THE JOURNAL OF
APPLIED POULTRY RESEARCH

Verify the effectiveness of various inclusions of butyrate on male broilers raised on used litter without antibiotics

C. J. Fritzlen, J. J. Maurer, E. A. Wong, and M. E. Persia [1]

The School of Animal Sciences at Virginia Tech, Blacksburg, VA, 24061, USA

Primary Audience: Researchers, Nutritionists

SUMMARY

An experiment was conducted to verify the effectiveness of butyrate (**BA**) in diets of broiler chickens raised without antibiotics and exposed to used litter. Dietary treatments included: negative control (**NC**), a nonsupplemented diet on fresh shavings; positive control (**PC**), the same nonsupplemented diet on used litter; 500 BA, similar diet with 500 ppm BA on used litter; 1,000 BA, similar diet with 1,000 ppm BA on used litter; 500/250 BA, similar diet with 500 ppm BA from 0 to 8 d and 250 ppm BA from 8 to 42 d on used litter; 1,000/250 BA, similar diet with 1,000 ppm BA from 0 to 8 d and 250 ppm BA from 8 to 42 d on used litter. From 0 to 8 d, the PC resulted in a 6.8 g decrease in BW gain (**BWG**) compared to NC, but this response was lost from 0 to 25 d or 0 to 42 d. There were no differences in mortality corrected, FCR (FCRm) between PC and NC. All BA treatments increased BWG in comparison to PC from 0 to 8 d, with no differences from NC. Butyrate improved 0 to 8 d FCRm compared to both PC and NC ($P \leq 0.05$), but these responses were lost over time ($P > 0.05$). Butyrate increased apparent ileal digestibility of energy and DM ($P > 0.05$). Butyrate had no effect on oocyst shedding compared to PC ($P > 0.05$). Butyrate was able to ameliorate the negative performance effects with reused litter over the 8-d starter period and was able to increase ileal digestibility of energy and DM.

Key words: broiler, performance, oocyst shedding, coccidiosis, feed additive

2024 J. Appl. Poult. Res. 33:100466
https://doi.org/10.1016/j.japr.2024.100466

DESCRIPTION OF PROBLEM

Avian coccidiosis persists as a challenge to the poultry industry, stemming from host-specific protozoa belonging to the genus *Eimeria* (Chapman et al., 2013). Coccidiosis may be classified into 3 levels: coccidiasis (mild infection, no adverse effects), subclinical coccidiosis (appear normal, reduced performance), and clinical coccidiosis (severe infection, diarrhea, high mortality), the latter 2 being of concern (Williams, 2005). In 2016, the worldwide impact of subclinical and clinical coccidiosis was estimated at approximately US $14.5 billion (Blake et al., 2020). Subclinical coccidial infections are the most prevalent and inflict damage to epithelial cells, diminish nutrient absorption, increase FCR, and reduce growth performance (Persia et al., 2006; Amerah and Ravindran, 2015; Chalvon-Demersay et al., 2021; de Freitas et al., 2023). Historically, the

[1]Corresponding author: mpersia@vt.edu

control of coccidiosis has relied on the use of vaccines, ionophores and chemical anticoccidials as part of a rotation of products. However, the ability to use ionophores have been increasingly constrained or eliminated due to regulatory measures and marketing claims, removing options for rotation. Production systems where poultry are raised without antibiotics (**RWA**) or no antibiotics ever (**NAE**) are restricted from using antibiotics, including ionophores, as FSIS recognizes ionophores as an antibiotic (Singer et al., 2019). Consequently, cost-effective alternatives to antibiotics are needed to effectively and efficiently manage coccidiosis in the commercial poultry industry.

Butyric acid, a short-chain fatty acid, is primarily produced through microbial fermentation processes (Xiong et al., 2022). Due to the rapid volatility and pungent smell, butyric acid is commonly supplemented in the form of butyrate (**BA**) bound to calcium or sodium (Kaczmarek et al., 2016). To regulate the release time and effectiveness of BA, it is often encapsulated in vegetable fat (Liu et al., 2017). This protective coating allows more of the BA to reach the small intestine of broilers. Supplementation of 200, 300, and 400 ppm of a coated BA improved FCR, nutrient digestibility, and increased ileal villus height compared to control broilers without BA supplementation (Kaczmarek et al., 2016). Similarly, 1,000 ppm of a coated BA improved FCR and increased villus height in the jejunum and ileum of male broilers at 21 d of age (Zhao et al., 2022). The protective effects of BA on the intestinal system are important considering the potential intestinal damage (decreased villus height) and reduced absorptive surface caused by coccidial infection (Calik et al., 2019).

Supplementation of an encapsulated BA at 400 and 800 ppm increased BW and 800 ppm further reduced mortality corrected FCR (FCRm) over the 42-d experiment (Sizmaz et al., 2022). Additionally, 400 ppm of the encapsulated BA had some efficacy in reducing litter oocyst counts. Younger broiler chicks are typically more susceptible to coccidiosis in comparison to older broilers that have had a chance to build immunity (Lawal et al., 2016). As such, it is possible that the effectiveness of BA supplementation may vary over time as the birds age and mature. Additionally, broilers consume more feed as they grow heavier, potentially allowing for lower inclusions of BA in the grower and finisher phases as a method to reduce feeding costs. Therefore, the current experiment was conducted to verify the effectiveness of BA on broiler chickens raised without antibiotics and exposed to used litter, and explore cost effective feeding strategies of higher BA concentrations fed when broilers are younger before transitioning to a lower dose later in the production cycle.

MATERIALS AND METHODS

Animals and Experimental Diets

This experiment was conducted according to the guidelines and review of the Virginia Tech Institutional Animal Care and Use Committee. A total of 2,520 male Ross 708 broiler chicks were selected from a larger group of chicks obtained from a commercial hatchery (Myers Hatchery, South Fork, PA). Broilers chicks received a brief inspection for general health before they were selected for similar BW across experimental treatments. Experiment treatments were randomly assigned to pen within a block, resulting in 6 experimental treatments with 12 replicate pens of 35 chicks each. Initially, the floor pens (1.372 m × 1.372 m) were stocked with birds at a density of 0.05 m^2 per broiler. By the end of the experiment, the final stocking density was 0.09 m^2 per broiler (maximum of 21 birds per pen after 14 birds were euthanized for sample collection on d 14). Treatments consisted of a negative control (**NC**), chickens receiving a nonsupplemented diet and housed on clean pine shavings; a positive control (**PC**), chickens receiving the same diet but housed on used pine shaving litter; and 4 treatments in which chickens received various inclusions of coated sodium BA (Ultramix C, Adisseo, Alpharetta, GA) at: 500 ppm from d 0 to 42 (500 BA), 1,000 ppm from d 0 to 42 (1,000 BA), 500 ppm d 0 to 8 and 250 ppm from d 8 to 42 (500/250 BA), or 1,000 ppm from d 0 to 8 and 250 ppm from d 8 to 42 (1,000/250 BA) with all chickens receiving BA treatments housed on used litter. All diets were

generated using a basal diet of common ingredients, including titanium dioxide used in the grower diet as an inert dietary marker, before BA additions to generate complete experimental diets. Dietary formulation for starter (D 0 −8), grower (D 8−25), and finisher diets (D 25 −42) are described in Table 1. Starter feed was provided as crumbled pellets, while grower and finisher diets were provided as pellets. Broilers were provided *ad libitum* access to experimental feed and water over the duration of the experiment.

The used litter model employed in this experiment was based on previous research (Sizmaz et al, 2022). In each pen designated for used litter, 30 d old male chicks, off males from a female broiler breeder line, were provided approximately 600 g of feed treated with a coccidial vaccine via a cardboard tray for the first 3 d. This treated diet was prepared by mixing 1 vial of Coccivac B-52 (Merck Animal Health, Rahway, NJ) with a small quantity of mash feed, which was then mixed with the remaining mash feed to produce 45 kg of treated mash feed. Following the initial 3 d, the seeder birds were transitioned to standard starter and grower diets until reaching 21 d of age, at which point both the seeder birds and feed were removed from all pens. All used litter was moved to the center of the house, thoroughly mixed manually

Table 1. Formulation and nutrient composition of experimental diets fed to male Ross 708 broiler chickens provided of various concentrations of dietary butyrate (BA) raised on fresh pine shavings or used litter.[1]

Ingredient	Starter (0−8 d)	Grower (8−25 d)	Finisher (25−42 d)
	(%)		
Corn	60.55	62.84	64.01
Soybean meal (48% CP)	27.32	21.61	18.39
Poultry by-product meal	4.00	6.00	6.00
Dried distillers grains with solubles	3.00	3.43	5.56
Soy oil	0.53	1.52	2.83
Salt (sodium chloride)	0.09	0.07	0.09
Sodium Bicarbonate	0.35	0.35	0.30
DL-Methionine	0.35	0.29	0.24
L-Lysine•HCl (78.5%)	0.38	0.32	0.24
L-Threonine	0.13	0.09	–
Limestone	1.02	0.91	0.85
Dicalcium phosphate	1.12	0.72	0.45
Choline chloride (60%)	0.10	0.10	0.10
Vitamin and mineral premix[2]	0.63	0.50	0.50
Titanium dioxide[3]	0.00	0.30	0.00
Phytase[4]	0.01	0.01	0.01
Nutrient Composition[5]		(%)	
Crude protein	22.00 (21.70)	20.65 (20.82)	19.57 (18.47)
Metabolizable energy (kcal/kg)	3000	3100	3200
Calcium	0.86	0.77	0.68
Nonphytate phosphorus	0.38	0.34	0.29
Crude fat	4.06 (2.52)	5.35 (3.39)	6.78 (4.75)
Crude fiber	2.66 (2.27)	2.60 (2.16)	2.65 (2.32)
Digestible methionine + cysteine	0.95	0.87	0.80
Digestible lysine	1.28	1.15	1.02
Digestible threonine	0.86	0.77	0.66

[1]Treatment diets were obtained by supplementing Ultramix C at 0.106%, 0.211%, and 0.422% to provide 250, 500, and 1,000 ppm of butyrate, respectively.

[2]Vitamin and mineral premix: provided per kg of premix: cobalt, 34 mg; copper, 540 mg; iodine, 134 mg; iron, 6,750 mg; manganese, 8,580 mg; zinc, 6,500 mg; vitamin A, 881,849 IU; vitamin D3, 295,419 ICU; vitamin E, 220 IU; vitamin B12, 0.88 mg; menadione, 154 mg; riboflavin, 551 mg; D-pantothenic acid, 811 mg; niacin, 2,646 mg; choline, 51,030 mg.

[3]Titanium Dioxide was added as an inert marker for digestibility determination.

[4]Phytase was formulated to provide 0.10% Ca and nonphytate phosphorus, Quantum BLUE 5 G, AB Vista, Marlborough, UK.

[5]Values within parenthesis are analyzed values for complete diets.

using a pitchfork, and redistributed into the designated used litter pens. The pens designated as NC remained empty until the used litter was redistributed to other pens, and fresh, clean pine shavings were added. Experimental broilers were placed into the pens and the formal experiment began on the same d as seeder birds were removed. The pen temperature was initially set to 35˚C for the first 3 d and gradually decreased until 20˚C was reached and maintained. Continuous lighting and supplemental heat were provided through heat lamps from 0 to 4 d of age, after which a lighting schedule of 20 h of light and 4 h of darkness was implemented. Health checks and mortality removal occurred at least twice daily to ensure the well-being of the birds throughout the experimental period.

Growth Performance

Birds were weighed on a pen basis on d 0, 8, 25, and 42 to align with changes in the diet phases. BW gain (**BWG**) and feed intake (FI) were calculated for the 0 to 8, 0 to 25, and 0 to 42 d periods. BW gain was determined by subtracting the initial pen weight from the respective pen weights and expressed on a per bird basis for each period. Feed intake was calculated by the difference of feed offered and refused over the same periods. Broilers that died or were culled for health or sample collection were weighed, recorded, and utilized to correct the FCR. Mortality corrected FCR (FCRm) was calculated by dividing the total pen feed intake by sum of the pen BWG plus the mortality BWG.

Ileal Apparent Digestibility

On d 14, fourteen broilers from each pen were weighed and euthanized for the collection of ileal contents. The contents from the posterior half of the ileum, defined by Meckel's diverticulum to the ileal-cecal junction, were gathered by gentle physical manipulation. Ileal contents were pooled by pen into WHIRL-PAK bags (Filtration Group, Austin, TX) and kept on ice until frozen for later analysis. The ileal samples were weighed before drying, then dried in a forced air oven at 55˚C for 24 h to determine ileal DM. Dried ileal content and feed were subsequently ground using a coffee grinder. The gross energy of both the ileal contents and feed were measured in duplicate using a bomb calorimeter (Parr 6400 Calorimeter, Parr Instrument Company, Moline, IL). Both feed and ileal content were digested with sulfuric acid to determine titanium content following the procedures outlined by Leone (1973). Approximately 0.3 g of dried ileal contents and 0.5 g of dried feed were weighed in duplicate for ileal contents or quadruplicate for feed. Apparent ileal energy digestibility (**AIDe**) and apparent ileal DM digestibility (AIDdm) were calculated using the following equation (Gautier and Rochell, 2020):

$$AID\% = (((diet\ component\ in\ diet\ /\ TiO_2\ in\ diet) - (diet\ component\ in\ ileal\ /TiO_2\ in\ ileal)) / (diet\ component\ in\ diet\ /\ TiO_2\ in\ diet)) * 100$$

Intestinal Morphology

One of the fourteen broilers euthanized for ileal content was selected to obtain a histology sample on d 14. A 1-inch segment from the center point of the jejunum was carefully flushed with formalin to remove contents and placed into a 5 mL conical tube containing 10% formalin. Once fixed, the method for handling and examining the jejunum followed procedures described by Vasanthakumari and colleagues (2023). Crypt depth was determined based on morphological differences between the crypt and villus structures, while VH was measured from the apex of the functional crypt to the tip of the villus. The VH/CD ratio was calculated based on these measurements. For each sample, approximately 40 to 50 measurements of CD and 10 to 15 measurements of VH were measured for 6 replicate samples for each treatment.

Intestinal Permeability

On d 15, 1 bird per pen was selected and orally gavaged with 1 mL of 8.32 mg/kg of fluorescein isothiocyanate-dextran (**FITC-d**) dissolved into double distilled water following the methods of Baxter and coworkers (2017).

The concentration of serum fluorescence was determined using a standard curve of FITC-d, generated by adding FITC-d into serum from broilers in the same experiment that were not administered FITC-d.

Quantitative Real-Time PCR

Intestinal tissues were gently scraped with a glass microscope slide to collect the mucosa from the underlying muscle layer of a 5 cm section in the center of the duodenum. These samples were then promptly frozen in liquid nitrogen and stored at −80°C for preservation. Mucosal scrapings from the duodenum were later homogenized in TriReagent (Molecular Research Center Inc., Cincinnati, OH), and total RNA extraction was performed using the Direct-ZOL RNA MiniPrep Kit (Zymo Research, Irvine, CA). Reverse transcription polymerase chain reaction (**RT-PCR**) was performed following the procedures of Cloft and coworkers (2023). Each sample was amplified in duplicate, and amplification efficiency was determined by standard curves to ensure equal efficiency between target genes and the reference gene. Relative fold change was calculated using the $2-\Delta\Delta C$ T method (Schmittgen and Livak, 2008) with glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) and ribosomal protein lateral stalk subunit P1 (**RPLP1**) serving as the reference genes. The genes measured and primers used for tight junction proteins (Claudin-1, CLDN-1; junctional adhesion molecule A, JAMA), host defense peptides (Avian β-defensin 10, AvBD10; Avian β-defensin 11, AvBD11), and inflammation markers (Myeloid differentiation primary response protein 88, MyD88; lipopolysaccharide-induced TNF-alpha factor, LITAF) are provided in Table 2.

Body Composition

On d 42, 5 birds from each pen were weighed before being euthanized via cervical dislocation, scalded at 60°C (Brower AM 48, Houghton, IA), and de-feathered (Ashley SP23, Greensburg, IN). Carcasses were frozen at −20°C until analysis. Upon thawing, the 5 carcasses per pen were scanned as a single sample utilizing dual energy X-ray absorptiometry (DXA, General Electric Healthcare, Madison, WI). Fat mass (g), lean mass (g), and total mass (g) were calculated and reported (Murugesan and Persia, 2013).

Oocyst Shedding

On d 5 to 7, 11 to 13, 17 to 19, and 22 to 24, fresh excreta were gathered from each pen to assess oocyst shedding. Each d, approximately 2 to 4 fresh excreta samples were collected, and these samples were combined over 3 consecutive d to form 1 composite sample per pen for each collection period. The method for handling and storing the fresh excreta followed procedures outlined by Long (1970). These

Table 2. Sequence of primers for gene expression using real-time PCR.

Gene	GenBank accession number	Amplicon size (bp)	Forward/reverse primers (5'→3')
CLDN1[1]	NM_001013611.2	115	TGGAGGATGACCAGGTGAAGA/ CGAGCCACTCTGTTGCCATA
JAMA[1]	NM_001083366.1	90	GAAAACCAACCCGTGGACAT/ GGAAGAGCCCTTCTGGAACTT
AvBD10[2]	NM_001001609.2	64	CAGACCCACTTTTCCCTGACA/ CCCAGCACGGCAGAAATT
AvBD11[2]	NM 001001779.1	56	GGTACTGCATCCGTTCCAAAG/ GCATGTTCCAAATGCAGCAA
MyD88[3]	NM_001030962.5	64	GGATGTCTTGCCAGGAACGT/ CCGACACCTTCTTTCTATGAGTTCT
LITAF[3]	NM_204267.2	62	CCCCTACCCTGTCCCACAA/ ACTGCGGAGGGTTCATTCC

[1]Primers′ sequence obtained from Jia et al. (2023).
[2]Primers′ sequence obtained from Garcia et al. (2021).
[3]Primers′ sequence obtained from Kinstler (2023).

composite samples were later standardized to 40 mL within conical tubes. Oocysts were quantified in quadruplicate using a counting chamber (McMaster; JA Whitlock & Company, New South Wales, Australia) and a microscope (Olympus CX21-FS1, Tokyo, Japan) set at 10x magnification (Dalloul et al., 2003). The total number of oocysts (both sporulated and unsporulated) were estimated using the formula:

$$\text{Total oocysts/g of excreta} = (\text{oocyst counted} / \text{chamber volume}) \times \text{dilution factor} \times (\text{composite sample volume/g of excreta used}).$$

Statistical Analysis

All data were analyzed using JMP Pro 16 (SAS Institute Inc., Cary, NC) by 1-way ANOVA. Each pen or sample within a pen was considered an experimental unit. Statistical differences were considered significant if ANOVA was $P \leq 0.05$. If significant ANOVA differences were noted, means were separated using Student's t-test (Sarsour and Persia, 2022). One replicate pen from the 1,000 BA treatment was excluded from analysis due to a water issue.

RESULTS AND DISCUSSION

There were no differences in initial broiler BW across experimental treatments with an average of 44.3 g per chick and a range of 41.5 to 46.1 g per chick ($P = 0.94$; SEM = 0.23). The use of reused litter seeded with coccidial vaccine resulted in a 6.8 g or a 6.5% decrease in 0 to 8 d BWG in comparison to the NC ($P \leq 0.05$) birds raised on clean pine shavings (Table 3). This response agrees with previous research as broiler chicks are more susceptible to stress and potentially disease during the first wk of life as the immune system is underdeveloped (Panda et al., 2015; Yerpes et al., 2020). Despite the reduced BWG, the differences in litter did not result in differences in feed utilization as there was no difference in FCRm between the PC and NC from 0 to 8 d ($P > 0.05$). It appears that the subclinical coccidial

Table 3. Effects of various concentrations of dietary butyrate (BA) on BW gain (BWG) and mortality corrected FCR (FCRm) of 0 to 42 d-old male Ross 708 broiler chickens raised on fresh pine shavings or used litter.[1]

	D 0−8		D 0−25		D 0−42	
Treatments[2]	BWG (g/bird)	FCRm[3] (g/g)	BWG (g/bird)	FCRm[4] (g/g)	BWG (g/bird)	FCRm[5] (g/g)
NC	107.5[a]	0.931[a]	1,066	1.304	2,825	1.660
PC	100.7[b]	0.944[a]	1,026	1.303	2,803	1.653
500 BA	107.1[a]	0.883[b]	1,051	1.295	2,789	1.654
1,000 BA	105.4[a]	0.881[b]	1,048	1.296	2,799	1.646
500/250 BA	108.3[a]	0.851[b]	1,062	1.285	2,784	1.653
1,000/250 BA	106.1[a]	0.857[b]	1,034	1.299	2,808	1.646
Pooled SEM	1.53	0.015	12.3	0.008	31.7	0.011
P-value	≤0.01	≤0.01	0.11	0.64	0.95	0.95

[1]Dietary treatments consisted of 12 replicate pens of 35 birds. One replicate from 1,000 B was excluded due to aberrant replicate value. Average initial BW = 44.3 g per chick ($P = 0.94$, SEM = 0.23).

[2]NC = nontreated diet with fresh pine shaving litter; PC = nontreated diet with used litter; 500 BA = diet with 500 ppm of BA on used litter; 1,000 BA = diet with 1,000 ppm of BA on used litter; 500/250 BA = diet with 500 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter; 1,000/250 BA = diet with 1,000 ppm of BA from 0 to 8 d and 250 ppm of butyrate from 8 to 42 d on used litter.

[3]Total mortality % per treatment for D 0 to 8: NC = 1.9%; PC = 0.7%; 500 BA = 0.5%; 1,000 BA = 0.5%; 500/250 BA = 0.7%; 1,000/250 BA = 1.7%.

[4]Total mortality % per treatment for D 0 to 25: NC = 2.4%; PC = 1.7%; 500 BA = 2.1%; 1,000 BA = 1.6%; 500/250 BA = 1.4%; 1,000/250 BA = 3.1%.

[5]Total mortality % per treatment for D 0 to 42: NC = 3.3%; PC = 2.6%; 500 BA = 2.9%; 1,000 BA = 2.3%; 500/250 BA = 2.6%; 1,000/250 BA = 3.3%.

[a-b]Columns without a common superscript are different ($P \leq 0.05$).

exposure noted in the starter phase moderated resulted in coccidiasis as performance differences noted early were lost from 0 to 25 and 0 to 42 d ($P > 0.05$). This moderation of the coccidial exposure is further supported by the lower shedding of coccidial oocysts noted over 2 to 3 wk of exposure as outlined below.

Treatment of broilers with BA increased 0 to 8 d BWG by 6.8, 6.4, 4.7, 7.6, and 5.4 g for PC, 500 BA, 1,000 BA, 500/250 BA, and 1,000/250 BA, respectively, in comparison to the PC broilers ($P \leq 0.05$). This increased BWG in comparison to the PC treated birds resulted in BWG similar to the NC birds raised on clean litter ($P > 0.05$). Moreover, supplementation of BA improved 0 to 8 d FCRm to 0.883, 0.881, 0.851, and 0.857 for 500 BA, 1,000 BA, 500/250 BA, and 1,000/250 BA, respectively, in comparison to both the NC (0.931) and PC (0.944: $P \leq 0.01$). However, there were no longer differences with BA supplementation in BWG or FCRm over the 0 to 25 d and 0 to 42 d periods ($P > 0.05$). Supplementation of 700 ppm of a coated sodium BA to nonchallenged broilers increased BWG and improved FCR (Chamba et al., 2014). Butyrate coated with a mixture of mono-, di-, and triglycerides at 1,000, 2,000, and 4,000 ppm resulted in no significant impact on broiler performance when those broilers were vaccinated against coccidiosis at 1 d of age (Lesson et al, 2005). Similarly, diets supplemented with 1,000 ppm of BA in nonchallenged broilers had no effect on BWG or FCR (González-Ortiz et al., 2019). Furthermore, feed supplemented with sodium BA at 200, 400, 800 or 1,000 ppm to nonchallenged broilers resulted in no differences in BWG or FCR ($P > 0.05$) over both 1 to 21 or 1 to 42 d (Wu et al., 2018). In contrast to the current experiment, Sizmaz and coworkers (2022) observed increased overall BWG in broilers raised on used litter seeded with coccidia when encapsulated BA was supplemented at 400 and 800 ppm in broiler feed. Additionally, an improvement in FCRm was observed with BA supplementation at 800 ppm from d 1 to 42. The inconsistency of these findings generally supports the argument that BA is less effective at improving performance in broilers that are not challenged or only mildly challenged, and more effective under higher challenge conditions. Overall, the literature is inconsistent regarding the effects of BA on broiler growth performance, highlighting the importance of documenting the level of challenge provided to the birds when BA is used as a feed additive.

There were no differences observed among treatments for broiler jejunum VH, CD, or VH/CD ratio at 14 d of age ($P > 0.05$), regardless of litter or dietary BA (Table 4). Supplementation of sodium BA at 500, 1,000, and 2,000 ppm had no effect on the VH or CD of the jejunum in nonchallenged broilers at 21 d of age (Hu and Guo, 2007). Furthermore, sodium BA at 200, 400, 800, and 1,000 ppm had no effect on jejunum VH or CD, but it did tend to increase ileum VH (Wu et al., 2018). The lack of significant effects of BA supplementation on the jejunum could potentially be attributed to the encapsulation of BA, which may be influencing its distribution and absorption within the broiler gastrointestinal tract. Liu et al. (2017) conducted an experiment determining the effect of 3 sodium BA products, each with varying release times. It was observed that the effects on intestinal morphology at different sections of the gastrointestinal tract were influenced by the inclusion level along with the release time of the sodium BA. In the context of a mild challenge, such as in the current experiment, the broilers did not exhibit significant improvements with BA supplementation. In contrast, a subclinical to clinical challenge involving notable VH changes, as seen in other studies, showed that BA supplementation is more effective under higher stress conditions, supporting the idea that feed additives like BA might have greater responses when broilers face more substantial challenges.

There were no differences in serum FITC-d concentrations among treatments on d 15 ($P > 0.05$; Table 4). The similar serum FITC-d concentrations suggest that there were no substantial differences in intestinal structure and integrity among the treatments or controls. This response may be attributed to the mild challenge conditions generated in this experiment and are consistent with the lack of difference after the initial 8 d starter period in the performance data. Supplementation of 500 ppm of encapsulated BA to broiler chickens resulted in no differences in intestinal permeability, as

Table 4. Effects of various concentrations of dietary butyrate (BA) on D 14 jejunum morphology, nutrient digestibility, and D 15 intestinal permeability (FITC-d) of 0 to 42 d-old male Ross 708 broiler chickens raised on fresh pine shavings or used litter.[1]

	D 14					D 15
Treatments[2]	VH (μm)	CD (μm)	VH/CD (μm/μm)	AIDe[3] (%)	AIDdm[4] (%)	FITC-d (ng/mL)
NC	833	220.7	4.11	71.1[b]	65.7[b]	157
PC	887	193.8	4.76	71.1[b]	65.4[b]	156
500 BA	976	223.3	4.37	73.5[a]	68.5[a]	157
1,000 BA	802	230.2	3.49	74.6[a]	70.1[a]	157
500/250 BA	821	206.1	4.13	73.2[ab]	69.2[a]	156
1,000/250 BA	983	211.5	4.78	73.3[ab]	69.6[a]	152
Pooled SEM	64.0	16.88	0.46	N/A[5]	N/A	2.36
P-value	0.16	0.67	0.34	0.03	≤0.01	0.57

[1]Dietary treatments consisted of 12 replicate pens of 35 birds. One replicate from 1,000 B was excluded due to aberrant replicate value.

[2]NC = nontreated diet with fresh pine shaving litter; PC = nontreated diet with used litter; 500 BA = diet with 500 ppm of BA on used litter; 1,000 BA = diet with 1,000 ppm of BA on used litter; 500/250 BA = diet with 500 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter; 1,000/250 BA = diet with 1,000 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter.

[3]AIDe = Apparent ileal energy digestibility.

[4]AIDdm = Apparent ileal DM digestibility.

[5]Percentage data were arcsine transformed before analysis to ensure normality.

[a–c]Columns without a common superscript are different ($P \leq 0.05$).

measured by FITC-D, when the broilers were challenged with a 10x dose of an attenuated live coccidiosis vaccine (Naghizadeh et al., 2022). The lack of differences in intestinal permeability could be due to the timing of the challenge and sampling. Broilers orally gavaged with 2×10^5 sporulated *E. maxima* oocysts only showed compromised intestinal permeability on d 5 and 6 postinoculation (Schneiders et al., 2019). Similarly, Teng and colleagues (2020) assessed intestinal permeability on d 3, 5, 6, 7, and 9 postinoculation in broiler chickens orally gavaged with *E. maxima, E. tenella,* and *E. acervulina*, with differences in intestinal permeability observed on d 5, 6, and 7, but not on d 3 and 9. It is crucial to note that the natural inoculation method on used litter, as conducted in the current study, does not replicate the high single dose of coccidia provided by direct oral gavage. This natural inoculation likely leads to a more gradual and variable exposure to the pathogens, which might not result in the same peak periods of compromised intestinal permeability as observed in studies with direct gavage. This variability in exposure and the resulting differences in the timing and magnitude of intestinal challenges could explain the absence of detectable differences in intestinal permeability in the current study.

There were no significant differences in AIDe when broilers were raised on clean or used litter as NC and PC both had 71.1% AIDe (Table 4). However, the supplementation of 500 and 1,000 BA to broilers on used litter significantly increased AIDe by 2.4 and 3.5%-points in comparison to the NC and PC broilers. Meanwhile, 500/250 BA and 1,000/250 BA were intermediate with a 2.1 and 2.2%-point increase. This response in AIDe was mirrored with AIDdm as supplementation of BA was able to increase AIDdm for 500 BA, 1,000 BA, 500/250 BA, and 1000/250 BA, 68.5, 70.1, 69.2, and 69.6% AIDdm, respectively, in contrast to the controls that resulted in a 65.7% AIDdm for the NC and 65.4% AIDdm for the PC ($P \leq 0.01$). These results agree with previous research that reported that supplementation of sodium BA at 500 and 1,000 ppm increased ileal energy digestibility in nonchallenged broilers (Liu et al., 2017). Furthermore, calcium BA supplemented at 300 ppm increased total tract crude fat digestibility and AMEn, as well as ileal CP digestibility in broilers at 14 d of age (Kaczmarek et al., 2016). The observed

increase in AIDe in broilers supplemented with BA, suggests that BA likely increases energy digestibility by improving overall gastrointestinal integrity and potentially increasing nutrient absorption. This response indicates that BA mode of action may involve mechanisms beyond reducing gut permeability.

The effects of various inclusions of BA on gene expression of tight junction proteins, host defense peptides, and inflammation markers in the duodenum on d 15 are presented in Table 5. Gene expression for tight junction proteins was inconsistent. There were no differences in CLDN-1 expression (P = 0.92), but JAMA expression was trending (P = 0.06). The expression of JAMA was increased in the PC compared to the NC. All BA treatments reduced expression compared to the PC. This suggests that BA supplementation may reduce JAMA expression in broilers challenged with used litter. In previous research, broilers on a BA supplemented diet that were then orally gavaged with mixed strains of *Eimeria* and finally with an oral inoculation of *C. perfringens* may increase the gene expression of tight junction proteins, potentially strengthening the intestinal barrier function (Song et al., 2017). However, this response was not observed in this current experiment, potentially due to the mild *Eimeria* challenge. Similar to the variability in expression of tight junction protein genes, the expression of host defense peptides also exhibited inconsistency. Gene expression of AvBD10 was not significantly different among treatments, while AvBD11 gene expression exhibited a trend (P = 0.06). Similarly to JAMA, AvBD11 expression was increased in the PC compared to the NC. The 500 BA, 1,000 BA, 500/250 BA, and 1,000/250 BA treatments all showed decreased expression levels compared to the PC, indicating that BA supplementation may decrease AvBD11 expression in broilers challenged with used litter. Previous research has suggested that BA supplementation has the potential to up-regulate avian β-defensins within chicken jejuna and cecal explants *in vitro* (Sunkara et al., 2011; Yang et al., 2021). However, *in vivo*, avian β-defensins expression has been inconsistent. In 2 experiments by Su and colleagues (2017), avian β-defensins

Table 5. Effects of various concentrations of dietary butyrate (BA) on D 14 gene expression of tight junction proteins, host defense peptides, and inflammation markers in the duodenum of 0 to 42 d-old male Ross 708 broiler chickens raised on fresh pine shavings or used litter.[1]

	Tight Junction Proteins[3]		Host Defense Peptides[4]		Inflammation Markers[5]	
Treatments[2]	CLDN-1	JAMA	AvBD10	AvBD11	MyD88	LITAF
	(Fold change)[6]					
NC	1.16	1.10	0.97	1.09	1.29	1.05
PC	1.27	4.81	1.08	4.81	0.99	1.30
500 BA	1.06	2.66	0.83	2.65	0.60	0.78
1,000 BA	0.90	2.39	1.27	2.41	0.88	0.83
500/250 BA	1.33	1.27	1.04	1.26	1.01	1.14
1,000/250 BA	0.68	1.52	1.77	1.68	0.86	0.69
Pooled SEM	0.51	0.42	0.71	0.41	0.44	0.25
P-value[7]	0.92	0.06	0.98	0.06	0.56	0.29

[1]Dietary treatments consisted of 12 replicate pens of 35 birds. One replicate from 1,000 B was excluded due to aberrant replicate value.

[2]NC = nontreated diet with fresh pine shaving litter; PC = nontreated diet with used litter; 500 BA = diet with 500 ppm of BA on used litter; 1,000 BA = diet with 1,000 ppm of BA on used litter; 500/250 BA = diet with 500 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter; 1,000/250 BA = diet with 1,000 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter.

[3]Tight Junction Proteins = CLDN-1, Claudin-1; JAMA, junctional adhesion molecule A.

[4]Host Defense Peptides = AvBD10, Avian β-defensin 10; AvBD11, Avian β-defensin 11, AvBD11.

[5]Inflammation Markers MyD88, Myeloid differentiation primary response protein 88; LITAF lipopolysaccharide-induced TNF-alpha factor.

[6]The NC samples were used as the calibrator for calculating fold change.

[7]The fold change values for qPCR were logarithmically transformed to conform to normality.

expression in response to *Eimeria* infection was inconsistent across different *Eimeria* challenges and potentially dose-dependent. In their first experiment, down-regulation of multiple avian β-defensins was observed in the duodenum in response to *E. acervulina* and *E. maxima*, with no effect with *E. tenella* challenge. However, in the second experiment, which involved only an *E. maxima* challenge, there was no down-regulation of avian β-defensins in the duodenum. In the current experiment, no significant differences were observed in gene expression for inflammation markers regardless of used litter or BA treatment. Both MyD88 and LITAF are involved in signaling pathways that lead to the production of pro-inflammatory cytokines (Hong et al., 2006; Ahmed et al., 2021). The lack of significant differences observed in this current experiment's response to gene expression for inflammation markers may be attributed to the mild coccidiosis challenge. However, previous research suggests potential benefits of BA supplementation in the reduction of inflammation and pro-inflammatory gene expression. Broilers challenged with 5.0×10^3 sporulated oocysts of *E. maxima* and supplemented with 1,000 ppm sodium BA observed a reduction in pro-inflammatory cytokine gene expression (Badawy Nafaa et al., 2023). Overall, while previous studies have demonstrated the potential benefits of butyrate supplementation on tight junction proteins, host defense peptides, and inflammation markers, the gene expression results in this study were inconsistent and may have been influenced by the mild *Eimeria* challenge used.

Similar to BWG from 0 to 42 d, there were no significant differences among treatment groups in total mass, fat mass, and lean mass on d 42 (Table 6). The total mass for these treatments ranged from 2,388 g to 2,439 g (P = 0.98), fat mass from 472 g to 517 g (P = 0.17), and lean mass from 1,872 g to 1,936 g (P = 0.93). In contrast to the current experiment, previous research has observed beneficial effects of BA for increasing carcass weight, muscle accretion, and lipid metabolism in nonchallenged broilers (Panda et al., 2009; Yin et al., 2016; Bedford et al., 2017). Furthermore, in broilers challenged with coccidiosis and supplemented with coated BA, alterations in body composition were observed; however, the changes in body composition were consistent with the overall growth responses (Sizmaz et al., 2022). While previous research has shown differences in the effects of BA on broiler body composition, the current experiment did not observe any differences, suggesting potential variability of BA supplementation effects in different experimental conditions.

Table 6. Effects of various concentrations of dietary butyrate (BA) on D 42 body composition of 0 to 42 d-old male Ross 708 broiler chickens raised on fresh pine shavings or used litter.[1]

Treatments[2]	Total mass	Fat mass	Lean mass
	(g)		
NC	2,397	495	1,901
PC	2,426	490	1,936
500 B	2,389	517	1,872
1,000 B	2,413	488	1,925
500/250 B	2,388	472	1,916
1,000/250 B	2,439	516	1,923
Pooled SEM	52	13	43
P-value	0.98	0.17	0.93

[1]Dietary treatments consisted of 12 replicate pens of 35 birds. One replicate from 1,000 B was excluded due to aberrant replicate value.

[2]NC = nontreated diet with fresh pine shaving litter; PC = nontreated diet with used litter; 500 BA = diet with 500 ppm of BA on used litter; 1,000 BA = diet with 1,000 ppm of BA on used litter; 500/250 BA = diet with 500 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter; 1,000/250 BA = diet with 1,000 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter.

Regardless of collection period, the NC treatment resulted in the lowest coccidia oocyst counts (Figure 1). To maintain uniform experimental conditions, the pens were in close proximity to each other. This ensured that the birds experienced similar environmental factors and management practices throughout the study. However, this close proximity also increased the potential of contamination between pens, which could explain why a small level of contamination occurred, even in environments intended to be relatively clean, such as the NC treatment. During the 5 to 7 d period, oocyst shedding was greatest for treatments with 500/250 BA and lowest for the NC-fed birds with the remaining treatments being intermediate ($P \leq 0.05$). From 11 to 13 d, NC broilers resulted in the lowest oocyst shedding, while

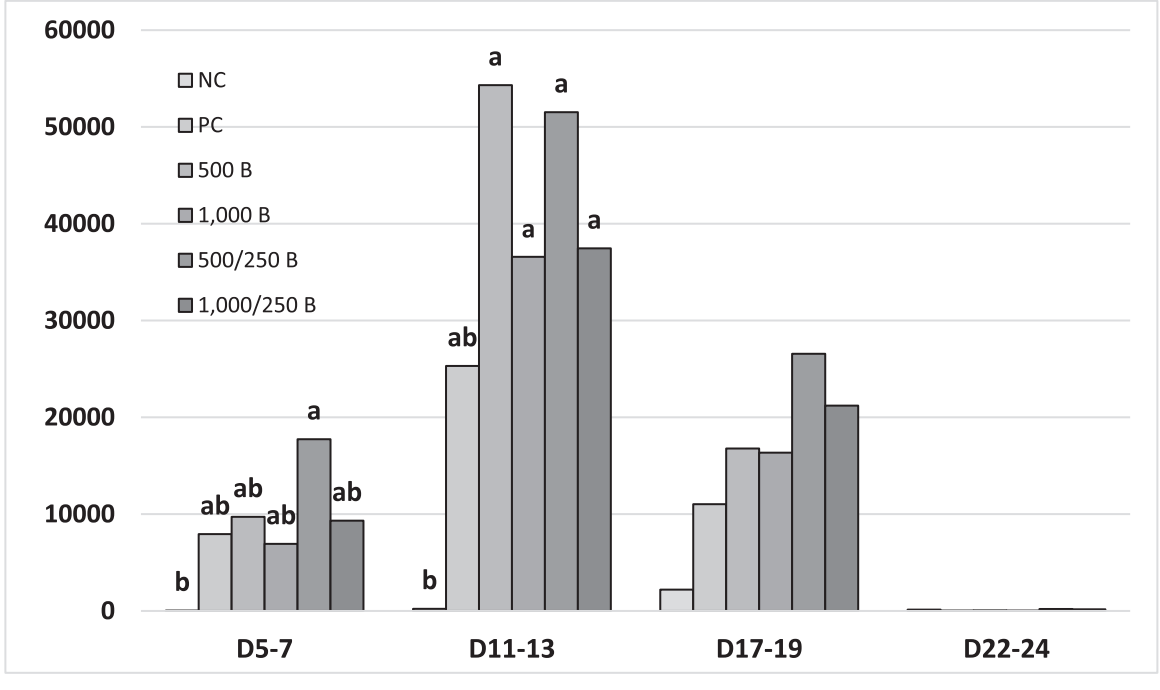

Figure 1. Effects of various concentrations of dietary butyrate (BA) on oocyst shedding from 5 to 7, 11 to 13, 17 to 19, and 22 to 24 d of age of male Ross 708 broiler chickens raised on fresh pine shavings or used litter. NC = nontreated diet with fresh pine shaving litter; PC = nontreated diet with used litter; 500 BA = diet with 500 ppm of BA on used litter; 1,000 BA = diet with 1,000 ppm of BA on used litter; 500/250 BA = diet with 500 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter; 1,000/250 BA = diet with 1,000 ppm of BA from 0 to 8 d and 250 ppm of BA from 8 to 42 d on used litter.

[a–b]Columns without a common superscript are different ($P \leq 0.05$).

all treatments with BA supplementation significantly increased oocyst shedding with PC treatment being intermediate. During the final 2 collection periods, 17 to 19 d and 22 to 24 d, oocyst shedding was similar among treatments ($P > 0.05$). In contrast to the current experiment, other researchers have reported effects of BA at reducing oocyst shedding. Wang and cohorts (2021) conducted 2 experiments supplementing 400 ppm of tributyrin (ester composed of butyric acid and glycerol) to 5 d old broilers where in Experiment 1 broilers were dosed with 1 × dose of coccidiosis vaccine and Experiment 2 broilers were dosed with 10 × dose of coccidiosis vaccine. In the first experiment, broilers given the 1 × dose of coccidiosis vaccine exhibited a reduction in oocyst shedding during d 20 to 27 with tributyrin whereas in the second experiment, broilers challenged with 10 × dose of coccidiosis vaccine, tributyrin reduced oocyst shedding on d 11. Neither of the coccidia doses had a significant reduction during other periods. Furthermore, broilers supplemented with 400 ppm BA significantly reduced litter oocysts compared to the control group at 14 d of age, while 200 and 800 ppm resulted in intermediate responses (Sizmaz et al., 2022). In this study, the observed reduction of coccidia oocyst shedding, particularly evident during the latter collection periods, aligns with the mitigated performance differences noted over time. It is possible that the results of this study reflect the transition of the subclinical challenge to coccidiasis, potentially explaining why BA had no opportunity to decrease oocyst shedding or improve performance.

CONCLUSIONS AND APPLICATIONS

1. Coated butyrate supplementation was able to ameliorate negative performance effects with reused litter over the 8-d starter period when a subclinical challenge was provided. However, when the subclinical coccidiosis resolved, butyrate did not alter broiler performance.
2. The supplementation of a coated butyrate improved apparent ileal digestibility of energy and DM from 0 to 14 d, suggesting benefits for nutrient absorption and utilization in young broilers.
3. Intestinal morphology, permeability, gene expression, body composition, and oocyst shedding were not significantly affected by butyrate supplementation suggesting its effects may be limited under the mild conditions of this experiment.
4. An initial high-dose followed by a lower maintenance dose of coated butyrate supplementation may be a cost-effective strategy to enhance broiler performance and nutrient absorption, maintaining benefits while reducing feed costs in antibiotic-free systems.

ACKNOWLEDGMENTS

This experiment was financially supported by the John Lee Pratt program, the Virginia Agricultural Experiment Station, and the Virginia Agricultural Council grant number 762. Cooper Fritzlen was supported by the John Lee Pratt Graduate Scholar program. Michael Persia was financially supported by USDA Multi-State Research Project NE-1942. The Authors wish to express gratitude and appreciation to Chris Coceano and Austin Funk for diet preparation and animal management and Alyssa Lyons and Matthew Hutchinson for data collection and Marya Miller, Samantha Huffer, Savannah Rounds, and Varun Sathyanarayanan for data collection, oocyst counting, and laboratory work.

DISCLOSURES

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Michael E. Persia reports financial support was provided by Virginia Agricultural Council. Cooper Fritzlen reports financial support was provided by John Lee Pratt Graduate Scholar Program. Michael E. Persia reports financial support was provided by USDA Multi-State Research Project NE-1942. If there are other authors, they declare that they have no known

competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Ahmed, H., M. A. Khan, U. D. Kahlert, M. Niemelä, D. Hänggi, S. R. Chaudhry, and S. Muhammad. 2021. Role of adaptor protein myeloid differentiation 88 (MyD88) in post-subarachnoid hemorrhage inflammation: A systematic review. Int. J. Mol. Sci. 22:.4185.

Amerah, A. M., and V. Ravindran. 2015. Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. Poult. Sci. 94:673–680.

Badawy Nafaa, R. E., F. A. M. Ahmed Gad, A. M. El-Mahmoudy, and A. S. Farid. 2023. Effects of dietary sodium butyrate on innate immunity and gut health of broiler chickens challenged with Eimeria maxima. Benha Vet. Med. J. 43:1–5.

Baxter, M. F., R. Merino-Guzman, J. D. Latorre, B. D. Mahaffey, Y. Yang, K. D. Teague, L. E. Graham, A. D. Wolfenden, X. Hernandez-Velasco, L. R. Bielke, and B. M. Hargis. 2017. Optimizing fluorescein isothiocyanate dextran measurement as a biomarker in a 24-h feed restriction model to induce gut permeability in broiler chickens. Front. Vet. Sci. 4:56.

Bedford, A., H. Yu, E. J. Squires, S. Leeson, and J. Gong. 2017. Effects of supplementation level and feeding schedule of butyrate glycerides on the growth performance and carcass composition of broiler chickens. Poult. Sci. 96:3221–3228.

Blake, D. P., J. Knox, B. Dehaeck, B. Huntington, T. Rathinam, V. Ravipati, S. Ayoade, W. Gilbert, A. O. Adebambo, I. D. Jatau, and M. Raman. 2020. Re-calculating the cost of coccidiosis in chickens. Vet. Res. 51:1–14.

Calik, A., I. I. Omara, M. B. White, W. Li, and R. A. Dalloul. 2019. Effects of dietary direct fed microbial supplementation on performance, intestinal morphology and immune response of broiler chickens challenged with coccidiosis. Front. Vet. Sci. 6:463.

Chalvon-Demersay, T., T. Yamphet, S. Srinongkote, H. Ohara, and W. Lambert. 2021. Supplementing functional amino acids and polyphenols at low dose can restore performance and amino acid digestibility in broilers challenged with coccidiosis. Livest. Sci. 254:104769.

Chamba, F., M. Puyalto, A. Ortiz, H. Torrealba, J. J. Mallo, and R. Riboty. 2014. Effect of partially protected sodium butyrate on performance, digestive organs, intestinal villi and E. coli development in broilers chickens. Int. J. Poult. Sci. 13:390.

Chapman, H. D., J. R. Barta, D. Blake, A. Gruber, M. Jenkins, N. C. Smith, X. Suo, and F. M. Tomley. 2013. A selective review of advances in coccidiosis research. Adv. Parasitol. 83:93–171.

Cloft, S. E., Z. Uni, and E. A. Wong. 2023. Profiling intestinal stem and proliferative cells in the small intestine of broiler chickens via in situ hybridization during the perihatch period. Poult. Sci. 102:102495.

Dalloul, R. A., H. S. Lillehoj, T. A. Shellem, and J. A. Doerr. 2003. Enhanced mucosal immunity against Eimeria acervulina in broilers fed a Lactobacillus-based probiotic. Poult. Sci. 82:62–66.

de Freitas, L. F. V. B., N. K. Sakomura, M. de Paula Reis, A. B. Mariani, W. Lambert, I. Andretta, and M. P. Létourneau-Montminy. 2023. Coccidiosis infection and growth performance of broilers in experimental trials: Insights from a meta-analysis including modulating factors. Poult. Sci. 102:103021.

Garcia, J. S., J. A. Byrd, and E. A. Wong. 2021. Tissue-, age-and dose-dependent changes in avian β-defensin and LEAP2 mRNA abundance in the intestines of Salmonella Typhimurium challenged broilers. Anim. Biotechnol. 32:637–645.

Gautier, A. E., and S. J. Rochell. 2020. Influence of coccidiosis vaccination on nutrient utilization of corn, soybean meal, and distillers dried grains with solubles in broilers. Poult. Sci. 99:3540–3549.

González-Ortiz, G., T. T. Dos Santos, K. Vienola, S. Vartiainen, J. Apajalahti, and M. R. Bedford. 2019. Response of broiler chickens to xylanase and butyrate supplementation. Poult. Sci. 98:3914–3925.

Hong, Y. H., H. S. Lillehoj, S. H. Lee, D. W. Park, and E. P. Lillehoj. 2006. Molecular cloning and characterization of chicken lipopolysaccharide-induced TNF-α factor (LITAF). Dev. Comp. Immunol. 30:919–929.

Hu, Z., and Y. Guo. 2007. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Anim. Feed Sci. Technol. 132:240–249.

Jia, M., K. L. Reynolds, and E. A. Wong. 2023. Effects of high incubation temperature on tight junction proteins in the yolk sac and small intestine of embryonic broilers. Poult. Sci. 102:102875.

Kaczmarek, S. A., A. Barri, M. Hejdysz, and A. Rutkowski. 2016. Effect of different doses of coated butyric acid on growth performance and energy utilization in broilers. Poult. Sci. 95:851–859.

Kinstler, S. R. 2023. The influence of necrotic enteritis, environmental factors, and genetics on intestinal development pathways and disease occurrence in broiler chickens. PhD Diss. Virginia Tech, Blacksburg.

Lawal, J. R., S. M. Jajere, U. I. Ibrahim, Y. A. Geidam, I. A. Gulani, G. Musa, and B. U. Ibekwe. 2016. Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri. Nigeria. Vet. World. 9:653–659.

Leeson, S., H. Namkung, M. Antongiovanni, and E. H. Lee. 2005. Effect of butyric acid on the performance and carcass yield of broiler chickens. Poult. Sci. 84:1418–1422.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Off. Anal. Chem. 56:535–537.

Liu, J. D., H. O. Bayir, D. E. Cosby, N. A. Cox, S. M. Williams, and J. Fowler. 2017. Evaluation of encapsulated sodium butyrate on growth performance, energy digestibility, gut development, and Salmonella colonization in broilers. Poult. Sci. 96:3638–3644.

Long, P. L. 1970. Studies on the viability of sporozoites of Eimeria tenella. Z Parasitenkd 35:1–6.

Murugesan, G. R., and M. E. Persia. 2013. Validation of the effects of small differences in dietary metabolizable energy and feed restriction in first-cycle laying hens. Poult. Sci. 92:1238–1243.

Naghizadeh, M., T. S. Dalgaard, L. Klaver, and R. M. Engberg. 2022. Effects of encapsulated butyrate and

salinomycin on gut leakage and intestinal inflammation in broilers. Br. Poult. Sci. 63:499–509.

Panda, A. K., S. V. Rama Rao, M. V. L. N. Raju, and G. Shyam Sunder. 2009. Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. Asian-Australas. J. Anim. Sci. 22:1026–1031.

Panda, A. K., S. K. Bhanja, and G. S. Sunder. 2015. Early post hatch nutrition on immune system development and function in broiler chickens. Worlds Poult. Sci. J. 71:285–296.

Persia, M. E., E. L. Young, P. L. Utterback, and C. M. Parsons. 2006. Effects of dietary ingredients and Eimeria acervulina infection on chick performance, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 85:48–55.

Sarsour, A. H., and M. E. Persia. 2022. Effects of sulfur amino acid supplementation on broiler chickens exposed to acute and chronic cyclic heat stress. Poult. Sci. 101:101952.

Schmittgen, T. D., and K. J. Livak. 2008. Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3:1101–1108.

Schneiders, G., J. Foutz, M. C. Milfort, A. Ghareeb, U. G. Sorhue, J. N. Richter, A. L. Fuller, S. M. Williams, R. Rekaya, and S. E. Aggrey. 2019. Ontogeny of intestinal permeability in chickens infected with Eimeria maxima: Implications for intestinal health. J. Adv. Parasitol. 6:41–50.

Singer, R. S., L. J. Porter, D. U. Thomson, M. Gage, A. Beaudoin, and J. K. Wishnie. 2019. Raising animals without antibiotics: US producer and veterinarian experiences and opinions. Front. Vet. Sci. 6:452.

Sizmaz, O., N. W. Barrett, J. Lewis, H. Yakout, and M. E. Persia. 2022. Effect of various concentration of butyric acid on growth performance, intestinal lesion scores, and body composition of broilers raised on used litter. J. Appl. Poult. Res. 31:100296.

Song, B., H. Li, Y. Wu, W. Zhen, Z. Wang, Z. Xia, and Y. Guo. 2017. Effect of microencapsulated sodium butyrate dietary supplementation on growth performance and intestinal barrier function of broiler chickens infected with necrotic enteritis. Anim. Feed Sci. Technol. 232:6–15.

Su, S., D. M. Dwyer, K. B. Miska, R. H. Fetterer, M. C. Jenkins, and E. A. Wong. 2017. Expression of host defense peptides in the intestine of Eimeria-challenged chickens. Poult. Sci. 96:2421–2427.

Sunkara, L. T., M. Achanta, N. B. Schreiber, Y. R. Bommineni, G. Dai, W. Jiang, S. Lamont, H. S. Lillehoj, A. Beker, R. G. Teeter, and G. Zhang. 2011. Butyrate enhances disease resistance of chickens by inducing antimicrobial host defense peptide gene expression. PLoS One 6:27225.

Teng, P. Y., S. Yadav, F. L. de Souza Castro, Y. H. Tompkins, A. L. Fuller, and W. K. Kim. 2020. Graded Eimeria challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. Poult. Sci. 99:4203–4216.

Vasanthakumari, B. L., K. R. Gedye, M. R. Abdollahi, M. Di Benedetto, D. G. Sanchez, A. Wealleans, and V. Ravindran. 2023. A new monocomponent xylanase improves performance, ileal digestibility of energy and nutrients, intestinal morphology, and intestinal microbiota in young broilers. J. Appl. Poult. Res. 32:100301.

Wang, J., S. Fu, X. Zou, C. Luo, D. Shu, and H. Qu. 2021. The impact of tributyrin on performance and intestinal health of broiler chickens post coccidiosis vaccination. Avian Dis. 65:493–499.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159–180.

Wu, W., Z. Xiao, W. An, Y. Dong, and B. Zhang. 2018. Dietary sodium butyrate improves intestinal development and function by modulating the microbial community in broilers. PloS One 13:0197762.

Xiong, R. G., D. D. Zhou, S. X. Wu, S. Y. Huang, A. Saimaiti, Z. J. Yang, A. Shang, C. N. Zhao, R. Y. Gan, and H. B. Li. 2022. Health Benefits and Side Effects of Short-Chain Fatty Acids. Foods. 11:2863.

Yang, Q., L. A. Fong, W. Lyu, L. T. Sunkara, K. Xiao, and G. Zhang. 2021. Synergistic induction of chicken antimicrobial host defense peptide gene expression by butyrate and sugars. Front. Microbiol. 12:781649.

Yerpes, M., P. Llonch, and X. Manteca. 2020. Factors associated with cumulative first-week mortality in broiler chicks. Animals (Basel) 10:310.

Yin, F., H. Yu, D. Lepp, X. Shi, X. Yang, J. Hu, S. Leeson, C. Yang, S. Nie, Y. Hou, and J. Gong. 2016. Transcriptome analysis reveals regulation of gene expression for lipid catabolism in young broilers by butyrate glycerides. PLoS One 11:0160751.

Zhao, H., H. Bai, F. Deng, R. Zhong, L. Liu, L. Chen, and H. Zhang. 2022. Chemically protected sodium butyrate improves growth performance and early development and function of small intestine in broilers as one effective substitute for antibiotics. Antibiotics (Basel) 11:132.