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Review: Exogenous butyrate: implications for the functional development of ruminal epithelium and calf performance

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The importance of the use of exogenous butyrate in calves' diets is due to its role as a factor stimulating the functional development of ruminal epithelium and improving calf performance during the transition from preruminant to ruminant status. Our review will first present results related to effects of the administration of butyrate in calves' diets on the development of ruminal epithelium toward a more effective absorption and metabolism of fermentation products from the rumen. The introduction of sodium butyrate at a level of about 0.3% of diet dry matter is accompanied by an increase to 35% in butyrate concentration in the rumen of 33-day-old calves. Mutual reliance between an enhanced ruminal concentration of butyrate and the activities of transcription factors, genes and proteins involved in cell proliferation, ketogenesis and the maintenance of cell pH homeostasis in the ruminal epithelial cells has been clearly confirmed in many experiments. Second, the review presents results related to the effects of the introduction of butyrate salts in the diet on calf performance. Of 11 studies a positive effect was found in six; five of these were obtained from the calves that started receiving butyrate supplement at a level of about 0.3% diet dry matter from the age of 3 to 5 days. Results indicate that when a supplement is given to calves soon after birth the functional development of ruminal epithelium in cooperation with the endocrine and digestion systems is transferred into improving the efficiency of rearing. There have been no studies on the effects of greater amounts of butyrate salts in milk replacer; butyrate constitutes about 1.2% of the whole cow's milk dry matter. In older calves, when butyrate administration is provided as a component of a starter concentrate at the increasing inclusion rate from 0.3% to 3.0%, the practical effect in calf performance relates to the risk of depression of rumen pH below 5.5 and accompanying disruption of the organization of the ruminal epithelial tissue. The higher risk is noted in calves received starter with substantial content of a rapidly degradable starch. At present, the insufficient number of positive results confirming the beneficial effect of butyrate supplements in terms of an improvement in performance does not allow their recommendation for use in the practical feeding of calves.

Keywords: calves, butyrate, rumen, epithelium, development

Implications

The functional development of the ruminal epithelium which covers the luminal surface of the rumen is crucial for proper solid feed utilization and body functions during the transition from a preruminant to a ruminant state in cattle. This review presents a discussion of recent investigations on the role of exogenous butyrate in the morphological and metabolic maturation processes in ruminal epithelial cells. In addition, the effects of the introduction of butyric acid salts into a diet in terms of improving calf performance are discussed, and some areas for future investigation are identified.

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Introduction

The luminal surface of the rumen is covered by a stratified epithelium which, during the transition from preruminant to ruminant status and due to dietary changes from liquid to solid feeds, undergoes a functional rebuild. The ruminal epithelium in preruminants undergoes maturation processes associated with the enlargement of the surface area for nutrient absorption and the development of ruminal epithelial cell capabilities for absorption and metabolism of short-chain fatty acids (SCFA) generated by the digestion of solid feed nutrients through the action of anaerobic microorganisms in the rumen (Bergman, 1990; Baldwin and Jesse, 1992). The functional development of rumen epithelium is

stimulated by butyric acid (Sander *et al*., 1959). An increase in butyric acid concentration in the rumen causes the enlargement of the ruminal epithelium absorptive surface area (Mentschel *et al*., 2001; Naeem *et al*., 2012; Malhi *et al*., 2013) and accelerates the oxidation of SCFA in the ketogenesis pathway (Connor *et al*., 2013; Yan *et al*., 2014). Recent reports support the hypothesis that an increase in butyric acid concentration is the basic factor influencing ruminal epithelium maturity.

With increasing prices of milk replacer ingredients, there is economic pressure to reduce the preweaning transition period for calves from their neonatal reliance on nutrients supplied from liquid feed to nutrients supplied from less expensive solid feed (Baldwin *et al*., 2004). For efficient solid feed utilization, the proper and rapid functional development of rumen epithelium is needed. The acceleration of epithelium development can be achieved by the addition of butyric acid salts into a calf's diet (Gorka *et al*., 2009; Malhi *et al*., 2013); however, the practical effects in terms of improvements in calf performance have been both positive (Górka *et al*., 2011a and 2011b; Nazari *et al*., 2012) and negative (Araujo *et al*., 2015; Wanat *et al*., 2015), and some results do not confirm these relationships (Kato *et al*., 2011). Therefore, it is equivocal as to whether exogenous butyrate could play a role as a factor stimulating the development of ruminal epithelium and that the consequences of these activities are improvements in calf performance.

This review presents results from studies on the effects of exogenous butyrate on the functional development of ruminal epithelium. In addition, we determine whether these effects are transferred into improving calf performance.

Butyric acid in calf rumen

Butyric acid (systematic name butanoic acid, structural formula $CH_3CH_2CH_2$-COOH), a weak acid with acid dissociation constant $pK_a = 4.82$, is a natural substance present in the sites of microbial fermentation in the gastrointestinal tract, and as a natural component of colostrum, milk, sweat and feces of most mammals, as well as a component of the cellular metabolism in all tissues. In the gastrointestinal tract, 90% to 99% of butyric acid is present as an anion, and for the purpose of the review the term 'butyrate' is used interchangeably for the acid and anion forms. Butyrate is one of the SCFA, the saturated aliphatic organic acids that consist of one to six carbons. Short-chain fatty acid concentrations in the rumen fluid of 8-week-old veal calves vary from 36 to 120 mM/l; and the acetate, propionate and butyrate (92% to 95%) are present at a molar ratio of 63 : 27 : 10 to 53 : 30 : 17 (Súarez *et al*., 2007). Among the most abundant SCFA, butyrate is recognized as the major stimulator of the functional development of ruminal epithelium, because it is the most extensively absorbed and, to a large extent, metabolized SCFA in the epithelium and its energy value is higher than that of propionate and acetate (Bergman, 1990; Rémond *et al*., 1995; Gäbel and Sehested, 1997). More recently, results have indicated that butyrate regulates the chromatin-remodeling activity of histone deacetylases and this process results in a modulation of gene expression in epithelial cell proliferation (Donohoe *et al*., 2012). The role of butyrate in the development of ruminal epithelial cells relates to its importance as a substrate of cell metabolism and as a regulator of gene activities. During the period from birth to weaning, the concentration of butyrate in rumen increases from 0.002 mM/l to a concentration between 5.1 and 17.3 mM/l (Lesmeister and Heinrichs, 2004; Súarez *et al*., 2007; Laarman *et al*., 2012). The elevation of the intraruminal concentration of butyrate within the normal physiological range accelerates the maturation processes in the ruminal epithelial cells in calves (Laarman *et al*., 2012; Connor *et al*., 2014). This concentration is modulated by varying the quality and quantity of feeds. A higher concentration of butyrate is found in the rumen of calves fed with milk in comparison with those receiving milk replacer without milk fat (Niwińska and Strzetelski, 2004). An increase in the concentration of butyrate has been noted in calf rumen as the consumption of solid feed increases and in those calves receiving starter concentrate with higher content of more rapidly fermentable carbohydrates (e.g. sugars and starches) in (Khan *et al*., 2008; Laarman *et al*., 2012; Khan *et al*., 2016). The rapidly fermentable carbohydrates present during rumen fermentation are an essential source of butyric acid. However, the provision of a butyrogenic starter diet could cause a low ruminal pH and may negatively affect the development of the rumen (Khan *et al*., 2008; Liu *et al*., 2013). Given this risk, the introduction of butyrate salts into the diet seems to be the simplest way to enhance ruminal butyrate concentration. Researchers have demonstrated a 25% to 35% increase in butyrate concentration in the rumen of 33-day-old calves as an effect of the introduction of butyrate salts in the form of sodium or calcium butyrate into diet at the level of 0.3% of ration dry matter (DM) (Gorka *et al*., 2009; Nazari *et al*., 2012). The results of studies have indicated that exogenous butyrate introduced in the form of butyrate salts in the diet contributes to an increase in the butyrate concentration in the rumen of calves.

Exogenous butyrate in the functional development of the ruminal epithelium

The ruminal epithelium overlays rumen papillae, the increase of which in terms of number and size results in an increased surface area and potential for nutrient absorption (Gäbel *et al*., 2002). The elevation of intraruminal concentrations of butyrate through its administration modulates the surface epithelium (Sakata and Tamata, 1978). The latest results regarding the effects of exogenous butyrate on the morphology of rumen papillae in preruminants are presented in Table 1. A substantial increase in papillary length has been observed as a consequence of the introduction of sodium butyrate at a level near 0.3% in ration DM, and there has been an accompanying increase in butyrate concentration in the ruminal content in calves (Mentschel *et al*., 2001; Gorka *et al*., 2009; Kato *et al*., 2011; Górka *et al*., 2011a and 2011b) and in lambs (Cavini *et al*., 2015) compared with animals receiving diets without butyrate supplementation.

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Table 1 *The effects of exogenous butyrate on the growth of rumen papillae in preruminants*

Effect[1]						
Rumen papillae						
Length	Width	Density	Surface area	Animals[2]	Na-butyrate supplementation	References
+**				Calves	3 g/kg BW	Mentschel *et al.* (2001)
+**	+**				0.3% (as fed)	Gorka *et al.* (2009)
+*	+*				0.3% DM	Górka *et al.* (2011b)
+#	+*				0.3% DM	Górka *et al.* (2011a)
+#					3 to 7 g/day	Kato *et al.* (2011)
+#	Ns	Ns		Lambs	0.36% DM	Cavini *et al.* (2015)
+**	+*	+#	+**	Goats	0.3 g/kg BW	Malhi *et al.*, 2013

DM = dry matter.
[1]The level of statistical significance in differences between effects on animals receiving butyrate supplemented *v.* unsupplemented feeds was declared (at *P*-value): Ns $P > 0.1$; #$P \leqslant 0.1$; *$P \leqslant 0.05$; **$P \leqslant 0.01$; (+) increase.
[2]The age of calves was from 26 to 42 days, of lambs 56 days and of goats 150 days.

Table 2 *The possible mechanisms of regulation of epithelial cell proliferation by an increase in the concentration of butyrate in the rumen*

			Butyrate			
Items		Effect[1]	Source	Concentration[2]	Animals[3]	References
Mitotic – apoptotic equilibrium	Mitotic index[4]	+*	Diet supplement	Sevenfold increase	Calves	Mentschel *et al.* (2001)
Activities of nuclear factors	Peroxisome proliferator-activated receptor-α,	+# IPA+[6]	Ruminal fermentation[5]			Naeem *et al.* (2012) Connor *et al.* (2013)
	Forkhead box protein O1	+*				Naeem *et al.* (2012)
	Transforming growth factor-β1	IPA+				Connor *et al.* (2014)
Regulation of the cell cycle	proportion of cells in G0/G1 to S state[7]	–#	Ruminal infusion	+*	Goats	Malhi *et al.* (2013)
	D-type cyclin, isoform 1	+*				
Regulation of the energy delivery	Ruminal vacuolar H^+-ATPase	+*	Ruminal fermentation	+**	Lambs	Kuzinski *et al.* (2012)

ATPase = adenosine triphosphatase.
[1]The level of statistical significance was declared (at *P*-value): #$P \leqslant 0.1$; *$P \leqslant 0.05$; **$P \leqslant 0.01$; (+) increase; (–) decrease.
[2]The increase in comparison with control groups.
[3]The age of calves was from 35 to 70 days, of goats 150 days and of lambs 7 months.
[4]The proportion of cells in a tissue undergoing mitosis.
[5]The source of butyrate was the rumen fermentation of starter concentrate.
[6]The results estimated by Ingenuity Pathway Analysis (Qiagen, Redwood City, CA, USA) on the base of statistics and algorithms of relationships among activities of nuclear factors in proliferation of epithelial cell and an increase in the concentration of butyrate in the rumen.
[7]The proportion of the fraction of cell in diploid resting state (G0/G1) to the fraction of cell in synthesis state double the amount of nuclear material (S) during the sequential phases of the cell cycle.

Similarly, significant and positive effects on papillary size, density and surface area (approximately by 82%) have been observed in goats after intraruminal butyrate infusion, when supporting a concentration within the normal physiological range (Malhi *et al.*, 2013). These results demonstrate that the administration of exogenous butyrate into the ration is accompanied by an increase in the number of papillae, together with their marked growth in terms of length and width. The mechanism responsible for the development of papillae resulting from elevated butyrate concentrations is explained in different ways (Table 2). Exogenous butyrate has been assigned the roles of a stimulator of mitotic rate, and an inhibitor of apoptosis (Mentschel *et al.*, 2001) and a modulator of cell cycle progression (Malhi *et al.*, 2013) in ruminal epithelial cells. The cell cycle is promoted by the faster transition from the diploid DNA resting state (G0/G1) to the synthesis state (S), double the amount of nuclear material of single epithelial cells (Shen *et al.*, 2005) and the activities of cyclin D-type, isoform1 (CCND1) and cyclin-dependent kinase type 4 (CDK4), the key regulators responsible for transition from G1 to S phase (King and Cidlowski, 1998). Exogenous butyrate decreases the proportion of the cellular fraction in G0/G1 phase to the cellular fraction in the S phase and increases the messenger RNA (mRNA) expression of CCND1 and CDK4 in the ruminal epithelial cells (Malhi *et al.*, 2013). In addition to regulation of the cell cycle, the relationship between the energy requirements, the intracellular pH (pHi) and the proliferation process of the ruminal epithelial cells is of particular importance. A higher activity of vacuolar-type H^+-adenosine

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triphosphatase (vH^+-ATPase), a sensor of substrate and energy availability participating in H^+ translocation across limiting membranes and of the peroxisome proliferator-activated receptor isoform-α (PPARα) involved in energy homeostasis and in cell cycle, has been estimated in proliferated epithelial cells in rumen with higher butyrate concentrations (Kuzinski *et al.*, 2012; Naeem *et al.*, 2012; Connor *et al.*, 2013). The process of cell division is connected with pHi changes which are induced metabolically (Madshus, 1988). This dependence is confirmed by the higher activities of transcription factors related to the proliferation pathways, such as the forkhead box protein O1 (FOXO1) and the transforming growth factor-β1, as well as those related to energy production, such as estrogen-related receptor α in cooperation with PPARα observed as an effect of enhancing nutrition levels in calves (Connor *et al.*, 2014). These results indicate that elevated butyrate concentration accelerates the proliferation of the ruminal epithelial cells by stimulation of the cell cycle, activities of proteins regulating cell division, pHi changes and the availability of energy to the dividing cells.

In addition to morphological development, the hallmark of metabolic development of ruminal epithelium tissue is ketogenesis, which is based on the ability to convert SCFA to the ketone bodies used as sources of oxidative fuels in the various tissues (Hegardt, 1999; Allen, 2014). In physiologically mature rumen epithelium between 75% and 90% of absorbed butyrate is metabolized and ~83% of metabolized butyrate is converted into β-hydroxybutyrate and acetoacetate (Rémond *et al.*, 1995; Gäbel and Sehested, 1997). The increase in butyrate concentration in calf rumen stimulates changes in the activities of genes and proteins involved in the ketogenesis pathway. The latest results concerning this area are summarized in Table 3. Acetyl-CoA acetyl transferase (ACAT) and 3-hydroxy-3-methylglutaryl CoA synthase (HMG-CoA synthase) play rate limiting roles in the ketogenesis process (Lane *et al.*, 2002). Increases in the mRNA abundance of ACAT isoform 1 (ACAT1), HMG-CoA synthase soluble isoform 1 (HMGCS1) and HMG-CoA synthase isoform 2 (HMGCS2) in response to a threefold increase in butyrate concentration in rumen liquid (Laarman *et al.*, 2012; Connor *et al.*, 2013) and in HMGCS1 in response to enhanced levels

Table 3 *The effects of elevated intraruminal concentration of butyrate on the activity of proteins involved in the metabolism of rumen epithelial cells*

Items			Effects[1]	Butyrate concentration in rumen[2]	References
Ketogenesis	Acetyl-CoA acetyl transferase, isoform 1		Ns		Naeem *et al.* (2012)
			+**		Connor *et al.* (2013)
	3-Hydroxy-3-methylglutaryl CoA synthase	Extramitochondrial isoform 1 (HMGCS1)	+**	+*	Laarman *et al.* (2012)
			−*		Naeem *et al.* (2012)
		Mitochondrial isoform 2 (HMGCS2)	+**		Connor *et al.* (2013)
			Ns		Naeem *et al.* (2012)
Intracellular pH regulation	Proton-linked monocarboxylate transporter	Isoform 1 (MCT1)	+**	+*	Yan *et al.* (2014)
			+**	+*	Laarman *et al.* (2012)
			Ns		Naeem *et al.* (2012)
		Isoform 4 (MCT4)	+*	+*	Yan *et al.* (2014)
			+*	+*	Malhi *et al.* (2013)
			Ns		Naeem *et al.* (2012)
	Na^+/H^+ exchanger	Isoform 1 (NHE-1)	+*	+*	Yan *et al.* (2014)
			Ns	+*	Laarman *et al.* (2012)
		Isoform 2 (NHE-2)	+**	+*	Yan *et al.* (2014)
			+*		Naeem *et al.* (2012)
			Ns	+*	Laarman *et al.* (2012)
		Isoform 3 (NHE-3)	+*	+*	Yan *et al.* (2014)
			+**	+*	Laarman *et al.*, 2012
	$SCFA^-/HCO_3^-$ exchangers	Downregulated in adenoma	+**	+*	Yan *et al.* (2014)
			Ns	+*	Laarman *et al.* (2012)
		Putative anion transporter 1	+**	+*	Yan *et al.* (2014)
			Ns	+*	Laarman *et al.* (2012)
		Anion exchanger 2	+**	+*	Yan *et al.* (2014)
			+*		Naeem *et al.* (2012)
	Ruminal vacuolar H^+-ATPase		+*	+**	Kuzinski *et al.* (2012)
			+**	+*	Yan *et al.* (2014)
	Na^+/K^+ ATPase		+**	+*	Yan *et al.* (2014)
			Ns		Naeem *et al.* (2012)

ATPase = adenosine triphosphatase.
[1]The level of statistical significance in differences was declared (at *P*-value): Ns $P > 0.1$; * $P \leqslant 0.05$; ** $P \leqslant 0.01$; (+) increase; (−) decrease.
[2]The increase in comparison with control groups.

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of nutrition and increasing butyrate concentrations (Naeem *et al*., 2012) have been observed in the ruminal epithelial cells of calves. The expression of HMGCS2 in the rumen has been found to be significantly higher postweaning than preweaning, thus supporting the existence of a positive relationship between ketogenesis and increasing rumen fermentation induced by intake of solid feed (Kato *et al*., 2016). In addition, on the basis of results of over-time and within diet analysis of differentially expressed genes (using the Permutation Analysis of Differential Expression), it has been proposed that transcription factor PPARα is a regulator of ACAT1 and HMGCS2 gene activities (Connor *et al*., 2013). PPARα is identified as a regulator of ketogenesis development in epithelial cells during the transition from pre-rumination to rumination state, and its activities are regulated by butyrate availability. In summary, these observations indicate a desirable relationship between an elevated concentration of butyrate in the rumen and the acceleration of ketone formation in the rumen epithelial cells.

The uptake and the metabolism of butyrate are rapid; the constant rate related to the butyrate flux from ruminal fluid to epithelial cells has been estimated at 0.97/h, and the absorption of butyrate by epithelial cells results in an elevation in pHi of about 0.45 pH units (Etschmann *et al*., 2006; Storm *et al*., 2012). After entry into the cells, the protonated form of butyrate readily dissociates, thereby delivering H^+ to the cell interior, and the intraepithelial butyrate metabolism gives rise to CO_2, the hydration of which supplies not only H^+ but also carbonic acid (Schweigel *et al*., 2000). The butyrate-induced acid load in the ruminal epithelial cells stimulates the activities of proteins involved in the pHi recovery to the values of cell homeostasis. These pH-homeostatic mechanisms include membrane-bound transporters belonging to the solute-carrier family, isoforms of (monocarboxylate transporter (MCT)), sodium–proton exchangers (NHEs) and also P-type cation transport adenosine triphosphatases (Graham *et al*., 2007; Kuzinski *et al*., 2012). It has been recognized that an increasing ruminal concentration of butyrate stimulates the activities of proton-linked monocarboxylate transporter isoform 1 (MCT1) and/or isoform 4 (MCT4) in transporting carboxylic acids in the ruminal epithelial cells in calves (Laarman *et al*., 2012), goats (Yan *et al*., 2014) and lambs (Malhi *et al*., 2013). The positive relationship between the activities of MCT1 and MCT4 and the intracellular concentrations of butyrate and ketone bodies indicates that these proteins mediate transruminal fluxes of butyrate and butyrate metabolites in the ruminal epithelium (Dengler *et al*., 2015). An increasing ruminal concentration of butyrate activates NHE isoforms 1, 2 and 3 in the ruminal epithelial cells in calves (Laarman *et al*., 2012; Naeem *et al*., 2012) and in goats (Yan *et al*., 2014). NHE-3 is very sensitive to changes in SCFA concentration. The full activity of NHEs has been recognized as a key transport element required for Na^+/K^+-ATPase and vH^+-ATPase functions in the regulation of pHi homeostasis (Albrecht *et al*., 2008). Activities of these both enzymes in the rumen epithelium are positively affected by an increasing ruminal concentration of butyrate in goats and lambs (Kuzinski *et al*., 2012; Yan *et al*., 2014). Butyrate exerts a positive effect on the pHi-homeostatic mechanisms allowing butyrate to pass from the rumen in the ruminal epithelial cells and butyrate metabolites into the capillary bed. The above literature collectively demonstrates that the provision of exogenous butyrate into the diet elevates butyrate concentrations in the calf rumen and positively affects the morphological and metabolic functions of the ruminal epithelial cells. These results suggested that metabolic development of the ruminal epithelial tissue driven by exogenous butyrate may positively affects calf performance.

Exogenous butyrate in calf rearing

The results of experiments investigating the effects of exogenous butyrate on calf performance is based on the daily weight gain, feed intake and feed conversion for BW gain (Table 4). In all the presented studies, the effects of butyrate supplementation on performance have been estimated in relation to those characterized in calves receiving unsupplemented diet. In these studies, sodium or calcium butyrate provided in milk replacer and/or starter concentrate at a rate of about 0.3% of the diet DM were used as a sources of exogenous butyrate. Positive effects on the daily weight gain, at the probability at least 0.1, have been reported in six studies. Five of them were obtained from the calves that started receiving butyrate supplement from 3 to 5 days of age as a component of milk replacer and/or starter concentrate et the level (Gorka *et al*., 2009; Górka *et al*., 2011a and 2011b; Nazari *et al*., 2012; Serbester *et al*., 2014). The results indicate that the improvement of performance is found during the 1st week of the preweaning period in calves that started receiving butyrate supplement from the 1st day of life. The improvement of performance was not observed when administration of butyrate as a component of milk replacer is started from day 12 of life (Guilloteau *et al*., 2010; Araujo *et al*., 2015). The results indicated that the practical effects in improvement of performance depend on the age at which the calf started to receive the supplementation. The ruminal concentration of butyrate in neonate calves is low, ~0.002 mM/l, similar to the plasma concentrations (Lesmeister and Heinrichs, 2004). The response to exogenous butyrate administration in calves aged 26 to 33 days is clearly noticeable in the elevated ruminal concentration of butyrate (Gorka *et al*., 2009; Nazari *et al*., 2012) and in the increase in the rumen area (Gorka *et al*., 2009; Górka *et al*., 2011a and 2011b). In accordance with the ontogenic development, the secretion of digestive enzymes soon after birth progressively increases and the exogenous butyrate additionally stimulates both the secretion of enzymes and their activities in the digestion of DM, protein and fat (Guilloteau *et al*., 2009; Guilloteau *et al*., 2010). Recent studies also point to the impact of butyrate on the activity of the endocrine system. The increase in the concentrations of gastrin, secretin, cholecystokinin, glucagon-like peptide 2, and IGF-1 in the blood and the increase in IGF-receptor 1 in the ruminal epithelial cells have been observed as a result of

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Table 4 *The effects of exogenous butyrate on calf performance*

	Supplements			Effects[1]			
Rearing periods	Included in	At level	Period (days of age)	Daily weight gain	Feed intake	Feed conversion[2]	References
Preweaning[3]	Milk replacer	3 g/day	3 to 48	+*	+*	+*	Nazari *et al.* (2012)
		3 to 7 g/day	4 to 42	Ns	Ns	Ns	Kato *et al.* (2011)
		0.3% (as fed)	5 to 26	+#4	Ns	Ns	Górka *et al.* (2011b)
		0.3% (as fed)	5 to 26	+#	Ns		Górka *et al.* (2011a)
		0.3% DM	12 to 45	Ns			Guilloteau *et al.* (2010)
		0.3% DM	12 to 47	Ns	Ns	−*	Araujo *et al.* (2015)
	Milk replacer and starter concentrate	0.3% (as fed)	5 to 26	+#	Ns		Gorka *et al.* (2009)
	Starter concentrate	0.3% (as fed)	5 to 26	Ns	+#		Górka *et al.* (2011a)
		0.3% (as fed)	4 to 35	+*	Ns	+*	Serbester *et al.* (2014)
		0.15%; 5.0% (as fed)		Ns	Ns	Ns	Ślusarczyk *et al.* (2010)
		0.3%; 1%; 3% DM	12 to 57	+*	+#		Wanat et al. (2015)
		0.3%; 0.6%; 0.9% (as fed)	13 to 62	−*	−**	Ns	
Postweaning[5]	Starter concentrate	0.3%; 1%; 3% DM	58 to 90	+#	+*		Ślusarczyk *et al.* (2010)
		0.15%; 0.3%; 5.0% (as fed)	35 to 70	Ns	Ns	Ns	Serbester *et al.* (2014)

DM = dry matter.
[1]The level of statistical significance in differences between effects on calves fed butyrate supplemented *v.* unsupplemented feeds was declared (at *P*-value): Ns $P > 0.1$; # $P \leqslant 0.1$; * $P \leqslant 0.05$; ** $P \leqslant 0.01$; (+) increase; (−) decrease.
[2]Estimated as a ratio: feed consumption/BW gain (kg/kg).
[3]Preweaning: from 3 to 62 days of age.
[4]Estimated during the period from 5 to 12 days of age.
[5]Postweaning: from weaning to 90 days of age.

butyrate supplementation (Shen *et al.*, 2005; Gorka *et al.*, 2009; Guilloteau *et al.*, 2010; Kato *et al.*, 2011; Shen *et al.*, 2012). All of these proteins and peptides are considered hormonal signals that regulate the nutrient absorption, metabolism and growth in mammalian tissues (Shen *et al.*, 2004; Siddle, 2011; Connor *et al.*, 2015). The presented investigations support the multifactorial mechanisms underlying the improvement of performance in calves receiving butyrate supplement. Exogenous butyrate stimulates these mechanisms and this positive effect is transferred into improving the efficiency of rearing when the supplement is given to calves from the 1st day of life. In light of these facts, it is important to answer the question as to whether the amount of supplied butyrate is optimal to enable its use as a stimulator of calf performance. It is commonly accepted that the most valuable liquid feeds for newborn calves are colostrum and whole cow's milk. Butyrate constitutes about 2.1% of colostrum DM (Garcia *et al.*, 2014) and ~1.2% of milk DM (Ceballos *et al.*, 2009). Twofold higher concentrations of butyrate in the rumen and near twofold higher weight gains are found in 36-day-old calves receiving whole cow's milk from the 1st week of life in comparison with that estimated in calves fed milk replacer without milk fat (Niwińska and Strzetelski, 2004). The quantity of butyrate in these natural liquid feeds is significantly greater than in experimental milk replacers contained about 0.3% of DM. The results suggested that the delivered amount of butyrate in experimental milk replacer may be too low to be considerably evident in improving calf performance. To our knowledge, the effects of the greater amounts of exogenous butyrate given to calves in milk replacer soon after birth have not been investigated. Further research on these aspects is needed.

There are conflicting reports regarding the practical effects in terms of improvements in calf performance when butyrate administration as a component of a starter concentrate is provided from the 2nd week of calf life. Within the period from 2 to 5 weeks of life, together with the greater solid feed intake, the ruminal fermentation increases, as is confirmed by the constant increase in butyrate concentration from 0.002 to 0.01 mM/l in calf rumen (Lesmeister and Heinrichs, 2004). The response to exogenous butyrate administration could be noticeable in the increase in the ruminal concentration of butyrate and subsequent calf performance. However, the presented effects have been both positive (Ślusarczyk *et al.*, 2010) and negative (Wanat *et al.*, 2015). An increase in daily weight gain and starter concentrate DM intake, at a probability of 0.05, is observed in calves receiving sodium butyrate at 0.3%, 1.0% and 3.0% of concentrate containing 30% of corn and 30% of wheat grains (Ślusarczyk *et al.*, 2010). In contrast, a linear decrease in daily weight gains and starter intake, at a probability of at least 0.02, are observed along with the increasing inclusion rate of sodium butyrate from 0.3%, 0.6% to 0.9% in the starter containing a twofold lower amount of corn and a 1.5-fold higher level of barley and wheat grains (Wanat *et al.*, 2015). The discrepancy in calf performance between the presented results could be explained by the differences in the

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rates and extents of ruminal starch fermentation existing among cereal grains included in the experimental concentrates. The starch of corn is digested in the rumen at 55% to 70%, with a degradation rate from 4.0% to 6.4%/h. The starches of barley and wheat are digested at 80% to 90%, with degradation rates from 14.7% to 24.5%/h in the mature rumen (Huntington, 1997). The rate of starch fermentation in developing calves affects the ruminal pH, which in 35-day-old calves is higher in calves fed a corn diet than in calves receiving barley or wheat diets (Khan *et al*., 2008). The increase in highly degradable starch content by up to 58% causes an increase in the ruminal butyrate concentration from 7.2 to 15.8 mM/l, but at the same time induces ruminal pH depression to below 5.3, accompanied by the cellular necrosis and disruption of tight junctions in the ruminal epithelial tissue (Liu *et al*., 2013). It is also worth noting that a rumen pH <5.5 predisposes calves to rumen acidosis (Wood *et al*., 2015; Khan *et al*., 2016). In response to a doubling in butyrate concentration, a dysfunction of the rumen microbial ecosystem is observed in adult cattle (Li *et al*., 2012). Presented results clearly indicate that the role of butyrate supplement is related to the risk of perturbation in the ruminal fermentation and the ruminal epithelial tissue organization in calves. The lower risk is noted in calves fed with starter concentrate with a slowly degradable starch and the higher in calves received starter with substantial content of a rapidly degradable starch. At present, only two studies have presented the practical effects of provision of butyrate as a component of starter concentrate before weaning on calf performance, and investigations results reveal contrasting effects. The problems are not sufficiently well recognized; further studies are required to establish the relation between butyrate supplementation and the starter diet composition.

Conclusions

Recent scientific reports clearly indicate that the enlargement of the ruminal epithelium surface area and the development of ruminal epithelial cells capabilities for oxidation of SCFA in the ketogenesis pathway is stimulated by dietary supplementation with butyrate. Such reports have presented the direct positive effects on the activities of genes, proteins and transcription factors related to the cell proliferation, butyrate uptake, ketone body formation, the maintenance of intracellular pH and energy homeostasis and also the transportation of butyrate metabolites into the capillary bed. These results indicate that exogenous butyrate is a factor stimulating functional development of ruminal epithelial cells. The transfer of the accelerated development of the ruminal epithelial tissue into improvement of calf performance is inconclusive. This is more pronounced in calves receiving a supplement in the form of sodium butyrate at the level of about 0.3% of diet DM not later than during the 1st week of life. A positive effect on the daily weight gain, at a probability of at least 0.1, has been reported. In light of the higher weight gains observed in calves fed whole cow's milk containing ~1.2% of butyrate in milk DM, the amount of butyrate in experimental milk replacer may be too low to be considerably evident in improving calf performance. The effects of greater amounts of exogenous butyrate given to calves in milk replacer soon after birth have not been investigated and further research on these aspects is needed. Reports regarding improvements in calf performance in terms of sodium butyrate administration as a component of starter concentrate indicate that this effect relates to the risk of depression of pH the rumen in calves. A high proportion of rapidly fermentable starch decreases ruminal pH below 5.3, accompanied by the cellular necrosis and disruption of tight junctions in the ruminal epithelial tissue. Currently, only two studies have presented practical effects of the provision of butyrate as a component of starter concentrate before weaning on the performance of calves, and investigations reveal the contrasting results. The relationship between the development of ruminal epithelium in calves and the improvement of calf performance is still largely unknown and this is an area for future investigation. The strategies for improving calf performance are not sufficiently well recognized. There is insufficient information to deserve a supplementary recommendation.

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