

Sodium butyrate in poultry - the importance of a proper protection

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There is more and more evidence that butyric acid possesses interesting characteristics that make it "not just an acid". In addition the well documented anti bacterial effect, sodium butyrate is known to stimulate the production of pancreatic secretions, including enzymes. It will also stimulate hormones, such as insulin, which in turn stimulates epithelium development. Sodium butyrate also improves the absorption of electrolytes and reduces the incidence of diarrhoea, providing the ileal and the hindgut mucosa with a preferred energy source. Recently, more and more data suggests positive impacts on the immune defence of the animal. However, not all butyrates are equal. Using a specific production process to protect sodium butyrate, it is possible to ensure a gradual release along the digestive tract. This will maximize the efficacy of this important additive.

The use of acids in poultry nutrition is quite well accepted. Besides the reduction of pH that limits the development of pathogens and helps in the digestion of proteins at crop level, some acids also have the ability to enter the gram-negative bacteria and disrupt their metabolism.

One of the organic acids, butyric acid, is a carboxylic acid with the chemical formula $\text{CH}_3\text{CH}_2\text{CH}_2\text{-COOH}$. Butyric acid is a natural product of the bacterial fermentation of the carbohydrates in the intestine of monogastrics, or in the rumen of ruminants. With acetic and propionic acids, butyric acid belongs to the group of volatile fatty acids (VFAs). Butyric acid is well known due to its antimicrobial activity, however, it is also known for its important function in intestinal epithelium development. This function is attributed to 3 facts: being high in caloric content, being preferably metabolized by the intestinal epithelium and being preferentially oxidized by the colonocytes. On top of that, butyric acid has been reported to have anti-inflammatory properties and help in immune defense.

However, butyric acid is volatile and corrosive, so when thinking in a practical way, we must use a salt, sodium butyrate, which is appropriate for use in pelleted feed, as it is stable during processing. Once sodium butyrate reaches the stomach of the bird (proventriculus and gizzard), it will quickly release the sodium and, due to the low pH, butyrate will be rapidly converted to the undissociated form, also termed butyric acid. This form is the one responsible for the antimicrobial activity, as butyric is strongly lipophilic and can diffuse across the membranes of bacteria (especially gram negative). As we go further down the digestive tract, pH increases and the proportion of butyric acid decreases while the proportion of butyrate increases. This is where we observe the trophic effect of butyrate

(and butyric) on the intestinal epithelium, because these 2 forms can be absorbed by the enterocytes thanks to different transporters.

Trophic effect of sodium butyrate

Sodium butyrate is a preferred source of energy for the enterocytes. This will result in better development of the intestinal villi, and also in stronger gut lining, as it was demonstrated in many species such as poultry, swine, but also calves or aquatic species. For instance, in swine, Ghol J. (2007) concluded that sodium butyrate reduces some of the negative effects of weaning by providing the ileal and the hindgut mucosa with a preferred energy source. Different publications by Galfi and Bokori show an increase in epithelial regeneration of the intestinal microvilli, together with an enlargement of the microvilli producing an increase of the intestinal absorption area when using sodium butyrate in the feed of piglets. In poultry, Leeson et al (2005) found numerically longer villus in the duodenum of birds receiving butyric acid in the diets than control birds.

Recently, a research project was conducted by Norel S.A. (Mallo et al., 48th AECA Scientific Poultry Symposium, 2011, pp 343-349) under the supervision of Dr. S.V. Rama Rao from the Project Directorate on Poultry, Rajendranagar, Hyderabad, India. In this experiment, a total of 750 Cobb 400 one day old broiler chickens were used with the main objective to evaluate the influence of sodium butyrate has on digestibility of a corn-soya diet, as well as villi development. The trial evaluated 3 treatments: control (no additives), 0.5 kg/t of Gustor B-92 (92% sodium butyrate) and 1 kg/t of Gustor B-92. The digestible energy of the diet and the digestibility of the protein were significantly improved by the addition of sodium butyrate ($P < 0.001$). The lengths and widths of the villis were also positively affected by the addition of butyrate in the diet (see table 1). From this trial and previous research, it was concluded that inclusion of butyrate in the diet improves the digestibility of energy and protein by increasing intestinal absorption surface.

Antibacterial effect of sodium butyrate

It has been demonstrated that VFAs can inhibit the growth of bacteria of the group of Enterobacteriaceae (*Salmonella*, *Escherichia coli* ...). This is because the undissociated form of these acids can freely diffuse across the bacterial membrane. Once inside the cytoplasm of the bacteria, the acid dissociates, thus releasing free hydrogen ion and reducing the pH, which causes internal cell damage. In this respect, butyric acid performs very well. Hume et al. (1993) showed that butyric acid has a higher diffusion coefficient than other acids with a shorter chain, which allows it to pass through the bacterial membrane more easily. In another experiment, Galfi and Neogradi (1995) found that the concentration of butyrate

required to reduce the growth of *E. coli* by 50% is much lower than the concentration of the other volatile fatty acids, acetate and propionate.

In poultry, Van Immerseel (2004) observed that butyric acid was the most effective acid to help controlling *Salmonella enteritidis* in broilers after a challenge (see Figure 1). It should be also noted that the antibacterial effect of butyrate is selective. For instance, in 1990, Galfi and Bokori demonstrated that butyric acid favours the development of lactic flora which competes against the enteropathogenic one (*Clostridia*, *E. coli*, *Coccidia*...). Leeson et al. (2005) also observed that birds receiving butyric acid before coccidial oocyte challenge showed higher weight gain following the challenge compared with birds that received a control feed.

Besides the direct antibacterial effect, one very unique feature of butyric acid is its ability to negatively affect the virulence of pathogenic bacteria such as *Salmonella*. To infect an animal host, *Salmonella* must penetrate the intestinal epithelial barrier. This very important step is called invasive phase and requires specific genes inside the bacterium. The invasion genes are located on the so-called "pathogenicity island" and are encoding some regulatory proteins, some structural components of a needle complex, plus some effector proteins, which make possible the entry of *Salmonella* into the epithelial cells. The pathogenicity island itself is activated by another protein called HilA, which can be environmentally regulated.

Different publications have shown that butyrate and propionate can reduce the pathogenicity of *Salmonella Typhimurium* or *Salmonella Enteritidis*, whereas other acids such as acetic or formic are actually promoting the invasion of epithelium cells by the bacteria (Durant et al., 2000 - Van Immerseel et al., 2003 - Lawhon et al., 2005 - Gantois et al., 2006 - Huang et al., 2008). The exact mode of action of butyrate in the bacterial cell is still unknown but it seems it can interfere with the genetic expression and results in a down regulation of the pathogenicity island, regulating the invasive phenotypes of *Salmonella*.

Sodium butyrate and immune defense

Research in various animal species indicates that adding sodium butyrate to the diet results in better resistance to a challenge, which can be explained by two mechanisms: anti-inflammatory effect, and reinforcement of the intestinal defence barrier. More specifically in poultry, recent studies reveal some additional features. For instance, in a recent work by Sunkara et al. (2011), the hypothesis that sodium butyrate is capable of inducing Host defense peptides (HDPs) and enhancing disease resistance in chickens was tested. Host

defense peptides are natural broad spectrum antimicrobials and an important first line of defense in almost all forms of life.

The authors reported that "butyrate is a potent inducer of several, but not all, chicken HDPs in HD11 macrophages as well as in primary monocytes, bone marrow cells, and jejuna and cecal explants". They also observed that butyrate treatment enhanced the antibacterial activity of chicken monocytes against *Salmonella enteritidis*, and that feed supplementation with 0.1% butyrate led to a significant increase in HDP gene expression in the intestinal tract of chickens, resulting in a nearly 10-fold reduction in the bacterial titer in the cecum following experimental infections with the pathogenic *Salmonella*. The research team concluded that dietary supplementation of butyrate has potential for further development as a convenient antibiotic-alternative strategy to enhance host innate immunity and disease resistance.

Other benefits of sodium butyrate

Although other organic acids also produced this effect, butyrate seems to be the one that causes the greatest stimulation of pancreatic secretions. For instance, Katoh and Tsudo (1984) observed that the injection of sodium butyrate in pigs resulted in greater stimulation of pancreatic fluid secretion and increased amylase secretion. Also, Katoh et al. (1989) demonstrated that intravenous injection of sodium butyrate in calves resulted in a higher secretion of pancreatic juice and greater release of proteins (particularly amylase) when compared to acetate and propionate. Sano et al. (1995) also observed that the administration of butyrate caused an increase in the plasmatic levels of insulin, which normally results in higher amount of energy and proteins deposited in the animal tissues. Recently, Kato et al.(2011) observed that calves fed with milk supplemented with sodium butyrate (Gustor, Norel) had higher levels of Insulin-like growth factor 1 (IGF-1), a polypeptide protein hormone playing an important role in growth.

Sodium butyrate also improves the absorption of electrolytes and reduces the incidence of diarrhoea (Galfi, 1989). Apparently, the mechanism by which butyrate affects the absorption of liquids and electrolytes in the colon consists of an energy-generating fuel effect, a favourable regulation of certain electrolyte carrier systems and probably some effects on neuroendocrine factors (Velázquez et al., 1997).

The importance of a proper protection

For several years already NOREL Animal Nutrition has been investigating the benefits of using butyric acid in different species (swine, poultry, ruminants but also aquaculture) as well as the production and handling aspects. With GUSTOR BP-70 (containing 70% of partially protected sodium butyrate), the third generation of butyrates is now available, ensuring an optimum efficacy.

The first generation of products have for main advantage to contain a high level of sodium butyrate (90 to 95%) but their main drawback is a strong and persistent smell. They can also cake in presence of moisture, and, more importantly, once ingested, the sodium butyrate is quickly absorbed by the enterocytes, so the activity is limited to the upper part of the digestive tract. In the second generation of butyrates, so-called coated butyrates, palm stearin is used to trap the active ingredient (sodium butyrate) into concentric layers of fat. Even if the issues of smell and caking are solved, using the coating technology has 2 consequences. First, the percentage of active ingredients in the product is quite low.

Usually, the products available in the market are containing 30% of sodium butyrate and 70% fat, requiring a higher dosage in feed to reach a sufficient level of sodium butyrate. Also, they require an effective lipase activity to release the sodium butyrate. This is especially an issue in young birds: when the fat layers are not degraded, it is possible to find the granules quite intact in the feces. We can therefore question the efficacy of such products.

Aware of the limitations of the first and second generations of butyrates, the R&D department of NOREL Animal Nutrition has continued its investigations with the aim of producing a more concentrated, though still protected, product to add to its range of slow-release, natural growth promoters. This has led to the development of GUSTOR BP-70, using a specific fat coating to protect the active ingredients. This allows the sodium butyrate to reach the distal sections of the GI tract, not only acting as a natural growth promoter but also reducing the levels of pathogenic bacteria, especially *Salmonella*.

This was demonstrated during a trial conducted at the University of Leon, Spain and published in Poultry Science by Fernández-Rubio et al. (2009). The objective of the experiment was to evaluate the effect of GUSTOR BP-70 (in comparison with a first generation product) as a preventing tool against *Salmonella enteritidis* infection at intestinal and systemic phases.

In this experiment, 3 groups of 50 broilers each were given either a standard diet (control), the same feed containing 1.0 kg per ton of an additive containing 92% of free sodium butyrate (Gustor B-92) or the standard broiler diet supplemented with 1.3 kg per ton of an

additive containing 70% of partially protected sodium butyrate (Gustor BP-70). The test lasted for 42 days. At day 1, all birds were confirmed being negative for *Salmonella enteritidis*. A few days later (day 5), 20% of birds from each group were orally infected with *Salmonella enteritidis*. Cloacal samples were taken on days 6, 9, 13, 27, and 41 to monitor infection levels. At day 42 all birds were slaughtered and crop, caecum, liver and spleen were sampled for bacteriological analyses.

Both butyrate-based additives showed a significant reduction ($P < 0.05$) of *Salmonella enteritidis* infection in birds from day 27 onward. However, the partially protected butyrate additive (GUSTOR BP-70) was more effective at the late phase of infection (see Figure 2). Partially protected butyrate treatment also successfully decreased infection not only in the crop and cecum, but also in the liver.

In another project presented during the XIIIth European Poultry Conference (Mallo et al., 2010), a feeding trial was carried out to evaluate the efficacy of two different presentations of sodium butyrate, GUSTOR BP-70 and GUSTOR B-COATED (30% sodium butyrate) in broilers. A total of 720 one-day-old (half male and half female) Ross 308 chicks were used and allocated at random to the 3 experimental treatments. All treatments consisted on a starter feed (from 0 to 21 days of age) and a finisher feed (from 22 to 42 days of age). Both diets were based on wheat, barley, maize and soybean meal. T2 and T3 were formulated to include the same amount of sodium butyrate in the feeds (see Table 2).

It was observed that, at the end of the trial, broilers receiving GUSTOR BP-70 had lower mortality rates than Controls (4.2 vs. 1.7 %; $P = 0.1005$; a near significant trend) with an intermediate result for GUSTOR B-COATED (2.5%). At 42 days of age, GUSTOR BP-70 supplemented birds grew more than Control chickens (2,241 grams vs. 2,301 ; $P = 0.1077$; again a near significant trend) while there was no observed effect on growth for GUSTOR B-COATED (final body weight = 2,257 grams). During this trial, histology of the gut was also evaluated. The most important information is that villus height was significantly higher with GUSTOR BP-70 than with the control or GUSTOR B-COATED (see Figure 3).

These results confirm that sodium butyrate partially protected with vegetable fats (GUSTOR BP-70) offers a unique balance of free and protected active substances, effective all along the gastrointestinal tract because it is slowly released during digestion. This is made possible thanks to the unique and specific production process developed by NOREL Animal Nutrition. Furthermore, this third generation sodium butyrate allows having a higher level of active substances (70% of sodium butyrate) when compared to the coated products, requiring a lower dose per kg of feed to achieve the same level of butyrate. GUSTOR BP-70 has a positive effect on the development of the intestinal villi and helps in controlling the gut pathogens such as *Salmonella*.

Figure 1: Number of colony forming units (CFU) per gram of ceca at day 8 after hatch of chickens orally inoculated with 1,000 CFU *Salmonella enteritidis* at days 5 and 6 and given feed supplemented with different organic acids (Adapted from Van Immerseel, 2004)

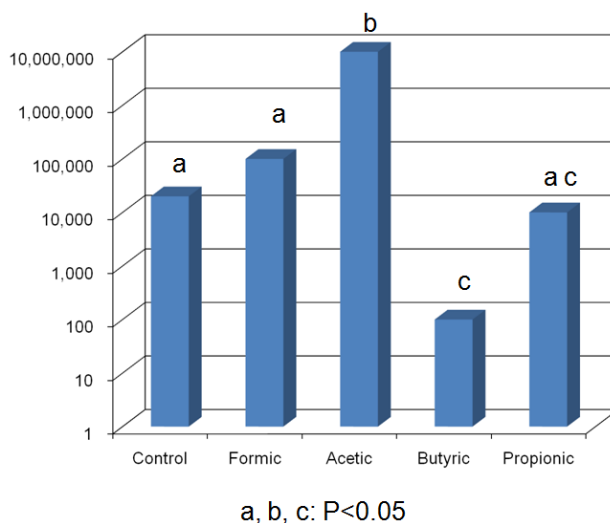
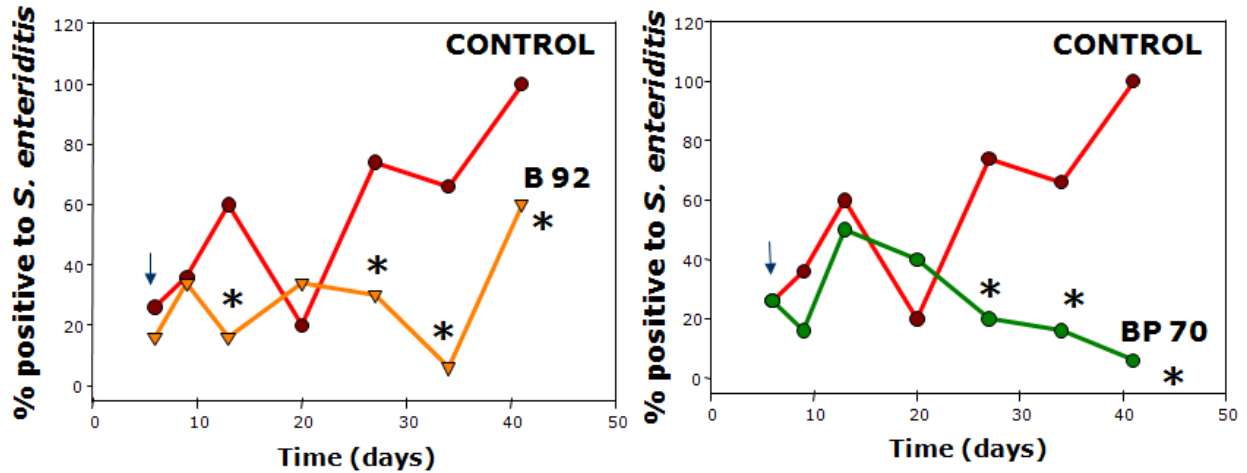


Table 1: Digestibility of energy and protein in commercial broilers fed different concentrations of sodium butyrate; Development of intestinal villi on 21 and 42 days of age

Treatment	Energy	Protein	Villi # 21d		Villi # 42d	
	kcal/kg	%	Length, mm	Width, μ m	Length, mm	Width, μ m
Control	3,105 ^B	64.69 ^C	0.977 ^C	224.7	0.944	138.5 ^B
Gustor 0.5 g/kg	3,264 ^A	65.81 ^B	1.516 ^A	260.8	1.314	314.2 ^A
Gustor 1.0 g/kg	3,285 ^A	67.70 ^A	1.243 ^B	197.7	1.116	317.2 ^A
P	0.001	0.001	0.001	0.137	0.068	0.001

Measure protocol scanning electron microscope (SEM)

Figure 2: Fecal shedding of Salmonella Enteritidis-infected broilers fed with the partially protected Gustor BP-70 and the unprotected Gustor B-92 butyrate-based additives



(*) Significant differences with control group

Table 2: Experimental treatments in the trial presented at the XIIIth European Poultry Conference (Mallo et al., 2010)

Treatment	Starter, 0-21 days	Finisher, 22-42 days
T1	Basal diet	Basal diet
T2	T1 + 1 kg/t GUSTOR BP-70	T1 + 0.5 kg/t GUSTOR BP-70
T3	T1 + 2.34 kg/t GUSTOR B-COATED	T1 + 1.17 kg/t GUSTOR B-COATED

Figure 3: Effect of the type of protection (partially protected sodium butyrate vs. coated butyrate) on the development of villi in the jejunum of broiler chickens

