [a]	23.892 [ab]	0.661	0.001	<0.001	0.017
ΣPUFA	20.153 [ab]	14.929 [b]	22.379 [a]	23.431 [a]	20.130 [ab]	0.903	0.004	0.834	0.027
PUFA/SFA	0.644 [ab]	0.579 [b]	0.749 [ab]	0.800 [a]	0.683 [ab]	0.025	0.018	0.332	0.015

[a,b,c] Means within a row with different letters differ ($p < 0.05$). Results are given as means, $n = 3$. [1] SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. [2] The treatments were as follows: CON, basal diet; AM: basal diet containing 150 mg/kg aureomycin; CBL, basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed; CBM, basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed; CBH, basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed. [3] SEM, standard error of the mean.

4. Discussion

The present study introduced an isolated *C. butyricum* strain with potent antioxidant properties as a possible novel feed additive for poultry [23,27]. Little research has evaluated *C. butyricum* effects on the meat quality of broilers. In this study, we mainly investigated the effects of dietary *C. butyricum* NF on carcass traits, antioxidant capacity, meat quality, and fatty acid composition of broiler chickens. The present study showed that supplementing *C. butyricum* NF in the broiler's diet enhanced liver antioxidant properties, improved the sensory qualities of meat in the breast and thigh muscle, and increased some MUFA and PUFA and total MUFA and PUFA concentrations of breast and thigh muscles.

Carcass traits occupy an essential position in broiler production. In the present study, no effects were found on slaughter performance with the inclusion of *C. butyricum* compared to the control treatment. It was found that dietary *C. butyricum* at 1×10^9 CFU/kg did not affect the abdominal in broilers [28]. In contrast, in our previous study, adding 1×10^9 CFU/kg *C. butyricum* in the broilers diet increased the breast muscle yield but decreased abdominal fat [23]. In other studies where a positive effect of *C. butyricum* on carcass traits has been reported, showing that synbiotics included 3×10^9 CFU/kg *C. butyricum* in broilers diets or cherry valley ducks diets reduced abdominal fat, and this dosage of *C. butyricum* in the feed also elevated breast muscle yield in broilers [29,30]. These inconsistent results may be due to strain-specific characteristics, administration level, diet composition, and animal species [31,32]. We speculated that a higher dose is required to improve the carcass traits of broilers regarding this isolated *C. butyricum*, compared with the level of inclusion in this study.

There is a well-managed balance between oxidation and reduction under physiological conditions. However, excess reactive oxygen species (ROS) are generated once the balance is disrupted, along with physiological changes [33]. Subsequently, irreversible detrimental consequences are triggered, including lipid peroxidation, protein, nuclear DNA and mitochondrial dysfunction [34]. With the help of enzymes and transition metals, ROS initiates oxidation of PUFA in meat products and further damages the body for its

nutritional and physiological characteristics [35,36]. As the body's first antioxidant defense, the three major antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), are termed key scavengers of ROS, whose concentration elevation means more potent resistance against oxidative stress [37,38]. On the contrary, malondialdehyde (MDA) is a secondary oxidation product involved in lipid peroxidation [39]. When it accumulates exceedingly in vivo, it will trigger deleterious, mutagenic and carcinogenic effects on organisms, among which one of the intuitionistic manifestations is compromising meat quality [40–43]. Pretreatment with *C. butyricum* could alleviate severe oxidation damage caused by carbon tetrachloride and reverse anomalous changes in SOD, CAT and MDA in mice [44]. Supplementing *C. butyricum* in the feed increased antioxidant enzymatic activity (T-SOD, CAT, and GSH-Px) but decreased the MDA content in the muscle of Peking ducks [17]. Our previous study had also revealed that *C. butyricum* elevated the hepatic SOD activity and serum GSH concentration but lowered the MDA concentration in both liver and serum of broilers [23]. In the present study, the addition of aureomycin to the feed triggered an imbalanced oxidative stress status in broilers at both the starter and grower phases. In our previous study [23], we also observed that dietary aureomycin led to a decline in the serum GSH but an increment in the serum MDA. We can speculate that aureomycin may cause a stress response in the broiler's body. Although there were no significant differences regarding serum antioxidative indices between the CON and *C. butyricum*-supplemented groups, no adverse effects were observed on serum antioxidative capacity in broilers with the inclusion of *C. butyricum*. Nevertheless, we found a linear increase in the concentrations of liver T-AOC, T-SOD, and CAT at the starter phase of broilers in response to increasing doses of *C. butyricum*. Moreover, the inclusion of *C. butyricum* to the feed resulted in the concentrations of liver T-SOD and CAT being linearly enhanced at the grower phase of broilers, while leading to the liver MDA contents being linearly reduced. The augmented antioxidant enzyme activities and decreased MDA contents in the body suggest a decrease in lipid peroxidation and improved whole-body antioxidant status [45]. As a consequence, the elevated antioxidant enzyme activities or lowered ROS can be a helpful indicator of meat quality. Our results suggested that *C. butyricum* could be a potential antioxidant to improve the productive performance of poultry.

With the increasing awareness of a healthy diet, the public has turned their attention to the nutritive chicken meat. Meat color can directly show meat quality and is a leading factor influencing consumer acceptance of food products [46]. The L*, a*, and b* values have been used to distinguish dark-colored from normal-colored broiler chicken. The L* value stands for lightness. Lightness was related negatively to pH and positively to drip and cook loss [47,48]. Myoglobin acts as the primary pigment accountable for the red color. Thus, higher a* value means more myoglobin accumulation in meat [49]. Contrary to the a* value, the b* value represents yellowness. It is generally thought that a lower b* value means less pale meat [50]. Supplementation of *C. butyricum* reduced the L* value of broilers but enhanced a* value of Peking ducks [17,51]. In our previous study [23], dietary *C. butyricum* showed no significant differences with respect to L*, a*, and b* values of broilers. In the current study, we observed that the redness of the breast muscle in broilers was only significantly increased with the inclusion of 4×10^8 CFU/kg *C. butyricum* in the feed, which indicated that administrating *C. butyricum* NF to the feed had a slight impact on meat color in broilers.

Another visual and sensory appeal determinant is the water-holding capacity (WHC). The more water that is retained, the higher the tenderness and juiciness in the meat [52]. In the present study, we employed drip loss and cooking loss to depict the WHC of meat. We luckily discovered that the shearing force was quadratically decreased in the breast muscle, and the cooking loss and shearing force were linearly reduced in the thigh muscle of broilers responded to dosages of *C. butyricum*. Previous studies found decreases in drip loss, cooking loss, and shear force in the breast muscle of poultry supplemented with *C. butyricum* in the diets [17,53]. Other probiotics, such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Bifidobacterium*, have been reported to improve meat quality [25,54]. In contrast, some

studies revealed that dietary probiotics slightly influenced meat sensory properties [55,56]. What we found in this study suggested that *C. butyricum* could improve the sensory quality of broilers, thus making it more appealing to consumers. The possible reasons that dietary *C. butyricum* improves meat quality may be associated with proteome alterations, variation of the nutrition metabolism, and activation of the glutathione and thioredoxin systems in the body [57–59]. The use of probiotics is complex because there are many factors, specific strains, optimal dosage, and an intricate network of interactions between probiotics and the gut microbiota. Further studies should investigate the mechanism of probiotics actions on the meat quality of broiler chickens.

The fatty acid profile of meat has been paid more attention to in recent years. Multiple fatty acids of meat contribute to nutritional value in daily diets and are beneficial for human health. High consumption of saturated fatty acids (SFAs) will lead to the elevation of serum cholesterol and low-density lipoproteins (LDL), which are likely to lead occurrence of some diseases, such as cardiovascular diseases (CDC) and type 2 diabetes [60]. Unsaturated fatty acid (UFA) can be further sorted into two types. One is monounsaturated fatty acids (MUFAs), and the other is polyunsaturated fatty acids (PUFAs). MUFAs can prevent CDC risk under high intake [61,62]. PUFAs have countless contributions to human health since they widely participate in diminishing inflammation, preventing the occurrence of CDC, and protecting the nerve [63–65]. In the current study, supplementing *C. butyricum* to the feed had a positive quadratic effect on the contents of some MUFA and PUFA and total PUFA in the breast muscle. Moreover, the concentrations of some MUFA and PUFA and total MUFA were linearly and quadratically increased, and the contents of total PUFA and the ratio of PUFA to SFA were quadratically augmented in the thigh muscle of broilers responded to the dosages of *C. butyricum*. With respect to the fatty acid profile, the inclusion of 4×10^8 CFU/kg *C. butyricum* had the optimum benefit on the breast muscles in broilers, and the supplementation of 4×10^8 CFU/kg or 8×10^8 CFU/kg in the diet exerted a better positive effect on the thigh muscle. Another remarkable result is that dietary *C. butyricum* has enhanced some PUFA contents, total PUFA, and the PUFA to SFA ratio compared to the aureomycin treatment. Similarly, our previous findings that dietary *C. butyricum* increased PUFA concentrations in the meat of broilers [23]. The comparable results appeared in other species. Dietary *C. butyricum* augmented MUFA and PUFA concentrations in breast muscle of Peking ducks, especially some long-chain PUFA (LC-PUFA) [17]. In recent years, several studies have shown that supplementation of other probiotics (*Lactobacillus johnsonii* and *B. amyloliquefaciens*) increase levels of PUFA and the ratio of PUFA to SFA in the meat of broilers, causing a reduction in abdominal fat in broilers [24,66,67]. The PUFA was proven to attenuate fat accumulation by activating peroxisomal beta-oxidation [68] and lower serum triglyceride concentration [69]. According to the current findings, we could speculate that the widespread dietary *C. butyricum* might be a viable technique for providing better health status for broilers.

Linoleic acid (LNA) and α-linolenic acid (ALA) cannot be synthesized by humans or other animals, so they are defined as essential fatty acids (EFAs). These EFAs subsequently transform into arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The latter two are n-3 PUFAs, which exert myriad health benefits and protect us against inflammatory diseases, cancer, cardiovascular diseases, diabetes and other diseases [70,71]. In recent years, there has been scanty information about *C. butyricum* works on the fatty acid profile of broilers. Previously, dietary *C. butyricum* decreased the ratio of n-6/n-3 fatty acids in the breast muscle and increased EPA and total n-3 fatty acids in broilers [72]. Our previous study showed that supplementation of *C. butyricum* increased broilers' EPA and DHA contents of the breast muscle [27]. In this study, the supplementation of *C. butyricum* in the diet had no positive or negative influence on DHA contents in broilers. Noticeably, the inclusion of 4×10^8 CFU/kg *C. butyricum* showed a significant difference compared to the aureomycin treatment. The different results may be explained by the differences among broilers' breeds. Two former studies were aimed at Arbor Acres broilers, while Ross 308 broilers were used in our experiments.

The mechanisms by which dietary *C. butyricum* regulates the fatty acid composition of meat are not fully explained. The increment in PUFA contents, such as LNA and ARA, observed in this study could be ascribed to an increase in the body's antioxidant activity. The antioxidant property of *C. butyricum* was supported in the present study, and this could hinder the peroxidation of tissue lipids, especially LC-PUFA. PUFAs are the preferred targets for ROS [39]. It is an excellent approach for employing antioxidants to maintain oxidant/antioxidant balance in animals and improve product quality by preventing lipid oxidation [35]. Some metabolites or bioactive substances from *C. butyricum* exert positive effects on inhibiting pathogens adhered to the intestines, modifying the gut microbial composition and protecting the integrity of the intestinal epithelial barrier, which could maintain and promote nutrients digestion and absorption [73–75]. The adjustment of fatty acid profiles may ascribe to the beneficial effects of metabolites from *C. butyricum*. In summary, it is well understood that dietary *C. butyricum* strongly affects animal health and meat quality by affecting PUFA deposition and strengthening the antioxidant status of broilers. Furthermore, our findings showed positive effects on the growth performance of broilers after dietary supplementation with *C. butyricum* (data not published) [76]. However, further studies are required to confirm the mechanism of *C. butyricum* supplementation works on broilers meat and investigate the association among its antioxidant properties, meat quality, and alteration of fatty acid profiles.

5. Conclusions

The results from our study indicated that *C. butyricum* as a natural feed additive in the broiler's diet improved liver antioxidant capacity, meat quality and fatty acid composition in the meat. Supplementation of *C. butyricum* in the broiler's diet has the potential to improve the nutritive value of meat and fatty acid profiles, thus benefiting human health. The positive alteration of fatty acid composition may be attributed to the enhanced antioxidant status of broilers. Therefore, this study demonstrated that *C. butyricum* could be successfully used as a potential antioxidant to in-feed additives for broiler chickens. It can be concluded that dietary *C. butyricum* supplementation at the level of 8×10^8 CFU/kg of diet have the benefit of enhancing the antioxidant capacity of broilers, while the dosages of 4×10^8 CFU/kg or 8×10^8 CFU/kg are more beneficial for altering the fatty acid composition of broilers meat. Further studies are required to confirm the mechanism of *C. butyricum* supplementation works on broiler meat and investigate the association between its antioxidant properties, meat quality, and alteration of fatty acid profiles.

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Data Availability Statement: The datasets during and/or analyzed during the current study available from the corresponding authors on reasonable request.

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Abbreviations

C. butyricum: *Clostridium butyricum*; CON: basal diet; AM: basal diet containing 150 mg/kg aureomycin; CBL: basal diet supplemented *C. butyricum* at 2×10^8 CFU/kg feed; CBM: basal diet supplemented *C. butyricum* at 4×10^8 CFU/kg feed; CBH: basal diet supplemented *C. butyricum* at 8×10^8 CFU/kg feed; CFU: colony-forming units; FAO: Food and Agriculture Organization; CGMCC: China General Microbiological Culture Collection Center; NRC: National Research Council; T-AOC: Total antioxidant capacity; T-SOD: total superoxide dismutase; CAT: catalase; GSH-Px: glutathione peroxidase; MDA: malonaldehyde; L*: lightness; a*: redness; b*: yellowness; pH_{45min}: muscle pH value at 45 min postmortem; pH_{24h}: muscle pH value at 24 h postmortem; WHC: water-holding capacity; SFA: saturated fatty acid; UFA: unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; LNA: linoleic acid; ARA: arachidonic acid; DHA: docosahexaenoic acid; DGLA: dihomo-gamma-linolenic acid; ROS: reactive oxygen species; LDL: low-density lipoproteins; CDC: cardiovascular diseases; LC-PUFA: long-chain PUFA; EFA: essential fatty acids; ALA: α-linolenic acid; EPA: eicosapentaenoic acid.

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