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Evaluation of Nutritional Factors that Influence the Efficacy of Tributyrin as a Feed Additive for Broilers

Brooke Bodle
University of Arkansas, Fayetteville

Evaluation of Nutritional Factors that Influence the Efficacy of Tributyrin as a Feed Additive for Broilers

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Brooke Bodle
Texas A&M University
Bachelor of Science in Poultry Science, 2016
Texas A&M University
Master of Science in Poultry Science, 2017

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University of Arkansas

This dissertation is approved for recommendation to the Graduate Council

_____
Samuel Rochell, Ph.D.
Dissertation Director

_____
Sami Dridi, Ph.D.
Committee Member

_____
Annie Donoghue, Ph.D.
Committee Member

_____
Brian Kerr, Ph.D.
Ex-Officio Committee Member

ABSTRACT

Tributyrin **(TB)** is a glyceride ester of butyrate that has the potential to improve broiler performance and intestinal development. Therefore, to fully evaluate this potential, three experiments were conducted to evaluate effect of tributyrin (TB) on broiler growth performance, nutrient digestibility, carcass characteristics, intestinal morphology, and gastrointestinal function in birds fed diets varying in composition reared in battery cages and floor pens. Experiment 1 explored the supplementation of graded doses of TB and its impact upon growth performance, nutrient digestibility, and carcass characteristics in a step-down program when added to a reduced energy and amino acid diet. A linear reduction in body weight gain and a quadratic response in feed conversion ratio was observed with increasing amounts of TB up to 5 times the recommended dose from d 0 – 35. In addition, a linear increase in fat pad yield and a linear reduction in breast meat yield were observed with increasing amounts of TB in the diet. When evaluating the 3 TB doses used in Experiment 1, growth performance was not negatively affected in birds fed 500 mg/kg of TB when compared to birds fed 3 to 5 times that level. Due to the differences between this study and previously published research, diet composition was subsequently evaluated as a potential source for differences observed in TB utilization among those studies. Therefore, in Experiment 2, TB supplementation, lipid source, lipid concentration, and corn particle size and their potential interactive effects on growth performance and nutrient digestibility were evaluated in 2 battery trials. Overall, TB efficacy was not consistently affected by dietary lipid source and lipid concentration. The same can be inferred regarding corn particle size as no differences were observed in broilers growth performance or gizzard function, with or without the inclusion of TB. Experiment 3 evaluated TB efficacy on growth performance, nutrient digestibility, and gastrointestinal pH in birds fed either animal or vegetable-based proteins reared in either battery cages or floor pens. Compared with diets containing animal

protein, all vegetable-based diets with elevated soybean meal levels may result in increased non-starch polysaccharide content leading to increased short chain fatty acid (SCFA) production. Therefore, increasing SCFA production may confer a synergistic effect between an exogenous source of butyrate and increased production of endogenous butyrate. However, no effects of TB or interaction with diet type were observed in growth performance, nutrient digestibility, or ileal and cecal pH throughout the experiment in 15 d battery cage trial or a 42 d floor pen trial. However, birds fed animal protein-based diets did have improved growth performance compared to birds fed vegetable based diets. Additionally, growth performance of birds fed animal protein or vegetable protein diets with or without the inclusion of TB, was not significantly different between the two rearing environments.

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I: INTRODUCTION

In the past 5 years, antibiotic free (ABF) production in broilers has grown by almost 40% in the poultry industry (Rennier Associates, Inc, 2020). Therefore, maintaining gut health in the absence of in-feed antibiotics has become increasingly important for poultry producers. Several alternatives to in-feed antibiotics have been widely researched on their effectiveness of providing the same advantages within the bird's system as antibiotics via increased resistance of pathogenic bacteria and improvement in growth performance. Numerous strategies have been evaluated including various feed additives and essential oils. One category of promising molecules is short chain, or volatile, fatty acids (SCFA) which have been shown to potentially alleviate some of the growth performance and gastrointestinal health issues that arise when in-feed antibiotics are not used (Van Immerseel et al., 2004). Endogenous SCFA are produced in the intestinal lumen of the bird by microbial fermentation of carbohydrates, especially nondigestible polysaccharides (Tan et al., 2014). The highest levels of SCFA are found in the ceca and are either metabolized by or transported across the intestinal epithelium and into the bloodstream. Short chain fatty acids have been reported to play a major role in the regulation of metabolism, inflammation, and disease (Tan et al., 2014).

The most abundant SCFA include acetate, butyrate, and propionate. Among these, butyrate has been a commonly used feed additive in the poultry industry for several years (Van Immerseel et al., 2004). Butyric acid can have a strong odor; therefore, it is often fed in several different forms such as anions, salts, or mono-, di-, or tributyrins. However, each form of butyrate has unique release characteristics within the bird's gastrointestinal tract. For example, unprotected butyrate has been reported to be active in the bird's crop and proventriculus where it is passively absorbed. On the other hand, coated or esterified forms, such as tributyrin (TB) have

been hypothesized to be released in the small intestine (Moquet et al., 2016). In TB, the butyrate glycerides are cleaved from the glycerol backbone by pancreatic lipase which primarily functions in lipolysis of the gastrointestinal tract (GIT). Therefore, due to the bird's lack of pre-duodenal lipolytic activity (Moreau et al., 1998), the main site of degradation and absorption of TB is thought to occur in the small intestine. Although some pancreatic lipase may be found in the gizzard due to reverse peristalsis that occurs between the duodenum and gizzard.

It is well established that lipids provide a concentrated source of energy and supply essential fatty acids in a poultry diet. However, the fatty acid profile and free fatty acid content can impact lipid digestibility and energy value, while the peroxidation status can influence the intestinal integrity of the animal (Mani et al, 2013; Rosero et al., 2015). In the US, soybean oil and poultry fat are commonly used as dietary fat sources in broiler diets due to their high digestibility and superior fatty acid profile. However, observational evidence from field and research trials have reported different effects of TB supplementation on broiler growth performance when included in diets containing soy oil or poultry fat. While information is sparse on the interaction between TB and lipid digestibility, several mechanisms within lipolysis could be responsible including pancreatic lipase activity, age of the bird, and bile acid concentration.

The use of soybean oil is increasing as the poultry industry adopts more all-vegetable based diets at the expense of animal-based products. For example, the elimination in animal protein by-products causes ingredients such as soybean meal to increase in the diet. An increase in soybean meal can lead to higher concentrations of oligosaccharides and potentially an increase in gut viscosity. On the other hand, an increase in fermentability may lead to increased fermentation in the ceca by the microbiota. Therefore, higher amounts of endogenous butyrate

could be produced, and when coupled with an exogenous butyrate source, may have a synergistic effect.

Moreover, the physical characteristics of the diet may also influence TB efficacy. The gizzard is responsible for many digestive functions in the bird's body but most notable is its role in the physical digestion of incoming feed particles (Svihus, 2011). In recent years, the importance of dietary particle size has attracted attention due to its effect upon the gizzard development and its functionality (Svihus et al., 2004). The physical act of breaking down feed particles can lead to secondary effects such as an increase in reverse peristalsis and higher enzymatic activity. If enzymatic activity is increased, higher rates of TB cleavage via pancreatic lipase activity could occur. However, this may lead to a reduction in gizzard pH due to the increase in butyric acid concentration (Moquet et al., 2016; 2018).

Currently, there is a lack of information on how nutritional factors influence TB efficacy. In addition, the optimal inclusion level of TB is inconsistent across previous literature. Therefore, in order to understand the complex interactions between nutritional factors and TB, a series of studies were conducted. The first experiment aimed to establish the optimal inclusion level of TB to achieve improvements in growth performance, carcass characteristics, and nutrient digestibility. The second experiments evaluated dietary factors such as lipid source, lipid concentration, and corn particle size, and their interactions with TB, on broiler growth performance, gastrointestinal parameters, and nutrient digestibility. The third experiment explored the differences between animal protein and vegetable-based diets in differing environments and its interaction with TB on growth performance, nutrient digestibility, and gastrointestinal pH.

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CHAPTER II: LITERATURE REVIEW

Short Chain Fatty Acids

In the ceca of the bird, the production of short chain fatty acids (SCFA) begins by bacteria converting a polysaccharide, such as arabinoxylan, pectin, or cellulose, to an oligosaccharide (Onrust et al., 2015). These oligosaccharides are then metabolized by *Firmicutes* and some *Bacteroidetes* species to produce lactic acid, hydrogen, and SCFA (Pryde et al., 2002). Acetate, propionate, butyrate, valerate, and isovalerate are produced through the fermentation process (Jozefiak et al., 2004). However, in most studies the common SCFA reported are acetate, butyrate, and propionate. Due to the low pKa (≤4.8) of SCFA and the neutral pH of the proximal small intestine, most SCFA are present as anions rather than free acids (Guilloteau et al., 2010). The mechanism by which SCFA are produced is unique and complex. The largest number of bacteria within the birds GIT is located within the ceca, most of which are anaerobes. Approximately 80% of total bacteria in the ceca belong to the phyla *Firmicutes* and *Bacteroidetes* (Onrust et al., 2015). In general, the *Firmicutes* phyla contain many species capable of fermenting substrates to butyrate. These can include *Ruminococcaceae* (*Clostridium* cluster IV) and *Lachnospiraceae* (*Clostridium* cluster XIVa) (Onrust et al., 2015). The *Bacteroidetes* phyla contains many polysaccharide-degrading bacteria species. Both *Firmicutes* and *Bacteroidetes* concentrations appear to be low in the first days of life but continue to increase over time and plateau around 15 days post-hatch (van Der Wielen et al., 2001).

The process in which bacteria convert substrates to products which can subsequently be utilized by other bacteria is known as cross-feeding (Duncan et al., 2004b). This can enhance certain SCFA, such as butyrate, production through the generation of intermediate metabolites. For example, Duncan et al. (2004a) noted that lactic acid is consumed by *Clostridium* cluster

XIV strains to produce butyrate. In rats, this conversion is beneficial due to the toxicity of lactic acid to bacteria when present in high concentrations (Hanstock et al., 2010). In addition, there is competition between sulfate-reducing bacteria and *Clostridium* cluster XIVa for lactate and butyrate. However, sulfate-reducing produce hydrogen sulfide which can cause cell death-inducing effects upon the intestinal epithelial cells (Carbonero et al., 2012). Therefore, Onrust et al. (2015) concluded that a complex interaction exists between different bacterial population for certain substrates, and the outcome of those interactions can determine if beneficial metabolites such as SCFA are produced.

Endogenous Butyrate Production

Butyric acid is a four-carbon molecule with a molecular weight of 88.12 g/mol, a density of 0.958 g/ml, and a pKa of 4.82, and belongs to the class of carboxylic acids known as butanoic acid (Ashan et al., 2016). These acids are characterized by both the carbon on the carboxyl group (-COOH) and the hydrogen ion of the hydroxyl group (-OH) being weakly bonded and replaceable. For example, butyric acid loses its hydrogen ion and forms butyrate when it is in solution ($CH_3CH_2CH_2COO$-) (Ashan et al., 2016). When produced endogenously, the most important butyrate producing bacteria belong to *Ruminococcaceae* and *Lachnospiraceae* families (Pryde et al., 2002) which are found in abundance along the broiler's small intestine and ceca. *Clostridium* clusters also produce butyrate, with clusters IV and XIVa producing significantly more butyrate than clusters I and XVI. The synthesis of butyrate occurs through several different pathways; however, the main pathway is known as the pyruvate and acetyl-conezyme A pathway (Vital et al., 2014). This occurs through the conversion of acetyl-CoA to the intermediate butyryl-CoA in a four-step pathway that resembles the β-oxidation of fatty acids. In the final step of this pathway, butryryl-CoA is converted to butyrate by either butyryl-Coa: acetate CoA

transferase or butyrate kinase (Vital et al., 2014). As such, the genes coding these 2 enzymes are commonly used as biomarkers for the detection of the butyrate producing bacteria communities within the distal intestine and ceca. Amino acids can also be used as substrates for butyrate production including glutarate and 4-aminobutyrate which are found in *Firmicutes* and *Bacteriodetes* phyla (Buckel and Barker, 1974, Barker et al., 1981, Gerhardt et al., 2000). However, these amino pathways often occur in low abundance and rarely without the presence of the acetyl-CoA pathway (Vital et al., 2014).

Butyrate and Gastrointestinal Health

It is well established that long villi and short crypt depths are indicators of a healthy and well-functioning gastrointestinal tract (Ferket et al., 2002). However, several other factors also play a role in the health status of the GIT such as mucosal enzyme activity, mucus layer and thickness, and goblet cell production (Moquet et al., 2018). Most of these indicators are influenced by the microbiome and its mutualistic relationship within the hosts GIT (Backhed et al., 2005). The intestinal wall is lined with epithelial cells that function to absorb water and nutrients for the maintenance and growth of the bird. The membrane of these epithelial cells combined with tight junctions make up a semi permeable barrier between the gut lumen and the internal tissues (Onrust et al., 2015). The permeability of the intestinal wall is regulated by several factors, including immune cells and cytokines, intestinal pathogens, environmental factors, and intestinal blood blow. However, any alteration in the factors mentioned previously, such as physiological stressors, may lead to an increase in permeability and result in gastrointestinal dysbiosis such as “leaky gut” (Stewart et al., 2017)

Morphology. Previous research has noted that when butyrate producing bacteria are in high abundance, an increase in the proliferation of epithelial cells and villi height occur. This

leads to a much stronger epithelial barrier integrity (Onrust et al., 2015). When looking at the role of unprotected butyrate in GIT health, Hu and Guo (2007) reported that birds fed 2,000 mg/kg of unprotected butyrate had a 32.3% improvement in jejunal villi height to crypt depth (VH:CD) ratio compared to broilers fed diets absent in butyrate at 21 days post-hatch. Similarly, Panda et al. (2009) found that broiler duodenal villi height and crypt depths at day 22 were also significantly increased by unprotected butyrate supplementation when included at 2,000, 4,000, and 6,000 mg/kg. Furthermore, Jerzsele et al. (2012) found that when butyrate was included at 1,500 mg/kg in the diet, jejunal villi height was increased by approximately 16% in 21-day old broilers but had no effect upon VH:CD. Whereas Smulikowska et al. (2009) reported that in broilers fed a fat coated sodium butyrate product at 1,000 mg/kg, no effect on villi height, width, or crypt depth in the jejunum or ileum was observed. Therefore, these studies suggest that butyrate's impact upon broilers morphology can depend on the inclusion level of butyrate in the diet as well as the form in which it is fed.

Tight Junctions. Integrity of the gut epithelial barrier can be partly attributed to the functioning of tight junctions located between the epithelial cells (Knudsen et al., 2018). Tight junctions are maintained by occludins, claudins, ZO-1, and plaque proteins which regulate the diffusion of water, ions, and nutrients between the cells. However, if any integrity is lost between the tight junctions, ion conductance increases across the paracellular route breaching the intestinal barrier and allowing pathogens and endotoxins to enter the bloodstream. Pathogens can diminish tight junctions via bacterial derived proteases or biochemical alterations (Awad et al., 2017). In humans, studies have shown that butyrate can downregulate expression of claudin-2, which may improve barrier function as claudin-2 is expressed in tight junctions of leaky epithelial cells (Daly et al., 2006).

Microbiota interaction. Exogenous butyrate supplementation has been reported in numerous studies to reduce the presence of pathogenic bacteria such as *Salmonella spp.*, *Escherichia coli*, and *Campylobacter jejuni* in the ceca and proximal intestine (Van Deun et al., 2008) through a direct bactericidal effect. This occurs when butyric acid diffuses through the lipophilic bacterial cell wall in its undissociated form causing a toxicity within the cell cytoplasm (Warnecke and Gill, 2005). This toxicity is a result of a reduction in cytoplasmic pH following the dissociation of butyrate acid into a proton and its corresponding anion (Fernandez-Rubio et al., 2009). This increased acidification can disrupt purine bases and compromise DNA integrity of the microbe. Furthermore, Fernandez-Rubio et al. (2009) noted that an increase in anion concentration can lead to a higher transport of potassium ions into the bacteria cell, leading to increased cell turgidity.

Endogenous and exogenous butyrate has shown to reduce *Salmonella* colonization in the ceca (Cox et al., 1994), crop, and liver. Orally ingested *Salmonella* moves down the crop and into the GIT where it grows anaerobically in a high fermentative environment such as the ceca (Ricke, 2003). Butyrate can directly inhibit *Salmonella's* invasion into the epithelial cells (Durant et al., 2000) by downregulating the expression of genes involved in the invasion (Van Immerseel et al., 2006) or indirectly affect bacteria by lowering the pH of the intestine. A lower intestinal pH enhances the growth of lactic acid producing bacteria such as *Lactobacilli* and *Bifidobacteria spp.* (Ahsan et al., 2016). In turn, these bacteria can compete with pathogenic bacteria for space and nutrients, helping moderate pathogenic bacterial proliferation.

Immune Function. Inflammation is a common defense mechanism to protect the host from infection or disease in the presence of a pathogen. When inflammation does occur, it is a well-regulated process involving several mechanisms and pathways (Kovarik et al., 2008).

However, excess inflammation can lead to damage to the host tissue. In the GIT, the first line of defense is the physical barriers established by the mucus layer and epithelial cells. The mucus layer is primarily composed of mucins which are glycoproteins that have a backbone encoded with MUC genes (Gaudier et al., 2004). Short chain fatty acids, but more specifically butyrate, have been reported to upregulate MUC genes resulting in increased mucin secretion (Gaudier et al., 2004). Additionally, butyric acid has been reported to stimulate the development of gut-associated lymphoid tissue (GALT) as well as increase the peptide production in the distal GIT (Cox et al., 2009). Wu et al. (2018) noted that broilers fed a diet supplemented with sodium butyrate had a higher secretion of mucus and larger concentrations of goblet cells in the jejunum and ileum. This coincided with longer villi lengths within the jejunum and ileum at 42 days, as well increased weight and length of the duodenum, jejunum, and ileum at 42 days. Therefore, it can be concluded that the supplementation of sodium butyrate promoted intestinal development and health in broilers by reducing inflammation via an increase in mucin production and stimulation of GALT.

Furthermore, G-protein-coupled receptors such as, GPR41, GPR43, and GPR109A can also be found along the intestinal epithelial lining and function as receptors for free fatty acids (Cox et al., 2009). In a human model, Chang et al. (2014) hypothesized that butyrate can bind to GPR41 and GPR43 which promote secretion of peptide YY (PYY). The secretion of PYY can lead to delays or inhibition of gastric emptying and intestinal passage rate, therefore suppressing appetite and increasing the secretion of glucagon-like peptide 1 (GLP-1) (Chang et al., 2014). Additionally, GPR109A signaling can promote anti-inflammatory properties in dendritic cells and macrophages, such as nitric oxide, resulting in the differentiation of regulatory T- cells and IL-10 producing T-cells (Thangaraju et al., 2009; Singh et al., 2014). The GPR109A is also

responsible for butyrate-mediated absorption of IL-18 cytokines into the colonic epithelium (Singh et al., 2014). Therefore, G-protein-coupled receptors are critical in butyrate signaling and its role in immunity.

Furthermore, butyrate and other SCFA have been reported to bind and activate PPARγ (Alex et al., 2013) which aids in the inhibition of NF-κβ, resulting in anti-inflammatory effect in the gut. The inhibition of NF-κβ causes a cascade of events including the inhibition of nitric oxide which is responsible for several physiological and pathological processes including inflammatory responses and vasodilation (Sharma et al., 2007; Taylor et al., 2008). During pathogenesis, bacteria interact with the host cells during which butyrate down regulates expression of invasion genes and decreases virulence of bacteria (Van Immerseel et al., 2004). As previously mentioned, macrophages can produce reactive oxygen species such as nitric oxide which stimulate an anti-inflammatory response to the pathogen during phagocytosis (Zhou et al., 2014).

Butyrate as a Feed Additive

The aforementioned positive effects for endogenous butyrate on broiler gastrointestinal health and immune system have led to its exogenous supplementation in poultry feed (Van Immerseel et al., 2004). However, because butyric acid is volatile and corrosive in nature, it is often fed in protected or coated forms for ease of handling (Ahsan et al., 2016). The different forms can include unprotected, fat-coated, salts, or esterified to a glycerol backbone as mono-, di- or tri glycerides (Moquet et al., 2018). However, the efficacy of butyrate depends upon the pKa value of butyric acid and the corresponding pH of the GIT. When ingested, the low pH of the crop, proventriculus, and gizzard causes butyrate to dissociate from its hydroxyl group and be converted into butyric acid. Once in its un-dissociated form, butyrate can be absorbed one of two

ways. The first method is through passive absorption into the enterocytes for use as an energy source (Ashan et al., 2016). The second method of absorption then occurs via bicarbonate ion (HCO3-) exchange method or active transport (McNeil et al., 1979; Velazquez et al., 1997; Ashan et al., 2016). The primary transporters involved in the active transport system are monocarboxylate transporter isoform 1 (MCT1) and sodium coupled mococarboxylate transporter 1 (SMCT1). It is important to note that butyric acid, rather than butyrate, is the preferred source of energy for enterocytes (Mahdavi and Torki, 2009) as butyrate requires active transport into the small intestine.

Therefore, when butyric acid is fed in the unprotected state, it is hypothesized that it is taken up rapidly via passive absorption by epithelial cells in the crop, proventriculus, and gizzard (Moquet et al., 2016). However, it is unclear if butyrate absorption in these areas impart any beneficial effects upon growth performance and gut morphology. Hu and Guo (2007) noted that birds fed unprotected butyrate had increased concentrations in the proventriculus region but not in the jejunal chyme. Thus, feeding in an unprotected state is rare as most research is protecting the butyric acid to achieve the release of butyrate within the small intestine (Moquet et al., 2018).

In addition, if fed in a protected form, butyric acid can reach the small intestine where it is then dissociated back into butyrate due to the higher pH. Protection of butyrate can occur in several ways, such as its attachment to a salt to improve its stability and reduce the odor that is often associated with butyric acid (Ashan et al., 2016). The most commonly available salt form is sodium butyrate which has been shown to improve the bird's gastrointestinal health by aiding in the development of the gut wall tissues and modulating the growth of the microflora present in the intestine (Van Immerseel et al., 2004; 2005; Leeson et al., 2005). As previously mentioned, butyrate contains a weakly bonded hydroxyl group, therefore in sodium butyrate the sodium

atom takes the place of the hydrogen of the –OH group. Sodium butyrate is converted to butyric acid upon ingestion due to the loss of the sodium ion. The acidic pH of the crop, proventriculus, and gizzard, allow the butyric acid molecule to stay in its undissociated form after losing its sodium atom (Ahsan et al., 2016), therefore, allowing entry into cells to impart bactericidal effects. However, similar to that of unprotected butyrate, it can be absorbed in the proximal gastrointestinal tract by enterocytes for energy. Thus, it must be protected from dissociation in the upper GIT to allow for a release in the distal portion of the bird's small intestine (Ashan et al., 2016).

One method of protection from absorption in the upper GIT, is embedding of butyrate in a fat matrix such as vegetable fat (Smulikowska et al., 2009; Zhang et al., 2011; Jerzsele et al., 2012). The inclusion of butyrate into these matrices can vary from 30% to 70% on a weight:weight basis (Smulikowska et al., 2009; Jerzsele et al., 2012) and these proportions of fat to butyrate may make it difficult to predict the exact release point within the birds GIT. Regardless, it partially protects the butyrate from absorption in the upper GIT and allows the release in the proximal portion of the small intestine (Moquet et al., 2018).

Another pathway to protect butyrate from absorption in the anterior GIT is through esterification. The esterification of butyrate to a glycerol backbone can create a mono-, di-, or tributyrin (Moquet et al., 2018). Due to the presence of the glycerol, butyric acid is degraded by the same pathway as lipids through pancreatic lipases. The chicken has very little pre-duodenal lipolytic activity (Moreau et al., 1988) therefore tributyrin can remain relatively stable in the upper GIT. However, reverse peristalsis from the duodenum into the gizzard may cause some degradation to occur if pancreatic lipase is present. This is also true for fat coated and microencapsulated butyrate products as well. Regardless, when it reaches the small intestine it is

cleaved by pancreatic lipases. Because lipases are only able to cleave the ester bonds of triacylglercol and the *sn-1* and *sn-3* positions, in a tributyrin, butyrate is likely absorbed as a 2-monobutyrin in the small intestine (Moquet et al., 2018).

Growth Performance. The efficacy of butyrate supplementation in broiler feeds appears to be dependent upon on the form fed, release site, age of birds, and diet composition. When sodium butyrate was supplemented in an unprotected state, Hu and Guo (2007) reported that broilers body weight gain (BWG) increased when fed 500 mg/kg and 2,000 mg/kg for 21 days compared to broilers fed diets absent in butyrate, however this effect disappeared by d 42. Similarly, Panda et al. (2009) observed a significant increase in BWG of broilers fed a much higher inclusion rate of unprotected butyric acid at 4,000 mg/kg during a 35-day grow out period, but with no additional benefits when supplemented at 6,000 mg/kg. Therefore, it appears that the lowest inclusion level of 2,000 mg/kg in the aforementioned studies improved broiler growth performance without any added benefit at a higher inclusion rate of 6,000 mg/kg.

Inconsistencies in the literature also appear when broilers are fed sodium butyrate. For example, Gonzalez-Ortiz et al. (2019) noted that the inclusion of a coated sodium butyrate product at 1,000 mg/kg in the diet negatively influenced growth performance at d 21 but increased butyrate concentration in the duodenum and jejunum digesta at d 42 with no impact upon growth performance. Kaczmarek et al. (2016) reported that encapsulated sodium butyrate included in the diet at 300 or 400 mg/kg improved feed conversion ratio (FCR) in broilers throughout a rearing period of 42 days. In addition, when supplemented with sodium butyrate at 1,000 mg/kg in a nutrient reduced diet, broilers had a 2.8% increase in BWG from day 1 to 28 compared to broilers fed a non-supplemented nutrient reduced diet (Bortoluzzi et al., 2017). However, Liu et al. (2019) suggested that sodium butyrate efficacy may not only be dependent

on inclusion level but also by the products targeted release time. These authors used an indigestible marker to evaluate differences in passage rate of two encapsulated sodium butyrate products. Liu et al. (2019) noted that broilers fed 250 and 750 mg/kg of an encapsulated sodium butyrate product (30% butyrate) with a pre-established 2-hour release time had higher ileal digestible energy compared to a pre-established 3 to 4 hour release time at a 750 and 1,000 mg/kg inclusion level.

Inconsistencies in butyrate's optimal dose for growth performance have also been reported. A study by Bedford et al. (2016) noted that when butyrate glycerides were supplemented at 500 or 2,000 mg/kg there was no effect on overall BWG or FCR, however both levels did reduce abdominal fat deposition. In a subsequent trial, Bedford et al. (2017) once again reported the lack of effect by butyrate glycerides supplemented at 500 mg/kg of the diet and 2,000 mg/kg of the diet on broilers BWG and FCR. Similarly, broilers fed 2,000 mg/kg or 4,000 mg/kg butyrate glycerides for 42 days had no differences in growth performance compared to broilers diets absent in butyrate (Leeson et al., 2005).

Nutrient digestibility. Data evaluating butyrate's impact on broiler nutrient digestibility is lacking. Kaczmarek et al. (2016) reported that dietary inclusion of calcium butyrate at 200 mg/kg led to significant improvement in apparent total tract crude fat digestibility and nitrogen corrected apparent metabolizable energy (AME_n). These improvements in nutrient digestibility may be attributed to butyrate's ability to increase cellular concentrations of Ca^{2+} ions in the pancreatic acinar cells, thus leading to activation of enzymatic secretion (Katoh and Tsuda 1987). Therefore, potentially improving nutrient digestibility and subsequently increasing AME_n within the diet. Smulikowska et al. (2009) also reported a significant increase in apparent total tract digestibility of nitrogen and organic matter without affecting crude fat digestibility when

broilers were fed 300 mg/kg of a fat coated butyrate. These authors suggested this improvement in nutrient digestibility was due to greater epithelial cell proliferation within the GIT. Moreover, Qaisrani (2015) reported a trend for increase proteolytic activity in broilers fed the same butyrate additive. However, in the aforementioned studies, the differences in nutrient digestibility, specifically within protein digestibility, were observed when a butyrate salt product was included in the diet. Therefore, butyrate glycerides impact upon nutrient and energy digestibility still needs to be investigated.

Diet Composition and Butyrate Efficacy

Dietary fermentation characteristics. Regardless of the form of butyrate fed, it is important to consider the diet composition in the studies previously mentioned. The fermentability of the diet could impact endogenous SCFA production, specifically butyrate. Because butyrate is produced through cecal fermentation of non-starch polysaccharides and oligosaccharides. Therefore, diet composition can play a large role in the production of endogenous butyrate production. Meat and bone meal (MBM) has been a commonly used feed ingredient in broiler diets for over 90 years. The addition of MBM can help reduce diet cost by providing valuable nutrients such as phosphorus and amino acids. A portion of the protein in MBM is not readily digestible by the broiler and will enter the hindgut undigested. When this occurs, an increase production of nitrogenous bacterial metabolites, such as amines and ammonia, can fuel pathogenic bacteria and potentially induce necrotic enteritis (Onifade et al., 1998; Zanu et al., 2020). In previous literature the inclusion of MBM from 5% to greater than 50% in poultry diets has led to increased concentrations of *C. perfringens* in ileal and cecal contents compared to birds fed vegetable-based proteins, thus resulting in higher incidences of necrotic enteritis and reduced growth performance (Wilkie et al., 2005; Zanu et al., 2020). It has

also been suggested that the inclusion of MBM in the diet can result in increased ileal and cecal pH due to the high calcium content that may interfere with amino acid and mineral utilization (Paiva et al., 2014). In a previous study conducted by Zanu et al. (2020), the addition of 6% MBM to wheat-based diets increased ileal and cecal pH of 16 d old broiler. Thus, the impact MBM has upon gastrointestinal health such as necrotic enteritis and its variable composition has led to the use of alternative feeding strategies such as all-vegetable based diets. To ensure that vegetable-based diets meet sufficient protein levels, vegetable proteins such as soybean meal (SBM) can be included up to 30% or more in the diet. In general, good quality soybean meal is well utilized by poultry, however, in a vegetable-based diet, the oligosaccharide content is greatly increased. Stachyose and raffinose, two common oligosaccharides found in SBM, have been shown to increase feed passage rate and reduce nutrient digestibility in roosters (Coon et al., 1990). Additionally, due to the potassium content in SBM, vegetable-based diets have been linked to an increase in wet litter and as a result, an increase in footpad dermatitis. On the other hand, SBM has been shown to confer some beneficial effects within the gastrointestinal tract. Yang et al. (2016) reported that the additional inclusion of 0.13% SBM oligosaccharides in a diet, which is comparable to a 1.9% increase in SBM, increased total SCFA in the excreta *in vivo* while in turn stimulating a change within the bacterial community in ceca of the bird in a diet containing 26.9% SBM. Additionally, if concentrations are high enough, volatile fatty acids can stimulate a neuro-hormonal response via peptide YY which can delay gastric emptying and duodenal digesta transit times (Cuche et al., 2000; Park et al., 2013), thus improving nutrient digestion and absorption (Singh et al., 2014). Therefore, differences in dietary compositions such as the inclusion of MBM or all-vegetable based diets may influence endogenous butyrate production and in turn its impact upon broiler nutrient utilization.

Lipid Sources. Lipids are included in poultry diets as a concentrated source of energy and to supply essential fatty acids. However, depending on the origin of the lipid and the processing methods it is subjected to, lipid quality can be highly variable (Kerr et al., 2016). The fatty acid profile and free fatty acid content of lipids can impact their digestibility and energy value, while the peroxidation status can influence the intestinal integrity of the animal (Mani et al, 2013; Rosero et al., 2015). Additionally, lipid source absorption and utilization can vary due to the bird's strain, age, or sex. A diverse array of lipid and oil sources are available for use in diets such as restaurant greases, rendered by-products, and acidulated soap stocks (Ravindran et al., 2016). Most poultry diets in the US use soybean oil or poultry fat due to their high digestibility, availability, and superior fatty acid profile making them ideal for use in the diet. However, soybean oil and poultry fat vary in many factors including their degree of saturation. Several studies have noted a negative correlation between the degree of saturation of a lipid and its utilization and apparent metabolizable energy in the bird (Renner and Hill, 1961a; Wiseman et al. 1991). The enzyme responsible for cleavage of the fatty acid off the glycerol backbone is known as pancreatic lipase. Lipase can be largely influenced by the degree of saturation of a fatty acid due to the difference in binding angle between unsaturated and saturated fatty acids (Ravindran et al., 2016). Pancreatic lipase has a binding affinity for a 141° angle between the double bond in unsaturated fatty acids. However, saturated fatty acids have a 180° angle due to the lack of a double bond, making them less digestible due to the reduced access of pancreatic lipase. Fatty acids such as oleic and linoleic significantly increase pancreatic lipase activity while saturated fatty acids such as stearic impart an inhibitory effect (Ravindran et al., 2016). Thus, the composition of the lipid source used in the diet can have a large impact on the ultimate absorption of the fatty acids.

In addition, the age of the bird may also impact the digestibility of different lipid sources. It has been reported that during the first week of life, enzyme secretion such as amylase, trypsin, and lipase are low (Tancharoenrat et al., 2013). The increase in amylase and trypsin appear to not increase until day 4 and continue to rise until 21 days post hatch (Noy and Sklan, 1995). In contrast, lipase activity increases much slower than the other enzymes (Noy and Sklan, 1995). Therefore, the ability of young birds to digest lipids is poor in general, and even more inefficient when fed lipids comprised of saturated fatty acids.

In addition to lipid composition, lipid digestibility is also influenced by the level in which it is included in the diet. A study conducted by Wiseman (1986) reported that with increasing concentrations of lipid in the diet, broilers experienced a negative and non-linear impact upon the dietary apparent metabolizable energy. This effect was most pronounced in diets containing saturated fatty acids in young broilers. In addition, a recent study noted that broilers fed increasing amounts of soybean oil (3.5% to 17.5%) from d 0 to 7, had a linear decrease in feed intake and a linear increase in FCR (Lamot et al., 2019). However, it is important to note that with the increase in soybean oil; amino acids, minerals, and the premix were also increased at the same ratio as the soybean oil. Interestingly, there was not a main effect of lipid level on fat and nitrogen digestibility. However, Ravindran et al. (2016) notes that lipid digestibility will be reduced with a higher degree of saturation, longer chain length, and higher inclusion levels.

Particle size. In a poultry diet, energy is not only derived from lipid sources but other ingredients such as cereal grains. Corn has been widely used in poultry diets due to its consistent high energy values and protein content, contributing up to 65% of the metabolizable energy and 20% of protein in poultry diets (Naderinejad et al. 2016). Most cereal grains in poultry diets are ground rather than included as a whole grain. However, several studies have debated the issue of

how finely to ground cereal grains to increase growth performance, nutrient utilization, and gizzard function.

The gizzard's most notable role is its ability to physically digest incoming feed particles (Svihus, 2011). The gizzard is comprised of muscles that move asymmetrically when contracted and crush the particles inside. Amerah et al. (2008, 2009) noted that the volume of the bird's gizzard increased significantly when structural components were added to the diet, indicating that the mean retention time will increase in correspondence with gizzard function (Svihus et al., 2011). Furthermore, the inclusion of structural components in the diet has shown to reduce the pH of the gizzard content (Gabriel et al., 2003; Engberg et al., 2004; Bjerrum et al., 2005). This occurs by an increase in hydrochloric acid secretion due to longer retention times and increased gizzard volume often associated with structural components (Svihus et al., 2011). Additionally, the increase in hydrochloric acid could impart positive benefits on the birds GIT by the reduction of pathogenic microflora and improved gastric digestion (Svihus et al., 2011).

Additionally, studies by Proudfoot and Hulan (1989) and Hamilton and Proudfoot (1995) reported improved BWG in broilers fed mash diets containing coarse corn particles or very coarse corn particles compared to broilers fed finely ground corn. One traditional view regarding nutrient utilization is that smaller particle sizes are associated with a larger surface area of the grain, leading to increased exposure to digestive enzymes thus resulting in higher digestibility (Preston et al., 2000). A study conducted by Kilburn and Edwards (2001) showed that when birds were fed mash diets containing finely ground corn (869 µm), the metabolizable energy was improved whereas a reduction in the magnitude of change in metabolizable energy was observed when birds were fed pelleted diets. Furthermore, Parsons et al. (2006) reported broilers fed coarse ground corn (2,242 µm) experienced an increase in lysine and nitrogen retention

compared to finely ground corn (781 µm). Therefore, it is important to consider particle size when formulating a diet to maximize the growth performance and nutrient utilization.

Lastly, conflicting data has been reported on the ability of particle size ability to influence intestinal morphology and digestive tract measurements. It has been suggested that the interaction between coarse feed particles and the intestinal mucosa allows for a reduction in gastrointestinal passage rate and thus an increase in time between the feed particle and mucosa (Sturkie, 2000; Warner, 1981). Therefore, an increase in villus length may lead to a larger absorptive surface area and result in higher enzymatic activity and increased transportation of nutrients at the villus surface (Cera et al., 1988). Previous research has noted a significant increase in broiler and layer small intestine villus height and villi height: crypt depth ratio when feeding diets containing coarse particles (Dahlke et al., 2003; Rohe et al., 2014; Gabriel et al. 2007). Furthermore, Amerah et al. (2007) observed an interaction of feed form and particle size on digestive tract measurements where broilers fed a mash diet containing a 839 µm particle size had heavier GIT components compared to the broilers fed a mash diet with 1,164 µm particle size. Additionally, the same interaction was observed in gastrointestinal lengths where broilers fed mash diets containing 1,164 µm particle size had shorter intestinal lengths compared to broilers fed mash diets containing a 839 µm particle size. However, in the same study, no impact of particle size was observed on duodenum or jejunal morphology. Additionally, Lv et al. (2015) reported a lack of interaction of feed particle size and feed form for all sections within the digestive tract when using 573 µm, 865 µm, and 1,027 µm corn particle size in a mash diet. Therefore, it appears that the impact of particle size on intestinal morphology and development and how it relates to nutrient digestibility needs to be further explored.

In conclusion, butyrate supplementation has the potential to improve broiler growth performance, nutrient utilization, and gastrointestinal health. The form in which to include butyrate must be taken into consideration, as different forms will have varying release points inside the gastrointestinal tract. In the current studies, butyrate glycerides, or tributyrin (TB), will be the primary molecule evaluated. In addition, nutritional factors will be investigated on their influence in TB efficacy. One of the nutritional factors will include dietary lipid source and level due to field experience and research trials reporting a difference in broiler growth performance in birds fed diets supplemented with TB containing either soy oil or poultry fat. Additionally, corn particle size will also be evaluated due to its impact upon gizzard function. An increase in gizzard function can result in improved growth performance, nutrient digestibility, and enzymatic secretion. This in turn may impact TB utilization due to the increase in pancreatic lipase activity that can occur with improved gizzard functionality.

Lastly, the fermentability of the diet is of great importance as different feeding programs are growing exponentially in the poultry industry. The elimination of animal protein in some diets has caused an increase in other ingredients such as soybean meal, resulting in higher fermentation of the diet. Thus, this increase in fermentation could lead to higher production of endogenous butyrate, allowing for a synergistic effect between exogenous butyrate supplementation and endogenous butyrate production. Therefore, these studies aim to evaluate nutritional factors influence upon TB efficacy and its impact on broiler growth performance, nutrient digestibility, carcass characteristics, and gastrointestinal development and morphology.

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CHAPTER III: EVALUATION OF GRADED DOSES OF TRIBUTYRIN ON BROILER LIVE PERFORMANCE, NUTRIENT DIGESTIBILITY, AND CARCASS CHARACTERISTICS

ABSTRACT

Tributyrin **(TB)** is a glyceride ester of butyrate that has previously been reported to improve broiler performance and improve carcass leanness. Therefore, two experiments were conducted to investigate the optimal inclusion level of tributyrin for broiler growth performance, apparent ileal nutrient **(AID)** and energy digestibility **(IDE)**, and carcass characteristics when fed in a step-down program and in a reduced energy and amino acid diet. Dietary treatments for both experiments consisted of a positive control **(PC),** negative control **(NC)**, and 3 inclusions of TB in the NC, which was formulated with a reduction of 100 kcal/kg in AMEn and 7% in digestible amino acids relative to PC. The 3 TB doses included 1x (500 mg/kg in starter and 250 mg/kg in the grower and finisher), 3x (1,500 mg/kg in starter and 750 mg/kg in the grower and finisher), and 5x (2,500 mg/kg in starter and 1,250 mg/kg in the grower and finisher) of the manufacturer's recommended dose. Dietary treatments were fed in three feeding phases in Experiment 1 starter, (0 to 12 d); grower, (13 to 26 d); and finisher, (27 to 35 d), whereas in Experiment 2 treatments were fed in a single feeding phase from d 0 – 14. Titanium dioxide was used as an indigestible marker in the feed for determination of AID and IDE following collection of ileal digesta at 14 d in Experiment 2. In Experiment 1, each treatment was replicated with 12 floor pens of 6 male and 6 female Ross 708 chicks and on d 35, 480 birds were processed and deboned. In

Experiment 2, each treatment was replicated with 6 floor pens of 12 male by-product chicks from a female Cobb 500 line. A linear reduction in BWG ($P = 0.002$) and a quadratic response in FCR ($P = 0.001$) with increasing amounts of TB from d 0 – 35 was observed in Experiment 1 while overall mortality was not impacted by increasing TB in the diet. In addition, a linear increase in fat pad yield ($P = 0.002$) and a linear reduction in breast meat yield ($P < 0.001$) was also observed with increasing TB doses. In Experiment 2, no significant differences were observed among treatments in growth performance at d 14. However, a linear increase in ether extract digestibility ($P = 0.022$) was observed with increasing TB inclusion. Lastly, a quadratic improvement was observed for both dry matter digestibility ($P = 0.042$) and IDE kcal/kg ($P = 0.012$) with the 1,500 mg/kg TB having the highest numerical values in both measurements. In conclusion, these data indicate that when evaluating the 3 TB doses used in Experiment 1, growth performance was not negatively impacted when fed at 500 mg/kg in the starter phase and stepped down to 250 mg/kg in the grower and finisher phases as compared to birds fed the higher inclusion levels of TB. In Experiment 2, 1,500 mg/kg of TB improved nutrient digestibility without affecting growth performance. Differences in genetic strains and age of birds at the termination of the trial may have influenced the differences in observations between the two studies.

INTRODUCTION

Butyric acid is a commonly used feed additive that has been well-studied regarding its effects on the gastrointestinal health and production performance of broiler chickens (Cox et al., 1994; Van Immerseel et al., 2004, Onrust et al., 2015). Potential benefits of exogenous butyric acid include improved intestinal morphology, immune function, and microbiota profile (Apajalahti and Vienola, 2016; Moquet et al., 2018). Collectively, these effects can increase

nutrient utilization, but data evaluating the effects of butyric acid on nutrient digestibility are lacking (Kaczmarek et al., 2016; Smulikowska et al., 2009). Various forms of butyric acid are used in poultry feeds and differ in their purity and sites of release within the gastrointestinal tract. One such form includes butyric acid esterified to a glycerol backbone, as mono-, di-, and tributyrins (Moquet et al., 2016). Glycerol bound butyrins may be more stable in feed and the anterior gastrointestinal tract when compared with unprotected forms of butyric acid such as anions and salts, which are often passively absorbed in the upper gastrointestinal tract (Ichikawa et al., 2002; Moquet et al., 2018). Therefore, microencapsulation or esterification of butyrate it is thought to release butyrate within the small intestine following exposure to pancreatic lipase which allows butyrate to provide energy to enterocytes and stimulate villi growth (Ahsan et al., 2016).

Current literature is inconsistent regarding the optimum level of butyrate glycerides to promote growth performance and carcass yield. In two studies conducted by Leeson et al. (2005), birds fed 100, 200, or 400 mg/kg of mixed butyrins had no difference in growth performance from 0 to 42 days, however, breast yield was the highest for birds fed the 200 mg/kg inclusion level. Bedford et al. (2017b) reported that a mixture of 500 mg/kg of mono- and tributyrin reduced abdominal fat deposition and improved breast yield with no impact upon growth performance. Furthermore, Bedford et al. (2017b) concluded that butyrate glycerides may not require feeding throughout the duration of growth in order to achieve an increase in carcass leanness. Thus, it is unclear if a reduction in butyrate glycerides between growth phases is beneficial for performance and carcass characteristics; and if so, at what magnitude is optimal for reduction. In a study conducted by Antongiovanni et al. (2009), straight run broilers were fed 2,000 mg/kg of a butyrate glyceride mixture in the starter phase and stepped down to 500 mg/kg

in the finisher phase which yielded a significant increase in FCR and a reduction in breast yield when compared to a control diet. It is noteworthy to mention, that in the aforementioned studies, only male broilers were used so it is unclear if TB is utilized differently between male and female broilers. Therefore, two experiments were designed to explore the supplementation of graded doses of tributyrin in a step-down program during the grower and finisher phases to establish an optimum inclusion level for use in subsequent studies. Furthermore, the two experiments aimed to evaluate TB in reduced nutrient dense diets while also establishing its impact on broiler growth performance, nutrient digestibility, and carcass characteristics in male and female broilers.

MATERIALS AND METHODS

All animal care and experimental procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

Animal Husbandry and Dietary Treatments (Experiments 1 and 2)

Experiment 1. In experiment 1, 720 Ross 708 straight-run chicks were obtained from a commercial hatchery on day of hatch. All chicks were vent-sexed by a trained professional and tagged according to sex. Birds were then group-weighed and distributed across 60 floor pens containing used litter top-dressed with fresh pine shavings. Each 0.91m by 1.22 m pen contained a total of 12 birds with 6 females and 6 males equipped with a commercial-type pan feeder and nipple waterers to provide free access to feed and clean water throughout the trial. Supplemental feed trays were placed in each pen from 0 to 7 d post-hatch. Lighting and temperature were maintained according to best practice appropriate for bird age as outlined in management guides published by the primary breeder.

On 0, 14, 28, and 35 d post-hatch, male and females were weighed separately, and feeder weights were recorded and used for the calculation of body weight gain **(BWG),** feed intake **(FI),** and mortality corrected feed conversion ratio **(FCR)**. Five dietary treatments were maintained across 3 feeding phases to 35 d post-hatch: starter (d 0 to 12), grower (13 to 26 d), and finisher (27 to 35 d). For each feeding phase, a common basal was mixed and experimental treatments were remixed with the appropriate ingredient additions according to treatment (Table 2.1). Diets contained corn, soybean meal, and distillers dried grains with solubles and were formulated to industry-relevant nutrient specifications. The negative control (NC) was formulated with a 100 kcal/kg reduction in nitrogen-corrected apparent metabolizable energy (AME_n) and a 7% reduction in digestible amino acids compared to the positive control (PC). The 3 TB doses included 1x (500 mg/kg in starter and 250 mg/kg in the grower and finisher), 3x (1,500 mg/kg in starter and 750 mg/kg in the grower and finisher), and 5x (2,500 mg/kg in starter and 1,250 mg/kg in the grower and finisher) of the manufacturer's recommended dose. For the 1x, 3x, and 5x doses, tributyrin was included at the expense of sand in the starter phase at 500, 1,500, and 2,500 mg/kg, and 250, 750, 1,250 mg/kg, in the grower and finisher phases: respectively.

At 35 d post-hatch, all birds from 8 replicate pens per treatment (480 minus mortality) were selected and fasted overnight. Birds were then transported in coops to the University of Arkansas Pilot Processing Plant. Live weights were measured immediately prior to live-hanging. Birds were processed and deboned by University of Arkansas research personnel. Processing measurements included hot carcass weights, hot fat pad weights, and chilled carcass, breast, tenders, wing, and leg quarters weights and yields. Yields were calculated for each bird relative to the live weights taken at the back dock.

Experiment 2. Experiment 2 was conducted to determine nutrient digestibility in response to graded doses of TB in a reduced nutrient dense diet. In Experiment 2, 360 Cobb 500 male by-product chicks from a Cobb 500 female line obtained from a commercial hatchery on day of hatch. The type of chicks used differed from Experiment 1 due to availability. Chicks were distributed among 30 floor pens (12 birds/pen) containing used litter top-dressed with fresh pine shavings and reared to 14 d post-hatch. Birds and feeders were weighed together at 0 and 14 d post-hatch for the calculation of BWG, FI, and FCR. The dietary treatments in Experiment 2 were the same as those used during the starter period.

At 14 d post-hatch, all birds from each pen were humanely euthanized. Digesta was flushed from all birds in 6 replicate pens of each treatment using deionized water from the lower one-third of the ileum (as defined by the section of small intestine between the Meckels diverticulum and ileo-cecal junction). Frozen ileal digesta samples were lyophilized and ground using an electric coffee grinder to provide an evenly ground sample while avoiding significant loss. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, and ether extract. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to the AOAC (2006) METHOD 920.39. Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (AID) of dry matter, gross energy, ether extract, and nitrogen were calculated using the following equation:

$$\text{AID, \%} = \{[(X / TiO_2)_{\text{diet}} - (X / TiO_2)_{\text{digesta}}] / (X / TiO_2)_{\text{diet}}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration to TiO_2 in the diet or ileal digesta. Energy digestibility (%) values obtained from the above equation were multiplied by the gross energy content of the feed to calculate ileal digestible energy (**IDE**) in kcal/kg.

Statistical Analysis

In both experiments, pen was considered the experimental unit with 12 replicate pens for Experiment 1 and 6 replicate pens for Experiment 2 for each of the 5 dietary treatments arranged in a randomized complete block design. Data were analyzed by a one-way ANOVA using the MIXED procedure in SAS 9.4. Orthogonal contrasts were used to compare the positive control versus negative control diets and the linear and quadratic effects of tributyrin inclusion. The main effect of sex and diet type interactions on body weight gain and carcass characteristics was analyzed by a two-way ANOVA using the procedure as described above. Statistical significance was considered at $P < 0.05$. Statistical outliers were defined as values exceeding 3 studentized residuals of the mean.

RESULTS

Growth performance

Experiment 1. During the starter phase, birds fed the NC diet had a higher BWG ($P = 0.033$) and a reduced FCR ($P = 0.009$) compared to birds fed the PC diet (Table 2.2). Furthermore, a quadratic response ($P = 0.049$) in BWG was observed with increasing amounts of TB, which was accompanied by a quadratic response in FI ($P = 0.020$) and increase in FCR ($P = 0.003$). Additionally, females had significantly higher body weights ($P < 0.001$) compared to male broilers, however, no interaction ($P > 0.05$) between diet and sex was observed (data not shown).

During the grower period, no main effects or interactions of sex ($P > 0.05$) during the grower period was observed for growth performance (data not shown). Furthermore, no differences ($P > 0.05$) were observed in BWG between birds fed the PC and NC diets (Table 2.2). However, birds fed the NC diet had higher ($P < 0.001$) FCR compared to birds fed the PC diet. There was also a linear decrease ($P = 0.015$) in BWG observed with increasing amounts of TB, although no differences were observed in FI or FCR in response to TB.

From 0 to 35 d post-hatch male broilers had significantly heavier body weights ($P < 0.001$) compared to female broilers (data not shown), however, there were no interactions ($P > 0.05$) between sex and diet observed. Additionally, there were no differences ($P > 0.05$) in BWG between birds fed the PC and NC diets, whereas birds fed the PC diet had lower ($P < 0.001$) FCR compared to birds fed the NC diet (Table 2.2). Furthermore, there was a linear reduction ($P = 0.002$) in BWG and a linear increase ($P = 0.001$) in FCR as TB dose increased, with no effects ($P > 0.05$) of TB on FI. There were also no differences ($P > 0.05$) in mortality among any of the treatments during the trial.

At 36 d post-hatch, females had increased carcass yield ($P < 0.001$), breast yield ($P < 0.001$), tender yield ($P < 0.001$), and leg quarter yield ($P = 0.003$) compared to males with no diet by sex interactions ($P > 0.05$) observed (Table 2.3). In addition, relative fat pad weight, which was higher ($P = 0.023$) for NC birds than for PC birds, was the only processing measurement observed to be different between the PC and NC groups (Table 2.3). Increasing dietary TB linearly reduced live bird weight and weights of hot carcasses, chilled carcasses, breast, tenders, wings, and leg quarters ($P < 0.01$) (data not shown). Linear reductions in chilled carcass ($P = 0.024$) and breast ($P < 0.001$) yield were observed with increasing TB concentration. Inversely, a linear increase ($P = 0.002$) in relative fat pad weight was observed

with increasing amounts of TB. Furthermore, a quadratic ($P = 0.022$) response in tender yield was observed with increasing TB.

Experiment 2. No differences ($P > 0.05$) in BWG, FI, or FCR were observed among any of the dietary treatments from 0 to 14 d post-hatch (Table 2.4). Birds fed the PC diet had greater ($P = 0.008$) nitrogen digestibility and IDE than birds fed the NC diet (Table 2.5). A linear increase ($P = 0.022$) was observed in ether extract digestibility as dietary TB increased. Additionally, there was a quadratic response of dietary TB observed for dry matter digestibility and IDE, with the greatest numerical value for TB-fed birds observed at the 3x dose.

DISCUSSION

In the current experiments, broilers were fed graded doses of TB in a step-down program in a reduced energy and amino acid diet to establish an optimal dose for broiler growth performance, nutrient digestibility, and carcass characteristics for subsequent experiments. In prior studies, the supplementation of butyrate glycerides have been inconsistent regarding their effects on growth performance and carcass characteristics (Bedford et al., 2017a; Antongiovanni et al., 2016; 2007; Leeson et al., 2005). In Experiment 1 and 2, birds were fed the same diets and reared under similar management and environmental conditions, however, different genetic lines were used due to chick availability at the time of the 2 experiments. It has previously been reported that broiler strains may influence responses to TB (Bedford et al., 2017a), therefore, the difference between genetic lines needs to be taken into consideration when evaluating the current experiments.

In Experiment 1, as expected, a reduction in energy and amino acid density in the negative control caused an increase in overall FI and FCR, ultimately leading to a reduction in carcass leanness and subsequent increases in abdominal fat. Tributyrin was added at 500, 1,500,

and 2,500 mg/kg in the starter phase and stepped down to 250, 750, and 1,250 mg/kg, respectively, in the grower and finisher phases of Experiment 1. During the starter phase, a quadratic response in FI and FCR was observed in Ross 708 birds with increasing amounts of TB. Additionally, feeding a step-down program in Experiment 1 led to a linear reduction in BWG and a linear increase in FCR with increasing amounts of TB from d 0 – 35. Bedford et al. (2017b) suggested that butyrate glycerides may not need to be fed at high levels throughout the growth phase. It is important to note, however, that in the current studies, the highest-level fed was a 5x dose (2,500S; 1,250 GF), which is a much lower dose compared to previous research (Leeson et al., 2005). Even so, there were no indication to suggest that the 5x dose (2,500S; 1,250GF) of TB was unsafe to the bird's health.

Furthermore, increasing the amount of TB in diets also impacted carcass characteristics. Specifically, birds fed the 5x dose ((2,500 starter (S); 1,250 grower and finisher (GF)) had significantly lower breast meat yield compared to birds fed the 1x dose of TB (500S; 250GF). Conversely, Leeson et al. (2005) reported that birds fed 2,000 mg/kg of mixed butyrins had higher carcass and breast weight at d 42 compared to birds fed 1,000 mg/kg. In agreement, Bedford et al. (2017b) observed a significant reduction in abdominal fat weight at 5 weeks of age in broilers fed a mixture of mono- and tributyrin, thus potentially indicating a shift in lipid metabolism with the addition of butyrate glycerides to the diet. One key difference between the current studies and previous research is the diet composition and nutrient levels. In the current studies, an excess of energy relative to the protein level may have occurred in the NC compared to the PC due to the relatively larger reduction in AME_n compared with AA density, thus birds may have overconsumed energy in order to meet their protein requirement (Griffiths et al., 1977). This overconsumption of energy by the broiler may have led to an increased accumulation

of abdominal fat. Whereas in previous research, the evaluation of butyrate glycerides on broiler growth performance and carcass characteristics has been in diets containing adequate nutrient levels, therefore overconsumption was likely not an issue. Furthermore, previous research has utilized diets containing higher dietary fat levels and included ingredients such as wheat by products (Leeson et al., 2005; Bedford et al., 2017b). Therefore, it is possible that dietary nutrient levels and diet composition may influence broiler's utilization of TB.

In Experiment 2, no significant differences in growth performance were observed from 0 – 14 days with increasing amounts of TB or between the PC and NC. An increase in nitrogen digestibility and IDE kcal/kg, however, was observed for PC fed birds compared to NC fed birds. The PC contained 4 times the amount of dietary lipid compared to the NC. Therefore, the higher lipid concentration may have potentially improved broiler nutrient absorption through an "extra-caloric effect" (Jensen et al., 1970). In Experiment 2, ether extract digestibility increased linearly with increasing TB supplementation and coincided with a quadratic increase in IDE kcal/kg, with the highest value reported at the 3x dose (1,500 mg/kg) which was exactly 100 kcal higher compared to birds fed the NC. This increase in energy utilization, with no increase in nitrogen digestibility, may have also contributed to an excess energy to protein ratio in Experiment 1, as evidenced by abdominal fat pad accumulation. Previous research has reported no difference in amino acid digestibility when TB was included in the diet at 1,000 mg/kg (Moquet et al., 2018) when compared to a control diet or other forms of butyrate. However, Liu et al. (2017) noted that when evaluating butyrate sources with different release points, an increase in IDE kcal/kg was observed at the 500 mg/kg level compared to a control diet.

In conclusion, increasing TB supplementation beyond the recommended dose of 500 mg/kg had a negative impact on broiler growth performance and carcass characteristics in

Experiment 1, including an increase in abdominal fat pad deposition. However, in Experiment 2, the same graded doses of TB had no influence upon growth performance or nitrogen digestibility, but improved ether extract and energy digestibility. This indicates that TB may have a greater potential to improve energy utilization than amino acid digestibility and that dietary energy level should be taken into consideration when using TB in practical feed formulations. Furthermore, the potential for TB to improve energy utilization may be influenced by the source, quality, and concentration of added lipids, and these factors warrant further investigation.

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TABLES

Table 2.1 Diet formulations for broilers fed graded doses of tributyrin (TB) in a step-down program from 0 to 35 d post-hatch (Experiment 1 and 2)

	Starter		Grower		Finisher	
Ingredient	PC	NC	PC	NC	PC	NC
Corn	52.10	58.53	55.62	62.47	60.41	66.66
Soybean meal	35.95	31.87	32.37	28.20	27.54	23.75
Corn DDGS[1]	5.00	5.00	5.00	5.00	5.00	5.00
Poultry fat	3.42	0.82	3.87	1.17	4.23	1.68
Limestone	1.21	1.23	1.14	1.17	1.09	1.11
Dicalcium phosphate	1.10	1.11	0.88	0.89	0.70	0.71
Sodium chloride	0.37	0.37	0.37	0.37	0.37	0.37
DL-methionine, 99%	0.30	0.27	0.28	0.24	0.24	0.21
L-lysine HCl, 78.8%	0.20	0.21	0.18	0.20	0.17	0.19
L-threonine, 98%	0.12	0.11	0.07	0.07	0.06	0.06
Trace mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride, 60%	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[4]	0.03	0.03	0.03	0.03	0.03	0.03
Selenium premix (0.06%)	0.02	0.02	0.02	0.02	0.02	0.02
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50
Builder's sand[5]	-	0.250	-	0.125	-	0.125
Calculated nutrient composition, % unless noted otherwise						
AME_n, kcal/kg	3,030	2,930	3,130	3,030	3,200	3,100
CP	23.00	21.50	21.50	20.00	19.50	18.14
Digestible Lys	1.25	1.16	1.12	1.04	1.00	0.93
Digestible TSAA	0.93	0.86	0.85	0.79	0.78	0.73
Digestible Thr	0.84	0.78	0.75	0.70	0.67	0.62
Total Ca	0.96	0.96	0.87	0.87	0.79	0.79
Available P	0.48	0.48	0.44	0.44	0.40	0.40
Analyzed nutrient composition, %						
Dry matter	89.12	89.25	90.00	89.71	89.53	89.93
Protein	23.00	22.20	21.60	20.70	20.00	18.60
Gross energy, kcal/kg	4,002	3,986	4,035	4,062	4,112	4,077

[1] Dried distillers grains with solubles.
[2] The mineral premix provided (per kg of diet): calcium, 55.5 mg, manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg copper, 10.0 mg; iodine, 1.0 mg.
[3] The vitamin premix provided (per kg of diet): vitamin A, 6,350 IU; vitamin D3, 4,536 ICU, vitamin E, 45 IU, vitamin B12 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.
[4] OptiPhos 2000, (Huvepharma Inc., Peachtree City, GA) provided 250 FTU/g of phytase activity.
[5] Tributyrin was added to the experimental diets at the expense of sand to achieve 500 mg/kg tributyrin.

Table 2.2 Live performance of broilers fed diets with graded doses of tributyrin (TB) in a step-down program from 0 to 12 d post-hatch (Experiment 1)[1]

	D 0 – 12			D 0 – 26			D 0 - 35		
Item	BWG, kg	FI, kg	FCR, kg:kg	BWG, kg	FI, kg	FCR, kg:kg	BWG, kg	FI, kg	FCR, kg:kg
PC	0.182	0.306	1.695	1.046	1.440	1.391	1.942	2.767	1.437
NC	0.194	0.305	1.582	1.053	1.491	1.436	1.918	2.845	1.500
NC + 1x TB	0.185	0.323	1.711	1.045	1.512	1.446	1.932	2.896	1.498
NC + 3x TB	0.181	0.309	1.698	1.001	1.468	1.453	1.839	2.812	1.521
NC + 5x TB	0.186	0.307	1.660	1.009	1.465	1.452	1.837	2.813	1.528
SEM	0.005	0.006	0.033	0.022	0.020	0.012	0.032	0.046	0.009
NC vs PC	0.033	0.857	0.009	0.753	0.064	<0.001	0.449	0.090	<0.001
TB Linear *P*-value	0.075	0.752	0.071	0.015	0.154	0.153	0.002	0.216	0.001
TB Quadratic *P*-value	0.049	0.020	0.003	0.602	0.551	0.523	0.714	0.448	0.516

[1]PC = positive control, NC = negative control (Reduction of 100 kcal/kg in AME_n and 7% digestible amino acids relative to PC), 1x = 500 mg/kg TB in starter; 250 mg/kg TB in grower and finisher, 3x = 1,500 mg/kg in starter, 750 mg/kg TB in grower and finisher, 5x = 2,500 mg/kg in starter, 1,500 mg/kg in grower and finisher

[2]ANOVA = overall *P* – value.

Table 2.3 Processing yields of broilers fed diets with graded doses of tributyrin (TB) fed in a step-down program from 0 to 36 d post hatch (Experiment 1)[1]

Item	Chilled Carcass Yield	Fat Pad Yield	Breast Yield	Tender Yield	Wing Yield	Leg Quarter Yield
	----------%					
Main effect of sex[2]						
Male	75.25[b]	1.36	19.10[b]	4.14[b]	8.06	22.57[a]
Female	76.15[a]	1.43	20.03[a]	4.41[a]	8.08	22.20[b]
SEM	0.13	0.03	0.12	0.03	0.03	0.09
Main effect of diet						
PC	75.80	1.24	19.81	4.35	8.04	22.38
NC	75.91	1.33	20.13	4.29	8.02	22.16
NC + 1x dose TB	76.09	1.35	19.86	4.35	8.11	22.32
NC + 3x dose TB	75.60	1.38	19.56	4.26	8.08	22.03
NC + 5x dose TB	75.41	1.47	19.07	4.10	8.10	22.09
SEM	0.27	0.04	0.21	0.06	0.05	0.28
P-values						
Sex	< 0.001	0.067	<0.001	<0.001	0.661	0.003
Sex x diet	0.668	0.957	0.537	0.525	0.708	0.295
NC vs PC	0.677	0.023	0.138	0.409	0.783	0.436
TB Linear *P*-value	0.024	0.002	<0.001	0.002	0.190	0.573
TB Quadratic *P*-value	0.340	0.208	0.471	0.022	0.294	0.820

[1]PC = positive control, NC = negative control (Reduction of 100 kcal/kg in AME_n and 7% digestible amino acids relative to PC), 1x = 500 mg/kg TB in starter; 250 mg/kg TB in grower and finisher, 3x = 1,500 mg/kg in starter, 750 mg/kg TB in grower and finisher, 5x = 2,500 mg/kg in starter, 1,500 mg/kg in grower and finisher.

[2] [a-b] Means within a row that do not share a common superscript are different ($P < 0.05$).

Table 2.4 Live performance of broilers fed diets with graded doses of tributyrin (TB) from 0 to 14 d post-hatch[1] (Experiment 2)

Item	D 14 BW, kg	BWG, kg	FI, kg	FCR
PC	0.399	0.354	0.409	1.359
NC	0.415	0.370	0.436	1.341
NC + 1x TB	0.410	0.366	0.438	1.354
NC + 3x TB	0.408	0.363	0.443	1.433
NC + 5x TB	0.405	0.361	0.422	1.374
SEM	0.015	0.015	0.019	0.035
NC vs PC	0.286	0.276	0.171	0.595
TB Linear *P*-value	0.501	0.485	0.548	0.106
TB Quadratic *P*-value	0.962	0.942	0.400	0.144

[1]PC = positive control, NC = negative control (Reduction of 100 kcal/kg in AME_n and 7% digestible amino acids relative to PC), 1x = 500 mg/kg TB in starter; 250 mg/kg TB in grower and finisher, 3x = 1,500 mg/kg in starter, 750 mg/kg TB in grower and finisher, 5x = 2,500 mg/kg in starter, 1,500 mg/kg in grower and finisher.
[2]ANOVA = overall *P* – value.

Table 2.5 Nutrient digestibility of broilers fed diets with graded doses of tributyrin (TB) from 0 to 14 d post-hatch[1] (Experiment 2)

Item	Dry Matter, %	Nitrogen, %	Ether Extract, %	IDE kcal/kg
PC	62.05	79.28	84.77	4,073
NC	57.95	75.19	81.71	3,479
NC + 1x TB	60.67	75.89	81.05	3,478
NC + 3x TB	62.65	77.88	84.80	3,579
NC + 5x TB	58.33	75.77	88.21	3,416
SEM	2.27	1.38	2.74	37
NC vs PC	0.072	0.008	0.270	<0.001
TB Linear *P*-value	0.669	0.407	0.022	0.437
TB Quadratic *P*-value	0.042	0.162	0.308	0.012

[1]PC = positive control, NC = negative control (Reduction of 100 kcal/kg in AME_n and 7% digestible amino acids relative to PC), 1x = 500 mg/kg TB in starter; 250 mg/kg TB in grower and finisher, 3x = 1,500 mg/kg in starter, 750 mg/kg TB in grower and finisher, 5x = 2,500 mg/kg in starter, 1,500 mg/kg in grower and finisher.
[2]ANOVA = overall *P*-value.

CHAPTER IV: EVALUATION OF TRIBUTYRIN SUPPLEMENTATION IN DIETS VARYING IN LIPID SOURCE, LIPID LEVEL, AND CORN PARTICLE SIZE ON LIVE PERFORMANCE AND NUTRIENT UTILIZATION IN BROILERS REARED TO 21 D

ABSTRACT

Two experiments were conducted to investigate the impact of tributyrin **(TB)** on broiler performance and apparent ileal and nutrient and energy digestibility **(IDE).** In Experiment 1, birds were fed diets containing either soy oil **(SO)** or poultry fat **(PF)** at either a standard or high level of inclusion. Diets that contained a higher level of fat increased by 1.88% and 100 kcal/kg compared to the standard diets. Whereas in Experiment 2, diets were fed containing either SO or PF with a 730 µm or 1,042 µm corn particle size and formulated to be isocaloric and isonitrogenous. In both experiments, dietary treatments were arranged in a 2 by 2 by 2 factorial for a total of 8 treatments and were fed and maintained across one phase (0 to 21 d). Titanium dioxide was used as an indigestible marker in the feed for the determination of ileal digestibility. Nine replicate battery cages of 8 Cobb 500 male broilers per treatment were placed for each experiment. In Experiment 1, a significant ($P = 0.001$) interaction of lipid level by TB inclusion in d 0 – 21 BWG was observed where birds fed a high lipid level diet with TB or fed a standard lipid level without TB significantly improved BWG compared to a standard lipid level with TB. This significant interaction (lipid level by TB) was also observed in FI. The birds fed a high lipid diet with TB had significantly ($P = 0.009$) higher FI compared to birds fed a high lipid diet without TB. Furthermore, the high level of lipid significantly ($P < 0.001$) improved nitrogen, fat, and ileal energy digestibility compared to the standard lipid level. In Experiment 2, a lipid source x TB inclusion interaction was observed (P = 0.005) for FCR, where an increase in FCR was

observed in broilers fed TB within SO diets but an inverse response on FCR in broiler fed PF diets. A 3-way interaction among lipid source, corn particle size, and TB inclusion was observed (P = 0.028) for N digestibility which was driven by a tendency for TB to increase N digestibility in SO diets with a 1,042 µm corn particle size that was not observed with the 730 µm corn particle size. A similar response led to a 3-way interaction for IDE (*P* = 0.014). In conclusion, the data from these two experiments indicate that dietary lipid source, lipid level, and corn particle size may not be a primary determinant of TB efficacy.

INTRODUCTION

In the past five years, the poultry industry has experienced a 40% increase in antibiotic free production (Rennier Associates Inc., 2019). Historically, antibiotics have helped to maintain flock health and subsequently ensure target performance is achieved. Therefore, causing a reduction in pathogenic bacteria while stimulating the growth of the intestinal epithelium, and in turn nutrient utilization, in the absence of in-feed antibiotics has become increasingly important for poultry producers (Truscott and Al-Sheikhly, 1977; Ferket et al., 2004). Several alternatives to in-feed antibiotics have been widely researched in their effectiveness and impact on broiler performance. One of the more popular alternatives includes short chain fatty acids (SCFA), specifically butyrate. Butyrate has been shown to improve broiler intestinal health by development of gut wall tissues and modulation of growth within the gastrointestinal microflora (Van Immerseel et al., 2004; Friedman and Barshira, 2005). Furthermore, butyrate has been reported to improve broiler body weight, feed conversion, and reduce pathogenic bacteria in the digestive tract (Chamba et al., 2014; Zhang et al., 2011; Hu and Guo, 2007). However, because butyrate can be fed in several different forms including unprotected, salt, or as a mono-, di-, triglyceride; the sites of digestion and activity can vary within the bird's gastrointestinal tract.

For instance, Moquet et al. (2018) reported that unprotected butyrate is rapidly absorbed via passive diffusion in the upper gastrointestinal tract, while absorption of esterified butyrate is thought to occur in the small intestine. Esterification can improve the component SCFA potential by delivering the fatty acids to the small intestine intact following cleavage by pancreatic lipase from the glycerol backbone, initiating lipolysis.

In the US, poultry fat **(PF)** and soy oil **(SO)** are commonly used dietary lipid sources due to their widespread availability, high digestibility, and low cost. Compared with PF, SO has 1.97 times lower levels of saturated fats and is typically more digestible (Tancharoenrat et al., 2013), resulting in higher metabolizable energy values. Dietary inclusion of soy oil has risen in recent years due to the increased adoption of all-vegetable based diets, however, the majority of diets formulated continue to use poultry fat. The dietary inclusion of these lipid sources can also vary depending on the stage of growth and other nutritional factors such as metabolizable energy. Another consideration when shifting from conventional to all-vegetable based, is the ingredient profile. When all animal by-products (i.e. by-product meals and poultry fat) are removed, the use of soy oil and cereal grains and legumes inclusion levels increase to maintain nutrient requirements. Previous studies have noted that lipid concentration can influence several aspects of the lipolysis process including pancreatic lipase secretion, colipase secretion, and bile salt formation (Krogdahl, 1985; Ravindran et al. 2016). Therefore, changes in dietary lipid source and concentration may also affect lipolysis of TB and its subsequent effects on broiler growth performance, nutrient utilization, duodenal pH, and intestinal morphology, especially in young broilers which may have limited secretion of enzymes such as pancreatic lipase.

In addition to lipid source and content, cereal grain particle size can have a direct bearing on gizzard function, reverse peristalsis, and pancreatic enzyme (e.g. lipase) secretion (Svihus,

2011; Naderinejad et al., 2016). A recent study by Qaisrani et al. (2015) noted that feeding a coarse particle size diet supplemented with an unprotected sodium butyrate butyric acid source resulted in improved feed intake, body weight gain, and feed conversion ratio, with little to no effect occurring in birds fed a fine particle size diet. Higher gizzard function can increase enzymatic activity, and when coupled with butyrate, may result in a synergistic effect upon nutrient digestibility and ultimately growth performance (Qaisrani et al., 2015). Thus, because TB requires cleavage by lipase, impacts of ingredient composition and particle size on passage rate and enzyme secretion may have an even greater influence on its utilization compared with other forms of butyric acid. These experiments aimed to evaluate the inclusion of tributyrin in diets varying in lipid source and concentration, and corn particle size and their effect on broiler growth performance, nutrient utilization, lipid metabolism, gizzard activity, and jejunal morphology.

MATERIALS AND METHODS

All animal care and experimental procedure were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

Common Husbandry Procedures (Experiments 1 and 2)

In both experiments, 576 male by-product chicks from a Cobb 500 female line were obtained from a commercial hatchery on day of hatch. All chicks were group-weighed and distributed to 72 battery cages. Each cage was equipped with a trough feeder and nipple drinkers. Eight birds per cage were placed (24 m by 24 m) and provided feed and water *ad libitum* throughout the experiment. The lighting schedule and temperature targets were adjusted according to management guidelines provided by the primary breeder. In both Experiment 1 and

2, birds and feeders were weighed at 0, 14, and 21 d post-hatch for calculation of body weight gain (**BWG**), feed intake (**FI**), and mortality corrected feed conversion ratio (**FCR**).
Birds fed corn and soybean meal-based mash diets formulated to meet or exceed nutrient recommendations (Cobb-Vantress, 2015) in a single feeding phase. For each experiment, common basal diets were mixed, aliquoted, and remixed with appropriate experimental ingredient additional for each experimental treatment. Titanium dioxide was included at 0.50% in all diets as an indigestible marker for determination of nutrient digestibility.

Experiment 1 Treatment Structure

Eight dietary treatments in Experiment 1 consisted of a factorial arrangement of 2 lipid sources (poultry fat or soy oil) by 2 lipid concentrations (standard and high) by 2 tributyrin concentrations (with or without 500 mg/kg tributyrin). For the high lipid diets, lipid supplementation was increased from 1.89% to 3.77% equating to a 100 kcal/kg increase in AME for the soy oil diets (Table 3.1); thus, diets were not isocaloric between lipid concentrations of sources.

Experiment 2 Treatment Structure

Dietary treatments consisted of a factorial arrangement of 2 lipid sources (poultry fat or soy oil) x 2 corn particle sizes (730 µm, and 1,042 µm) x 2 tributyrin concentrations (with or without 500 mg/kg tributyrin) (Table 3.2). Whole corn was ground with a hammer mill equipped with either a 3 or 9 mm screen to produce ground corn with geometric mean diameters of 730 and 1,042 µm, respectively. The geometric mean diameter was determined according to the American Society of Agricultural Engineers (2008). In addition, tributyrin was added at the expense of cellulose in treatment diets 2, 4, 6, and 8. Cellulose was used in soy oil diets to

maintain isocaloric levels between diets. All diets were analyzed for proximate nutrient composition.

Sampling and Laboratory Analyses

In both experiments, all birds from 9 replicate cages were humanely euthanized by CO_2 inhalation for sample collection on d 21. Ileal contents from all birds in each cage were collected by gently flushing the distal half of the ileum using deionized water. Digesta samples were pooled within cage and frozen (−20°C) until analysis. Two birds per cage were also randomly selected for blood, jejunal tissue collection, and pH determination of the duodenal lumen contents that were subsequently collected for lipase activity and volatile fatty acid (VFA) concentrations. Blood was collected via cardiac puncture into tubes containing EDTA, placed on ice, and centrifuged for 15 min at 1,300 × *g* and 4°C to separate plasma. Plasma from birds within a cage were pooled, aliquoted, and stored at -80°C until further analysis. Jejunal tissue samples (~ 2 cm in length) were collected at the midpoint of the jejunum between the end of the duodenal loop and the Meckel's diverticulum and rinsed with PBS to remove luminal contents and placed in scintillation vials containing 10% neutral-buffered formalin.

Organ weights, pH, and plasma analysis. In both experiments, liver and adipose tissue surrounding the gizzard and fat pad were collected and weighed from 2 birds per cage on d 21. All organ weights were adjusted to bird weight to achieve a relative organ weight. Additionally, plasma samples were pooled together from 2 birds per cage and analyzed for triglycerides using the instructions provided by the manufacturer (Kit# 10010303; Cayman Chemical, Ann Arbor, MI). To determine duodenal pH in Experiment 1, the duodenal loop was cut in a half and the digital pH meter (Mettler-Toledo, UK) and a spear tip piercing pH electrode (Sensorex S175CD) was directly inserted into the digesta of the distal duodenal loop for pH measurement. The probe

was rinsed with distilled water after each reading and the tip of the pH probe was stored in double distilled water when not in use. After pH was recorded, the digesta was gently squeezed into cryogenic vials and snap frozen using liquid nitrogen. Digesta was then stored at -80°C for further analysis for lipase activity and butyrate concentration. In Experiment 2, gizzard pH was measured using the procedure as described above. It was then emptied using deionized water and weighed.

Lipase activity and SCFA determination. Lipase activity of duodenal digesta samples was measured using the lipase activity assay kit according to the instructions provided by the manufacturer (Kit # MAK046; Sigma Aldrich, St. Louis, MO). Volatile fatty acid concentrations were determined using the method described by Weber et al. (2010) where duodenal digesta samples were thawed and thoroughly mixed for at least 30 s. Approximately 0.5 g of duodenal digesta was pooled from 2 birds/cage to total 1.0 g which was placed into 15-mL polypropylene centrifuge tubes and diluted with 5 mL of deionized water. Samples were then mixed overnight on a rocking platform. After mixing, samples were centrifuged at 4°C for 30 min at 21,000 x g to separate supernatant. Approximately 2.5 mL of clear supernatant was removed and placed into tubes and o-phosphoric acid was added to achieve a pH between 2 – 2.5. Exactly 1 mL of the pH-adjusted supernatant sampled was placed into 20 mL gas chromatography vials with 0.3 g of NaCl. Prepared samples were frozen and shipped to an external laboratory (USDA-ARS-MWA-NLAE, Ames, IA) for gas chromatography analysis as described by McCafferty et al. (2019) (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc, Wilmington, DE). Samples were analyzed in duplicates and total VFA concentrations measured included acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic, and heptanoic acid concentrations.

Nutrient digestibility. Frozen ileal digesta samples were lyophilized and ground using an electric coffee grinder. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, and ether extract. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to the AOAC (2006) method 920.39. Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (AID) of dry matter, gross energy, ether extract, and nitrogen were calculated using the following equation:

$$AID, \% = \{[(X / TiO_2)_{diet} - (X / TiO_2)_{digesta}] / (X / TiO_2)_{diet}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration to TiO_2 in the diet or ileal digesta. Energy digestibility (%) values obtained from the above equation, were multiplied by the gross energy content of the feed to calculate ileal digestible energy (**IDE**) in kcal/kg.

Jejunal histology. Jejunum tissue samples were embedded in paraffin, sectioned at 4 μm, set on a glass slide, and stained with hematoxylin and eosin. Photomicrographs of each jejunum sample were acquired using a light microscope (Nikon Eclipse) equipped with camera and software and used for morphometric analysis. Imaging software (Nikon's NIS Elements Basic Research Microscope Imaging) was used for measurement of villus height, crypt depth, and villus width under 4x magnification. For villus height, approximately 4 intact well-oriented villi per bird were randomly selected and measured. Villus height was measured from the tip of the villus to the villus-crypt junction, whereas crypt depth was defined as the depth of the invagination between adjacent villi. The width of the villus was measured at the basal (crypt-

villus junction) and apical ends (Iji et al., 2001). Apparent jejunal villus surface area was surface area was calculated using the following equation published by Iji et al. (2001):

Apparent villus surface area = ((villus basal width + villus apical width)/2 × villus height)).

Statistical analysis

In both experiments, cage was considered the experimental unit with 9 replicate cages for each of the 8 dietary treatments arranged in a randomized complete block design and the statistical model used pen location as a random blocking factor. Data were analyzed by a three-way ANOVA to evaluate the main effects and all interactions among lipid source, lipid level, and tributyrin inclusion in a 2 by 2 by 2 factorial arrangement for Experiment 1. In Experiment 2, data were also analyzed by a three-way ANOVA to evaluate the main effects and all interactions among lipid source, corn particle size, and tributyrin inclusion in a 2 by 2 by 2 factorial arrangement. Statistical significance was considered at $P \leq 0.05$ in all cases. Statistical outliers were defined as values exceeding 3 studentized residuals of the mean. Before removing statistical outliers, all raw data and calculations were confirmed to be correct.

RESULTS

Experiment 1

There were no three-way interactions ($P > 0.05$) observed among lipid source, lipid level, and TB inclusion on growth performance. Therefore, only two-way interactions and main effects will be discussed for all measurements (Table 3.3). For D 0 to 21, a main effect ($P < 0.001$) of lipid level was observed whereby birds fed a high lipid level diet had improved FCR compared to birds fed a standard lipid level diet (Table 3.3). For body weight gain during the same time period, a lipid level by TB inclusion interaction ($P = 0.001$) was observed where TB inclusion reduced BWG by 55 grams in broilers fed the standard lipid concentration but increased BWG

by 44 grams in broilers fed the high lipid concentration (Table 3.3). This interaction ($P = 0.009$) was also observed for FI whereby TB inclusion numerically decreased FI in broilers fed the standard lipid concentration and increased FI in broilers fed the high lipid concentration. This trend was also observed in FCR ($P = 0.053$). Additionally, broilers fed soy oil had lower FCR compared to broilers fed poultry fat ($P = 0.039$). Lastly, broilers fed the high lipid concentration had a 20% increase ($P = 0.038$) in relative adipose tissue weight compared with broilers fed the standard lipid concentration. There were no two-way interactions or main effects ($P > 0.05$) observed for relative liver weight (Table 3.3).

Birds fed the high lipid level had increased apparent ileal digestibility (AID) of dry matter ($P < 0.001$), nitrogen ($P = 0.002$), ether extract ($P = 0.018$), and ileal digestible energy ($P < 0.001$) compared to the birds fed the standard lipid level (Table 3.4). The high level of poultry fat increased broiler nutrient digestibility compared to the birds fed the standard level of poultry fat and soy oil and improved dry matter and ether extract digestibility compared to birds fed the high soy oil diets. Moreover, nitrogen digestibility and IDE kcal/kg was elevated in high poultry fat diets compared to birds fed high soy oil diet. Additionally, a lipid source by TB interaction was observed where TB inclusion increased AID of dry matter ($P < 0.001$), nitrogen ($P < 0.001$), and IDE kcal/kg ($P < 0.001$) in broilers fed soy oil but reduced all of these measurements in broilers fed poultry fat. There was also an interaction between lipid level and TB where TB inclusion increased AID of dry matter ($P < 0.001$) and nitrogen digestibility ($P < 0.001$) in broilers fed the standard lipid concentration but reduced digestibility in broilers fed the high lipid concentration. Conversely, for lipid digestibility, TB inclusion reduced AID of ether extract in broilers fed a standard lipid diet but increased AID of ether extract in broilers fed a high lipid diet (Level by TB, $P = 0.017$).

There were no main effects or interactions ($P > 0.05$) observed for duodenal pH (Table 3.4). Average concentrations of acetic acid, propionic acid, butyric acid, and isobutyric acid in the duodenal digesta were as follows: 229 mm/L, 6.5 mm/L, 9.0 mm/L, and 1.4 mm/L, respectively, and were not affected by dietary treatment. Interestingly, a two-way interaction of lipid source by TB ($P = 0.012$) was observed in pancreatic lipase activity whereby the inclusion of TB in soy oil diets decreased broiler lipase activity. Whereas no differences in broiler's pancreatic lipase activity was observed when fed poultry fat diets (Table 3.4).

Experiment 2

From D 0 to 21, no treatment effects ($P > 0.05$) were observed for BWG or FI (Table 3.5). An improvement in FCR ($P = 0.005$), however, was observed whereby the absence of TB in soy oil diets improved FCR compared to soy oil diets supplemented with TB. Conversely, TB inclusion in poultry fat diets did not influence FCR ($P > 0.05$).

Dietary treatment did not influence ($P > 0.05$) relative liver, adipose tissue, or gizzard weights (Table 3.5 and 3.7). There were also no treatment effects ($P > 0.05$) on gizzard pH (Table 3.7) or plasma triglycerides (data not shown). A main effect of lipid source was observed for jejunal villus height ($P = 0.010$) and crypt depth ($P = 0.042$) where broilers fed diets containing poultry fat had longer villi height and deeper crypt depths compared to broilers fed soy oil diets (Table 3.6). A corn particle size main effect was also observed for jejunal villus height ($P = 0.021$) and villus surface area ($P = 0.001$) where broilers fed diets containing 1,042 µm particle size had longer villi height and greater surface area compared to broilers fed diets containing 730 µm particle size. A lipid source by corn particle size by TB inclusion interaction ($P = 0.015$) was observed for crypt depth and villi height: crypt depth ratio, however, Tukey's test was unable to separate the means.

Three-way interactions among lipid source, corn particle size, and TB interaction were observed for dry matter ($P < 0.001$) and nitrogen ($P = 0.028$) digestibility and IDE ($P = 0.014$) (Table 3.7). In soy oil diets, the addition of TB increased dry matter digestibility ($P < 0.001$) and IDE kcal/kg ($P = 0.014$) in broilers fed 1,042 µm particle size but reduced these measurements in broilers fed 730 µm particle size, with no effect of TB among birds fed the poultry fat diets. A similar interaction was observed in the soy oil diets for nitrogen digestibility ($P = 0.028$) where the absence of TB at the 1,042 µm particle size resulted in improved broiler nitrogen digestibility compared to the 730 µm particle size soy oil diet absent in TB and poultry fat diet supplemented with TB.

DISCUSSION

In recent years, butyrate has been a commonly used feed additive in the poultry industry, but studies have been inconclusive regarding its impact on broiler growth performance and intestinal health (Van Immerseel et al., 2004; Leeson et al., 2005; Hu and Guo, 2007; Moquet et al., 2018). Tributyrin, a form of butyrate, is a triglyceride composed of three butyric acid molecules esterified to a glycerol backbone and is primarily thought to be released in the small intestine via pancreatic lipase. While butyric acid has been reported to play a role in several pathways, it primarily functions as an energy source for enterocytes which can accelerate enterocyte growth and in turn, can promote villus elongation and subsequent nutrient utilization (Cox et al., 2009; Ahsan et al., 2016). Because pancreatic lipase is the primary enzyme involved in lipolysis and TB cleavage, it is important to consider any nutritional factors that may influence its activity. In both experiments, different lipid sources were utilized to explore the impact, if any, on broiler growth performance, nutrient utilization, and jejunal morphology and as a result, TB efficacy. However, to ensure that the inclusion level of the dietary lipid source did not also

influence these parameters, lipid concentration was also taken into consideration in Experiment 1. In general, main effects and interactive effects of lipid source and level were observed on growth performance and nutrient utilization.

Previous research has noted that increasing dietary lipid concentration is thought to have an "extra-caloric" effect resulting in improved absorption of other nutrients (Jensen et al., 1970). This could be attributed to a reduction in passage rate and a subsequent longer retention time in the GIT due to the increase in lipid concentration (Mateos et al., 1982). In Experiment 1, increasing lipid concentrations improved growth performance, nutrient digestibility, and increased relative adipose tissue weight compared to birds fed a standard lipid level. Additionally, diets were formulated on a lipid level basis, resulting in slight differences in the AME_n of the diets containing an additional 1.9% of soy oil and poultry fat in Experiment 1. The elevated AME_n in diets containing soy oil improved FCR compared to broilers fed diets containing poultry fat. Whereas, in Experiment 2, diets were formulated to be isocaloric and no main effect was observed between lipid sources during growth performance. In Experiment 1, nutrient digestibility was influenced by lipid source and level. In general, an improvement in broiler nutrient digestibility was observed in both lipid sources when fed the higher inclusion level, however, the differences were significantly larger in the poultry fat diets compared to the soy oil diets.

It has been suggested that the release of TB in the small intestine may cause increased concentrations of butyrate and in turn, a reduction in pH. (Moquet et al., 2016; Moquet et al., 2018). However, In Experiment 1 TB inclusion had no effect on duodenal pH, SCFA concentration, or pancreatic lipase activity. In contrast, the addition of TB to diets containing soy oil reduced pancreatic lipase activity in the duodenum, and improved broiler dry matter

digestibility, nitrogen digestibility, and IDE kcal/kg compared to birds fed diets absent in TB. The opposite trend in nutrient digestibility occurred in the birds fed the poultry fat diets. This data suggests that pancreatic lipase may not act as a limiting factor in TB utilization as improvements were observed in nutrient digestibility when lipase activity was reduced in soy oil diets. Furthermore, the interaction in lipase activity did not appear to influence lipolysis as observed by the lack of effect in ether extract digestibility, suggesting that the lowest pancreatic lipase level supported sufficient ether extract digestibility in the bird. Additionally, a rapid absorption of butyrate is thought to take place once butyrate is cleaved from the glycerol backbone, which would explain the lack of effect observed in the SCFA concentration within the duodenum in Experiment 1 (Ahsan et al., 2016).

Tributyrin also interacted with the lipid concentration in the diet during broiler growth performance and nutrient digestibility. As previously mentioned, increasing lipid concentration is thought to have an "extra-caloric" effect (Mateos et al., 1982). This could be attributed to hormones such as cholecystokinin (CCK), ghrelin, and gastrin which have been reported to impact gastric emptying by inhibiting gastric motility and stimulating gastric muscles (Martinez et al., 1992). This is important to note because CCK acts in conjunction with the vagus nerve to stimulate pancreatic enzyme secretion (i.e. pancreatic lipase) for nutrient digestion (Li and Owyang, 1993). In Experiment 1, TB supplementation at the higher lipid level increased broiler BWG, FI, ether extract digestibility, and IDE kcal/kg compared to broilers fed the standard lipid level diet. However, as stated previously, pancreatic lipase activity appeared to not be a limiting factor in TB utilization. Therefore, the interaction that occurred in growth performance and nutrient digestibility between lipid level and TB activity was more than likely driven by the main effect of lipid concentration.

A similar main effect of lipid level was observed in broilers fed the higher lipid level having increased adipose tissue weight compared to birds fed the standard lipid level. It is well established that lipogenesis occurs primarily in the liver of chickens (Back et al., 1986) and the adipose tissue is considered to be the main lipid storage site (Griffin et al., 1992; Cogburn et al., 2004). Previous literature has reported that TB can reduce abdominal fat deposition in broilers within similar genetic strains (Bedford et al., 2017). However, in contrast to growth performance and nutrient digestibility, no interactive effects were observed between TB inclusion and lipid level of relative liver weight or adipose tissue weight. Therefore, due to the lack of effect or interaction by TB on adipose tissue and liver weight, it suggests that lipogenesis was not impacted.

It was anticipated that increasing corn particle size in Experiment 2 would alter gastrointestinal physiology, and subsequently, the effects of TB supplementation. However, there were generally minimal differences in indicators of gizzard function (i.e., weight or pH) between broilers fed 730 and 1,042 µm corn in this study. Svihus et al. (2011) noted that cereal grain particles should be larger than 1,000 µm, with at least 20% of particles within 1,500 µm to 2,000 µm, to markedly stimulate gizzard development and functionality, whereas only 10% of particles of the 1,042 µm corn were distributed between 1,500 µm and 2,000 µm in the current experiment. Nonetheless, broilers fed the 1,042 µm corn particle size had greater jejunal villi and length and surface area compared to broilers fed the 730 µm corn particle size. This agrees with previous research that has reported increased broiler and layer small intestine villus height and villi height: crypt depth ratios when feeding diets containing coarse particles (Dahlke et al., 2003; Rohe et al., 2014; Gabriel et al. 2008).

Several studies have suggested that coarser feed particles can positively affect nutrient digestibility and live performance (Nir et al., 1995; Amerah et al., 2008; Samu et al., 2010; Xu et al., 2015), possibly due in part to enhanced intestinal morphology elicited by coarse grain particles as observed in the current study (Garbriel et al., 2008). In Experiment 2, nutrient digestibility was influenced by a three-way interaction among lipid source, corn particle size, and TB inclusion. In general, TB supplementation positively affected AID of dry matter and nitrogen and IDE in broilers fed the soy oil diets with 1,042 µm corn, but adversely affected these measurements in soy oil diets with 730 µm corn, while values were similar between corn particle size and TB inclusion in diets containing poultry fat. Previous research has noted that birds fed a coarse particle size diet supplemented with butyric acid experienced an improvement in growth performance and duodenal morphology compared to a fine particle size diet (Qaisrani et al., 2016). Additionally, negative responses to 1 mg/kg TB supplementation reported by Moquet were observed in broilers fed finely ground corn and rapeseed meal, agreeing with the current experiment that TB supplementation may be most beneficial when broilers are fed coarser grain particles. This may be due to the ability of butyric acid ability to provide energy to enterocytes and in turn stimulate villus elongation (Czerwinski et al., 2012.).

Overall, inconsistencies in growth performance and nutrient digestibility were observed between the two experiments regarding lipid source and lipid level. While an overall lack of main effect was observed in corn particle size during Experiment 2. In addition, although interactions were observed, these nutritional factors may not be primarily responsible for TB efficacy as previously hypothesized. Therefore, other dietary factors need to be considered in their role of TB efficacy.

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TABLES

Table 3.1 Diet formulations of broilers fed diets varying in lipid source, lipid concentration, and tributyrin inlcusion for 21 d post-hatch (Experiment 1)

Ingredient	Soy oil – standard lipid	Soy oil – high lipid	Poultry fat – standard lipid	Poultry fat – high lipid
Corn	54.57	52.69	54.57	52.69
Soybean meal	34.76	34.76	34.76	34.76
Corn DDGS[1]	5.00	5.00	5.00	5.00
Soybean oil or poultry fat	1.89	3.77	1.89	3.77
Limestone	1.13	1.13	1.13	1.13
Dicalcium phosphate	0.86	0.86	0.86	0.86
Sodium chloride	0.33	0.33	0.33	0.33
DL-methionine, 99%	0.29	0.29	0.29	0.29
L-lysine HCl, 78.8%	0.15	0.15	0.15	0.15
L-threonine, 98%	0.14	0.14	0.14	0.14
Trace mineral premix[2]	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10	0.10
Choline chloride, 60%	0.10	0.10	0.10	0.10
Phytase[4]	0.01	0.01	0.01	0.01
Selenium premix (0.06%)	0.02	0.02	0.02	0.02
Titanium dioxide	0.50	0.50	0.50	0.50
Builder's sand[5]	0.05	0.05	0.05	0.05
Calculated nutrient composition, % unless noted otherwise				
AME_n, kcal/kg	2,975	3,075	2,969	3,063
CP	22.27	22.13	22.27	22.13
Digestible Lys	1.18	1.18	1.18	1.18
Digestible TSAA	0.89	0.88	0.89	0.88
Digestible Thr	0.80	0.80	0.80	0.80
Total Ca	0.90	0.90	0.90	0.90
Available P	0.60	0.59	0.60	0.59
Analyzed nutrient composition, %				
Dry matter	89.89	90.18	90.56	91.20
Protein	24.40	24.20	23.90	24.10
Gross energy, kcal/kg	4,087	4,171	4,059	4,234

[1] Distillers dried grain with solubles

[2] The mineral premix provided (per kg of diet): calcium, 55.5 mg; manganese, 100 mg; magnesium, 27.0 mg; zinc, 100 mg; iron, 50.0 mg; iodine, 1.0 mg.

[3] The vitamin premix provided (per kg of diet): vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3,5 mg; biotin, 0.33 mg.

[4] OptiPhos 2000 (Huvepharma Inc., Peachtree City, GA) provided 250 FTU/g of phytase activity.

[5] Tributyrin was added to the experimental diets at the expense of sand to achieve 500 ppm tributyrin.

Table 3.2 Diet formulations of broilers fed diets varying in lipid source, corn particle size, and tributyrin (TB) inclusion for 21 d post-hatch (Experiment 2)

Ingredient	Soy oil – 730 µm	Soy oil – 1,042 µm	Poultry fat – 730 µm	Poultry fat – 1,042 µm
Corn	55.70	55.70	55.93	55.93
Soybean meal	33.36	33.36	33.32	33.32
Corn DDGS[1]	5.00	5.00	5.00	5.00
Soybean oil or poultry fat	2.03	2.03	2.03	2.03
Limestone	1.14	1.14	1.14	1.14
Dicalcium phosphate	0.87	0.87	0.87	0.87
Sodium chloride	0.33	0.33	0.33	0.33
DL-methionine, 99%	0.26	0.26	0.26	0.26
L-lysine HCl, 78.8%	0.14	0.14	0.14	0.14
L-threonine, 98%	0.10	0.10	0.10	0.10
Trace mineral premix[2]	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.10	0.10	0.10	0.10
Choline chloride, 60%	0.10	0.10	0.10	0.10
Phytase[4]	0.01	0.01	0.01	0.01
Selenium premix (0.06%)	0.02	0.02	0.02	0.02
Titanium dioxide	0.50	0.50	0.50	0.50
Cellulose[5]	0.24	0.24	0.05	0.05
Calculated nutrient composition, % unless noted otherwise				
AME_n, kcal/kg	2,975	2,975	2,975	2,975
CP	22.00	22.00	22.00	22.00
Digestible Lys	1.18	1.18	1.18	1.18
Digestible TSAA	0.89	0.89	0.89	0.89
Digestible Thr	0.80	0.80	0.80	0.80
Total Ca	0.90	0.90	0.90	0.90
Available P	0.45	0.45	0.45	0.45
Analyzed nutrient composition, %				
Dry matter	91.07	91.10	90.97	92.81
Protein	23.20	23.75	24.40	23.85
Gross energy, kcal/kg	4,143	4,117	4,157	4,143

[1] Distillers dried grains with solubles

[2] The mineral premix provided (per kg of diet): calcium, 55.5 mg; manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg; copper, 10.0 mg; iodine, 1.0 mg.

[3] The vitamin premix provided (per kg of diet): vitamin A, 30,863 IU; vitamin D3, 22,045 ICU; vitamin E, 220 IU; vitamin B12, 0.05 mg; menadione, 6.0 mg; riboflavin, 26 mg; d-pantothenic acid, 40 mg; thiamine 6.2 mg; niacin, 154 mg; pyridoxine, 11 mg; folic acid, 3,5 mg; biotin, 0.33 mg.

[4] OptiPhos 2000 (Huvepharma Inc., Peachtree City, GA) provided 250 FTU/g of phytase activity.

[5] Tributyrin was added to the experimental diets at the expense of cellulose to achieve 500 ppm tributyrin.

Table 3.3 Growth performance and relative liver and adipose tissue weight of broilers fed diets varying in lipid source, lipid concentration, and tributyrin (TB) inclusion from 0 to 21 d post-hatch (Experiment 1)

Item	D 0 - 21 BWG, kg	FI, kg	FCR, kg:kg	Relative liver wt. (%)	Relative adipose tissue wt. (%)
Main effect of lipid source					
Soy oil	0.798	1.041	1.356[b]	2.94	0.64
Poultry fat	0.780	1.018	1.379[a]	2.82	0.57
SEM	0.015	0.024	0.011	0.09	0.04
Main effect of lipid level					
Standard	0.770[b]	1.024	1.390[a]	2.81	0.55[b]
High	0.807[a]	1.035	1.344[b]	2.94	0.66[a]
SEM	0.015	0.024	0.011	0.09	0.04
Main effect of TB[1]					
0 ppm	0.791	1.012	1.364	2.91	0.61
500 ppm	0.786	1.047	1.371	2.85	0.60
SEM	0.015	0.024	0.011	0.09	0.04
Two way interaction of lipid source x lipid level					
Soy oil + std	0.782	1.053	1.380	2.88	0.58
Soy oil + high	0.814	1.028	1.332	2.99	0.70
Poultry fat + std	0.759	0.995	1.401	2.74	0.53
Poultry fat + high	0.800	1.042	1.357	2.90	0.62
SEM	0.021	0.034	0.015	0.13	0.05
Two way interaction of lipid source x TB					
Soy oil + 0 ppm TB	0.804	1.019	1.355	2.99	0.65
Soy oil + 500 ppm TB	0.791	1.062	1.357	2.88	0.62
Poultry fat + 0 ppm TB	0.779	1.005	1.372	2.82	0.57
Poultry fat + 500 ppm TB	0.781	1.032	1.385	2.81	0.58
SEM	0.021	0.034	0.015	0.13	0.05
Two way interaction of lipid level x TB					
Std + 0 ppm TB	0.798[a]	1.040[ab]	1.376	2.71	0.51
Std + 500 ppm TB	0.743[b]	1.008[ab]	1.405	2.89	0.59
High + 0 ppm TB	0.785[ab]	0.984[b]	1.351	3.10	0.71
High + 500 ppm TB	0.829[a]	1.086[a]	1.338	2.79	0.61
SEM	0.021	0.034	0.015	0.13	0.05

Table 3.3 (Cont.)

	D 0 – 21 BWG, kg	FI, kg	FCR, kg:kg	Relative liver wt. (%)	Relative adipose tissue wt. (%)
P-values					
Lipid source	0.222	0.364	0.039	0.363	0.231
Lipid level	0.015	0.649	<0.001	0.297	0.038
TB inclusion	0.710	0.156	0.483	0.646	0.836
Source x level	0.75	0.149	0.842	0.867	0.770
Source x TB	0.616	0.766	0.581	0.699	0.730
Level x TB	0.001	0.009	0.053	0.064	0.098
Source x Level x TB	0.756	0.769	0.853	0.897	0.313

[a,b] Means within a row that do not share a common superscript are different ($P < 0.05$).

Table 3.4. Nutrient utilization of broilers fed diets varying in lipid source, lipid concentration, and tributyrin (TB) inclusion from 0 to 21 d post-hatch (Experiment 1)

	Duodenum					
	pH	Lipase activity (milliunits/ g digesta)	Dry matter, %	Nitrogen, %	Ether extract, %	IDE kcal/kg
Main effect of lipid source						
Soy oil	6.33	40.90	67.94	82.82	85.01	2,883
Poultry fat	6.34	41.35	68.50	82.74	84.83	2,933
SEM	0.02	3.16	0.41	0.33	0.80	20
Main effect of lipid level						
Standard	6.32	43.31	65.92[b]	81.29[b]	81.69[b]	2,742[b]
High	6.35	38.94	70.52[a]	84.27[a]	88.15[a]	3,075[a]
SEM	0.02	3.16	0.42	0.33	0.80	20
Main effect of TB						
0 ppm	6.32	43.73	68.53	82.76	84.76	2,920
500 ppm	6.35	38.52	67.90	82.79	85.08	2,896
SEM	0.02	3.16	0.42	0.32	0.80	20
Two way interaction of lipid source x lipid level						
Soy oil - std	6.30	43.71	66.74[c]	82.08[bc]	83.15[bc]	2,780[c]
Soy oil - high	6.35	38.10	69.13[b]	83.56[ab]	86.87[ab]	2,986[b]
Poultry fat - std	6.34	42.91	65.09[c]	80.50[c]	80.23[c]	2,703[c]
Poultry fat - high	6.34	39.78	71.91[a]	84.97[a]	89.43[a]	3,164[a]
SEM	0.03	4.54	0.60	0.45	1.13	29
Two way interaction of lipid source x TB						
Soy oil + 0 mg/kg TB	6.33	49.25[a]	65.47[b]	81.28[b]	83.54	2,791[b]
Soy oil + 500 mg/kg TB	6.33	32.56[b]	70.40[a]	84.36[a]	86.48	2,976[a]
Poultry fat + 0 mg/kg TB	6.32	38.21[ab]	71.59[a]	84.25[a]	85.99	3,050[a]
Poultry fat + 500 mg/kg TB	6.37	44.49[ab]	65.40[b]	82.22[b]	83.67	2,817[b]
SEM	0.03	4.54	0.60	0.46	1.16	28

Table 3.4 (Cont.)

	pH	Lipase activity (millinuts/ g digesta	Dry matter, %	Nitrogen, %	Ether extract, %	IDE kcal/kg
Two way interaction of lipid level x TB						
Std + 0 mg/kg TB	6.31	45.10	64.96[c]	80.40[c]	82.92[bc]	2,724[b]
Std + 500 mg/kg TB	6.33	41.52	66.87[bc]	82.18[b]	80.46[c]	2,759[b]
High + 0 mg/kg TB	6.33	42.35	72.11[a]	85.13[a]	86.60[ab]	3,117[a]
High + 500 mg/kg TB	6.36	35.53	68.93[b]	83.40[b]	89.69[a]	3,034[a]
SEM	0.03	4.54	0.60	0.46	1.13	29
P-values						
Lipid source	0.613	0.920	0.343	0.859	0.873	0.083
Lipid level	0.265	0.329	<0.001	<0.001	<0.001	<0.001
TB inclusion	0.285	0.245	0.289	0.956	0.779	0.396
Source x level	0.296	0.781	<0.001	0.002	0.018	<0.001
Source x TB	0.307	0.012	<0.001	<0.001	0.023	<0.001
Level x TB	0.897	0.717	<0.001	<0.001	0.017	0.042
Source x level x TB	0.680	0.431	0.826	0.815	0.357	0.817

[a-c] Means within a row that do not share a common superscript are different ($P < 0.05$).

Table 3.5 Growth performance and relative liver and adipose tissue weight of broilers fed diets varying in lipid source, corn particle size, and tributyrin (TB) inclusion from 0 to 21 d post-hatch (Experiment 2)

Treatment	D 0 – 21 BWG, kg	FI, kg	FCR, kg:kg	Relative liver wt. (%)	Relative adipose tissue wt. (%)
Main effect of lipid source					
Soy oil	0.779	1.048	1.349	2.97	0.65
Poultry fat	0.769	1.029	1.352	2.88	0.59
SEM	0.008	0.010	0.011	0.07	0.03
Main effect of corn particle size					
730 µm	0.768	1.027	1.345	2.91	0.60
1,042 µm	0.780	1.050	1.356	2.95	0.65
SEM	0.008	0.010	0.011	0.07	0.03
Main effect of TB					
0 mg/kg	0.769	1.027	1.343	2.97	0.62
500 mg/kg	0.779	1.050	1.358	2.89	0.63
SEM	0.008	0.010	0.011	0.07	0.03
Two way interaction lipid source x TB					
Soy oil + 0 mg/kg TB	0.778	1.026	1.320[b]	2.98	0.65
Soy oil + 500 mg/kg TB	0.780	1.070	1.379[a]	2.97	0.65
Poultry fat + 0 mg/kg TB	0.760	1.028	1.367[ab]	2.96	0.58
Poultry fat + 500 mg/kg TB	0.779	1.031	1.336[ab]	2.81	0.60
SEM	0.011	0.015	0.016	0.09	0.04

Table 3.5 (Cont)

Treatment	D 0 - 21 BWG, kg	FI, kg	FCR, kg:kg	Relative liver wt. (%)	Relative adipose tissue wt. (%)
Two way interaction of corn particle size x TB					
730 µm + 0 mg/kg TB	0.767	1.015	1.331	2.94	0.57
730 µm + 500 mg/kg TB	0.769	1.039	1.358	2.87	0.62
1,042 µm + 0 mg/kg TB	0.770	1.038	1.355	2.99	0.66
1,042 µm + 500 mg/kg TB	0.789	1.062	1.357	2.90	0.64
SEM	0.011	0.015	0.016	0.09	0.04
P-values					
Lipid source	0.397	0.210	0.875	0.339	0.078
Corn particle size	0.317	0.118	0.469	0.638	0.122
TB inclusion	0.352	0.110	0.362	0.386	0.717
Source x corn particle size	0.173	0.115	0.953	0.814	0.314
Source x TB	0.451	0.166	0.005	0.466	0.729
Corn particle size x TB	0.431	0.988	0.431	0.914	0.330
Source x corn particle size x TB	0.102	0.373	0.166	0.383	0.129

[a,b] Means within a row that do not share a common superscript are different ($P < 0.05$).

Table 3.6 Jejunal mucosal morphology of broilers fed diets varying in lipid source, corn particle size, and tributyrin (TB) inclusion from 0 to 21 d post-hatch (Experiment 2)

Treatment	Villus height, µm	Crypt depth, µm	Villus height to crypt depth	Villus surface area, mm^2
Main effect of lipid source				
Soy oil	1,657[b]	175[b]	9.88	5.36
Poultry fat	1,772[a]	190[a]	9.53	5.34
SEM	32.05	5.43	0.31	0.02
Main effect of corn particle size				
730 µm	1,664[b]	180	9.58	5.32[b]
1,042 µm	1,765[a]	184	9.83	5.39[a]
SEM	31.89	5.42	0.31	0.02
Main effect of TB				
0 mg/kg	1,750	189	9.68	5.35
500 mg/kg	1,679	176	9.73	5.36
SEM	30.66	5.19	0.30	0.02
Two way interaction of lipid source x corn particle size				
Soy oil + 730 µm	1,629	171	9.96	5.32
Soy oil + 1,042 µm	1,684	179	9.80	5.40
Poultry fat + 730 µm	1,698	190	9.20	5.32
Poultry fat + 1,042 µm	1,846	190	9.86	5.37
SEM	50.07	8.61	0.50	0.02

Table 3. 6 (Cont)

	Villus height, µm	Crypt depth, µm	Villus height to crypt depth	Villus surface area, mm^2
Two way interaction of lipid source x TB				
Soy oil + 0 mg/kg TB	1,666	178	9.88	5.33
Soy oil + 500 mg/kg TB	1,648	172	9.88	5.39
Poultry fat + 0 mg/kg TB	1,835	200	9.49	5.37
Poultry fat + 500 mg/kg TB	1,711	180	9.58	5.32
SEM	47.38	7.91	0.46	0.02
Two way interaction of corn particle size x TB				
730 µm + 0 mg/kg TB	1,662	192	9.11	5.33
730 µm + 500 mg/kg TB	1,667	169	10.06	5.31
1,042 µm + 0 mg/kg TB	1,839	186	10.26	5.37
1,042 µm + 500 mg/kg TB	1,692	183	9.40	5.40
SEM	47.01	8.08	0.47	0.02
Three way interaction of lipid source x corn particle size x TB				
Soy oil + 730 µm + 0 mg/kg TB	1,609	172	10.03	5.31
Soy oil + 730 µm + 500 mg/kg TB	1,649	170	9.89	5.32
Poultry fat + 730 µm + 0 mg/kg TB	1,715	212	8.18	5.34
Poultry fat + 730 µm + 500 mg/kg TB	1,683	168	10.22	5.29
Soy oil + 1,042 µm + 0 mg/kg TB	1,724	185	9.73	5.35
Soy oil + 1,042 µm + 500 mg/kg TB	1,645	173	9.87	5.46
Poultry fat + 1,042 µm + mg/kg TB	1,955	188	10.79	5.39
Poultry fat + 1,042 µm + 500 mg/kg TB	1,738	192	8.94	5.35
SEM	75.68	13.01	0.75	0.04
P-values				
Lipid source	0.010	0.042	0.410	0.396
Corn particle size	0.021	0.581	0.551	0.001
TB inclusion	0.097	0.070	0.915	0.687
Source x corn particle size	0.280	0.587	0.323	0.360
Source x TB	0.226	0.340	0.910	0.011
Corn particle size x TB	0.080	0.183	0.036	0.174
Source x corn particle size x TB	0.695	0.048	0.015	0.162

[a-c] Means within a row that do not share a common superscript are different ($P < 0.05$)

Table 3.7 Nutrient digestibility and gizzard activity of broilers fed diets varying lipid source, corn particle size, and tributyrin (TB) inclusion from 0 to 21 d post-hatch (Experiment 2)

Treatment	pH	Gizzard Relative wt. %	Dry matter, %	Nitrogen, %	Ether extract, %	IDE kcal/kg
Main effect of lipid source						
Soy oil	2.09	2.35	75.28	84.28	89.41	3,158
Poultry fat	2.07	2.36	75.12	84.74	88.63	3,179
SEM	0.11	0.05	0.45	0.33	0.64	21
Main effect of corn particle size						
730 µm	2.11	2.33	75.22	84.86	89.53	3,182
1,042 µm	2.05	2.39	75.18	84.17	88.50	3,155
SEM	0.11	0.05	0.45	0.33	0.64	21
Main effect of TB						
0 mg/kg	2.10	2.35	74.50	84.13	88.04^{b}	3,155
500 mg/kg	2.06	2.37	75.90	84.90	90.00^{a}	3,182
SEM	0.11	0.05	0.45	0.33	0.63	21
Two way interaction of lipid source x corn particle size						
Soy oil + 730 µm	2.11	2.32	75.49	84.83	88.55^{ab}	3,196
Soy oil + 1,042 µm	2.07	2.41	75.06	83.73	90.26^{a}	3,120
Poultry fat + 730 µm	2.12	2.34	74.94	84.89	90.51^{a}	3,168
Poultry fat + 1,042 µm	2.03	2.36	75.30	84.60	86.74^{b}	3,190
SEM	0.15	0.08	0.66	0.47	0.94	30
Two way interaction of lipid source x TB						
Soy oil + 0 mg/kg TB	2.14	2.39	74.86	83.91	88.64	3,139
Soy oil + 500 mg/kg TB	2.03	2.34	75.70	84.65	90.17	3,178
Poultry fat + 0 mg/kg TB	2.06	2.31	74.14	84.35	87.43	3,171
Poultry fat + 500 mg/kg TB	2.09	2.40	76.11	85.14	89.82	3,186
SEM	0.15	0.08	0.64	0.47	0.91	30
Two way interaction of corn particle size x TB						
730 µm + 0 mg/kg TB	2.19	2.34	75.68^{a}	84.95	88.65	3,215^{a}
730 µm + 500 mg/kg TB	2.04	2.32	74.76^{ab}	84.77	90.41	3,149^{ab}
1,042 µm + 0 mg/kg TB	2.01	2.36	73.32^{b}	83.31	87.42	3,095^{b}
1,042 µm + 500 mg/kg TB	2.09	2.41	77.05^{a}	85.03	89.58	3,215^{a}
SEM	0.15	0.08	0.64	0.47	0.91	30
Three way interaction of lipid source x corn particle size x TB						
Soy oil + 730 µm + 0 mg/kg TB	2.17	2.33	77.46^{a}	85.46^{a}	87.10	3,261^{a}
Soy oil + 730 µm + 500 mg/kg TB	2.05	2.31	73.53^{b}	84.20^{ab}	90.00	3,131^{ab}
Poultry fat + 730 µm + 0 mg/kg TB	2.20	2.35	73.90^{ab}	84.44^{ab}	90.20	3,169^{ab}
Poultry fat + 730 µm + 500 mg/kg TB	2.03	2.34	75.99^{ab}	85.34^{a}	90.82	3,167^{ab}
Soy oil + 1,042 µm + 0 mg/kg TB	2.11	2.45	72.26^{b}	82.36^{b}	90.19	3,017^{b}
Soy oil + 1,042 µm + 500 mg/kg TB	2.02	2.36	77.86^{a}	85.11^{ab}	90.34	3,224^{a}
Poultry fat + 1,042 µm + 0 mg/kg TB	1.91	2.27	74.38^{ab}	84.25^{ab}	84.66	3,174^{ab}
Poultry fat + 1,042 µm + 500 mg/kg TB	2.15	2.46	76.23^{ab}	84.94^{ab}	88.83	3,206^{a}
SEM	0.21	0.11	0.93	0.66	1.33	42

Table 3.7 (Cont)

Treatment	pH	Gizzard Relative wt. %	Dry matter, %	Nitrogen, %	Ether extract, %	IDE kcal/kg
P-values						
Lipid source	0.929	0.870	0.808	0.329	0.388	0.491
Corn particle size	0.674	0.782	0.956	0.145	0.258	0.369
TB inclusion	0.813	0.818	0.533	0.108	0.033	0.374
Source x corn particle size	0.888	0.686	0.031	0.393	0.003	0.105
Source x TB	0.647	0.346	0.374	0.956	0.628	0.695
Corn particle size x TB	0.452	0.680	<0.001	0.047	0.825	0.003
Source x corn particle size x TB	0.526	0.373	<0.001	0.028	0.084	0.014

[a-c] Means within a row that do not share a common superscript are different ($P < 0.05$).

CHAPTER V: THE EVALUATION OF TRIBUTYRIN IN ANIMAL PROTEIN AND VEGETABLE BASED DIETS REARED IN BATTERY CAGES AND FLOOR PENS AND ITS IMPACT UPON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY

ABSTRACT

Tributyrin **(TB)** is glyceride ester of butyrate that has the potential to improve broiler performance and intestinal development. Previous research conducted by our lab noted inconsistent effects of lipid source and concentration on TB utilization. The presence of animal protein **(APM)** or an increase in vegetable proteins **(VEG),** however, may increase the fermentability of the diet and in turn increase endogenous butyrate production. Additionally, the evaluation of TB efficacy in our lab has been in birds reared in battery cages, thus the exposure to commercial challenges, such as dirty litter and a coccidiosis vaccine, may influence TB utilization. Therefore, two experiment were conducted simultaneously to investigate the interactive effects of TB and diet type on broiler growth performance and apparent ileal nutrient **(AID)** and energy digestibility **(IDE).** Dietary treatments were arranged in a 2 by 2 factorial arrangement of 2 diet types (animal protein or vegetable based) x tributyrin supplementation (with or without 500mg/kg tributyrin) in either floor pens or battery cages. Dietary treatments were fed in one feeding phases in Experiment 1: starter (0 – 15 d), and three feeding phases in Experiment 2: starter (0 – 14 d), grower (15 – 28 d), and finisher (29 – 42 d). Diets were formulated to be isocaloric and isonitrogenous. Titanium dioxide was used in the starter feeds as an indigestible marker for determination of AID and IDE following collection of ileal digesta at 15 d in Experiment 2. Off-sex male chicks from a Cobb 500 breeder line was used for both

experiments, and birds in Experiment 1 were sprayed with a live coccidiosis vaccine at day of hatch and placed in 12 replicate floor pens of 22 birds/pen. Birds in Experiment 2 were non-vaccinated and placed in 9 replicate battery cages of 12 birds/pen. Cumulative FI was reduced at d 14 ($P = 0.032$), d 28 ($P = 0.043$), and d 42 ($P < 0.001$) and FCR was improved at d 28 ($P = 0.001$) and d 42 ($P = 0.003$) in APM fed birds compared to VEG fed birds in Experiment 1. Similarly, birds fed APM diets in Experiment 2 had reduced FI ($P < 0.001$), higher AID dry matter digestibility ($P < 0.001$), increased nitrogen digestibility ($P = 0.013$), and higher IDE kcal/kg ($P < 0.001$) compared to VEG fed birds. An underestimation of the digestibility coefficients in the meat and bone meal may be responsible for this significant increase in growth performance and nutrient utilization in the APM fed birds. Furthermore, there were no main effects or interactive effects ($P > 0.05$) of TB observed in growth performance or nutrient utilization in either trial. These findings suggest that TB supplementation had no effect in birds reared in different environments or fed APM or VEG diets.

INTRODUCTION

The poultry industry has experienced a recent shift toward increased antibiotic free production and the use of all-vegetable based diets. Nonetheless, animal proteins such as meat and bone meal (MBM) are still commonly used to replace soybean meal (SBM) and provide valuable nutrients such as phosphorus and amino acids to the diet. In vegetable-based diets, vegetable proteins such as SBM are included at levels exceeding 30% to achieve sufficient essential amino acid levels. An increase in SBM increases concentrations of oligosaccharides and non-starch polysaccharides present in the diet (Choct, 1997). Yang et al. (2016) reported that an additional 0.13% of dietary soybean oligosaccharides in a diet, which is comparable to an 1.9% increase in SBM, to a diet containing 26.9% SBM increased total volatile fatty acids in the

excreta of broilers and altered the cecal microbiota. Therefore, reduction of dietary SBM with MBM may reduce overall SCFA production from soy-derived carbohydrates. Furthermore, fermentation of undigested nitrogen derived from animal proteins such as MBM can increase the production of nitrogenous bacterial metabolites in the hindgut (Zanu et al., 2020) which has been shown to fuel pathogenic bacteria growth and exacerbate necrotic enteritis infections when exceeding levels beyond 5% (Onifade et al., 1998; Zanu et al., 2020).

Changes in ingredient composition, and in turn, endogenous SCFA production may influence the response to exogenous SCFA supplementation. Tributyrin is an esterified form of butyrate and has targeted release site in the small intestine following cleavage by pancreatic lipase. Although tributyrin supplementation has been reported to provide some benefits in the absence of antibiotics through its bactericidal activity and enhancements to gut morphology, studies have been inconsistent regarding the effect of tributyrin supplementation effect upon growth performance and carcass characteristics (Leeson et al., 2005; Bedford et al., 2017). Therefore, it is important to understand how changes in ingredient composition that may influence overall SCFA production can affect responses to dietary tributyrin supplementation.

In addition to ingredient composition, responses to gut health promoting feed additives such as tributyrin are likely influenced by bacterial load, pathogenic challenge, and environmental stress experienced by the bird (Kim et al. 2014). It has been suggested that beneficial effects of organic acids, including butyric acid, are more apparent when birds encounter environmental stressors compared to birds raised in environments with minimal challenges (Sayrafi et al., 2011). Therefore, in this study, two experiments were conducted simultaneously to evaluate TB efficacy in birds fed animal protein or all-vegetable based diets and exposed to two different environments (i.e. battery cages vs floor pens) and its influence on growth performance, nutrient

digestibility, and gastrointestinal pH. In Experiment 1, non-vaccinated birds were reared in battery cages for 15 d. Whereas in Experiment 2, birds were vaccinated with a commercial coccidiosis and reared in floor pens on used litter to 42 d. In both experiments, birds were fed vegetable-based or animal protein-based diets with or without the inclusion of tributyrin at 500 mg/kg.

MATERIALS AND METHODS

All animal care and experimental procedure were approved by the University of Arkansas Institutional Animal Care and Use Committee before initiation of the experiment.

Common Procedures and Dietary Treatments (Experiments 1 and 2)

A total of 1,488 male by-product chicks from a Cobb 500 female line were obtained from a commercial hatchery on day of hatch. In Experiment 1, 482 non-vaccinated chicks were reared in battery cages, whereas in Experiment 2, a commercial coccidiosis vaccine was administered at the hatchery in the remaining 1,056 chicks and placed in floor pens. Birds were reared simultaneously in both experiments and fed 4 experimental diets based on a factorial arrangement of 2 protein sources (vegetable or animal) in combination with 2 tributyrin concentrations (with or without 500 mg/kg tributyrin). Dietary treatments were maintained across 1 feeding phases in Experiment 1: starter (0 to 15 d); and 3 feeding phases in Experiment 2: starter (0 to 14 d), grower (15 to 28 d), and finisher (29 to 42 d). For the starter phase, a common basal was mixed, quartered, and experimental treatments were remixed with the appropriate ingredient additions according to treatment (Table 1) treatments were then allocated and fed to birds in Experiment 1 and 2. The subsequent feeding phases for Experiment 2 followed the same procedure. Diets were corn and soybean meal based and formulated on an isocaloric and digestible amino acid basis. In addition to soybean meal, the diets with animal

protein-included pork meat and bone meal and poultry fat, while vegetable-based diets included soybean oil as the source of added lipid. Titanium dioxide was also included in the starter diets at 0.5% as an indigestible marker for determination of nutrient digestibility.

Animal Husbandry

Experiment 1. All chicks were group-weighed and distributed to 36 battery cages on day of hatch. Each cage was equipped with a trough feeder and nipple drinkers. Twelve birds per cage were placed (24 cm by 24 cm) and birds were provided *ad libitum* throughout the experiment. The lighting schedule and temperature targets were adjusted according to the management guidelines provided by the primary breeder. Birds were reared up to 15 d post-hatch and on d 0 and 15, birds and feeders were weighed for calculation of body weight gain **(BWG),** feed intake **(FI)**, and mortality corrected feed conversion ratio **(FCR)**.

Experiment 2. Birds were group-weighed on day of hatch and distributed among 48 floor pens (22 birds/pen) containing used litter and top-dressed with fresh pine shavings and reared to 42 d post-hatch. Each pen contained a total of 22 birds equipped with a commercial-type pan feeder and nipple waterers to provide free access to feed and clean water throughout the trial. Supplemental feed trays were placed in each pen from 0 to 7 d post-hatch to facilitate access to feed for young chicks. Lighting and temperature were maintained according to best practice appropriate for bird age as outlined in management guides published by the primary breeder. On 0, 14, 28, and 42 days post-hatch, birds and feeders were weighed and recorded and used for the calculation of BWG, FI, and FCR.

Analysis Procedures

In Experiment 1, on d 15, all birds from 9 replicate pens were humanely euthanized by CO_2 inhalation. Ileal contents from all birds in each pen were collected by gently flushing the

distal half of the ileum using deionized water. Digesta samples were pooled within pen and frozen (-20°C) until analysis. On d 42 in Experiment 2, ileal and cecal pH was recorded from 2 birds/pen.

Ileal and cecal pH. In Experiment 2, to determine ileal and cecal pH, an incision was made on the lower section of the ileal and the rounded tip of the ceca, and the digital pH meters (Mettler-Toledo, UK) with a spear tip piercing pH electrode (Sensorex S175CD) were directly inserted into the digesta of ileum and ceca and the pH was recorded. The probes were rinsed with distilled water after each reading and the tip of the pH probes were stored in double distilled water when not in use.

Nutrient digestibility. In Experiment 1, frozen ileal digesta samples were lyophilized and ground using an electric coffee grinder to provide an evenly ground sample while avoiding significant loss. Diet and digesta samples were analyzed for dry matter, gross energy, nitrogen, and ether extract. Gross energy was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Nitrogen was determined using the combustion method (Fisions NA-2000, CE Elantech, Lakewood, NJ) standardized with EDTA (method 990.03, AOAC International 2006) and ether extract was determined according to the AOAC (2006) method 920.39. Titanium dioxide was included in the feed at 0.5% as an indigestible marker, and diet and digesta TiO_2 concentrations were determined in duplicate following the procedures of Short et al. (1996). Apparent ileal digestibility (AID) of dry matter, gross energy, ether extract, and nitrogen were calculated using the following equation:

$$\text{AID, \%} = \{[(X / TiO_2)_{diet} - (X / TiO_2)_{digesta}] / (X / TiO_2)_{diet}\} \times 100,$$

where (X/TiO_2) = ratio of nutrient concentration to TiO_2 in the diet or ileal digesta. Energy digestibility (%) values obtained from the above equation, were multiplied by the gross energy content of the feed to calculate ileal digestible energy (**IDE**).

Statistical analysis

In each experiment, pen was considered the experimental unit with 9 replicate pens in Experiment 1 and 12 replicate pens for Experiment 2 for each of the 4 dietary treatments arranged in a randomized complete block design. Data were analyzed by a two-way ANOVA to evaluate the main effects and interactions among the diet type and tributyrin inclusion in a 2 by 2 factorial arrangement. Statistical significance was considered at $P \leq 0.05$ in all cases. Statistical outliers were defined as values exceeding 3 studentized residuals of the mean. Before removing statistical outliers, all raw data and calculations were confirmed to be correct.

RESULTS AND DISCUSSION

The goal of the current experiment was to simultaneously evaluate tributyrin (TB) efficacy in growth performance, nutrient utilization, and gastrointestinal pH in birds fed animal protein (APM) or all-vegetable based diets (VEG) while exposed to two different environments (i.e. battery cages vs floor pens). Even though diets were formulated on an isocaloric and digestible amino acid basis, diet analyses indicated a 7.7% increase in CP levels during the starter phase and a 4.5% and 3.8% increase during the grower and finisher phases, respectively, for the APM diets as compared to VEG diets (Table 4.1). Although the increase in CP values did not impact growth performance in Experiment 1, an improvement in AID dry matter digestibility ($P < 0.001$), increased nitrogen digestibility ($P = 0.013$), and higher IDE kcal/kg ($P < 0.001$) was observed in Experiment 1 for APM fed birds compared to VEG fed birds, with no impact of TB supplementation (Table 4.2 and 4.3, respectively). It is possible that the digestibility coefficients

used for MBM were underestimated, therefore increasing the digestible amino acid content in the APM diets compared to the VEG diets.

Similar to the starter phase in Experiment 1, no differences in body weight gain (BWG) were observed between birds fed APM or VEG diets with or without the inclusion of TB during the starter period in Experiment 2 (Table 4). There was however a reduction in the starter phase FI ($P = 0.032$) in APM fed birds compared to VEG fed birds (Tables 4). Even though feed conversion (FCR) was not affected by diet type during the starter phase, FCR was improved at d 28 ($P = 0.001$) and d 42 ($P = 0.003$) in Experiment 2 in APM fed birds compared to VEG fed birds, regardless of TB supplementation (Table 4). It has been suggested that the inclusion of MBM can increase the occurrence of necrotic enteritis (Wilkie et al., 2005). In previous literature the inclusion of MBM from 5% to greater than 50% in poultry diets has led to increased concentrations of *C. perfringens* in ileal and cecal contents compared to birds fed vegetable-based proteins, thus resulting in higher incidences of necrotic enteritis and reduced growth performance (Wilkie et al., 2005; Zanu et al., 2020). Comparatively, birds were fed 4% MBM in the current studies and experienced better growth performance compared to VEG fed birds. It should be noted that birds in Experiment 1 were non-vaccinated and reared in battery cages whereas in Experiment 2, birds were exposed to a commercial coccidiosis and placed in floor pens containing used litter. Previous research has noted that birds raised in battery cages exhibit improved performance compared to birds raised in floor pens which may be attributed to floor pens birds being directly exposed to dirty litter which increases exposure to pathogenic bacteria and parasites (Reece et al., 1971; Kim et al., 2014). Regardless, in Experiment 2, birds administrated a coccidiosis vaccine and placed on used litter had improved growth performance when fed APM diets compared to VEG fed birds, suggesting that in the current experiments,

even under mildly challenged conditions and increased CP values, MBM did not induce necrotic enteritis or impair growth performance as seen in previous studies.

Increased SBM concentrations in the VEG diets in the current studies was thought to increase the fermentability of the diet and in turn, enhance SCFA production in the ceca via anaerobic fermentation. However, no effect ($P > 0.05$) was observed in cecal or ileal pH between the VEG and APM diets (Table 5). This is in contrast to Yang et al. (2016) who reported that the additional inclusion of SBM oligosaccharides by 0.13% in a diet, which is comparable to a 1.9% increase in SBM, in a diet containing 26.9% SBM increased total SCFA in the excreta *in vivo* while in turn stimulated a change within the bacterial community in ceca of the bird. Qaisrani et al. (2015) also noted that supplementing fermentable carbohydrates in the diet could decrease hindgut protein fermentation which in turn could improve gut health and promote beneficial microbiota growth. Additionally, the inclusion of MBM is also thought to stimulate fermentation in the ceca of the broiler, however, an increase in pathogenic bacteria via biogenic amine formation has been reported (Sharma et al., 2017). In a previous study conducted by Zanu et al. (2020), the addition of 6% MBM to wheat-based diets increased ileal and cecal pH of 16 d old broilers. In the current experiment, however, a reduction in nitrogen fermentation via pathogenic bacteria may have been a result of the increase in digestibility of MBM thus leading to a lack of effect on cecal pH. In addition to changes in hindgut fermentation, it has been suggested that the inclusion of MBM in the diet can result in increased ileal and cecal pH due to the high calcium content that may interfere with amino acid and mineral utilization (Paiva et al., 2014). It is noteworthy to mention, however, the birds sampled in the current study were 42 d old broilers and were fed lower levels of MBM in the finisher phase at 2.5%. Therefore, the lack of effect on

ileal and cecal pH may be attributed to the stability of the gastrointestinal tract at 42 days and the relatively lower inclusion of MBM during this period.

There were no main effect or interactive effects of TB inclusion at 500 mg/kg in the Experiment 1 and 2. Kim et al. (2014) noted that when evaluating possible alternative to antibiotics, the housing system may influence the efficacy of the product as differences in bacterial load, pathogenic challenge, and environmental stress exist between birds reared in battery cages and floor pens. However, in Experiment 2, the coccidiosis vaccination that broilers received and the used litter that birds were reared on appeared to not impact TB efficacy and its effects on growth performance or nutrient utilization. These findings agree with a previous study conducted by Leeson et al. (2005) who reported no differences in overall growth performance in birds fed 200 or 400 mg/kg of butyrate glyceride esters when given a coccidiosis vaccine on day of hatch and reared in floor pens for 42 days compared to antibiotic fed birds and control fed birds. Tributyrin supplementation in Experiment 1 did not impact non-vaccinated birds reared in battery cages. These observations suggest that the housing system and assumed pathogenic and bacterial load that is associated with floor pen rearing and coccidiosis vaccine, did not influence the utilization of TB.

The composition between the two diet types was hypothesized to influence TB efficacy. In an effort to create isocaloric diets in the current studies, different concentrations of soy oil and poultry fat were used. Upon doing so, the dietary lipid level was increased in VEG diets approximately three times the amount used in APM diets. Even so, a general lack of interaction by TB within each diet type was observed in performance and nutrient digestibility. Additionally, it was anticipated that a synergistic effect of TB as an exogenous source of butyrate and an increase of endogenous butyrate production would occur. The lack of effect ($P > 0.05$),

however, on ileal and cecal pH suggest that TB supplementation did not influence SCFA production (Table 5) (Moquet et al., 2016). The lack of effect on ileal and cecal pH is likely due to TB being degraded and absorbed with no accumulation in the small intestine, thus resulting in no differences among ileal and cecal pH.

In conclusion, birds fed APM diets had improved growth performance and nutrient digestibility compared to VEG fed birds. However, this is more than likely attributed to an underestimation of the digestibility coefficients which in turn resulted in higher digestible amino acids in the APM diets. Moreover, TB efficacy did not appear to be impacted by APM or VEG diets in a battery cage system or floor pens. A lack of effect by TB supplementation was also noted within environment types with the addition of a coccidiosis vaccine and exposure to used litter having no impact on bird performance or nutrient utilization. Further studies should be conducted to evaluate TB efficacy in a challenged model to enhance the stress on the GIT as well as in diets containing viscous cereals to promote higher ceaecal fermentation.

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TABLES

Table 4.1 Diet formulations for broilers fed vegetable based (VEG) or animal protein based (APM) diets with or without the inclusion of tributyrin (TB) for 15 or 42 d post-hatch (Experiment 1 and 2)

	Starter		Grower		Finisher	
Ingredient	VEG	APM	VEG	APM	VEG	APM
Corn	60.50	62.98	62.15	64.07	67.60	69.00
Soybean Meal	33.92	29.83	32.08	28.77	26.12	23.56
MBM[1]	-	4.00	-	3.25	-	2.50
Soybean oil or poultry fat	1.55	0.57	2.67	1.95	3.32	2.86
Limestone	1.09	0.60	1.04	0.67	1.05	0.74
Sodium bicarbonate	-	0.13	-	0.10	-	0.08
Dicalcium phosphate	0.98	-	0.83	-	0.60	-
Sodium chloride	0.44	0.33	0.44	0.36	0.45	0.38
DL-methionine, 99%	0.33	0.33	0.28	0.28	0.27	0.27
L-lysine HCl, 78.8%	0.23	0.25	0.16	0.17	0.21	0.22
L-threonine, 98%	0.14	0.14	0.06	0.07	0.08	0.08
Trace mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[3]	0.06	0.06	0.05	0.05	0.05	0.05
Choline chloride, 60%	0.05	0.07	0.03	0.05	0.04	0.05
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01
Titanium dioxide	0.50	0.50	-	-	-	-
Builder's sand[5]	0.10	0.10	0.10	0.10	0.10	0.10
Calculated nutrient composition, % unless noted otherwise						
AME_n, kcal/kg	3,000	3,000	3,100	3,100	3,200	3,200
CP	21.33	21.56	20.41	20.60	18.02	18.15
Digestible Lys	1.20	1.20	1.10	1.10	1.00	1.00
Digestible TSAA	0.90	0.90	0.84	0.84	0.78	0.78
Digestible Thr	0.82	0.82	0.72	0.72	0.65	0.65
Total Ca	0.90	0.90	0.84	0.84	0.77	0.77
Available P	0.46	0.46	0.43	0.43	0.38	0.38
Analyzed nutrient composition, %						
Dry Matter	89.08	90.27	88.01	89.36	88.77	89.69
Protein	21.73	23.41	20.00	20.90	18.10	18.80
Gross energy, kcal/kg	3,872	3,956	3,970	4,014	3,928	4,024

[1] Meat and bone (MBM) containing 49.99% CP, 13% fat, 6.05% phosphorus, and 12.13% calcium.

[2] The mineral premix provided (per kg of diet): calcium, 55.5 mg, manganese, 100.0 mg; magnesium, 27.0 mg; zinc, 100.0 mg; iron, 50.0 mg copper, 10.0 mg; iodine, 1.0 mg.

[3] The vitamin premix provided (per kg of diet): vitamin A, 6,350 IU; vitamin D3, 4,536 ICU, vitamin E, 45 IU, vitamin B12 0.01 mg; mendadione, 1.24 mg; riboflavin, 5.44 mg; d-pantothenic acid, 8.16 mg; niacin, 31.75 mg; folic acid, 0.73 mg; pyridoxine, 2.27 mg; thiamine, 1.27 mg.

[4] OptiPhos 2000, (Huvepharma Inc., Peachtree City, GA) provided 250 FTU/g of phytase activity.

[5] Tributyrin was added to the experimental diets at the expense of sand to achieve 500 mg/kg tributyrin.

Table 4.2. Nutrient digestibility of non-vaccinated broilers fed vegetable based (VEG) or animal protein based (APM) diets with or without the inclusion of tributyrin (TB) from 0 to 15 d post hatch (Experiment 1)

Item	Dry matter, %	Nitrogen, %	Ether extract, %	IDE, kcal/kg
Main effect of diet type				
VEG	75.57[b]	73.74[b]	96.44	2,964[b]
APM	80.15[a]	76.34[a]	96.18	3,211[a]
SEM	0.40	0.72	0.44	16
Main effect of TB				
0 mg/kg TB	77.52	74.19	96.44	3,086
500 mg/kg TB	78.21	75.89	96.18	3,090
SEM	0.41	0.70	0.46	17
Two way interaction of diet type x TB				
VEG + 0 mg/kg TB	75.51	73.69	96.90	2,978
VEG + 500 mg/kg TB	75.64	73.79	95.98	2,950
APM + 0 mg/kg TB	79.52	74.68	95.98	3,194
APM + 500 mg/kg TB	80.78	77.99	96.39	3,230
SEM	0.60	1.05	0.64	25
P-values				
Diet type	< 0.001	0.013	0.684	< 0.001
TB inclusion	0.232	0.092	0.685	0.856
Diet type x TB	0.330	0.111	0.297	0.181

[a,b] Means within a row that do not share a common superscript are different ($P < 0.05$).

Table 4.3. Growth performance of non-vaccinated broilers fed vegetable based (VEG) or animal protein based (APM) diets with or without the inclusion of tributyrin (TB) from 0 to 15 d post hatch (Experiment 1)

Item	D 0 - 15 BWG, kg	FI, kg	FCR, kg:kg
Main effect of diet type			
VEG	0.537	0.661[a]	1.237
APM	0.529	0.623[b]	1.213
SEM	0.007	0.006	0.009
Main effect of TB			
0 mg/kg TB	0.525	0.635	1.229
500 mg/kg TB	0.540	0.649	1.220
SEM	0.007	0.006	0.009
Two way interaction of diet type x TB			
VEG + 0 mg/kg TB	0.530	0.657	1.244
VEG + 500 mg/kg TB	0.543	0.664	1.229
APM + 0 mg/kg TB	0.521	0.613	1.214
APM + 500 mg/kg TB	0.536	0.633	1.212
SEM	0.010	0.009	0.013
P-values			
Diet type	0.437	< 0.001	0.086
TB inclusion	0.162	0.135	0.523
Diet type x TB	0.951	0.429	0.611

[a,b] Means within a row that do not share a common superscript are different ($P < 0.05$).

Table 4.4. Growth performance of vaccinated broilers fed vegetable based (VEG) or animal protein based (APM) diets with or without the inclusion of tributyrin (TB) from 0 to 42 d post hatch (Experiment 2)

	D 0 - 14			D 0 - 28			D 0 - 42		
Item	BWG, kg	FI, kg	FCR, kg:kg	BWG, kg	FI, kg	FCR, kg:kg	BWG, kg	FI, kg	FCR, kg:kg
Main effect of diet type									
VEG	0.405	0.519[a]	1.286	1.624	2.365[a]	1.475[a]	3.229	5.201[a]	1.639[a]
APM	0.398	0.506[b]	1.279	1.614	2.328[b]	1.451[b]	3.193	5.005[b]	1.619[b]
SEM	0.004	0.004	0.006	0.009	0.013	0.005	0.018	0.028	0.005
Main effect of TB									
0 mg/kg TB	0.404	0.512	1.275	1.628	2.346	1.461	3.219	5.082	1.628
500 mg/kg TB	0.399	0.512	1.290	1.611	2.348	1.465	3.204	5.124	1.630
SEM	0.004	0.004	0.006	0.009	0.013	0.005	0.018	0.028	0.005
Two way interaction of diet type x TB									
VEG + 0 mg/kg TB	0.407	0.519	1.280	1.626	2.362	1.474	3.233	5.168	1.640
VEG + 500 mg/kg TB	0.403	0.519	1.293	1.623	2.368	1.475	3.226	5.233	1.637
APM + 0 mg/kg TB	0.401	0.506	1.271	1.629	2.329	1.448	3.204	4.996	1.615
APM + 500 mg/kg TB	0.395	0.505	1.286	1.599	2.327	1.454	3.182	5.014	1.622
SEM	0.006	0.006	0.008	0.013	0.018	0.007	0.026	0.039	0.007
P-values									
Diet type	0.217	0.032	0.339	0.463	0.043	0.001	0.155	< 0.001	0.003
TB inclusion	0.370	0.967	0.076	0.212	0.903	0.564	0.560	0.293	0.788
Diet type x TB	0.925	0.956	0.860	0.311	0.827	0.668	0.771	0.559	0.425

[a,b] Means within a row that do not share a common superscript are different ($P < 0.05$)

Table 4.5. Ileal and cecal pH of vaccinated broilers fed vegetable based (VEG) or animal protein based (APM) diets with or without the inclusion of tributyrin (TB) from 0 to 42 d post hatch. (Experiment 2)

Item	Ileal pH	Cecal pH
Main effect of diet type		
VEG	5.37	6.83
APM	5.61	6.86
SEM	0.11	0.03
Main effect of TB		
0 mg/kg TB	5.49	6.85
500 mg/kg TB	5.48	6.84
SEM	0.11	0.03
Two way interaction of diet type x TB		
VEG + 0 mg/kg TB	5.47	6.81
VEG + 500 mg/kg TB	5.27	6.85
APM + 0 mg/kg TB	5.52	6.88
APM + 500 mg/kg TB	5.69	6.83
SEM	0.16	0.05
P-values		
Diet type	0.123	0.605
TB inclusion	0.921	0.849
Diet type x TB	0.230	0.367

[a,b] Means within a row that do not share a common superscript are different ($P < 0.05$).

CHAPTER VI: GENERAL CONCLUSIONS

The overall focus of these studies was to explore the interactive effects between tributyrin (TB) and nutritional factors when fed in broiler diets. Experiment 1 revealed that feeding TB beyond 500 mg/kg can potentially negatively influence broiler growth performance and carcass characteristics. Experiment 2 indicated, however, that lipid source nor lipid concentration were primary driving factors that impact TB efficacy on broiler growth performance and nutrient utilization. Even so, TB supplementation in soybean oil diets did improve nutrient utilization when compared to birds fed a diet absent in TB. Interestingly, the opposite trend in nutrient utilization was observed in birds fed diets containing poultry fat and TB. These differences, however, were not observed in the second experiment trial within Experiment 2; therefore, suggesting that the interaction between TB and the dietary lipid source is variable. Additionally, corn particle size did not impact gizzard function as indicated by its weight or pH of its contents, nor was an interactive effect observed with TB supplementation on growth performance or nutrient utilization in Experiment 2. Therefore, Experiment 3 sought to evaluate TB efficacy in birds fed animal protein or vegetable-based diets reared under different housing environments. Non-vaccinated birds reared in battery cages and vaccinated broilers reared in floor pens and fed animal-protein based diets had improved growth performance and nutrient utilization compared to vegetable fed birds. These differences did not influence ileal or cecal pH as previously hypothesized, suggesting that the increase in soybean oligosaccharide and non-starch polysaccharides did not stimulate an increase in short chain fatty acid production such as butyrate. Lastly, TB efficacy did not affect broiler growth performance or nutrient utilization when birds were reared in battery cages or in floor pens with exposure to mild challenges (used litter and coccidial vaccine).

Collectively, the data from these studies suggest that feeding TB beyond the recommended dose (500 mg/kg in the starter and 250 mg/kg in subsequent feeds) can lead to a negative impact upon growth performance and carcass characteristics. However, it is not impacted by dietary lipid source, lipid concentration, corn particle size, or the presence of MBM and increased soybean oligosaccharides. While some differences in TB efficacy were observed among varying lipid sources, inconsistencies in these results suggest that lipid source and level are not primary drivers of TB efficacy. Additionally, the presence of a mild challenge in the form of a coccidiosis vaccine combined with exposure to used litter did not influence TB efficacy. Further research should be conducted to determine whether other environmental challenges or diet compositions will affect tributyrin's role within the gastrointestinal tract and its subsequent effects on growth performance and nutrient utilization in broilers.