Sodium-butyrate as a growth promoter in milk replacer formula for young calves¹

P. Guilloteau,^{*2} R. Zabielski,[†] J. C. David,^{*} J. W. Blum,^{‡3} J. A. Morisset,[§] M. Biernat,[†] J. Woliński,[#] D. Laubitz,[#] and Y. Hamon^{||}

^{*}INRA, UMR 1079, Système d'Élevage, Nutrition Animale et Humaine (SENAH), Domaine de la Prise, 35590 Saint-Gilles, France

[†]Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, 02-766 Warsaw, Poland

[‡]Division of Physiology, Faculty of Veterinary Medicine, University of Bern, CH-3012 Bern, Switzerland

[§]Département Médecine, Faculté de Médecine, Université de Sherbrooke, Sherbrooke, Québec, J1H 5N4, Canada

[#]The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, 05-110 Jablonna, Poland

^{||}SEVO, 3 rue de la Miltière, 85480-Bournezeau, France

ABSTRACT

In milk-fed calves, the effects of sodium-butyrate (Na-butyrate) to replace flavomycin on growth performance and some mechanisms involved were studied. Pancreatic and intestinal morphology, digestive enzyme activities, plasma gut regulatory peptide concentrations, and expression of their receptors in the gastrointestinal tract were measured. Gastrointestinal tract defense systems were examined by measuring protein levels of 2 heat-shock proteins (HSP27 and HSP70). The calves were randomly allocated into 2 groups fed the same basic diet with flavomycin as an antimicrobial growth promoter or with Na-butyrate (3 g/kg of dry matter). Sodium-butyrate disappeared quickly in the upper gut and was not found in circulating blood. Supplementation with Na-butyrate enhanced growth rate and improved feed conversion into body weight gain compared with the flavomycin group. Supplementation with Na-butyrate was likely associated with an improvement in efficacy of the gastrointestinal tract digestive capacities expressed by enhanced production of digestive enzymes and increased absorptive capacities in the upper small intestine. The effects could have been controlled by insulin-like growth factor-1 but probably not by any of the cholecystokinin/gastrin peptide family. Concentrations of HSP27 and HSP70 were increased in stomach and colon of calves receiving Na-butyrate, thereby assuring protection of cells with intensive metabolism (chaperone function). In conclusion, beneficial effects of Na-butyrate on maturation of gastrointestinal func-

tions were shown in milk-fed calves and may be applied to young mammals of other species.

Key words: sodium-butyrate and flavomycin as growth promoters, maturation of gastrointestinal tract, cholecystokinin/gastrin family peptides and insulin-like growth factor-I, heat shock protein

INTRODUCTION

Soon after the introduction of antimicrobial agents to cure bacterial infections in humans and animals, their growth-promoting effects were discovered. Since the beginning of the 1950s antimicrobial agents have been included in feeds for animal production and they soon became a popular means to improve growth performance and reduce production costs (Vissek, 1978; Hardy, 2002). However, antimicrobial resistance emerged following extensive introduction of antimicrobials for growth promotion, compromising the clinical effectiveness of antibiotic effects in both humans and animals, and this antimicrobial resistance became dangerous for public health (Witte, 1997; Aarestrup, 2000; Kühn et al., 2005). The European Union banned the use of antimicrobial additives starting in 2006; therefore, other substances are needed to replace these antimicrobial substances. In this context, we have compared the effects of sodium butyrate (Na-butyrate) with that of flavomycin (regarded as the reference growth promoter) on animal performance in young calves. Until the ban, flavomycin was widely used as a growth promoter in milk replacer formula in most countries in the European Union. Flavomycin (bambermycin, flavophospholipol, moenomycin) is a phosphoglycolipid antibiotic that has been used as an antimicrobial agent (Edwards et al., 2005a, b) and growth promoter in livestock production (Rebolini et al., 1982). Flavomycin was believed to reduce bacterial growth in the gastrointestinal tract (GIT) and thereby increase the amount of nutrients available for the animal as well as to prevent pathogenic

Received March 31, 2008.

Accepted November 5, 2008.

¹Partially supported by Bonilait, Chasseneuil du Poitou and Sevo, Bournezeau, France. Dr. Morisset's research is financed by le Conseil de Recherches en Sciences Naturelles et au Genie du Canada (CRSNG).

²Corresponding author: Paul.Guilloteau@rennes.inra.fr

³Present address: Division of Physiology, Vetsuisse Faculty, University of Bern, CH-3012 Bern, Switzerland.

bacterial infections (Vissek, 1978). The protein turnover rate of GIT wall tissues was reduced as well (Edwards et al., 2005a, b). In *in vivo* studies in germ-free chickens, flavomycin addition failed to promote growth (Reidel et al., 1974), thus eliminating the possibility that flavomycin may also promote growth through a direct effect on tissue metabolism. Moreover, flavomycin did not affect the total production of short-chain fatty acids (SCFA) and their proportions at any point in the GIT (Edwards et al., 2005a), which is crucial for the design of the present study.

Butyric acid, a VFA, is a natural substance present in forestomach of ruminants and in the colon of monogastric species. It is also present in the milk of most mammals (0.16 g/L in cow milk) with the exception of sow milk, and only traces have been found in human milk (Alais, 1984). This molecule has widespread effects on growth, digestibility, and feed efficiency through modulation of proliferation, differentiation, and function in tissues of the GIT, especially mucosal epithelial cells and on defense systems (barrier function, antimicrobial potency, immune system) in healthy and sick animals (Pouillart, 1998; Partanen and Mroz, 1999; Gauthier, 2002; Scheppach and Weiler, 2004; Franco et al., 2005; Mroz, 2005; Manzanilla et al., 2006; Mazzoni et al., 2008). In animal studies and in practice, salts of butyric acid (Na-butyrate or Ca-butyrate) are often used instead of butyric acid itself because the salts are more stable and less odorous. Studies on the mechanisms involved indicate that Na-butyrate may act through IGF (Tsubaki et al., 2001) or other endocrine or growth factor systems (Simon et al., 1997; Bartholome et al., 2004). Sodium-butyrate may also stimulate defense systems through heat-shock proteins (HSP) and modify immune and inflammatory responses (Ren et al., 2001; Millard et al., 2002; Böcker et al., 2003). Heat-shock proteins protect cells against environmental stress (e.g., elevated temperature, oxidative stress, pH and osmolarity changes, electromagnetic fields) and function as chaperone proteins, thereby protecting cells with intensive metabolism (Sikora and Grzesiuk, 2007). All HSP possess chaperone activities and the most important role is attributed to the HSP70 family (Żylicz and Wawrzynow, 2001). Finally, Na-butyrate may influence the intestinal microflora (Biagi et al., 2007). Although data have been obtained in many different species (vole, mice, rat, rabbit, guinea pig, calf, ruminant, human), the combined effects of Na-butyrate on the animal's performance, digestive function, health, and the regulatory mechanisms involved have not yet been performed in one single species.

In this study, we hypothesized that Na-butyrate ingested in low doses (close to physiological range) could be a good growth promoter in milk replacer-fed calves

by improving the morphology of the GIT and digestive functions of the pancreas and intestinal mucosa. The aim was to assess, in milk-fed calves held under standard dairy cow farm conditions, the effects of Na-butyrate supplementation as a replacement for flavomycin on growth performance and to study some of possible mechanisms involved. We evaluated modifications in morphology of the small intestine and pancreas, activities of pancreatic and intestinal enzymes, plasma of some gut regulatory peptide concentrations, and the expression of their receptors in GIT tissues. Finally, the expression of 2 HSP (HSP27 and HSP70) in the GIT was analyzed.

MATERIALS AND METHODS

Animals and Diets

Treatments and experiments were conducted according to European Union regulations concerning the protection of experimental animals. Animal studies were performed under conditions of a standard mid-size dairy farm (Pays de Loire region, France) in 2002 and 2003 (Veissier et al., 2003). Eighty-eight Holstein milk-fed calves were selected from 104 male calves bought at a mean age of 5 d (range 3 to 7 d) and held in individual stalls in the same building. They were divided into 2 groups (flavomycin-supplemented group, **F**; Na-butyrate-supplemented group, **B**) from 12 d after birth until slaughter. On d 12, calves with clinical problems during the preceding 7-d period were excluded from the study. The remaining animals were allocated as pairs based on their birth date, BW, and hematocrit. Thus, 44 pairs were randomly dispatched in the stalls and were chosen among the animals with a BW nearest to the average weight of the entire group of animals of the same age on this farm. From each pair, 1 animal was allocated to the F group and the other to the B group. At d 12, BW were 43.9 ± 0.4 and 43.7 ± 0.4 kg and hematocrit were 34.6 ± 0.9 and $32.3 \pm 1.1\%$, respectively, in the F and B groups (difference between groups not significant). The 2 groups received 3 milk replacers (Bonilait, Chasseneuil du Poitou, France). From d 12 to 47 of life, calves were fed a starter diet and from d 48 to 55, they received a growth diet. Finally, from d 56 until slaughter, calves were fed a mixture of the growth diet that was gradually replaced from 97% (ninth week of life) to 65% (last week before slaughter) by a fattening diet (Table 1). In using this feeding design, the level of performance desired in the F-group calves was 1,150 g/d for BW gain (**BWG**) and 1.5 for feed/BWG ratio (kg/kg). Milk replacer for the F group contained 16.5 mg of flavomycin/kg of DM. Because the study was performed on a commercial farm, no permission

Table 1. Composition of the diets

Item	Starter diet	Growth diet	Fattening diet
Ingredient, g/100 g of powder			
Lard	6.25	10	5.78
Vegetable oil	13.85	10.38	7.68
Skim milk powder	0	50.5	0
Delactosed whey powder	15	0	0
Whey powder	30.65	23.12	59.59
Whey protein concentrate	11	0	0
Pregelatinized starch	5	0	20
Wheat flour	0	3	4
Wheat gluten	5	0	0
Soybean concentrate	10	0	0
L-Lysine	0.6	0.25	0.2
DL-Methionine	0	0.1	0
Minerals and trace elements ¹	2.65	2.65	2.75
Chemical composition, g/100 g of DM			
ME, MJ	11.0	11.2	9.7
CP (N $\times$ 6.25)	21.7	22.4	8.1
Crude fat	21.8	21.6	14.4
Nitrogen-free extract	48.5	49.3	72.6
Crude ash	8.0	6.7	4.9
Ca	0.54	0.88	0.50
P	0.65	0.78	0.52

¹Mineral and trace elements was a proprietary formula from Bonilait Company (Chasseneuil du Poitou, France) that contained also flavomycin or Na-butyrate.

was obtained to compose a true control group of calves without any growth promoter or antimicrobial protection. Milk replacer for the B group contained 3 g of Na-butyrate/kg of DM (Na-butyrate, Sigma-Aldrich, St. Louis, MO) instead of the antibiotic.

A standard feeding schedule was applied for both groups of calves (Sevo, Bournezeau, France): calves were fed twice daily at 0830 and 1630 h (except on Sundays, when they received only the morning meal) and the amounts of milk substitute were gradually increased from 650 to 2,300 g of DM/calf per day from the age of 2 to 22 wk. Moreover, each calf received a fiber-enriched diet complement from wk 2 (50 g/d) until wk 22 (250 g/d). All animals were weighed at 12, 59, 124, and 145 d of age and killed at the slaughterhouse at the age of 151 d by stunning. Only 16 calves were weighed immediately before slaughter.

Sample Collection and Analyses

Plasma Concentrations of Gut Regulatory Peptides and BHBA. At 89 and 116 d of age, 10 mL of blood was withdrawn from the left or right jugular vein of each calf 1 h before and 1 h after their morning meal for gastrin, cholecystokinin (CCK), and somatostatin analysis according to the methods described by Le Dréan et al. (1997). Moreover, on d 145, a series of blood samples was obtained at -1.0 , -0.75 , 0.5 , 1.0 , 1.5 , 2.0 , 3.0 , 5.0 , and 7.0 h relative to the morning meal. Samples were collected in tubes containing heparin (500 IU/mL, Choay Heparin, Sanofi Winthrop,

Gentilly, France) and aprotinin (10,000 IU/mL, Iniprol, Sanofi Winthrop). Plasma concentrations of gastrin and somatostatin were measured by RIA as described previously with the exception that in the present study, carbon dextran was used to extract nonantibody-bound hormone instead of a secondary antibody (Le Dréan et al., 1997). Cholecystokinin was determined using a commercially available kit (EURIA_CCK, Eurodiagnostica, Malmö, Sweden). Results are expressed as picomoles of standard per liter of plasma. Because most absorbed butyrate is metabolized in the liver and transformed to BHBA, we measured BHBA enzymatically with a commercial kit (# 310A, Sigma Chemical Co.). Plasma samples were aliquots of those used to analyze the gut regulatory peptides in blood samples obtained from the jugular vein.

Morphometry and Enzymology. All calves were slaughtered 16 to 17 h after their last meal given the day before slaughter. Among the 88 animals, 16 were selected (8 per group) on the basis of BW, BWG, and feed efficiency to obtain 2 subgroups that were the most representative of the 2 experimental groups (F and B). Thus, for each parameter cited and for each treatment, the mean of the subgroup was similar to the means of the groups B and F, respectively [e.g., for BWG (g/d), the mean and SEM were $1,142 \pm 11$ vs. $1,181 \pm 16$ for F and B subgroups, respectively, and $1,139 \pm 14$ vs. $1,177 \pm 14$, respectively, for all data obtained from F and B groups; difference not significant]. Immediately after slaughter, the entire GIT was removed and the weights and the length of the small intestine and pancreas were

measured. One-centimeter-long pieces of the pancreas (right lobe) and whole-thickness samples of the duodenum (50% of its length), the proximal, middle, and distal jejunum (25, 50, and 75%, respectively, of the jejunum length), and the ileum (50% of its length) were immediately fixed in Bouin's solution for histological analyses. Paraffin-embedded serial histological sections (5 μ m thickness) were stained with hematoxylin and eosin. Histomorphometrical analyses involved the size of the pancreatic acini (cross-section area), the number of cells per acinus, and in the small intestine, the depth of crypts, length of villi, the thickness of the tunica mucosa, and the mitotic index as described previously (Biernat et al., 1999).

Three pieces of the pancreas and small intestinal mucosa, scraped from the sites corresponding to sampling for histological analyses, were gently collected (about 1 g per sample) and frozen in liquid nitrogen for enzyme activity analysis. After thawing, the pancreatic tissue and intestinal mucosa samples were homogenized in cold distilled water (1 g of tissue/5 mL of distilled water) and centrifuged for 5 min at $1,000 \times g$ at 4°C. The protein contents were then determined as described by Hartree (1972) using BSA as standard, and DNA was determined using the Wizard Genomic DNA purification kit (no. A1120, Promega, Madison, WI). In pancreatic tissue, the activity of trypsin was assayed using the modified method by Erlanger et al. (1961), and that of α -amylase according to Bernfelt (1955). Activities of elastases I and II and lipase were measured using L-alanyl-L-alanyl-L-alanine methyl ester, succinyl-L-alanyl-L-alanyl-L-prolyl-L-leucine-*p*-nitroanilide, and tributyrin as substrates, respectively (Gestin et al., 1997a). In mucosa homogenates, activities of lactase and maltase were measured as described by Dahlquist (1964) with minor modifications. The activities of aminopeptidases A and N were measured with L-glutamyl-*p*-nitroanilide and L-leucyl-*p*-nitroanilide as substrates, respectively (Maroux et al., 1973), and the activity of dipeptidyl-peptidase IV was measured with glycyl-L-prolyl-*p*-nitroanilide (Nagatsu et al., 1976). The results were expressed as international units (IU) per milligram of protein (specific activity) for all the enzymes, as well as per kilogram of BW (only for pancreatic enzymes) and per milligram of DNA.

CCK/Gastrin Family Receptors and Expression of IGF Receptors. Aliquots of the same samples of the pancreas and small intestine as used for enzyme determination and abomasal tissue samples were analyzed for the presence of CCK₁ and CCK₂ receptors using indirect immunofluorescence with specific CCK₁ and CCK₂ antibodies as well as Western blots, as described in detail previously (Morisset et al., 2000)

To measure IGF-1 receptor mRNA levels, total RNA extraction was performed from full-thickness walls of duodenum, mid-jejunum, and ileum using TRIzol reagent (Gibco BRL, Basel, Switzerland) according to the instructions of the manufacturer and was resuspended in RNase-free water, treated with diethyl pyrocarbonate (DEPC, Sigma-Aldrich Vertriebs GmbH, Deisenhofen, Germany). Purity of RNA was acceptable if the ratio of optical density (OD) measurements at 260 and 280 nm (OD 260 nm/280 nm) was greater than 1.9. Electrophoresis using ethidium bromide staining was used to check for possible RNA breakdown. Reverse transcription (RT)-PCR was performed using random hexamer primers. The RT-PCR quantification was performed with the LightCycler System (Roche Molecular Biochemicals, Rotkreuz, Switzerland) using software package 3.3 (Pfaffl et al., 2002). Ubiquitin, GAPDH, β -actin, and 18S were included in the assays as housekeeping genes. The mRNA levels of IGF-1 receptor mRNA levels were calculated based on standard curves (Pfaffl et al., 2002). The maximal efficiency of PCR is 2 if PCR products are doubled in each cycle. In this study the efficiency, calculated as efficiency = $10^{[-\text{slope}]}$ (Rasmussen, 2001), ranged from 1.78 to 1.9 and was therefore close to 2.

HSP. One-hundred-milligram pieces of tissue were also collected from antrum, duodenum, mid-jejunum, ileum, and distal colon and frozen in liquid nitrogen for analysis of HSP27 and HSP70. The preparation of protein homogenates, Western blotting, and standardization of HSP expressions were performed as described in detail previously for HSP27 (David et al., 2001a), and HSP70 (David et al., 2001b). In Western blots, the density of each band of interest was expressed as a percentage of the density of the β -actin band in the same gel.

Statistical Analysis

The Shapiro-Wilk normality test (v.4.03, GraphPad Prism software, San Diego, CA) was used for statistical data analysis; data are expressed as mean $\pm$ standard error of the mean (SEM). For histometry, enzyme activities, and plasma gut regulatory peptides data, statistical analyses were made using unpaired *t*-test, nonparametric Mann-Whitney test, one-way ANOVA followed by Tukey test, and area under the curve analysis, when appropriate. In statistical analyses, $P < 0.05$ was taken as the level of significance and $P < 0.1$ was considered to indicate a tendency. The results obtained for HSP expression were tested with global ANOVA and the mean values were compared by a nonparametric analysis (Kruskal-Wallis test). When significant,

Table 2. Growth and feed intake in calves fed with milk formula with flavomycin (F group) and with Na-butyrate (B group)¹

Growth parameter	Group	Period ²			
		13 to 59 d	60 to 124 d	125 to 145 d	13 to 145 d
BW, ² kg at the end of the period	F	67.1 ± 0.5	151.8 ± 1.6	195.1 ± 1.9	—
	B	68.6 ± 0.6	156.9 ± 1.3	200.5 ± 1.8	—
<i>P</i> -value		0.05	0.01	0.02	
BW gain, g/d	F	608 ± 14	1,303 ± 20	2,064 ± 53	1,139 ± 14
	B	644 ± 17	1,359 ± 15	2,073 ± 43	1,177 ± 14
<i>P</i> -value		0.052	0.01	0.45	0.02
Feed/BW gain ratio, kg/kg	F	1.70 ± 0.62	1.58 ± 0.03	1.49 ± 0.05	1.54 ± 0.02
	B	1.62 ± 0.36	1.51 ± 0.01	1.42 ± 0.04	1.48 ± 0.01
<i>P</i> -value		0.24	0.01	0.25	0.02

¹Data are means ± SEM; *P*-values are based on (unpaired) Student *t*-test.

²Each period is defined by the age (d) at the beginning and at the end.

³BW at 12 d of age: 43.7 ± and 43.6 ± kg, respectively, for groups F and B (*P* > 0.05).

2 by 2 comparisons were made according to Conover procedure for the highly significant multiple comparison tests with *P*-values < 0.01 indicating significance (David et al., 2001a, b).

RESULTS

Feed Efficiency, Growth, and Morphometric Measurements

The calves of both groups remained clinically healthy during the entire study. The amounts of feed refused were low with no difference between the 2 groups. During the entire experiment, total DMI was similar in both groups (256.5 kg). During the experiment, calves from the B group had greater BW than those of the F group, mainly during the last 2 mo (Table 2). The effect of Na-butyrate on BWG was greater in the B than in the F group (*P* = 0.02) and greatest during the first 2 mo (+5.9%). After the age of 2 mo, this difference was smaller (+4%) and did not change up to the end of

the study. During the whole experiment, feed efficiency was improved in the B group compared with the F group, but the greatest efficiency was found between d 60 and 124 (Table 2). Pancreatic weight (Table 3) and length of the small intestine segments (Table 4) were not different between groups except for the duodenum, which tended to be longer in the B group than in the F group (*P* = 0.09).

Histomorphometry of the Pancreas and Small Intestine

The histomorphometric measures of the pancreas and small intestine of B group were different from those of the F group. In the pancreas (Table 3), although the acinus area was not affected, the number of cells per acinus was smaller (−8%, *P* = 0.02) in the B group compared with the F group. Protein concentration tended to be reduced in the B group, whereas total contents of protein and DNA, expressed per kilogram

Table 3. Pancreas weight, morphometry (area of pancreatic acini, number of cells per acinus), and protein, DNA, and pancreatic enzyme content of calves fed a milk formula with flavomycin or Na-butyrate¹

Pancreas	Flavomycin	Na-butyrate	<i>P</i> -value
Morphometry			
Pancreas, g	145 ± 28	128 ± 24	0.31
Pancreas, g/kg of BW	0.74 ± 0.06	0.65 ± 0.05	0.25
Acinus area, μm ²	833 ± 63	783 ± 23	0.16
Acinar cells per acinus, n	8.10 ± 0.29	7.61 ± 0.33	0.02
Content			
Protein concentration, mg/g of tissue	168 ± 5	155 ± 5	0.06
DNA, mg/g of pancreatic tissue	3.58 ± 0.54	4.23 ± 0.47	0.35
Trypsin, IU/kg of BW	6.83 ± 0.67	5.95 ± 0.43	0.26
Elastase I, IU/kg of BW	0.46 ± 0.05	0.51 ± 0.09	0.64
Elastase II, IU/kg of BW	9.16 ± 0.75	14.24 ± 2.43	0.05
Lipase, IU/kg of BW	8.7 ± 1.6	5.8 ± 0.5	0.09
Amylase, IU/kg of BW	6.3 ± 0.7	5.1 ± 1.0	0.31

¹Data are means ± SEM; *P*-values are based on (unpaired) Student *t*-test.

Table 4. Intestinal length and histomorphometry of the small intestine of calves fed a milk formula with flavomycin (F) and with Na-butyrate (B)¹

Tissue/group		Histomorphometric measure				
		Intestine length, cm	Crypt depth, μm	Villus length, μm	Tunica mucosa, μm	Mitotic index
Duodenum	F	58.2 $\pm$ 3.5	277 $\pm$ 27	649 $\pm$ 64	918 $\pm$ 89	2.36 $\pm$ 0.14
	B	65.1 $\pm$ 1.3	279 $\pm$ 24	869 $\pm$ 111*	1156 $\pm$ 134**	2.53 $\pm$ 0.27
Total jejunum	F	1,840 $\pm$ 60				
	B	1,830 $\pm$ 90				
Proximal jejunum	F		256 $\pm$ 24	1,133 $\pm$ 144	1,419 $\pm$ 176	3.29 $\pm$ 0.27
	B		291 $\pm$ 24*	1,115 $\pm$ 68	1,407 $\pm$ 155	3.90 $\pm$ 0.35*
Middle jejunum	F		305 $\pm$ 26	944 $\pm$ 301	1,262 $\pm$ 374	4.00 $\pm$ 0.23
	B		258 $\pm$ 19**	788 $\pm$ 111	1,107 $\pm$ 120	4.51 $\pm$ 0.21*
Distal jejunum	F		274 $\pm$ 32	674 $\pm$ 118	1,011 $\pm$ 111	3.06 $\pm$ 0.25
	B		352 $\pm$ 54**	848 $\pm$ 175	1,235 $\pm$ 219	3.61 $\pm$ 0.31
Ileum	F	79.1 $\pm$ 5.0	259 $\pm$ 13	628 $\pm$ 32	938 $\pm$ 54	2.16 $\pm$ 0.12
	B	74.5 $\pm$ 6.5	244 $\pm$ 16	664 $\pm$ 53	985 $\pm$ 87	2.23 $\pm$ 0.24

¹Data are means $\pm$ SEM; *P*-values are based on (unpaired) Student *t*-test.

*Asterisk indicates statistical differences between flavomycin and butyrate groups: **P* < 0.05, ***P* < 0.01.

of BW (data not shown), were not modified by Na-butyrate supplementation (Table 3).

In the duodenum (Table 4), the length of villi, and thereby the thickness of the tunica mucosa, were greater in the B group than in the F group. In the other parts of the small intestine there were no significant differences of the length of villi and mucosa thickness. In the jejunum, crypt depth was increased in the proximal and distal jejunum by 14 and 28%, respectively, but reduced by 15% in mid jejunum in the B group compared with the F group (*P* < 0.05). There were no significant differences in the ileum between the 2 groups of calves. The mitotic index was always greater in the B group for all examined segments of the small intestine, but the difference was significant (*P* < 0.05) only in the proximal and mid jejunum.

Pancreatic and Brush Border Enzyme Activities

There were no significant differences in pancreatic enzyme activities between the treatments, except for

greater elastase II activity in the B group than in the F group (Table 3). The contents of DNA and total protein of the duodenal mucosa and activities of 5 brush border enzymes are shown in Table 5. Activities in proximal jejunum and ileum were not different between the groups (data not shown). In the duodenum, the activities of aminopeptidase N and dipeptidase IV were greater in the B group than in the F group (*P* = 0.04).

Plasma Butyrate, CCK, Gastrin, and Somatostatin Concentrations

Concentrations of BHBA were below the level of detection (0.1 mmol/L) in all samples examined (data not shown). There were no age-related differences in the concentrations of the determined gut regulatory peptides between the 2 groups of calves, except for preprandial CCK concentrations that decreased with age in the F group, although these were higher compared with preprandial CCK in the B group (Table 6). Feeding resulted in an increase of CCK and gastrin concen-

Table 5. Concentrations of DNA and protein and activities of 5 brush border enzymes in the duodenal mucosa¹ of calves fed a milk formula with flavomycin or Na-butyrate²

Item	Flavomycin	Na-butyrate	<i>P</i> -value
DNA, mg/g mucosa of tissue	5.22 $\pm$ 0.81	4.56 $\pm$ 0.41	0.12
Protein, mg/g mucosa of tissue	135 $\pm$ 19	133 $\pm$ 6	0.87
Protein, mg/mg of DNA	26.6 $\pm$ 2.1	29.8 $\pm$ 2.9	0.12
Lactase activity, IU/kg of BW	0.50 $\pm$ 0.17	0.76 $\pm$ 0.20	0.09
Maltase activity, IU/kg of BW	0.11 $\pm$ 0.02	0.14 $\pm$ 0.02	0.06
Aminopeptidase A activity, IU/mg of DNA	0.079 $\pm$ 0.024	0.094 $\pm$ 0.017	0.32
Aminopeptidase N activity, IU/mg of DNA	0.15 $\pm$ 0.04	0.10 $\pm$ 0.02	0.04
Dipeptidase IV activity, IU/mg of DNA	0.056 $\pm$ 0.021	0.091 $\pm$ 0.024	0.04

¹Similar data were measured for the proximal jejunum and ileum. These data are not shown because there were no significant differences (*P* > 0.05) between the groups.

²Data are means $\pm$ SEM; *P*-values are based on (unpaired) Student *t*-test.

Table 6. Plasma concentration of cholecystokinin (CCK), gastrin, and somatostatin 1 h before and 1 h after feeding (−1 and +1), in 89-, 116-, and 145-d-old calves fed a milk formula with flavomycin (F) and with Na-butyrate (B)¹

		Age, d					
		89		116		145	
Enzyme/group		−1	+1	−1	+1	−1	+1
CCK, pmol/L	F	1.88 ± 0.43 ^a	9.81 ± 1.56	0.93 ± 0.07 ^b	7.11 ± 0.77	1.09 ± 0.17 ^b	8.05 ± 0.68
	B	0.49 ± 0.13	9.02 ± 1.32	0.57 ± 0.18	8.78 ± 2.04	0.56 ± 0.13	8.20 ± 1.03
<i>P</i> -value ²		0.005	0.35	0.047	0.23	0.02	0.45
Gastrin, pmol/L	F	9.66 ± 0.67	34.71 ± 4.81	8.58 ± 1.76	37.33 ± 7.58	9.43 ± 1.62	35.20 ± 6.57
	B	8.44 ± 1.10	23.30 ± 3.06	6.76 ± 1.48	16.76 ± 2.57	6.81 ± 1.05	21.62 ± 5.52
<i>P</i> -value		0.14	0.10	0.22	0.01	0.10	0.08
Somatostatin, pmol/L	F	27.32 ± 6.65	22.93 ± 2.99	28.05 ± 5.30	29.15 ± 3.78	26.65 ± 5.00	36.77 ± 7.87
	B	26.28 ± 4.09	13.72 ± 4.39	30.43 ± 7.01	27.32 ± 4.70	29.27 ± 3.78	20.80 ± 3.84
<i>P</i> -value		0.45	0.05	0.40	0.38	0.34	0.04

^{a,b}Means in rows with different superscript letters indicate the statistical differences of the peptide level at different ages ($P < 0.05$) in fasted calves.

¹Data are means ± SEM; *P*-values are based on (unpaired) Student *t*-test.

²*P*-value indicates the level of significance when peptide levels are compared between B and F groups, before or after feeding.

trations but not of somatostatin concentration in both groups (Table 6). Plasma concentration of somatostatin did not show any postprandial peak in calves of the B group (Figure 1) as was seen in the F group ($P < 0.05$). When the kinetics of plasma gut regulatory peptides in response to food were considered (Figure 1), the areas under the concentration curve were smaller ($P < 0.05$) for CCK in the B group than in the F group.

Expression of CCK/Gastrin Family Receptors and IGF Receptors

Evaluation of the CCK₁ and CCK₂ receptor concentrations by immunofluorescence or Western ligand blotting showed no significant difference in the pancreas and intestinal mucosa between the 2 groups (data not shown). Expression of IGF-1 receptor (Figure 2) was also not different between groups, but there was a significant tissue and group × tissue interaction ($P = 0.026$). The level of IGF-1 receptor mRNA was greater in the jejunum of the B group than the F group ($P < 0.05$), but no difference between B and F groups was found in duodenum and ileum. The IGF-1 receptor mRNA levels in jejunum were higher than in duodenum, and those in duodenum were higher than in ileum ($P < 0.05$).

HSP27 and HSP70 Concentrations

As shown in Figure 3, HSP27 was present in all studied parts of the GIT (abomasum, duodenum, mid-jejunum, ileum, and colon). There was no difference between groups in the small intestinal concentrations of HSP27 and HSP70. In the abomasum and colon,

however, the concentrations of HSP27 and HSP70 were greater in the B group compared with the F group (Figure 3).

DISCUSSION

In the present study we have shown improved growth performance and several modifications in GIT morphology and functions in calves fed a diet supplemented in Na-butyrate as compared with calves treated with flavomycin. The suggested growth promoting mechanism of flavomycin is the modification of gut microflora resulting in modifications of metabolic activities of the gut (Edwards et al., 2005a). Studies on the effects of Na-butyrate on the GIT functions in calves are lacking. On the other hand, studies in neonatal (Kotunia et al., 2004) and weaned piglets (Le Gall et al., 2007; Mazzoni et al., 2008) showed that ingestion of Na-butyrate resulted in improved performance, which was further improved if Na-butyrate was fed sooner rather than later after birth. A similar pattern was also found in the present study. We used Na-butyrate at a very low dose (i.e., 3 g/kg of DM; 28 mmol/kg of DM). Therefore, the effects of Na-butyrate were barely due to enhanced acidity of the ingesta as suggested by Manzanilla et al. (2006). The amounts chosen in our study were in the range of those (1 to 4 g of Na-butyrate/kg of DM) used by Biagi et al. (2007), who observed similar effects on growth performance in pigs as we did in calves.

Our data are in agreement with previous studies concerning growth performance in other farm animals (Partenen and Mroz, 1999; Gauthier, 2002; Franco et al., 2005; Mroz, 2005; Biagi et al., 2007), because the addition of 0.3% Na-butyrate improved BWG as well

Figure 1. Postprandial changes of plasma cholecystokinin (CCK), gastrin, and somatostatin concentrations in calves fed a formula with flavomycin or Na-butyrate. Data are means $\pm$ SEM. The area under the concentration curve for CCK was smaller ($P < 0.05$), and no postprandial peak was observed in plasma somatostatin concentration in calves that received Na-butyrate in comparison with flavomycin.

as the feed conversion ratio. Our results agree with improved BWG previously observed during a 6-d period following weaning in piglets fed a solid diet supplemented with Na-butyrate (Galfi and Bokori, 1990; Galfi et al., 1993). In contrast with previous studies in piglets, we did not observe any increase of feed intake (256.5 kg of total DMI during the entire experiment in both groups). Therefore, one reason for enhanced growth performance by Na-butyrate in our study was probably improved digestibility of the diet. A previous study in

milk-fed calves chronically implanted with catheters for collection of pancreatic juice demonstrated stimulation of pancreatic juice volume and trypsin secretion by Na-butyrate (Guilloteau et al., 2004). In the present study we observed a smaller plasma CCK response to food intake in the B group compared with the F group. However, physiological stimulation of pancreatic exocrine secretion acting through a CCK-vagal-dependent neurohormonal mechanism is not reflected by plasma CCK (Zabielski et al., 1998). Previous studies in milk-fed calves have shown that, unlike in adult animals, the amount of secreted pancreatic juice can limit intestinal digestibility. Thus, digestibility was improved by duodenal infusion of pancreatic juice (Guilloteau et al., 1999; P. Guilloteau and R. Zabielski; unpublished data). Similar conclusions can be drawn from a study by Botermans and Pierzynowski (1999) who compared the feed conversion ratio in piglets with high and low secretion of pancreatic trypsin. Thus, it seems that the enhanced BWG of calves during long-term feeding with milk formula supplemented with Na-butyrate could be due to increased efficiency of digestion by pancreatic enzymes.

Although pancreas size was not modified along with its total protein content, which tended to decrease (possibly due to enhanced pancreatic secretion as discussed above), Na-butyrate supplementation was accompanied by a 50% increase in activity of elastase II. This finding strongly supports the idea of enhanced digestibility of proteins, because elastase II, closely related to the chymotrypsin family, exhibits broad specificity for substrates containing medium and large hydrophobic amino acids in the P1 position. Moreover, the specificity of this enzyme is complementary to elastase I, trypsin, and chymotrypsin (Gestin et al., 1997b). The activity of brush border dipeptidylpeptidase IV was also increased, and there was a tendency toward an increase in lactase and maltase activities. Similar results were reported in other species (i.e., mice, rat, guinea pig, vole, pig, sheep, and goat) when Na-butyrate was used in vitro preparations, as an additive in the diet as well as after intravenous (in conscious or anesthetized animals) or intraduodenal administration. Although it is realized that the response can be different among species (Demol and Sarles, 1978; Harada and Kato, 1983; Harada, 1985; Kato et al., 1989; Katoh and Yajima, 1989; Galfi et al., 1993; Claus et al., 2003), these data as a whole suggest that Na-butyrate in mammalian species acts directly at the GIT level rather than after its absorption. Our study strongly confirms this mechanism because no Na-butyrate was found in the circulation. Furthermore, no direct effects of Na-butyrate could be expected on the intestinal epithelium because in early-weaned piglets, butyrate included in the diet was detected only in the

Figure 2. Level of IGF-1 receptor mRNA in duodenum, jejunum, and ileum in calves fed a milk formula with flavomycin or Na-butyrate. Data are means $\pm$ SEM; ^{a,b} means are significantly different from flavomycin group ($P < 0.05$); ^{x,z} means are significantly different between the segments of the small intestine ($P < 0.05$).

stomach but not in the proximal parts of the jejunum (Manzanilla et al., 2006).

Histomorphometry of the GIT and Digestive Enzymes

Sodium-butyrate supplementation modified some digestive enzyme activities in the calves in our study as well as in other studies (Demol and Sarles, 1978; Harada and Kato, 1983; Harada, 1985; Kato et al., 1989; Katoh and Yajima, 1989; Galfi et al., 1993) and modified the microstructure of the small intestine in animals and humans (Galfi and Bokori, 1990; Frankel et al., 1994; Bartholome et al., 2004; Kotunia et al., 2004), thus, serving as a basis for enhanced digestibility and absorption. Sengupta et al. (2006) found that butyrate may enhance proliferation, differentiation, and maturation, and reduce apoptosis of normal enterocytes, mediated through its influence on gene expression and protein synthesis. In our study, the enterocyte proliferation rate was increased in the upper jejunum, and a tendency toward increase was found in duodenal crypts. The duodenum was the only part of the small intestine in which a statistically significant trophic effect on the villi was observed. Our histomorphometry findings coincided with Na-butyrate effects on lactase and aminopeptidase N activities, which were only observed in the upper small intestine. This suggests that the direct effects of Na-butyrate are only in the upper part of the small intestine. Villus height, but not crypt depth, was shown to directly correlate with animal performance (Vente-Spreewenbergh et al., 2003). The

Figure 3. Relative levels of heat-shock protein (HSP) 27 and 70 mRNA in stomach, duodenum, jejunum, ileum, and colon in calves fed milk formula with flavomycin or Na-butyrate. A total of 100 μ g of protein was loaded on each lane. The last representative membrane shows the level of β -actin in the stomach. Data are means $\pm$ SEM of 6 determinations; * indicates a value significantly different from the flavomycin group ($P < 0.001$).

increase in crypt size reflects an enhanced maturation of the mucosa and more intensive crypt secretion. Our results are in agreement with those of Manzanilla et al. (2006) in piglets, but not with those of Biagi et al. (2007), probably because in this study Na-butyrate was supplemented too late in the life of growing pigs.

HSP

This article, to our knowledge, is the first to present data on concentrations of HSP in the GIT mucosa of young mammals under nonstress conditions. Our analytical approach followed that previously presented in neonatal piglets (David et al., 2001a, b). We described mucosal distribution of HSP27 and HSP70 levels along the GIT in preruminant calves and we demonstrated that Na-butyrate enhanced HSP27 and HSP70 levels in the abomasum and colon compared with the action of flavomycin. Removal of flavomycin from the diet results in substantial modification of intestinal microflora. Moreover, members of HSP family have been implicated in an ever-growing number of cellular activities including effects of microbial agents (Narberhaus, 2002). It is reasonable to think that different gut bacteria can induce different levels of HSP activation or expression. Thus, in our experiment, the enhancement of HSP levels with butyrate compared with flavomycin could be related to gut microflora composition. Thus, cell protection was possibly enhanced because chaperone activity is mostly ascribed to HSP70 family members (Sikora and Grzesiuk, 2007). It was shown previously that HSP25/27 need SCFA for basal expression in intestinal epithelial cells of the colon and the distal small intestine *in vivo* in the rat (Ren et al., 2001; Arvans et al., 2005), which is in agreement with our results in the stomach mucosa obtained in calves of the B group. *In vitro*, SCFA enhanced the expression of HSP25/27 in a dose- and time-dependent manner, an effect that is transcriptionally regulated (Ropeleski et al., 2003) and protected rat intestinal epithelial cells from injury (Ren et al., 2001). In the calves of the B group, the concentration of HSP70 followed the distribution pattern found for HSP27. The HSP response is a universal response to cell injury (Musch et al., 1996) that could be associated with a stress response of the GIT epithelium. However, HSP have also been reported to have wound healing and cytoprotective effects (Wischmeyer et al., 1997). Thus, increased expression of HSP70 in the rat stomach was considered to protect gastric mucosa against gastric ulcers (Shichijo et al., 2003), which are frequently found in calves. Interestingly, it was recently demonstrated that butyrate fed at a low dose in piglets has a positive effect on gastric morphology and func-

tion, presumably in relation to its action on mucosal maturation and differentiation (Mazzoni et al., 2008).

Taken together, these findings suggest that HSP may also enhance cytoprotection of the abomasal and colonic mucosa. Changes in abomasal mucosa HSP expression were probably a direct effect of ingested Na-butyrate, whereas the effects in the colon remain unclear. Heat-shock proteins are known to improve cell survival in a variety of stress conditions (Morimoto et al., 1990; Welch and Gaestel, 1998), but in the GIT, the defense system includes the mucous layer in the gut (physical barrier), the innate and adaptive immune systems, and HSP.

Mechanisms Involved

The pre- and postprandial concentrations of plasma gastrin, a regulatory peptide known for its trophic effects in the upper gut, were not changed, although a reduction or a tendency toward a reduction was observed in Na-butyrate-supplemented compared with flavomycin-supplemented calves. Postprandial plasma concentrations of CCK, which also has a trophic effect on the proximal small intestine (Biernat et al., 1999; Guilloteau et al., 2006), were also reduced in Na-butyrate-supplemented compared with flavomycin-supplemented calves. Moreover, the expression of CCK/gastrin receptors was not influenced. This finding is in opposition to an enhanced efficiency of duodenal digestive functions. These data suggest that the effects of Na-butyrate observed in the duodenum and proximal jejunum were not mediated by gastrin or CCK. This finding disagrees with data obtained from *in vitro* cell culture studies showing increases in gastrin mRNA levels and in intracellular and secreted gastrin during butyrate treatment (Simon et al., 1997). Inhibition of postprandial somatostatin responses (coinciding with a reduction of the plasma CCK level) and a tendency toward reduction in plasma gastrin concentration (not significant) may be explained by a direct effect of Na-butyrate on somatostatin-secreting cells. The effects of Na-butyrate on the upper small intestine could, however, involve glucagon-like peptide-2 (Bartholome et al., 2004). There was an increase in the expression of IGF-1 receptors in the jejunum of Na-butyrate-supplemented calves. Our results suggest that part of the effects of Na-butyrate were likely mediated by IGF-1 (Tsubaki et al., 2001).

In conclusion, supplementation of a milk replacer with Na-butyrate enhanced the growth performance of young calves held under practical farm conditions. Our data suggest that Na-butyrate did not act directly on lower gut microflora because it was utilized in the

upper GIT. Digestibility and feed efficiency could be improved when using Na-butyrate because of enhanced maturation of the GIT (such as enhanced villus size and modified activity of digestive enzymes). Plasma gut regulatory peptide concentrations (CCK/gastrin family and their corresponding intestinal receptors) were apparently not involved, but glucagon-like peptide-2 could be a candidate. Moreover, IGF-1 may play an important role. Finally, Na-butyrate could stimulate cytoprotection through HSP acting as chaperone proteins. The application of Na-butyrate as a feed additive in calf nutrition seems to be very promising. It could act as a growth factor and we propose that supplementation should begin as soon as possible after birth.

ACKNOWLEDGMENTS

We thank A. Barbeau and J. Chevalier (INRA-SE-NAH, St-Gilles, France) for their help in bibliographical research and presentation of illustrations, respectively, as well as A. Sahar for English revision of the manuscript. We also thank the Augereau family (La Fusellerie, Saint Christophe du Bois, France) for their help and comprehension.

REFERENCES

- Aarestrup, F. M. 2000. Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. *APMIS Suppl.*, 101:1–48.
- Alais, C. 1984. Science du lait. Principe des Techniques Laitières. Société d'Édition et de Promotion Agro-alimentaires, Industrielles et Commerciales, Paris, France.
- Arvans, M., S. R. Vavricka, H. Ren, M. W. Musch, L. Kang, F. G. Rocha, A. Lucioni, J. R. Turner, J. Alverdy, and E. B. Chang. 2005. Luminal bacterial flora determines physiological expression of intestinal epithelial cytoprotective heat shock proteins 25 and 72. *Am. J. Physiol.* 288:G696–G704.
- Bartholome, A. L., D. M. Albin, D. H. Baker, J. J. Holst, and K. A. Tappenden. 2004. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunioileal resection in neonatal piglets. *JPEN J. Parenter. Enteral Nutr.* 28:210–222.
- Bernfelt, P. 1955. Amylase α and β . *Methods Enzymol.* 1:149–155.
- Biagi, G., A. Piva, M. Moschini, E. Vezzali, and F. X. Roth. 2007. Performance, intestinal microflora, and wall morphology of weanling pigs fed sodium butyrate. *J. Anim. Sci.* 85:1184–1191.
- Biernat, M., R. Zabielski, P. Sysa, B. Sosak-Swidarska, I. Le Huërou-Luron, and P. Guilloteau. 1999. Small intestinal and pancreas microstructures are modified by an intraduodenal CCK-A receptor antagonist administration in neonatal calves. *Regul. Pept.* 85:77–85.
- Böcker, U., T. Nebe, F. Herweck, L. Holt, A. Panjas, C. Jobin, S. Rossol, R. B. Sartor, and M. V. Singer. 2003. Butyrate modulates intestinal epithelial cell-mediated neutrophil migration. *Clin. Exp. Immunol.* 131:53–60.
- Botermans, J. A., and S. G. Pierzynowski. 1999. Relations between body weight, feed intake, daily weight gain, and exocrine pancreatic secretion in chronically catheterized growing pigs. *J. Anim. Sci.* 77:450–456.
- Claus, R., M. Lösel, M. Lacorn, J. Mentschel, and H. Schenkel. 2003. Effects of butyrate on apoptosis in the pig colon and its consequences for skatole formation and tissue accumulation. *J. Anim. Sci.* 81:239–248.
- Dahlquist, A. 1964. Assay of intestinal disaccharidases. *Scand. J. Lab. Invest.* 44:169–172.
- David, J. C., J. Landry, and J. F. Grongnet. 2001b. Perinatal expression of HSP 27 in brain regions and non-neuronal tissues of the piglet. *J. Mol. Neurosci.* 15:109–120.
- David, J. C., R. M. Tanguay, and J. F. Grongnet. 2001a. Perinatal expression of heat shock proteins HSC 70 and HSP 70 in neural and non neural tissues of the piglet. *Brain Res. Dev.* 128:91–99.
- Demol, P., and H. Sarles. 1978. Action of fatty acids on the exocrine pancreatic secretion of the conscious rat: Further evidence for a protein pancreatic inhibitory factor. *J. Physiol.* 275:27–37.
- Edwards, J. E., B. J. Bequette, N. McKain, N. R. McEwan, and R. J. Wallace. 2005a. Influence of flavomycin on microbial numbers, microbial metabolism and gut tissue protein turnover in the digestive tract of sheep. *Br. J. Nutr.* 94:64–70.
- Edwards, J. E., N. R. McEwan, N. McKain, N. Walker, and R. J. Wallace. 2005b. Influence of flavomycin on ruminal fermentation and microbial populations in sheep. *Microbiology* 151:717–725.
- Erlanger, B. F., N. Kokowsky, and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.* 05:271–278.
- Franco, L. D., M. Fondevila, M. B. Lobera, and C. Castrillo. 2005. Effect of combinations of organic acids in weaned pig diets on microbial species of digestive tract contents and their response on digestibility. *J. Anim. Phys. Anim. Nutr.* 89:88–93.
- Frankel, W., J. Lew, B. Su, A. Bain, D. Klurfeld, E. Einhorn, R. P. MacDermott, and J. Rombeau. 1994. Butyrate increases colonocyte protein synthesis in ulcerative colitis. *J. Surg. Res.* 57:210–214.
- Galfi, P., and J. Bokori. 1990. Feeding trial in pigs with a diet containing sodium n-butyrate. *Acta Vet. Hung.* 38:3–17.
- Galfi, P., G. Gabel, and H. Martens. 1993. Influences of extracellular matrix components on the growth and differentiation of ruminal epithelial cells in primary culture. *Res. Vet. Sci.* 54:102–109.
- Gauthier, R. 2002. The mode of action of acidifiers and the interest they generate in the growing-finishing phase. Current developments in Pig Production. French Association of Swine Practitioners, Maisons-Alfort, France.
- Gestin, M., I. Le Huërou-Luron, J. Peinau, G. Le Drean, V. Rome, A. Aumaitre, and P. Guilloteau. 1997b. Diet modifies elastase I and II activities and mRNA levels during postnatal development and weaning in piglets. *J. Nutr.* 127:2205–2211.
- Gestin, M., I. Le Huërou-Luron, J. Peinau, E. Thioulouse, C. Desbois, G. Le Drean, D. Feldman, A. Aumaitre, and P. Guilloteau. 1997a. Method of measurement of pancreatic elastase II activity and postnatal development of proteases in human duodenal juice and bovine and porcine pancreatic tissue. *Dig. Dis. Sci.* 42:1302–1311.
- Guilloteau, P., I. Le Huërou-Luron, V. Romé, and M. Plodari. 1999. Nutrient absorption is related to quantity of pancreatic enzyme secretion: Preliminary results. *S. Afr. J. Anim. Sci.* 29:241–242.
- Guilloteau, P., V. Le Meuth-Metzinger, J. Morisset, and R. Zabielski. 2006. Gastrin, cholecystokinin and gastrointestinal tract functions in mammals. *Nutr. Res. Rev.* 19:254–283.
- Guilloteau, P., V. Romé, L. Le Normand, G. Savary, and R. Zabielski. 2004. Is Na-butyrate a growth factor in preruminant calf? Preliminary results. *J. Anim. Feed Sci.* 13(suppl. 1):393–396.
- Harada, E. 1985. Comparison of pancreatic digestive enzyme secretion induced by volatile fatty acids in mice, Japanese field voles and goats. *Comp. Biochem. Physiol. A* 81A:539–543.
- Harada, E., and S. Kato. 1983. Effect of short-chain fatty acids on the secretory response of the ovine exocrine pancreas. *Am. J. Physiol.* 7:G284–G290.
- Hardy, B. 2002. The issue of antibiotic use in the livestock industry: What have we learned? *Anim. Biotechnol.* 13:129–147.
- Hartree, E. F. 1972. Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.* 48:422–427.
- Kato, S., N. Asakawa, H. Mineo, and J. Ushijima. 1989. Effect of short-chain fatty acids on pancreatic exocrine secretion in calves aged 2 weeks and 13 weeks. *Jpn. J. Vet. Sci.* 51:123–127.

- Katoh, K., and T. Yajima. 1989. Effects of butyric acid and analogues on amylase release from pancreas segments of sheep and goats. *Pflügers Arch.* 413:256–260.
- Kotunia, A., J. Wolinski, D. Laubitz, M. Jurkowska, V. Romé, P. Guilloteau, and R. Zabielski. 2004. Effect of sodium butyrate on the small intestine development in neonatal piglets fed by artificial sow. *J. Physiol. Pharmacol.* 55(Suppl. 2):59–68.
- Kühn, I., A. Iversen, M. Finn, C. Greko, L. G. Burman, A. R. Blanch, X. Vilanova, A. Manero, H. Taylor, J. Caplin, L. Dominguez, I. A. Herrero, M. A. Moreno, and R. Mollby. 2005. Occurrence and relatedness of vancomycin-resistant enterococci in animals, humans, and the environment in different European regions. *Appl. Environ. Microbiol.* 71:5383–5390.
- Le Dréan, G., I. Le Huërou-Luron, J. A. Chayvialle, V. Philouze-Romé, M. Gestein, C. Bernard, R. Toullec, and P. Guilloteau. 1997. Kinetics of pancreatic exocrine secretion and plasma gut regulatory peptide release in response to feeding in preruminant and ruminant calves. *Comp. Biochem. Physiol.* 117A:245–255.
- Le Gall, M., B. Sève, A. Sahar, M. Leborgne, J. P. Lallès, and P. Guilloteau. 2007. Effect of sodium butyrate on growth, appetite and gastrointestinal tract development in piglet. *Ann. Nutr. Metab.* 51(Suppl. 1):113.
- Manzanilla, E. G., M. Nofrarias, M. Anguita, M. Castillo, J. F. Perez, S. M. Martin-Orue, C. Kamel, and J. Gasa. 2006. Effects of butyrate, avilamycin, and a plant extract combination on the intestinal equilibrium of early-weaned pigs. *J. Anim. Sci.* 84:2743–2745.
- Maroux, S., D. Louvard, and J. Baratti. 1973. The aminopeptidase from hog intestinal brush border. *Biochim. Biophys. Acta* 321:282–295.
- Mazzoni, M., M. Le Gall, S. De Filippi, L. Minieri, P. Trevisi, J. Wolinski, G. Lalatta-Costerbosa, J. P. Lallès, P. Guilloteau, and P. Bosi. 2008. Supplemental sodium butyrate stimulates different gastric cells in weaned pigs. *J. Nutr.* In press.
- Millard, A. L., P. M. Mertes, D. Ittelet, F. Villard, P. Jeannesson, and J. Bernard. 2002. Butyrate affects differentiation, maturation and function of human monocyte-derived dendritic cells and macrophages. *Clin. Exp. Immunol.* 130:245–255.
- Morimoto, R. I., A. Tissières, and C. Georgopoulos. 1990. The stress response, function of the proteins, and perspectives. Pages 1–36 in *Stress Proteins in Biology and Medicine*. Cold Spring Harbor Press, Cold Spring Harbor, NY.
- Morisset, J., H. Wong, J. H. Walsh, J. Lainé, and J. Bourassa. 2000. Pancreatic CCKB receptors: Their potential role in somatostatin release and δ cell proliferation. *Am. J. Physiol.* 279:G148–G156.
- Mroz, Z. 2005. Organic acids as potential alternative to antibiotic growth promoters for pigs. *Adv. Pork Prod.* 16:169–182.
- Musch, M. W., M. J. Ciancio, K. Sarge, and E. B. Chang. 1996. Induction of heat shock protein 70 protects intestinal epithelial IEC-18 cells from oxidant and thermal injury. *Am. J. Physiol.* 270:429–436.
- Nagatsu, T., M. Hino, H. Fuyamada, T. Hayakawa, and S. Sakakibara. 1976. New chromogenic substrates for X-prolyl dipeptidyl-aminopeptidase. *Anal. Biochem.* 74:466–476.
- Narberhaus, F. 2002. α -Crystallin-type heat shock proteins: Socializing minichaperones in the context of a multichaperone network. *Microbiol. Mol. Biol. Rev.* 66:64–93.
- Partanen, K. H., and Z. Mroz. 1999. Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev.* 12:117–145.
- Pfaffl, M. W., T. M. Georgieva, I. P. Georgiev, E. Ontsouka, M. Hageleit, and J. W. Blum. 2002. Real-time RT-PCR quantification of insulin-like growth factor (IGF)-1, IGF-1 receptor, IGF-2, IGF-2 receptor, insulin receptor, growth hormone receptor, IGF-binding proteins 1, 2 and 3 in the bovine species. *Domest. Anim. Endocrinol.* 22:91–102.
- Pouillart, P. R. 1998. Role of butyric acid and its derivatives in the treatment of colorectal cancer and hemoglobinopathies. *Life Sci.* 63:1739–1760.
- Rasmussen, R. 2001. Quantification on the LightCycler. Pages 21–34 in *Rapid Cycle Real-time PCR, Methods and Applications*. S. Meuer, C. Wittwer, and K. Nakagawara, ed. Springer, Heidelberg, Germany.
- Rebolini, O., D. Gallazzi, and L. Valerani. 1982. Use of flavophospholipol as a growth promotant in feeds for fattening rabbits. *Coniglicoltura* 19:57–60.
- Reidel, G., H. Reiter, and U. Losch. 1974. Effect of oral flavophospholipol (FPL) on the growth of germfree chickens. *Z. Tierphysiol.* 32:328–334.
- Ren, H., M. W. Musch, K. Kojima, D. Boone, A. Ma, and E. B. Chang. 2001. Short-chain fatty acids induce intestinal epithelial heat shock protein 25 expression in rats and IEC 18 cells. *Gastroenterology* 121:631–639.
- Ropeleski, M. J., J. Tang, M. M. Walsh-Reitz, M. W. Musch, and E. B. Chang. 2003. Interleukin-11-induced heat shock protein 25 confers intestinal epithelial-specific cytoprotection from oxidant stress. *Gastroenterology* 124:1358–1368.
- Scheppach, W., and F. Weiler. 2004. The butyrate story: Old wine in new bottles? *Curr. Opin. Clin. Nutr. Metab. Care* 7:563–567.
- Sengupta, S., J. G. Muir, and P. R. Gibson. 2006. Does butyrate protect from colorectal cancer? *J. Gastroenterol. Hepatol.* 21:209–218.
- Shichijo, K., M. Ihara, M. Matsuu, M. Ito, Y. Okumura, and I. Sekine. 2003. Overexpression of heat shock protein 70 in stomach of stress-induced gastric ulcer-resistant rats. *Dig. Dis. Sci.* 48:340–348.
- Sikora, A., and E. Grzesiuk. 2007. Heat shock response in gastrointestinal tract. *J. Physiol. Pharmacol.* 58(S3):43–62.
- Simon, B., J. L. Merchant, R. Eissele, K. Kohler, and R. Arnold. 1997. Transient transcriptional activation of gastrin during sodium butyrate-induced differentiation of islet cells. *Regul. Pept.* 70:143–148.
- Tsubaki, J., W. K. Choi, A. R. Ingermann, S. M. Twigg, H. S. Kim, R. G. Rosenfeld, and Y. Oh. 2001. Effects of sodium butyrate on expression of members of the IGF-binding protein superfamily in human mammary epithelial cells. *J. Endocrinol.* 169:97–110.
- Veissier, I., G. Bertrand, and R. Toullec. 2003. Le veau de boucherie: Concilier bien-être animal et production. INRA, Paris, France.
- Vente-Spreuwenberg, M. A., J. M. Verdonk, M. W. Verstegen, and A. C. Beynen. 2003. Villus height and gut development in weaned piglets receiving diets containing either glucose, lactose or starch. *Br. J. Nutr.* 90:907–913.
- Visek, W. J. 1978. The mode of growth promotion by antibiotics. *J. Anim. Sci.* 46:1448–1469.
- Welch, M. J., and M. Gaestel. 1998. Small heat shock protein family: Function in health and disease. *Ann. N. Y. Acad. Sci.* 851:28–35.
- Wischmeyer, P. E., M. W. Musch, M. B. Madonna, R. Thisted, and E. B. Chang. 1997. Glutamine protects intestinal epithelial cells: Role of inducible HSP70. *Am. J. Physiol.* 272:G879–G884.
- Witte, W. 1997. Impact of antibiotic use in animal feeding on resistance of bacterial pathogens in humans. *Ciba Found. Symp.* 207:61–71.
- Zabielski, R., V. Leśniewska, J. Borlak, P. C. Gregory, P. Kiela, S. G. Pierzynowski, and W. Barej. 1998. Effects of intraduodenal administration of tarazepide on pancreatic secretion and duodenal EMG in neonatal calves. *Regul. Pept.* 78:113–123.
- Żylicz, M., and A. Wawrzynow. 2001. Insight into the function of Hsp70 chaperones. *IUBMB Life* 51:283–287.