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Effects of Sodium Butyrate on the Intestinal Morphology and DNA-Binding Activity of Intestinal Nuclear Factor-κB in Weanling Pigs

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Abstract: A total of 96 pigs weaned at day 21 (Duroc x Landrace x Yorkshire) with an average initial body weight of 6.42 kg were used to investigate the effects of sodium butyrate on growth performance, intestinal morphology and DNA-binding activity of Nuclear Factor-κB (NF-κB) in the small intestine. The pigs were allocated to three dietary treatments in a randomized complete block design. Each treatment was replicated four times with eight pigs per replicate and the trial lasted for 21 days. The dietary treatments were: basal diet, basal diet+500 mg kg^{-1} sodium butyrate basal diet+1000 mg kg^{-1} sodium butyrate. The results showed that supplementation with 1000 mg kg^{-1} sodium butyrate in diets improved ($p<0.05$) growth performance, increased ($p<0.05$) villous height and the villus height to crypt depth ratio at the small intestinal mucosa, reduced ($p<0.05$) the total viable counts of proximal colon Clostridium and *Escherichia coli*, decreased ($p<0.05$) Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6) level in the serum and DNA-binding activity of the intestinal NF-κB ($p<0.05$) as compared with control or 500 mg kg^{-1} sodium butyrate. Supplementation with 500 mg kg^{-1} sodium butyrate in diets increased ($p<0.05$) villus height and the villus height to crypt depth ratio at the jejunum, decreased ($p<0.05$) TNF-α and IL-6 level in the serum as compared with control. The results indicated that sodium butyrate is effective on improving growth performance and enhancing the intestinal morphology of weanling pigs, this effect might be through the pathway of decreasing the TNF-α, IL-6 level in serum and inhibition of DNA-binding activity of the intestinal NF-κB.

Key words: Sodium butyrate, intestinal morphology, nuclear factor-κB, DNA-binding activity, weanling pigs

INTRODUCTION

Short-Chain Fatty Acids (SCFAs) which produced in the hindgut of mammals by microbial fermentation, play great importance in the gastrointestinal tract of pigs (Malbert *et al*., 1994). Weaning had a significant effect on SCFAs production and this effect may impact upon microflora population, feed intake and ultimately on the health of the young pigs (Mathew *et al*., 1996). Changes in intestine morphology and function of piglets throughout weaning have received great attention. Today, organic acids have received much attention and considerable research confirms positive effects on growth performance in all classes of pigs (Witte *et al*., 2000). Several studies have proved that supplementation of butyrate in diets has beneficial effects on the digstive tract health of weanling pigs (Piva and Morlacchini, 2002; Kotunia *et al*., 2004; Wang *et al*., 2005; Lu *et al*., 2008). Butyrate has been shown to provide an important energy substrate for the intestinal cells metabolism (Jozefiak *et al*., 2004), inhibit mucosal apoptosis (Mentschel and Claus, 2003), induce absorption of water and sodium and proliferation of intestinal cells (Kripke *et al*., 1989; Friedel and Levine, 1992), stimulate intestinal blood flow (Binder and Mehta, 1989) and the synthesis of gastrointestinal hormones (Mortensen *et al*., 1990).

But the mechanisms of the effects of sodium butyrate in weanling pigs are not completely understood. It would be of interest to investigate the effecs of the addition of butyrate directly to diets on modifying the morphology and function of the intestine in weanling pigs.

Nuclear Factor-κB (NF-κB), a transcription factor crucial to the regulation of the inflammation response, plays a key role in the intestinal injury and inflammation by regulating the transcription of pro-inflammatory cytokines (De Plaen *et al*., 1998). One study found that butyrate inhibited the activity of NF-κB in human colonic

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epithelial cell (Inan *et al*., 2000). It was wondered whether or not that the intestinal butyrate would exert a modulatory effect on the small intestine mucosa through the NF-κB pathway in weanling pigs. Detailed information of butyrate may provide valuable information relevant to the maintenance of digestive tract health in the young pigs. In the present study, researchers examined the effects of sodium butyrate on growth performance, intestinal morphology and NF-κB DNA-binding activity of the small intestine in weanling pigs.

MATERIALS AND METHODS

Sodium butyrate (99.9%) was obtained from the Huadong Medicine Company (Zhejiang province, China) whose molecular formula is $C_4H_7O_2Na$. Gel Shift Assay System kits and NF-κB oligonucleotides were purchased from Promega, Madison, WI, USA. Poly dI-dC was obtained from Pharmacia Biotech, Piscataway, NJ, USA. [γ-^{32}P] ATP (3000 Ci mmol^{-1}, 10 mCi mL^{-1}) was purchased from Amersham, Arlington Heights, IL, USA.

Experimental design and sampling procedure: All procedures were approved by the University of Zhejiang Institutional Animal Care and Use Committee. A total of 96 pigs (Duroc x Landrace x Yorkshire) weaned at day 21st with an average initial body weight of 6.42 kg were allocated to three dietary treatments in a randomized complete block design, each treatment was replicated four times with eight pigs per replicate and the trial lasted for 21 days. The dietary treatments were:

- Basal diet
- Basal diet+500 mg kg^{-1} sodium butyrate
- Basal diet+1000 mg kg^{-1} sodium butyrate

Diets were formulated according to the requirements suggested by the NRC for 5-10 kg pigs. All diets were fed as mash and no antibiotics were included (Table 1). All pigs were given *ad libitum* access to feed and water. Feed intake was recorded per pen. At the start and end of the feeding trial, the pigs were weighed individually and growth performance results as Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), average Gain/Feed (G/F) were calculated for all pigs. At the end of the feeding trial, eight pigs from each treatment (2 pigs per pen), selected randomly based on similar body weight were slaughtered under general anaesthesia and then immediately eviscerated. Blood samples were centrifuged at 3,000 g for 10 min and serum was separated and packed in eppendorf tubes and kept at -70°C until analysis. Samples of the contents from the small intestine (from the distal end of the duodenum to the ileo-caecal junction) and proximal colon were collected into glass containers

Table 1: Ingredient and chemical composition of the basal diet on an as-fed basis

Items	Percenatge
Ingredient	
Maize	54.27
Soyabean meal (45% CP)	19.00
Soyabean oil	1.60
Extruded full-fat soyabean	12.00
Fish meal (63% CP)	4.00
Dried whey	4.00
Dicalcium phosphate	2.00
Limestone	1.00
Sodium chloride	0.25
L-lysine HCl (78%)	0.28
Methionine (98.5%)	0.60
Vitamin-mineral premix[1]	1.00
Analysed chemical composition as feed	
DE (MJ kg^{-2})	14.40
Crude protein	20.39
Lysine	1.30
Met+Cyst.	0.82
Threonine	0.90
Calcium	0.90
Total phosphorus	0.63

[1]The vitamin-mineral premix provided, per kg feed, IU: Vit. A 4000, Vit. D_3 800, Vit. E 10; mg: Vit. K_3 0.5, biotin 0.05, folic acid 0.3, niacin 10, d-pantothenic acid 10, riboflavin 3.6, thiamine 1.0, pyridoxine 1.5, cobalamin 15, Mn ($MnSO_4 \cdot H_2O$) 10, Zn ($ZnSO_4 \cdot 7H_2O$) 80, Fe ($FeSO_4 \cdot 7H_2O$) 80, Cu ($CuSO_4 \cdot 5H_2O$) 15.0, I (KI) 0.14 and Se (Na_2SeO_3) 0.15; [2]DE (Digestible Energy) was based on calculated values

under CO_2, sealed and put on ice until they were transported to the lab for enumeration of microbial populations. Small intestine removed and washed with ice-cold saline. The specimens from the middle part of duodenum, jejunum and ileum segment were excised, flushed with physiological saline and fixed in 10% formalin. Approximately, 20 cm of ileum was isolated and the Peyer's patches were dissected and removed. Ileum was chosen because a study indicated that NF-κB DNA-binding activity is much higher in the ileum than in the jejunum (De Plaen *et al*., 1998). The tissue samples were then weighted and nuclear extracts were prepared.

Intestinal microbial populations: About 10 g of mixed contents were blended under CO_2 in 90 mL of Anaerobic Dilution Solution (ADS). Further, serial dilutions were made in ADS for anaerobic bacterial enumeration. The initial dilution in ADS was also used as a source for serial dilutions in phosphate buffer solution for enumeration of aerobic bacterial populations. Triplicate plates were then inoculated with 0.1 mL samples and incubated at 37°C aerobically or anaerobically as appropriate. Three dilutions were plated for each medium. Bacteria were enumerated on Wilkins chalgren agar (Oxoid; total anaerobes), brain heart infusion agar (Oxoid; total aerobes), MRS agar (Oxoid; Lactobacillus), reinforced clostridial agar plus supplements (Munoa and Pares,

1988; Bifidobacterium), sulphite-polymyxin milk agar (Mevissen-Verhage *et al*., 1987; Clostridium) and MacConkey's No. 2 (Oxoid; *E. coli*). Single colonies were removed from selective media plates and grown in Peptone Yeast Glucose (PYG) broth. Subsequently, the bacteria were characterized to genus level on the basis of colonial appearance, gram reaction, spore production, cell morphology and fermentation end-product formation (Holdeman *et al*., 1977).

Histomorphometry: Three cross-sections for each intestinal sample were prepared after staining with hematoxylin and eosin using standard paraffin embedding procedures (Xu *et al*., 2003). A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section (30 measurements for each sample, total of 240 measurements per dietary treatment). Villus height and crypt depth were determined using image processing and analysis system (Version 1, Leica Imaging Systems Ltd., Cambridge, England).

Measurement of Tumor Necrosis Factor-α (TNF-α) and Interleukin-6 (IL-6) level in the serum: Levels of TNF-α and IL-6 in serum were measured separately using Quantikine ELISA kits (R and D Systems, Minneapolis, MN. 21 pg mL^{-1} detection limit for IL-6 and 5 pg mL^{-1} for TNF-α, respectively) according to the manufacturer's instructions. The reaction was terminated with a stop buffer and absorbance read at 450 nm using an MRX Revelation (Dynex Technologies, Chantilly, VA) multiwell plate reader. Experiments were carried out in triplicate and results are shown as pg mL^{-1}.

Preparation of nuclear extracts: Approximately 500 mg of frozen ileum tissue were ground into powder with a mortar in liquid nitrogen (Deryckere and Gannon, 1994). The powder was then briefly homogenized in 5 mL of cushion buffer (0.6% Nonidet p-40, 150 mM NaCl, 10 mM HEPES, pH 7.9, 1 mM EDTA, 0.5 mM Phenymethylsulfonyl Fluoride (PMSF) and centrifuged for 1 min at 2,000 g to remove any large debris. The supernatant was placed on ice for 5 min. Nuclei were obtained by centrifugation at 5000×g for 5 min and lysed in 0.5 mL of buffer containing 25% glycerol, 20 mM HEPES, pH 7.9, 420 mM NaCl, 1.2 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM Dithiotheitol (DTT), 0.5 mM PMSF, 2 mM benzamidine and 5 μg mL^{-1} each of pepstatin, leupeptin and aprotinin. After centrifugation at 10,000 g, the supernatant (nuclear extract) was stored at -70°C. Protein concentration was determined by Bradford'method (Bio-Rad).

Determination of NF-κB DNA-binding activity by Electrophoretic Mobility Shift Assay (EMSA): The oligonucleotide containing the NF-κB specific binding sites (5'-TAGTTGAGGGGACTTTCCCAG-3'; 3'-ACTCCCCTGAAAGGGTCCGTT-5') was labeled by [γ-^{32}P] ATP (3000 and 10mCi mL^{-1}) with T4 polynucleotide kinase. Equal amounts of nuclear extracts (10 μg/10 μL) were incubated with 5 μL of gel shift binding buffer (40% glycerol, 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM EDTA, 1 mM $MgCl_2$, 0.5 mM DTT, 0.01 mg mL^{-1} Poly dI-dC) for 15 min at room temperature. DNA nuclear protein binding reaction was initiated by adding 1 μL of ^{32}P-labelled oligonucleotide probe and the mixture was incubated at room temperature for 20 min. The sample was mixed with 1.5 μL of loading buffer and analyzed by electrophoresis in 6% polyacrylamide gel (Kravchenko *et al*., 1995). Following the binding reaction, free DNA and DNA-protein complexes were separated under nondenaturing conditions for 2 h at 300 V in 0.25×TBE (22.25 mM Tris, 22.25 mM boric acid, 0.5 mM EDTA, pH8). After electrophoresis, the gel was dried and analyzed using a phosphorimaging system (Storm 860-Imagequant, Molecular Dynamics, Sunnyvale, CA, USA). Individual retarded band density values of NF-κB binding complex was expressed as Relative Density Unit (RDU). To confirm the specificity of NF-κB DNA-binding activity, a competitive experiment tested by incubating samples with 50 and 100 fold molar excess of the unlabeled oligonucleotide probe.

Statistical analysis: All data were analyzed by analysis of variances using the General Linear Model (GLM) procedures of SAS (Version 6.12). Duncan's multiple range test was applied to treatment means which showed a statistically significant variation in the samples. Differences were considered significant at $p<0.05$.

RESULTS

The growth performance of weanling pigs is shown in Table 2. Pigs fed with 1000 mg kg^{-1} sodium butyrate diets had higher ($p<0.05$) ADG, ADFI and feed conversion than those fed with 500 mg kg^{-1} sodium butyrate diets or control. Supplementation with 500 mg kg^{-1} sodium butyrate had no significant ($p>0.05$) effect on growth performance as compared with the control.

Intestinal microflora of weanling pigs is shown in Table 3. Supplementation with 1000 mg kg^{-1} sodium butyrate reduced ($p<0.05$) the total viable counts of Clostridium and *E. coli* in the small intestine and proximal colon of weanling pigs as compared with the control. Pigs fed with 1000 mg kg^{-1} sodium butyrate had lower ($p<0.05$)

Table 2: Effects of sodium butyrate on growth performance of weanling pigs[1]

Items	Control	500 mg kg^{-1} SB[2]	1000 mg kg^{-1} SB	SEM[3]
Initial weight (kg)	6.43	6.42	6.43	0.130
Final weight (kg)	12.20^{b}	12.55^{b}	13.85^{a}	0.410
ADG (g)	275.00^{b}	292.00^{b}	353.00^{a}	14.000
ADFI (g)	400.00^{b}	421.00^{b}	476.00^{a}	19.000
Gain:feed	0.69^{b}	0.69^{b}	0.74^{a}	0.011

Means within a row with different letters (a-b) differ significantly ($p<0.05$) when tested with Duncan's new multiple-range test following analysis of variance; [1]Data are means of four replicate pens of eight pigs each; n = 32 for ADG, n = 4 for ADFI and F/G per treatment. The trial lasted 21 days; [2]SB: Sodium Butyrate; [3]Standard error of the mean

Table 3: Effect of dietary Sodium Butyrate (SB) on intestinal microflora of weanling pigs[1, 2]

Site and microflora	Control	500 mg kg^{-1} SB[3]	1000 mg kg^{-1} SB	SEM[4]
Small intestine				
Total aerobes	7.9	7.5	7.3	0.20
Total anaerobes	8.7	8.9	9.0	0.31
Bifidobacterium	7.3	7.3	7.4	0.23
Lactobacillus	8.1	8.2	8.5	0.29
Clostridium	6.2^{a}	5.5^{ab}	5.3^{b}	0.30
Escherichia coli	7.2^{a}	6.5^{ab}	6.2^{b}	0.24
Proximal colon				
Total aerobes	9.4	9.2	9.2	0.29
Total anaerobes	10.4	10.4	10.3	0.20
Bifidobacterium	7.8	8.1	8.0	0.21
Lactobacillus	8.8	9.1	9.3	0.28
Clostridium	7.5^{a}	7.1^{a}	6.4^{b}	0.25
Escherichia coli	8.3^{a}	8.2^{a}	7.3^{b}	0.18

Means within a row with different letters (a, b) differ significantly ($p<0.05$); [1]Bacterial numbers are expressed as log10 colony-forming units per gram of DM; [2]Data are the means of four replicates of two pigs per replicate; [3]SB: Sodium Butyrate; [4]Standard error of the mean

Table 4: Effects of Sodium Butyrate (SB) on the morphology of the intestinal mucosa in different sites of the small intestine of weanling pigs[1]

Items	Control	500 mg kg^{-1} SB[2]	1000 mg kg^{-1} SB	SEM[3]
Villus height (μm)				
Duodenum	510^{b}	531^{ab}	588^{a}	21.0
Jejunum	711^{c}	802^{b}	883^{a}	20.0
Ileum	493^{b}	507^{ab}	567^{a}	20.0
Crypt depth (μm)				
Duodenum	345	330	326	17.0
Jejunum	421^{a}	382^{ab}	354^{b}	18.0
Ileum	319	312	311	14.0
Villus height:Crypt depth				
Duodenum	1.48^{b}	1.61^{ab}	1.80^{a}	0.05
Jejunum	1.69^{c}	2.10^{b}	2.49^{a}	0.09
Ileum	1.55^{b}	1.63^{b}	1.82^{a}	0.06

Means within a row with different letters (a-c) differ significantly ($p<0.05$); [1]Data are the means of four replicates of two pigs per replicate; [2]SB: Sodium Butyrate; [3]Standard error of the mean

viable counts of Clostridium and *E. coli* in colonic contents than those fed with 500 mg kg^{-1} sodium butyrate. The total aerobes, total anaerobes, Bifidobacterium and Lactobacillus in the small intestinal and colonic contents of pigs were not affected by the dietary treatments. Morphological measurement of the small intestinal mucosa of weanling pigs is shown in Table 4.

Table 5: Effects of Sodium Butyrate (SB) on the level of TNF-α and IL-6 in serum, the DNA- binding activity of NF-κB in the ileum mucosa of weanling pigs[1]

Items	Control	500 mg kg^{-1} SB[2]	1000 mg kg^{-1} SB	SEM[3]
TNF-α (pg mL^{-1})	789.00^{a}	433.00^{b}	181.00^{c}	35.00
IL-6 (pg mL^{-1})	390.00^{a}	253.00^{b}	101.00^{c}	42.00
NF-κB (RDU)	1.61^{a}	1.28^{a}	0.43^{b}	0.32

Means within a row with different letters (a-c) differ significantly ($p<0.05$); [1]Data are the means of four replicates of two pigs per replicate; [2]SB: Sodium Butyrate; [3]Standard error of the mean; [4]RDU: Relative Density Unit

Supplementation with 1000 mg kg^{-1} sodium butyrate had higher ($p<0.05$) villus height and the villus height to crypt depth ratio at the small intestinal mucosa as compared with the control and increased ($p<0.05$) villus height at the jejunum and the villus height to crypt depth ratio at the jejunum and ileum as compared with 500 mg kg^{-1} sodium butyrate. Supplementation with 500 mg kg^{-1} sodium butyrate increased ($p<0.05$) villus height and the villus height to crypt depth ratio at the jejunum as compared with the control. The results were consistent with previous study reported by Galfi and Bokori (1990) and Wang *et al.* (2005).

Level of TNF-α and IL-6 in the serum of weanling pigs is shown in Table 5. Supplementation with 1000 mg kg^{-1} sodium butyrate reduced ($p<0.01$) the level of TNF-α and IL-6 in serum of weanling pigs as compared with the control or 500 mg kg^{-1} sodium butyrate group. Supplementation with 500 mg kg^{-1} sodium butyrate reduced ($p<0.05$) the level of TNF-α and IL-6 in the serum of weanling pigs as compared with the control.

The specificity of the NF-κB binding was confirmed by a competitive experiment using unlabelled oligonucleotide. The radio-labelled NF-κB-DNA-complex formation was almost completely blocked by the addition of unlabelled oligonucleotide. Supplementation with 1000 mg kg^{-1} sodium butyrate decreased ($p<0.05$) the DNA-binding activity of ileum NF-κB in weanling pigs as compared with control or 500 mg kg^{-1} sodium butyrate. Supplementation with 500 mg kg^{-1} sodium butyrate had no significant effects ($p>0.05$) on the DNA-binding activity of ileum NF-κB as compared with control.

DISCUSSION

The current results proved the beneficial effects of sodium butyrate supplementation in diets on growth performance and gastrointestinal tract health in weanling pigs which was consistent with the previous reports of Galfi and Bokori (1990) and Piva and Morlacchini (2002). The observation of decreased level of TNF-α and IL-6 in the serum, low activity of intestinal NF-κB in pigs supplemented with 1000 mg kg^{-1} sodium butyrate, indicated that sodium butyrate plays an important role in keeping the completeness of structure of intestinal membrane in addition to serve as a major energy substrate

in weanling pigs. Experiments have demonstrated that butyrate plays an important role in the homeostasis of the intestinal mucosa because it stimulates cell proliferation (Scheppach, 1994), sodium absorption (Kripke *et al*., 1989; Friedel and Levine, 1992) and an increase in mucosal blood flow ((Binder and Mehta, 1989). It showed that butyrate appears to regulate immune function, contributing to the maintenance of homeostasis of the intestine mucosa (D'Argenio and Mazzacca, 1999) which include inhibition of intestinal NF-κB activation (Inan *et al*., 2000).

It was found that the diet containing 0.17% sodium butyrate markedly reduced the percentile proportion of Coliform bacteria and increased the counts of Lactobacillus in the ileum and caecum (Galfi and Bokori, 1990). The effect of sodium butyrate might be mainly due to its extensive biological action which resist invasion of opportunistic bacteria including direct inhibition of bacterial growth and/or interference with adhesion to host tissues (Mathew *et al*., 1996). However, determination of Coliform profiles would likely require further studies that address serotypic identification of adherent *E. coli* on the mucosal lining. Because some Enterotoxigenic *E. coli* (ETEC) serogroups characteristically adhere to the gut mucosa as a prerequisite to virulence (Gaastra and de Graaf, 1982), their numbers might not be detected by the assays used in the current study.

The structure of the intestinal mucosa reveals some information on gut health. In the present study, an increase in villus height and villus height to crypt depth ratio at the small intestinal mucosa of the piglets supplemented with 1000 mg kg^{-1} sodium butyrate was observed. Such improved intestinal mucosal morphology could be explained by increased ADFI, low level of serum cytokines (TNF-α, IL-6) and inhibition of the intestinal NF-κB activity in weanling pigs.

As one of the most potent stimuli of intestinal proliferation, the oral intake of food and its physical presence in the gastrointestinal tract are necessary for structural and functional maintenance of the intestinal mucosa (Kelly *et al*., 1991). The absence of nutrients from the gut lumen occurs after weaning will have marked effects on the rate of cell differentiation and cell turnover. The exclusion of nutrients from the lumen of the small intestine either by starvation, dietary restriction or intravenous feeding, results in villous atrophy and a decrease in crypt-cell production rate. Since, these changes have been reported in the gut of the newly-weaned pig, it is likely that daily feed intake plays a strong role in the integrity of the structure and function of the small intestine after weaning.

Some evidences have demonstrated that serum cytokines are important regulators of intestinal immunity (Kramer *et al*., 1995) and major regulators of epithelial cell growth and development including intestinal inflammation and epithelial restitution following mucosal damage (Ferreira *et al*.,1990; Lionetti *et al*., 1993). The role of such cytokine in either epithelial integrity or mucosal immune function is not yet fully understood.

In the study, decreased TNF-α and IL-6 levels in the serum were observed in pigs fed with sodium butyrate. Because the expression and sythesis of TNF-α and IL-6 was up-regulated by the DNA-bounding activity of the intestinal NF-κB, the low level of serum TNF-α and IL-6 in pigs supplemented with sodium butyrate might be the result from decreased activity of the intestinal NF-κB. But the mechanism of interaction between singnificant decrease of TNF-α and IL-6 level and no significant decrease of the intestinal NF-κB activity in pigs supplemented with 500 mg kg^{-1} sodium butyrate was not clear.

Nuclear factor-κB was first identified as a protein bound to a sequence in the immunoglobulin κ light chain enhancer in B cells stimulated with lipopolysaccharide (Sen and Baltimore, 1986). NF-κB exits in the cytoplasm in an inactive state, linked to a potent inhibitor IκB (Inhibitor κB) and sequestered in the NF-κB/IκB complex. Stimulatory signals including bacterial products, viral proteins, reactive oxygen species, radiation, ischemia/ reperfusion and oxidative stress, induce the phosphorylation of IκB by an ubiquitindependent protein kinase and proteolytic degradation. Then, NF-κB dissociates from its inhibitor and rapidly translocates to the nucleus. There, it binds to a 10 bp sequence (κB sequence) and regulates the gene transcription of diverse inflammatory and immune response mediators including cytokines (TNF-α, IL-6), chemokines and cell adhesion molecules (Baeuerle and Henkel, 1994). The abnormal, constitutive activation of the intestinal NF-κB has been associated with Inflammatory Bowel Disease (IBD) and Ulcerative Colitis (UC) (Barnes and Karin, 1997; Jobin and Sartor, 2000).

The study of Crohn's disease showed that butyrate inhibits inflammatory responses through NF-κB inhibition (Segain *et al*., 2000). In the present study, it was found that high DNA-bounding activity of NF-κB in the small intestine of weanling pigs was associated with decreased villus height at the jejunum and the villus height to crypt depth ratio at the jejunum and ileum. Several *in vivo* observations suggest an inverse correlation between mucosal NF-κB activity and the integrity of the intestinal epithelium (Thiele *et al*., 1999). But the mechanisms were not understood.

Experiment has demonstrated that colonocytes NF-κB activation could be inhibited by sodium butyrate (Inan *et al*., 2000). In this study, several mechanisms were

proposed to explain sodium butyrate-induced inhibition of the intestinal NF-κB as follows. First, the inhibition of NF-κB activity might be resulted from the changes of intestinal microflora populations with the supplementation of sodium butyrate because the mucosal NF-κB could be activated by pathogenic stimuli (bacterial products). The importance of fermentation acids in resisting enteric pathogens has been suggested (Blomberg *et al*., 1993). The decrease in ileum SCFAs after weaning coincided with an increase in total *E. coli*, a greater proportion of K_{88} pathogenic *E. coli* and a decrease in total lactobacilli in the ileal contents (Mathew *et al*., 1993). The supplementation of sodium butyrate in diets may increase resistance to opportunistic organism including pathogenic *E.coli* in the intestinal tract. Galfi and Bokori (1990) found that the diet containing sodium butyrate markedly reduced the percentile proportion of coliform bacteria and increased the counts of Lactobacillus in the ileum and caecum. Second, sodium butyrate may induce synthesis of the NF-κB inhibitor IκB or inhibit the degradation of IκB (Jobin and Sartor, 2000) by suppressing signalinducible IκB proteolysis through inhibiting IκB Kinase which traps activated NF-κB, thereby preventing its translocation into the nucleus (Yin, 1998). Third, sodium butyrate may directly bind to the NF-κB subunit and interfere with NF-κB DNA-binding activity (Yang *et al*., 1995). Finally, sodium butyrate may inhibit NF-κB-dependent transcription by scavenging Reactive Oxygen Species (ROS) which are required for NF-κB activation (Epinat and Gilmore, 1999). This effect was discussed on numerous natural and synthetic antioxidants such as vitamin E and C (Yin, 1998). However, all these proposals remain to be further investigated.

CONCLUSION

As an endogenesis produced by microbial fermentation in the intestine not alien to the body, sodium butyrate plays an important role on improving the growth performance and keeping the completeness of structure of intestinal membrane in weanling pigs. This effect of sodium butyrate in weanling pigs might be through the pathway of decreasing the TNF-α, IL-6 level in serum and inhibition of DNA-binding activity of the intestinal NF-κB.

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