



Review Article

Implications of butyrate and its derivatives for gut health and animal production



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ARTICLE INFO

Article history:

Received 20 July 2017

Accepted 10 August 2017

Available online 13 September 2017

Keywords:

Butyrate

Butyrins

Antibiotic alternatives

Gut health

Nutrition

Animal production

ABSTRACT

Butyrate is produced by microbial fermentation in the large intestine of humans and animals. It serves as not only a primary nutrient that provides energy to colonocytes, but also a cellular mediator regulating multiple functions of gut cells and beyond, including gene expression, cell differentiation, gut tissue development, immune modulation, oxidative stress reduction, and diarrhea control. Although there are a large number of studies in human medicine using butyrate to treat intestinal disease, the importance of butyrate in maintaining gut health has also attracted significant research attention to its application for animal production, particularly as an alternative to in-feed antibiotics. Due to the difficulties of using butyrate in practice (i.e., offensive odor and absorption in the upper gut), different forms of butyrate, such as sodium butyrate and butyrate glycerides, have been developed and examined for their effects on gut health and growth performance across different species. Butyrate and its derivatives generally demonstrate positive effects on animal production, including enhancement of gut development, control of enteric pathogens, reduction of inflammation, improvement of growth performance (including carcass composition), and modulation of gut microbiota. These benefits are more evident in young animals, and variations in the results have been reported. The present article has critically reviewed recent findings in animal research on butyrate and its derivatives in regard to their effects and mechanisms behind and discussed the implications of these findings for improving animal gut health and production. In addition, significant findings of medical research in humans that are relevant to animal production have been cited.

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1. Introduction

Short-chain fatty acids (SCFA) are organic fatty acids with 1 to 6 carbons produced within the intestinal lumen by bacterial fermentation of undigested dietary carbohydrates, and to a lesser extent, dietary and endogenous proteins such as sloughed epithelial cells and mucous, entering the colon (Topping and Clifton,

2001). The SCFA that are most abundant in the gastrointestinal tract (GIT) are acetate, propionate, and butyrate. The production of these SCFA allows the salvage of energy mainly from carbon sources that are not digested in the small intestine. It has been estimated that SCFA can contribute 5% to 15% of the total caloric requirements of humans (Bergman, 1990).

Despite being the least abundant of the 3 primary SCFA listed, butyrate is important as it is a major metabolite for the colonic epithelial cells: as much as 90% of butyrate is metabolized by colonocytes (Hamer et al., 2008). Colonocytes are instrumental in the absorption of water, sodium, and chloride from the intestinal lumen. Butyrate has also been shown to have multiple beneficial effects in the GIT as well as the peripheral tissues, and acts through multiple mechanisms, but many of them are related to its regulatory effects on gene expression (Canani et al., 2011). Butyrate is part of a class of epigenetic substances known as histone deacetylase inhibitors (HDI). Histone deacetylases act by

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



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removing the acetyl groups from lysine residues leading to the formation of a condensed and transcriptionally silent chromatin. The HDI block this action and can result in hyperacetylation of histones, impacting a large amount of gene expression (Marks et al., 2000).

The SCFA, including butyrate, possess antimicrobial activity, and have been widely used as feed additives in an effort to control pathogenic bacteria. Fatty acids and their monoglycerides have also been shown to be effective in inhibiting bacterial growth (Kabara et al., 1972; Thormar et al., 2006). Being supplemented into the diets of newly hatched chicks, butyric acid significantly reduced *Salmonella* colonization in the ceca (Cox et al., 1994). In an *in vitro* study by Namkung et al. (2011), butyric acid and its derivatives, monobutyric and a mix of mono-, di-, and tri-butyric, were tested for their antimicrobial activities at different concentrations against *Salmonella* Typhimurium and *Clostridium perfringens*. They found that *Salmonella* was best inhibited by butyric acid followed by mono-butyric, with tributyrin having minimal inhibition without the addition of lipase, and that *C. perfringens* growth was attenuated by both butyric acid and its glycerides in the same manner (Namkung et al., 2011). Sodium butyrate supplementation *in vitro* has been shown to induce the expression of host defence peptides, including β -defensins and cathelicidins, in a variety of chicken cell types, such as HD11 macrophage cells, primary monocytes, and bone marrow cells, as well as jejunal and cecal explants (Sunkara et al., 2011). Very recently, Rivera-Chavez et al. (2016) reported that streptomycin treatment depleted commensal, butyrate-producing *Clostridia* from the mouse intestinal lumen, leading to decreased butyrate levels, increased epithelial oxygenation, and aerobic expansion of *Salmonella enterica* serovar Typhimurium. Epithelial hypoxia and *Salmonella* restriction could be restored by tributyrin treatment.

There have been many investigations into the effects of butyrate supplementation into diets on animal performance, in addition to the studies of its potential applications in human health. One of the major problems in the application of butyrate is the difficulty in handling. Butyrate has an offensive odor making it unpleasant to work with, and can deter animals from consuming feed with free butyrate incorporated. Moreover, free butyrate has been shown to be largely absorbed in the upper GIT, meaning that the majority would not reach the large intestine, including the colon, where butyrate would exert its major functions (Pituch et al., 2013). In this regard, butyrate glycerides, butyrate salts, and different encapsulation techniques have been developed and used in order to ease the handling and prevent the release of butyrate in the upper GIT.

Butyrate glycerides, including mono-, di-, and tri-butyric, consist of a varied number of butyric acid molecules attached to glycerol backbone. In the small intestine, the butyrate is liberated from the glycerol through the action of lipase. In this form, the butyrate is protected from absorption in the upper GIT. Sodium butyrate is the sodium salt of butyric acid. It can be supplemented freely to stimulate development in the upper GIT or in a protected form, e.g., in a triglyceride matrix, to allow a slower release and target the lower GIT.

Because of their antimicrobial activity, function as HDI, and effect on host immune response, butyrate and its derivatives are considered to be potential substitutes for in-feed antibiotics for animal production, as well as promising treatments for inflammatory bowel diseases in humans. This review will summarize and critically evaluate previous studies on the effects of butyrate and its derivatives on animal performance with the effects on human health as a reference. In addition, their future applications in these 2 fields, including both potential and challenges, will be discussed along with connections between species.

2. Butyrate in the human colon

The importance of butyrate for human colon health has been demonstrated in many studies with patients suffering from colon inflammatory diseases. The colonocytes of these patients present with an impaired capacity to oxidize butyrate (Hamer et al., 2010; De Preter et al., 2011). Through its function as an HDI, butyrate can exert actions related to cellular homeostasis including anti-diarrhetic, anti-oxidant, anti-carcinogenic, and anti-inflammatory functions (Williams et al., 2003; Mathew et al., 2014; Jahns et al., 2015).

The absorption of butyrate has been shown to promote the absorption of sodium, potassium, and water, the effects that give it antidiarrhetic properties (Ruppin et al., 1980). This is significant as diarrhea is a recognized complication in critically ill patients. Additionally, patients with short bowel syndrome often experience significant loss of water and sodium due to the lack of absorption; butyrate supplementation can improve this absorption and reduce the requirement for intravenous electrolyte replacement (Tappenden, 2010). Dysbiosis, a primary cause of diarrhea, is caused by an antibiotic disturbance of the gut microbiota that suppresses their fermentation and production of butyrate (Whelan and Schneider, 2011). With the supplementation of fiber into the diets of jejunal feeding critically ill patients, an increase in butyrate-producing bacteria was observed, and 75% of patients exhibited a cessation of diarrhea (O'Keefe et al., 2011).

The anti-inflammatory properties of butyrate have been shown to be mediated by several mechanisms including: the reduction of pro-inflammatory cytokine expression (interferon gamma [*IFN- γ*], tumor necrosis factor- α [*TNF- α*], interleukin-1B [*IL-1B*], *IL-6*, *IL-8*), the induction of *IL-10* and transforming growth factor-B (*TGF-B*) expression and signaling, the induction of nitric oxide synthase and metalloproteinases, and the reduction of lymphocyte proliferation and activation (Kyner et al., 1976; Segain et al., 2000; Matsumoto et al., 2006; Meijer et al., 2010; Fung et al., 2012). The most studied anti-inflammatory pathway of butyrate is via the inhibition of nuclear factor kappa B (NF- κ B). This pathway controls the expression of genes encoding pro-inflammatory cytokines, inflammation-inducing enzymes, growth factors, heat shock proteins, and immune receptors (Vinolo et al., 2011).

Several studies have linked impaired butyrate metabolism with mucosal damage and inflammation in patients with inflammatory bowel diseases including ulcerative colitis and Crohn's disease (Roediger, 1980; Den Hond et al., 1998; Duffy et al., 1998; De Preter et al., 2011; Kovarik et al., 2011; De Preter et al., 2012; Morgan et al., 2012), suggesting that treatments to increase butyrate in the GIT of these patients can prove to be beneficial. More data have indicated that intestinal inflammation also affects butyrate transport, and thus its oxidation (Thibault et al., 2007). Various experimental models have shown that monocarboxylate transporter 1 (*MCT1*) transports butyrate into colonic epithelial cells (Tamai et al., 1995; Ritzhaupt et al., 1998; Cuff et al., 2005), and that *MCT1* down-regulation is common in patients with ulcerative colitis (Thibault et al., 2010; De Preter et al., 2011). Butyrate intake has shown a positive effect in experimental studies on inflammatory bowel disease (Hamer et al., 2010; Komiyama et al., 2011; Vieira et al., 2012), though clinical studies have shown inconsistent results (Russo et al., 2012). Butyrate irrigation has been shown to improve inflammation symptoms in biopsies from inflammatory bowel disease patients; however, high concentrations are required to illicit these improvements (Segain et al., 2000).

There are several mechanisms by which butyrate can control oxidative stress. In a study with healthy human subjects, the administration of a daily butyrate enema (10,000 mg/kg) for 2 weeks resulted in an increase in the anti-oxidant glutathione, and a

decrease in uric acid production compared to those without butyrate treatment (Hamer et al., 2009). However, when a similar study was performed on patients with ulcerative colitis in remission, butyrate showed only minor effects on inflammatory and oxidative stress parameters that seem to be dependent on the level of inflammation (Hamer et al., 2010). *In vitro*, physiological levels of butyrate have been found to reduce H₂O₂-induced DNA damage, increase catalase expression (one of the key defense systems against oxidative stress), and reduce cyclooxygenase-2 (COX-2) expression (an indicator of inflammation) in human colonocytes (Sauer et al., 2007). These results also support the potential of butyrate in cancer treatment and prevention, as COX-2 over-expression is found in colon tumors.

Butyrate has been linked to the prevention and inhibition of colon carcinogenesis, largely through the increased intake of dietary fiber, resulting in increased fermentation and butyrate production (Trock et al., 1990; Howe et al., 1992; Bingham et al., 2003). A role for butyrate in colon cancer treatment has been supported by the findings of downregulated butyrate transporters (MCT1 and sodium-coupled monocarboxylate transporter 1 [SMCT1]) in human colon cancer tissue (Lambert et al., 2002; Li et al., 2003), resulting in reduced uptake and metabolism of butyrate in colonocytes. Several rat models have been used to demonstrate a protective effect of butyrate on colorectal carcinogenesis (McIntyre et al., 1993; Kameue et al., 2004; Bauer-Marinovic et al., 2006), but direct evidence for a protective effect of butyrate on carcinogenesis in humans is lacking. One study has investigated the relationship in humans between butyrate and G-protein-coupled receptor GPR109A in the colon (Thangaraju et al., 2009). It was found that butyrate binding to GPR109A can induce apoptosis in colon cancer cells as well as blocking activation of the NF-κB inflammation pathway, potentially mediating inflammatory bowel disease (IBD). Butyrate has also been shown to enhance the effects of anticancer drug therapy including vincristine, celecoxib, cisplatin, and etoposide via its HDI activity, increasing the cytotoxicity of the drugs (Ramos et al., 2004; Kang et al., 2012; Maruyama et al., 2012).

The overall aim for the use of butyrate in humans differs greatly from that for animal production. Although the use of butyrate in humans is desired mainly for the treatment of disease and in animal production is for disease prevention and growth promotion, the functions in enhancing GIT health, releasing stress, and controlling inflammation are commonly desired for both humans and animals. Thus, the mechanisms of butyrate effects revealed by human research can be valuable references to promote the research and application of butyrate and its derivatives in animal production.

3. Butyrate supplementation in poultry

With the dramatic improvements in growth rate and feed conversion in chicken production over the past 40 years, the nutrition and health care of chickens are becoming more critical and demanding (Yegani and Korver, 2008; Cooper and Songer, 2009). The nutritional and health status of birds is largely influenced by their gut health, which affects digestion, absorption, and metabolism of nutrients, as well as disease resistance and immunity (Kelly and Conway, 2001; Yegani and Korver, 2008). There are a number of disorders associated with the gut health of chickens, including diarrhea, malabsorption syndrome, coccidiosis, and necrotic enteritis (caused by an overgrowth of *C. perfringens*) (M'Sadeq et al., 2015). In-feed butyrate has been studied as a possible additive to combat GIT disorders, and ultimately enhance chicken gut health and improve bird performance.

The first week of a broiler chick's life is accompanied by many changes in developmental processes, including changes in organ

growth patterns and development of immunocompetence (Nitsan et al., 1991), which is critical for high producing broiler chickens. Given the functions of butyrate and its derivatives, supplementation of these additives can be one effective approach to enhance chicken gut development and health, including the development of immunity, to possibly improve the quality of the chicken carcass. In one previous study, Hu and Guo (2007) observed a dose response in body weight gain to sodium butyrate at tested concentrations of 500, 1,000, and 2,000 mg/kg through 21 days of treatment. They did not detect a significant difference in the absorptive function of the jejunum, but an increase in the concentrations of DNA, RNA, and protein in the duodenal mucosa in response to increased sodium butyrate levels was observed. This suggests that sodium butyrate stimulated the growth of the duodenum, and was largely absorbed here before entering the jejunum to exert its function. The authors also found that the jejunal villus height to crypt depth ratio was increased in a dose responsive action with dietary sodium butyrate inclusion, which suggests improved digestive tract maintenance and could be the reason behind the improved growth performance. Nevertheless, it remains to be determined why this jejunal histomorphological improvement was not linked to an increased absorptive function.

The effect of sodium butyrate supplementation on bacterial infection in broilers has also been reported. One example is the investigation by Fernandez-Rubio et al. (2009) into the effect of feeding either free or partially protected sodium butyrate in vegetable fats (both at 920 mg/kg) on *Salmonella enteritidis* colonization in broilers. In their study, birds were challenged with *Salmonella* on day 5 post-hatch and fecal shedding was assessed periodically from day 6 through day 41. After 42 days, all birds were euthanized and tissues were sampled for bacteriological analysis. They found that both protected and free sodium butyrate were effective in reducing *Salmonella* burden in birds from 27 days onward, with free butyrate being more effective in the early stage of infection (at day 13) and protected sodium butyrate having more effect in late stage infection (at day 41). Protected sodium butyrate was more effective at preventing colonization in the crop and cecum, and infection in the liver, compared to the free form. The growth performance of these birds was not reported. These results suggest that the slow and varied release of the protected sodium butyrate is possibly more effective in preventing bacterial infection, as it is released and active all along the digestive tract.

The effect of sodium butyrate on host-defense peptide (HDP) expression and disease resistance has also been studied. In a study reported by Sunkara et al. (2011), 5-day-old birds were separated into 2 groups on diets with or without sodium butyrate (1,000 mg/kg), and challenged with *S. Enteritidis* at 7 days of age. Sodium butyrate supplementation resulted in a significant decrease in *S. Enteritidis* in the cecal digesta, and induced the expression of HDP Avian beta-defensin 9 in the crop. Sodium butyrate has also been shown to moderate the immune response of broiler chickens. When supplemented with 1,000 mg/kg sodium butyrate, birds challenged with *Escherichia coli* lipopolysaccharide (LPS) had a reduced level of serum IL-6 and TNF-α, and increased serum superoxide dismutase and catalase activities (Zhang et al., 2011). This result agrees with the anti-inflammatory effects observed in humans, in which butyrate reduced proinflammatory cytokine expression (Segain et al., 2000). Also of interest, sodium butyrate supplementation prevented a reduction in growth that was observed in the control challenged birds. These protective attributes of sodium butyrate support its potential inclusion as a substitute for antibiotics in broiler diets.

Sodium butyrate supplementation has also been applied to layer hens, focusing on the enhancement of egg production and egg characteristics. Nollet et al. (2014) tested different inclusion rates of

sodium butyrate into the diets of layer hens. They found that the highest inclusion level tested, 500 mg/kg, had no effect on the average egg weight. However, lay efficiency and feed conversion were improved, as was the daily egg mass output in g/(bird·d). Additionally, inclusion levels over 100 mg/kg reduced the percentage of eggs having egg bending, indicating stronger shells (Nollet et al., 2014). In another study with layer hens, the effect of the addition of 2,000 mg/kg sodium butyrate on laying performance, with or without phytase in the diet (500 U/kg), was investigated (Vieira et al., 2011). Contrary to the previous study, they did not observe any changes in egg production, egg characteristics, or calcium balance with the addition of sodium butyrate. Further research thus appears to be required to determine if sodium butyrate supplementation is beneficial for laying hens, and how the observed affects in broiler chickens can be exploited for egg production.

As with encapsulated sodium butyrate, butyrate glycerides have been used in an effort to target butyrate release in the lower GIT. Interested in their effect on small intestine morphology and growth performance, Antongiovanni et al. (2007) tested a butyrate glyceride mix (mono-, di-, and tri-glycerides) at 4 different levels (2,000, 3,500, 5,000, and 10,000 mg/kg) into the diets of broiler chickens. Birds treated with the butyrate glyceride mix had a higher live weight at slaughter, and an improved feed conversion rate. Additionally, birds receiving the lowest inclusion levels of butyrate glycerides (2,000 mg/kg) had shorter villi, longer microvilli, and deeper crypts in the jejunum, a sign in increased cell turnover (Zhang et al., 2005). This result is similar to what was observed in birds supplemented with sodium butyrate, suggesting that both butyrate derivatives are effective in promoting intestinal development (Hu and Guo, 2007). With a mixture of butyric acid glycerides (mono-, di-, and triglycerides) fed at 2,000 or 4,000 mg/kg, Leeson et al. (2005) observed no change in ADG, but treatment birds had significantly improved breast muscle and carcass weight. Further, upon challenge with coccidiosis, butyrate glyceride supplemented birds showed improved growth post-challenge compared to unsupplemented birds (Leeson et al., 2005). In agreement with Leeson et al. (2005), one of our studies investigating the effects on broiler performance of SILO Health 104 (a butyrate glyceride product, claimed by the manufacture to contain 65% monobutyryl, 5% dibutyryl, and 30% a mix of mono- and diglycerides of lauric, caprylic, capric, and propionic acids) showed an increase in relative breast muscle weight (to body weight) in a dose responsive manner ($y = 18.428 + 0.000450x$; $P = 0.0074$) to the increase of SILO Health 104 supplementation from 500 to 3,000 mg/kg, although ADG was unaffected (unpublished data). Additionally, across multiple studies, we have observed a decrease in relative abdominal fat weight with the supplementation of either monobutyryl (Bedford et al., 2017a), tributuryl (Bedford et al., 2017b), or their mixture (Yin et al., 2016) at tested concentrations. These results, to be discussed in the following section, provide additional evidence on the beneficial effects of butyrate glycerides on the performance, carcass traits, and gut morphology of broiler chickens.

More recent studies have been performed in an attempt to determine the mechanism of action behind the effects of butyrate glycerides on broiler chickens. One effort was to elucidate the pathways related to energy expenditure and lipid metabolism that are affected by butyrate glyceride supplementation, Yin et al. (2016) performed RNA-seq analysis on the liver and jejunum from broilers treated with butyrate glycerides. In these birds, RNA-seq analysis revealed 79 and 205 differentially expressed genes (DEG) in the jejunum and liver, respectively. Further, 255 and 165 treatment specifically expressed genes (TSEG) were found in butyrate glyceride birds in the jejunum and liver, respectively.

Among these genes, bioinformatic analysis determined a significant enrichment of DEG and TSEG involved in the biological processes for reducing synthesis, storage, transportation and secretion of lipids in the jejunum, and enhancing the oxidation of ingested lipids and fatty acids in the liver. In particular, transcriptional regulators of thyroid hormone responsive (*THRSP*) and early growth response gene-1 (*EGR-1*) as well as several DEG involved in the peroxisome proliferator-activated receptors (PPAR) signaling pathway were significantly affected by dietary intervention of butyrate glyceride for lipid catabolism. Also in this study, serum triglycerides and total cholesterol were lowered in butyrate glyceride birds. Fatty acid synthase levels were lowered in the serum, liver and adipose tissue of butyrate glyceride fed birds, while lipoprotein lipase was decreased in the jejunum, liver and adipose of the same birds. These results suggest that the reduced body fat deposition observed was due to the regulation of gene expression influenced by butyrate glycerides and provide a valuable reference for future studies on the regulation of ingested energy distribution and how it can be used to improve animal production.

Following the study by Yin et al. (2016), Bedford et al. (2017b) investigated the effects of tributuryl glycerides on the performance of 2 different broiler strains (Ross 308 and Ross 708). Although no overall changes in average daily gain or feed efficiency were observed, tributuryl glyceride supplementation significantly lowered abdominal fat deposition, as well as fat deposition in the breast muscle in both strains compared to control birds. Supporting the changes in lipid metabolism, significant differences in the expression of hepatic sterol regulatory element-binding protein 1, peroxisome proliferator-activated receptor alpha, and ATP citrate lyase were observed between tributuryl glyceride treated birds and controls. Very recently, Yang et al. (unpublished data) investigated butyrate glycerides-induced changes in the chicken intestinal microbiota and serum metabolites as well as their links via pyrosequencing of bacterial 16S rRNA genes and nuclear magnetic resonance (NMR)-based metabolomics analysis. They found that dietary treatment with butyrate glycerides did not affect overall diversity of the intestinal microbiota, but altered its composition. *Bacillus* was the only genus in the ileal microbiota that was significantly modulated by butyrate glyceride supplementation. In contrast, there were several changes in the cecal microbiota composition, including a group of butyrate-producing bacteria (*Subdoligranulum*). In particular, *Bifidobacterium* demonstrated a considerable increase in not only the abundance but also the species diversity upon dietary intervention with butyrate glycerides. The NMR-based analysis also revealed changes in serum concentrations of metabolites, including those of bacteria-derivation, such as choline, glycerophosphorylcholine, dimethylamine, trimethylamine, trimethylamine-N-oxide, lactate, and succinate (Nicholson et al., 2012). The coincidence of the shift in the cecal microbiota composition, particularly the increase in the abundance and species diversity of *Bifidobacterium*, with elevated serum concentrations of choline metabolites suggests a contribution from intestinal bacteria to lipid metabolism/energy homeostasis in broilers, which may have partially contributed to the decrease in abdominal fat deposition described above. These findings can improve our understanding of the molecular mechanisms underlying the effect of butyrate on chicken performance.

In the broiler duck, the addition of sodium butyrate into the basic ration at 350, 700, and 1,050 mg/kg was reported to improve feeding efficiency in a dose responsive manner, compared to the control group of ducks (Liu et al., 2011). The 2 higher butyrate concentration groups had increased average daily gains compared to control birds. In addition to the growth parameters, they

investigated the effect of sodium butyrate supplementation on the fecal content of the ducks in regards to pollutants. Sodium butyrate at 700 mg/kg was the best at reducing the levels of total nitrogen, total phosphorus, and ammonia nitrogen in the feces. These environmental parameters that have not been considered in relation to butyrate supplementation in any other study, but could be an additional, area of interest for future butyrate research in animal production.

4. Butyrate supplementation in pigs

The weaning transition is a critical time period for piglets. Shifting from liquid to solid feed, changes in environment, and mixing with new pen mates are stressful, often resulting in a post-weaning growth lag. A significant factor in this growth lag is the underdeveloped GIT due to early weaning, leading to the inability to properly digest and absorb nutrients. There have been many studies investigating different strategies and feed additives, including SCFA, to ease the transition of piglet weaning (Ravindran and Kornegay, 1993; Lalles et al., 2007; de Lange et al., 2010; Heo et al., 2013; Thacker, 2013).

Early studies on the inclusion of organic acids in the diets of weaned pigs have demonstrated that their inclusion can improve growth performance, and increase digestibility of the diet (Falkowski and Aherne, 1984; Henry et al., 1985). A further study on the role of butyrate in the intestinal metabolism was reported by Piva et al. (2002a). In the study, 6-week-old piglets were divided into 2 groups fed an antibiotic-free conventional diet with or without the inclusion of sodium butyrate (800 mg/kg) (Piva et al., 2002a). Piglets fed sodium butyrate had a significantly higher ADG after 14 days of treatment compared to control pigs, but this advantage did not carry through to 35 days of treatment. It is proposed that this occurs due to the fact that butyrate has a positive effect on cell proliferation of the intestinal epithelium, which is of greater biological value in the early weaning period when the small and large intestine are rapidly increasing in size (Sakata and Setoyama, 1997). Additionally, pigs fed the sodium butyrate diet had an increased feed intake compared to control pigs through 35 days of treatment. The study by Sakata and Setoyama (1997) suggests that sodium butyrate could encourage solid feed intake, although the beneficial effects may not be carried through to growth performance over time, and perhaps an earlier addition of butyrate to the diets may illicit increased beneficial responses. A later study by the same group included sodium butyrate into the diets of 32-day-old weaned piglets at 1,000, 2,000, or 3,000 mg/kg for 6 weeks (Biagi et al., 2007). No significant differences were observed between treatments in growth performance, intestinal morphology, or intestinal microbiota throughout the trial. Authors indicated that the lack of response in these parameters may have been due to a different dietary composition or gut maturation status compared to the previous a trial, in which they did observe changes in growth performance. Nonetheless, differences were observed in the cecum of sodium butyrate fed animals, including increased cecal pH, increased cecal chime ammonia concentration, and increased cecal isobutyric acid concentration. These results suggest that sodium butyrate can influence the activity of the cecal microbiota and may present a possibility to negate the negative effects of early weaning through the manipulation of energy sources in the hindgut.

Targeting the small intestine and lower GIT may be advantageous in weaned piglets to help stimulate intestinal development, improve digestive capabilities, and prevent post-weaning diarrhea. Mallo et al. (2012) compared the effects of the inclusion of encapsulated sodium butyrate and monobutyrate glycerides on

21-day-old weaned piglets. They observed no differences in growth performance, but higher concentrations of butyric acid and VFA in the colon in encapsulated sodium butyrate fed animals compared to monobutyrate glyceride fed animals. This result suggests that certain encapsulation techniques could facilitate an easier release of butyric acid than from monobutyrate glycerides, allowing more butyric acid to reach the distal GIT. When supplemented into artificial milk formulas of 2-week-old piglets for 7 days, sodium butyrate (3,000 mg/kg) was shown to increase crypt depth, villi length, and mucosa thickness in the jejunum and ileum compared to unsupplemented pigs (Kotunia et al., 2004). By further investigating the increased gut maturation, Mazzoni et al. (2008) supplemented sodium butyrate (3,000 mg/kg) to piglets through the suckling (days 4 to 28), weaning (day 28), and/or postweaning period (days 29 to 40). Sodium butyrate supplementation increased parietal cell number. In particular, after weaning the supplementation specifically increased the number of enteroendocrine and somatostatin positive cells in the oxyntic mucosa, in addition to the increase of gastric mucosa thickness (Mazzoni et al., 2008). This effect on increasing the mucosa thickness was also previously observed in the jejunum, ileum, colon, and cecum of piglets given a cecal infusion of butyrate (Kien et al., 2007). These studies show the potential proliferative effects of butyrate on the porcine GIT.

There have also been studies on the effects of dietary supplementation of tributyrate glycerides on the growth, intestine development, and immune function of weaned piglets. Tributyrate glycerides (10,000 mg/kg) with a sweetener (lacticol, 3,000 mg/kg) has been shown to improve the average daily gain of piglets through 42 days of age when supplemented from weaning (d 28) onwards (Piva et al., 2002b). Intrauterine growth restriction (IGR), a common problem in animal production and a recognized issue in human health, is a condition where a fetus is growing at an abnormally slow rate inside the womb, leading to the risk of health problems during gestation, delivery, and after birth (Wang et al., 2008). When supplemented to piglets suffering from IGR, tributyrate glyceride was shown to improve body weight, as well as increase spleen and small intestine development compared to unsupplemented IGR piglets (Dong et al., 2016). In addition, tributyrate glyceride supplementation was shown to reduce the expression of pro-inflammatory cytokines and improve tight junction formation in the colon, a benefit to intestinal health (Tugnoli et al., 2014). Recently, Hou et al. (2014) reported that tributyrate glyceride supplementation (1,000 mg/kg) was able to alleviate intestinal injury, possibly by inhibiting apoptosis, promoting tight-junction formation, and activating EGFR signaling, in a study with the porcine model of ulcerative colitis. These results support the previously discussed benefits of butyrate for the treatment of intestinal disease in humans. Tributyrate glycerides appear to be an option for relief of digestive dysfunctions, as well as mediating immune response and improving growth performance in swine.

The term boar taint refers to an off-putting odor and taste that can be evident during the cooking or eating of pork from non-castrated male pigs. It is caused by the accumulation of androstenone and skatole in the fat of these pigs: androstenone is produced in the testes, whereas skatole is produced by intestinal bacteria then absorbed (Deslandes et al., 2001). Increased skatole formation has been linked with debris from cell apoptosis. Intestinal butyrate has been shown to decrease apoptosis, leading to the studies to determine whether butyrate has an effect on skatole formation and tissue accumulation (Hass et al., 1997; Mentschel et al., 2001). Barrows fed diets containing resistant potato starch, a substrate known to increase butyrate formation in the colon, were found to have significantly increased butyrate and

decreased rates of apoptosis in the colon (Govers et al., 1999; Topping and Clifton, 2001; Claus et al., 2003). Consequently, skatole was decreased in both the feces and blood plasma, and the concentration of skatole in the fat tissue was below the detection limit (0.8 ng/g), a significant reduction compared to control pigs (167 ng/g) (Claus et al., 2003). These decreased levels suggest potential for butyrate in lessening boar taint and improving the sensory quality of pig meat.

Early studies on monobutyrate glycerides have revealed a unique function that is relevant to animal production. The 1-Butyryl-glycerol (monobutyrate glyceride) is a simple lipid secreted by adipocytes showing angiogenic activity when tested in the chick chorioallantoic membrane assay (Dobson et al., 1990; Wilkison et al., 1991). The biosynthesis of monobutyrate glycerides is tightly linked to lipolysis associated with changes in blood flow (Ailhaud et al., 1992). Moreover, synthetic monobutyrate glycerides have shown the same spectrum of biological activities as the adipocyte-derived factor monobutyrate glyceride (Wilkison and Spiegelman, 1993). The 2 types of monobutyrate glycerides, α -monoglyceride and β -monoglyceride, originate from dietary sources or tributyrates glyceride digestion, and are transported into the blood stream from the small intestine. Absorbed monobutyrate glycerides can stimulate the growth of endothelial cells in the development of new blood vessels, which are required for the development of any new tissue under normal or pathological conditions. Thus, monobutyrate glycerides may represent a therapeutic opportunity for stimulating the growth of intestinal tissue through its angiogenic activity in food-producing animals, especially when there are wounds or damages in the intestinal epithelia. Nonetheless, further studies are required to confirm the concept on the potential effects of monobutyrate glyceride in poultry and pigs.

5. Butyrate supplementation in ruminants

Sodium butyrate is found in the milk of most animals, with the exception of sow milk (Alais, 1984), and is naturally found in the forestomach of ruminants. An early study by Sander et al. (1959) showed that the administration of a sodium butyrate solution (100,000 mg/kg) directly into the rumen of cannulated calves for the first 11 weeks after weaning (at 2 to 5 weeks of age) resulted in an increased rate of rumen papillae development (Sander et al., 1959). This is the only study using such a high inclusion level, perhaps owing to the fact that it was one of the first butyrate studies and the dosage was likely desired to maximize the chance of observing a response. Similar results were observed with adult sheep, in which sodium butyrate (2 g/kg body weight per day) administered intraruminally for 6 days resulted in increased rumen epithelium development (Sakata and Tamate, 1978).

Volatile fatty acids, including butyrate, have been established to be significant factors in the postnatal development of the ruminal epithelium (Sakata and Tamate, 1978). The rumen epithelium is responsible for many important physiological functions including absorption, transport, and SCFA metabolism (Graham and Simmons, 2005). Improving the rumen epithelium development could lead to enhanced animal performance, especially in early life.

Flavomycin is a phosphoglycerolipid antibiotic that has been used exclusively and extensively as an antimicrobial agent and growth promoter in livestock production (Edwards et al., 2005). A study by Guilloteau et al. (2009) investigated the effect of replacing flavomycin (16.5 mg/kg) with sodium butyrate (3,000 mg/kg) in the diets of milk fed calves from 12 days of age to slaughter. They found that compared to controls, calves supplemented with sodium

butyrate had significantly improved body weight, average daily gain, and feed conversion ratio from 60 to 124 days of age (Guilloteau et al., 2009). Enterocyte proliferation in the upper jejunum and duodenal villi height were also improved in sodium butyrate calves. Additionally, sodium butyrate enhanced the levels of heat shock proteins (HSP) 27 and 70 in the abomasum and colon, and expression of insulin-like growth factor 1 (*IGF-1*) receptors in the jejunum compared with the action of flavomycin. As the removal of flavomycin resulted in substantial modulation of the intestinal microbiota, the increase of these HSP could be related to the change of microbiota composition, and have a protective effect on the GIT. Increased expression of the receptors suggests that *IGF-1* was likely one mediator of the observed growth effects, along with the improved GIT development.

For economic reasons, the early weaning of calves from whole milk or a milk replacer as early as 3 or 4 weeks of age is often practiced. This transition requires the rapid development of the GIT, especially the rumen, as it directly affects solid feed intake, and thus, the growth and health of calves post-weaning (Greenwood et al., 1997; Baldwin et al., 2004). Prior to weaning, the choice of supplied liquid feed determines the growth and health of the animal, and therefore the success of the transition onto solid feed including rumen development (Khan et al., 2007). It has been shown that the use of milk replacer instead of whole milk as a liquid feed slows the small intestine development, which impairs performance, decreases solid feed intake, and in turn, slows rumen development (Blattler et al., 2001; Gorka et al., 2011b). One previous study investigated the effect of feeding calves whole milk, milk replacer, or milk replacer supplemented with sodium butyrate (3,000 mg/kg) on rumen development in calves (Gorka et al., 2011b). Results from the study showed that feeding calves milk replacer instead of whole milk from 5 to 26 days of age slowed down small intestine development, and negatively affected the metabolic status of the animals. However, the addition of sodium butyrate stimulated small intestine development and partially negated the negative effects of milk replacer on rumen development. A further study by the same group investigated the addition of sodium butyrate into the milk replacer (at 3,000 mg/kg), as well as into the dry starter mixture (at 6,000 mg/kg), offered to calves from the trial's onset (Gorka et al., 2011a). They found that the addition of sodium butyrate to both the milk replacer and the starter mixture had a positive effect on rumen development, indicated by an increased percentage of the whole stomach weight and increased papillae length and width. Furthermore, both body weight gain and general calf health were improved by the addition of sodium butyrate. Tributyrates glycerides have also been studied as an additive in milk replacer for calves and was shown to modulate glucose and insulin dynamics when supplemented at 3,000 mg/kg from 12 days of age, but did not increase growth performance (Araujo et al., 2013). These studies indicate that butyrate derivatives could be particularly beneficial in improving rumen development and easing the weaning transition of calves.

A recent shift in lamb production to an intensive fattening system means that in some countries, lambs are fed a high concentrate diet from 2 weeks of age to assure fast growth and high productivity (Cavini et al., 2015). Failing to adapt to this sudden dietary change can lead to complications such as inadequate rumen development and slow growth. Cavini et al. (2015) investigated the effect of incorporating sodium butyrate (3,500 mg/kg) into the concentrate diet during suckling, weaning, or both, on lamb growth performance and rumen characteristics. They found that sodium butyrate supplementation during the suckling period led to significant increases in hot carcass weight and dressing percentage, without having an effect on rumen characteristics. However,

sodium butyrate supplementation during the fattening period had no significant effects. There have been limited studies investigating the inclusion of butyrate derivatives into ovine diets; more research is required to determine if a similar result as observed in calves could be achieved.

6. Major benefits and potential cross species effects

The main common thread with the supplementation of butyrate and its derivatives across animal production species is their benefit on the development of the GIT, including improved morphology and cell proliferation, thus on animal gut health. The result of these improvements is often associated with an observed increase in growth performance, including changes in carcass composition, although it may depend on the age of the animals. Thus, earlier supplementation may result in a better chance for observed improvements.

Butyrate supplementation has shown potential for alleviating the symptoms of inflammatory bowel diseases in human patients, most notably through the inhibition of inflammatory pathways via the inhibition of NF- κ B. Although the application of butyrate derivatives in feed for animal production is mainly subtherapeutic, and not intended for disease treatment, this anti-inflammatory response was also observed in broiler chickens and weaned piglets, which is one of expected properties for the alternatives to antibiotics in feed. The theme of butyrate being an alternative to antibiotics is evidently strong within the studies using food-producing animals. This is especially apparent in the studies where the use of a butyrate derivative is used as a direct replacement for an antibiotic, but also observed in studies where butyrate-fed animals are challenged with a pathogen. Thus, butyrate and its derivatives can be expected to have a role in the post-antibiotic era of animal production.

7. Conclusions

The work completed thus far with butyrate and its derivatives in feed for animal production has laid a foundation for future studies, as well as for the extension of application. The various reported beneficial effects, such as antimicrobial and anti-inflammatory activities, enhancement of growth performance (including carcass composition) and gut tissue development/maturation, and modulation of immune response and intestinal microbiota, grant butyrate and its derivatives the potential to develop into valuable supplements across species and as an alternative to in-feed antibiotics for animal production. Although the benefits in human health applications show its promise in the treatment of disease, such as IBD, the positive effects of different forms of butyrate on multiple food-producing animal species demonstrate its ability to be a diverse product for livestock production. Given that the benefits appear to be more evident in young animals, it is important to maximize the potential of butyrate and its derivatives from young to adult animals.

Acknowledgments

This work was supported by Agriculture and Agri-Food Canada and Canadian Poultry Research Council through the Poultry Research Cluster Program (AAFC J-000264). Andrea Bedford was a NSERC Visiting Fellow to Canadian Federal Government Laboratories.

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