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Technical Bulletin

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Butyric acid forms and their Enteric release

Introduction

A healthy gut is essential for efficient conversion of feed into constituents for optimal nutrient absorption. Gut health is a complex process combining nutrition, microbiology, immunology and physiology. When gut health is compromised, digestion and nutrient absorption are affected, which in turn has a detrimental effect on feed conversion and production. This leads to economic losses and a greater susceptibility to disease.

A characteristic feature of a healthy gut is a large surface area covered with long healthy villi having deep crypts. Deep crypts are indicative of rapid tissue turn over in order to permit regeneration of damaged villi. Longer villi and deeper crypts provide a larger surface area for the absorption of nutrients, allowing efficient enzyme production and maturation of intestinal cells.

In commercial poultry production, it is said that profit comes from a healthy chicken. Healthy chicken comes from healthy gut. Healthy intestinal villi leads to better absorption of nutrients. Even if a high quality feed is given to birds with damaged or underdeveloped villi, desired performance cannot be achieved which is important for profitability.

Under modern systems of production, poultry birds are inevitably exposed to considerable stress during their productive lifetime. Post hatching is a period of considerable stress. The gastrointestinal tracts of newly hatched chicks are immature and sterile. The GIT of the bird begin to develop and function when the chicks start to ingest feed. At this stage, chicks are very susceptible to pathogenic microorganisms. Under such circumstances, anti-microbial feed additives such as antibiotics are often used to suppress or eliminate harmful organisms in the intestine and to improve growth and feed efficiency.

However, the use of antibiotics as feed additives has been banned in the recent years in most countries due to public health concerns over possible antibiotic residual effects and the development of drug resistant bacteria. Several other countries are working to reduce or ban the usage of antibiotics as growth promoters.

As a consequence, the development of alternatives to antibiotics received considerable attention. Ideally, alternatives to antibiotics should have the same advantageous properties. Isolated nutrients (amino acids, fatty acids, minerals, and vitamins), dietary supplements (probiotics, prebiotics, symbiotic, organic acids, antioxidants, and enzymes), plant derivatives (polyphenols, herbs, and spices) and genetically modified foods have been extensively studied in search for alternatives.

Organic acids are appropriate for in-feed use to maintain gut health. These are natural products of the microbial metabolism or fermentation of the carbohydrates in the intestines of animals. Commonly known organic acids are acetic acid, propionic acid, butyric acid and lactic acid. These are also known as volatile fatty acids (VFAs) or short chain fatty acids (SCFAs).

Among these, butyric acid is an organic acid with four carbons known for its ability to improve intestinal health. It acts as a preferred biofuel for epithelial cells lining the intestinal tract, which leads to increase in the density and the length of villi thereby the enlargement of the absorptive surface area of the intestine. Butyric acid reduces production of pro-inflammatory cytokines, stimulates enzyme secretion and helps in stabilising microflora in the gut, resulting in more efficient nutrient digestibility and better colonisation resistance.

Benefits of Butyric acid

Butyrate is a preferred energy source for the epithelial cells of the intestine. Different studies have proven that Butyric acid improves performance, increases length of villi of intestinal mucosa and digestive and absorptive capacity of the intestine.

The crypt contains Ki67 cells, which represent the proliferative stem cells of the intestinal epithelium. The inclusion of Butyrate in animal nutrition increases the number of Ki67 cells and has a beneficial effect on the villi absorptive surface and in the rate of villi per plica.

Immunity

During the process of pathogenesis, there is an interaction between bacteria and host cells where Butyrate regulates the expression of invasion genes and decreases the virulence of bacteria (Van Immerseel et al.,2004). It facilitates the creation and maintenance of cellular tight junctions or zonula occludens (Peng et al., 2007) which leads to better nutrient absorption and higher resistance.

In addition to cellular barrier function, when Butyrate is present in the blood stream or in the proximal parts of the intestinal tract, it induces the production of host defence peptides (HDPs) (Guilloteau et al., 2009). These peptides stimulate the development and repair of the intestine (Bartholome et al., 2004) and have a role in innate immunity (Sunkara et.,2011), (Brogden et al. 2003).

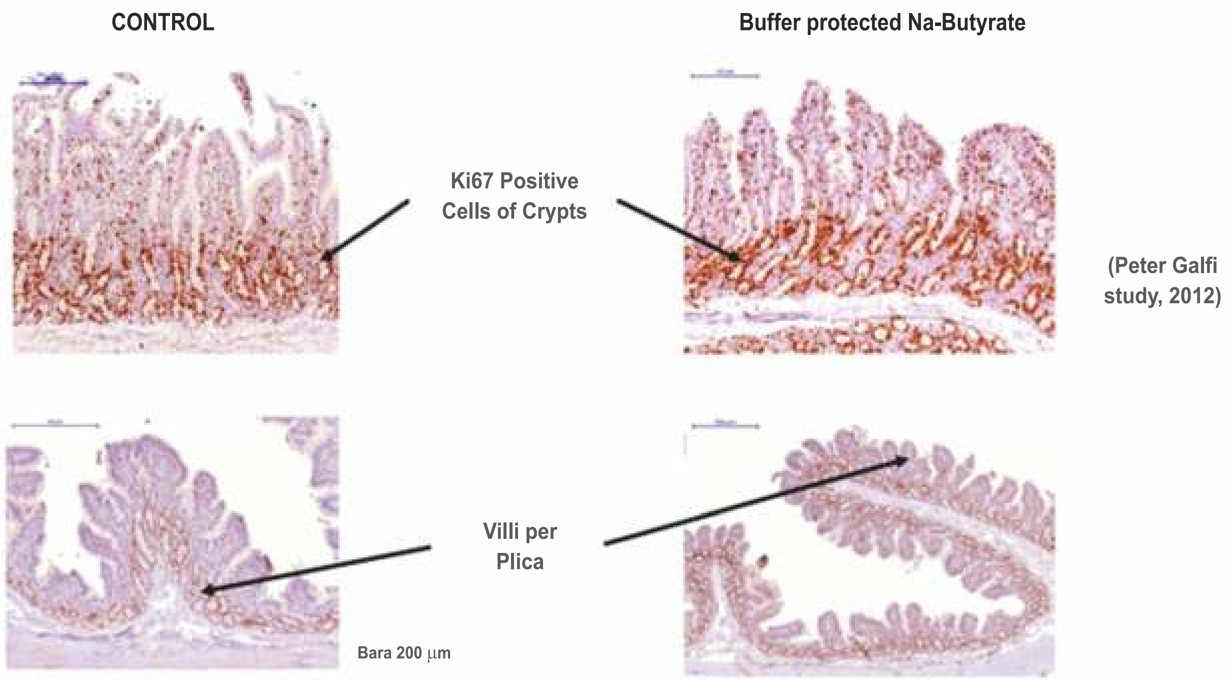
Researchers have also shown Butyrate to enhance disease resistance to Salmonella enteritis and Salmonella typhimurium in chickens by inducing synthesis of various HDPs (Sunkara et., 2011).

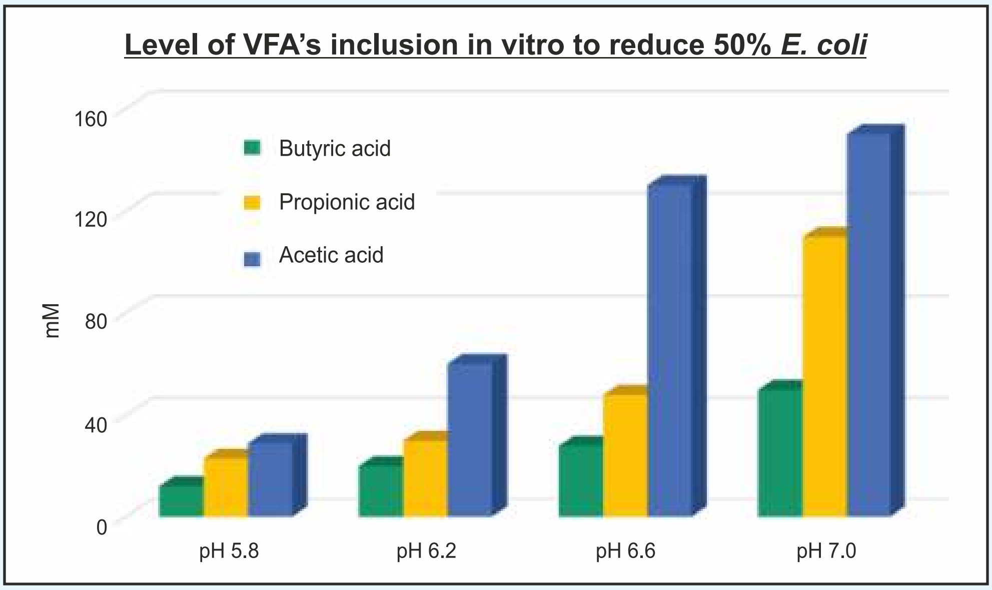
Other research has shown that Butyrate has a multifactorial role in intestinal health and controlling diseases such as necrotic enteritis and coccidiosis.

Antimicrobial effect

Volatile Fatty Acids (VFAs) can inhibit the growth of bacteria under the group of Enterobacteriaceae (Salmonella, Escherichia coli etc.). This is because the un-dissociated form of these acids is strongly lipophilic, and able to diffuse across bacterial cell walls. Once inside, VFA liberate H+ which reduces the

Effects of buffer protected Butyrate on proliferation of intestinal cells





internal pH . The reduction in cytoplasmic pH of the bacterial cell in turn affects the purine bases and results in denaturing essential enzymes inside the cell, ultimately leading to death of bacteria (Salmond et al. 1984).

In an experiment, Galfi and Neogardi (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 such as acetate and propionate.

The antibacterial activity of Butyrate is selective as it kills pathogenic bacteria and promotes the growth of beneficial bacteria. Butyrate lowers the pH of the intestine, which favours the growth of lactic acid bacteria such as Lactobacilli and Bifidobacterium Spp. as they require an acidic medium for their growth.

Additional benefits

Butyrate stimulates the production of VFAs and lactic acid in entire intestinal tract which leads to acidification of digesta in the tract. This acidification increases the calcium absorption from the intestine, in turn inhibiting phytic acid from formation of calcium -phytate complex (Boling et al.,2000; Rafacaz Livingston et al., 2005). Some authors have documented increased serum phosphorus and magnesium levels in broilers after supplementation of Butyrate (Adil et al;2010; Kamal and Ragaa,2014).

Butyrate increases the absorption of water and sodium (Friedel and Levine,1992) reducing the risk of diarrhoea and stained eggs.

The use of Butyrate in animal feed has been generally accepted as a tool to stimulate feed intake. Butyrate stimulates insulin secretion, which in turn stimulates the entry of glucose from blood into the cell. When the blood glucose level decreases, the brain stimulates an increase in feed intake. Butyrate also promotes a more efficient digestive process by enhancing the absorption of feed through a healthy villi. The increased absorption and utilization of feed leads to a lower Feed Conversion Ratio. This has encouraged the use of Butyrates as an alternative to antibiotics.

Katoch and Tusdo (1984, 1985) observed that the injection of Sodium Butyrate in pigs resulted in greater stimulation of pancreatic fluid secretions and increased secretion of digestive enzymes like amylase and lipase.

Also, Katoch et al. (1989) demonstrated that intravenous injection of Sodium Butyrate in calves resulted in higher secretion of pancreatic juice and greater release of amylase than acetate and propionate. A positive effect on rumen papillae in small ruminants was also observed.

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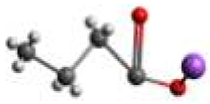
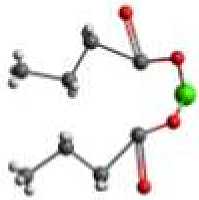
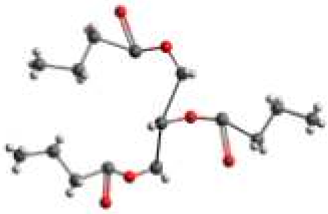
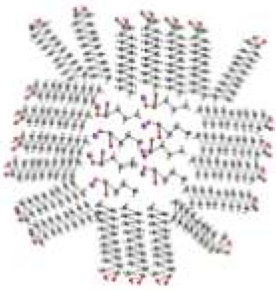
Common commercial sources of Butyric acid

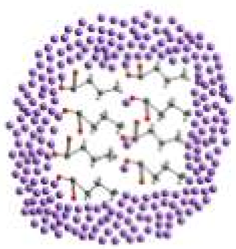

Butyric acid

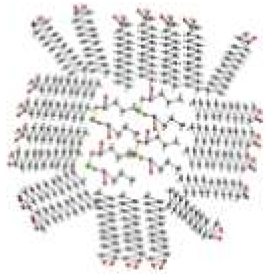

Na-Butyrate


Ca-Butyrate


Glycerides of Butyric acid - Butyrins


Coated Na-Butyrate


Buffer protected Na-Butyrate


Coated Ca-Butyrate

In its pure form, Butyric acid is corrosive and has a rancid smell. Butyric acid is a weak acid with a pKa value of 4.82, which means that in its pure form, it will be rapidly metabolised in crop and upper gastrointestinal tract.

To overcome the corrosiveness and rancid smell, salts of Butyric acid, primarily sodium and calcium butyrate, came into common use. These salts are in solid form and odourless, making them easier to handle. The main advantage of these type of Butyrates is that they contain high levels of Butyric acid. However, the problem with pure Butyrate salts is that they are absorbed in the upper parts of the GIT. This implies that the Butyrate activity is mainly in the upper part of the digestive tract, with a very small impact in the intestine. This can be overcome by coating the Butyrates to ensure its release in the intestinal area.

Technologies to tackle the challenge of enteric butyrate delivery

1. Coated Butyrates
2. Butyrins
3. Buffer Protected Butyrate

Coated Butyrates

Coated products are typically composed of beads containing Butyrate embedded in a protective matrix of vegetable fat. The rationale behind this protection is that a significant part of Butyrate content will be released the moment lipase is secreted in the duodenum, breaking down the lipid matrix.

This implies Lipase requires an activity time to break down the coating. This means that in order for the Butyrate to be absorbed, there needs to be enough Lipase and enough time for the Lipase to break down the coating. If these conditions are not met, the coated Butyrate will pass further down the GIT and be released in the hindgut and eventually in the faeces.

In younger chicks, the inability of the pancreas to produce sufficient quantities of lipase enzyme (Ravindran, 2003) may result in decreased release of fat coated Butyrate leading to lower villi height and crypt depth in the starter phase.

Another common problem with coated Butyrates is that the ability of the fat coating to withstand pelleting temperatures is questionable, as the feed is pelleted at temperatures higher than the melting point of fat.

Butyrins

Monobutyrins, dibutyrins and tributyrins are composed of a glycerol backbone to which one, two or three Butyrate units are bound, respectively.

Glycerides are produced by esterifying molecules of butyric acid to a glycerol. As these are glycerol esters, they need to be digested like oils/fats making lipase essential for their release in the GIT.

As the availability of lipase in chicks is limited, the Butyrate release from Butyrins is incomplete.

Butyrins, most advanced form - Triglyceride, contains relatively high amount of butyric acid which is produced by esterifying three molecules of butyric acid to a glycerol, resulting in 'tributyrin'. Thereby, for its release lipase has to cleave three ester bonds for butyric acid in the intestine but the pancreatic lipase has been described to preferentially cleave the ester bond of first and third butyrate , leaving the second position intact (Brocherhoff,2012).

Butyrins reliance on the presence of Lipase and the lack of data on its heat stability makes it an uncompetitive choice when choosing a Butyrate for poultry.

Buffer Protected Butyrate

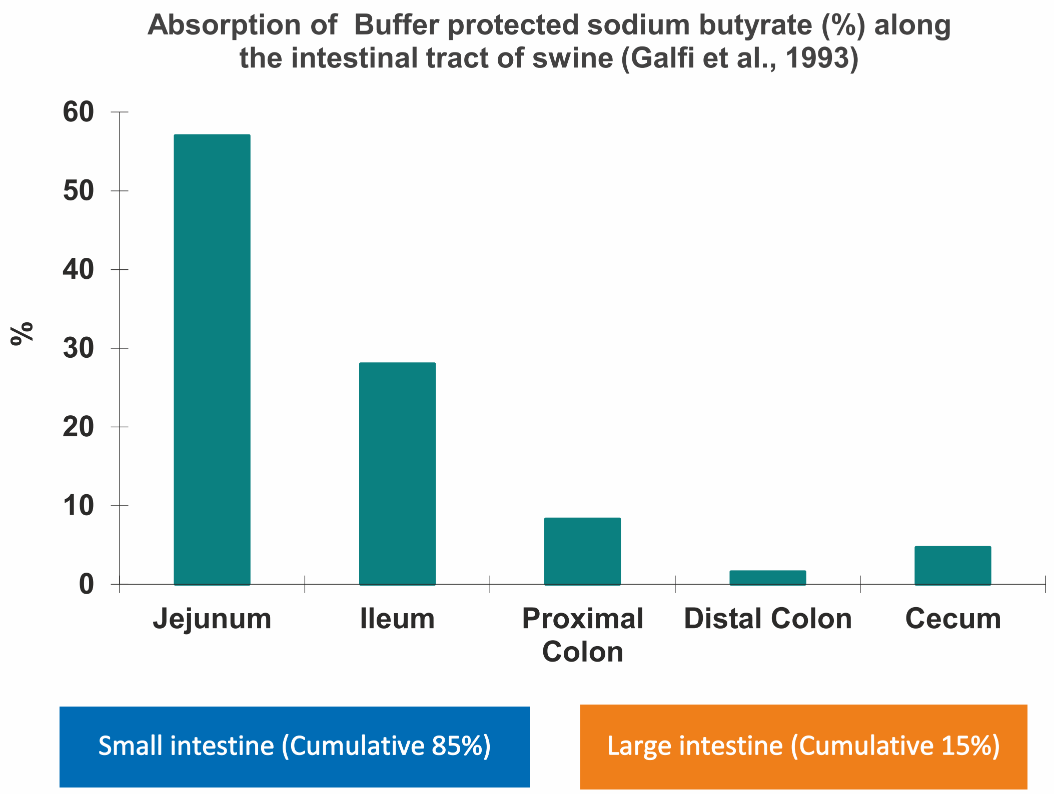
It is the most advanced technology of enteric Butyrate at present.

In this form, the Butyrate salt is protected by a physical chemical structure of buffering salts, where each salt has a different pKa value. These salts prevent it from dissolving in the initial part of the enteric system thereby ensuring that it reaches the intestine.

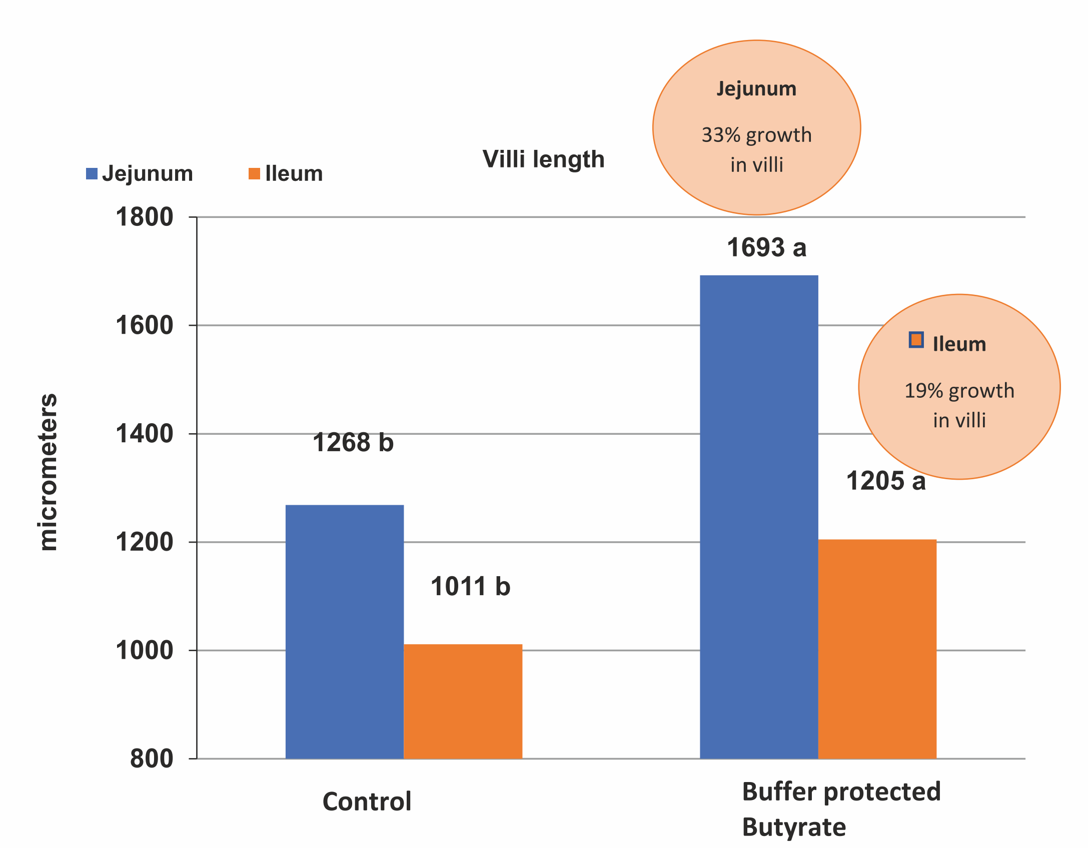
This Buffer matrix protects butyrate from solubilising and dissociating in the initial part of the GIT. Buffer protected forms also contain a higher level of Butyrate salt.

Buffer protected Butyrates have been proven stable at pelleting temperatures.

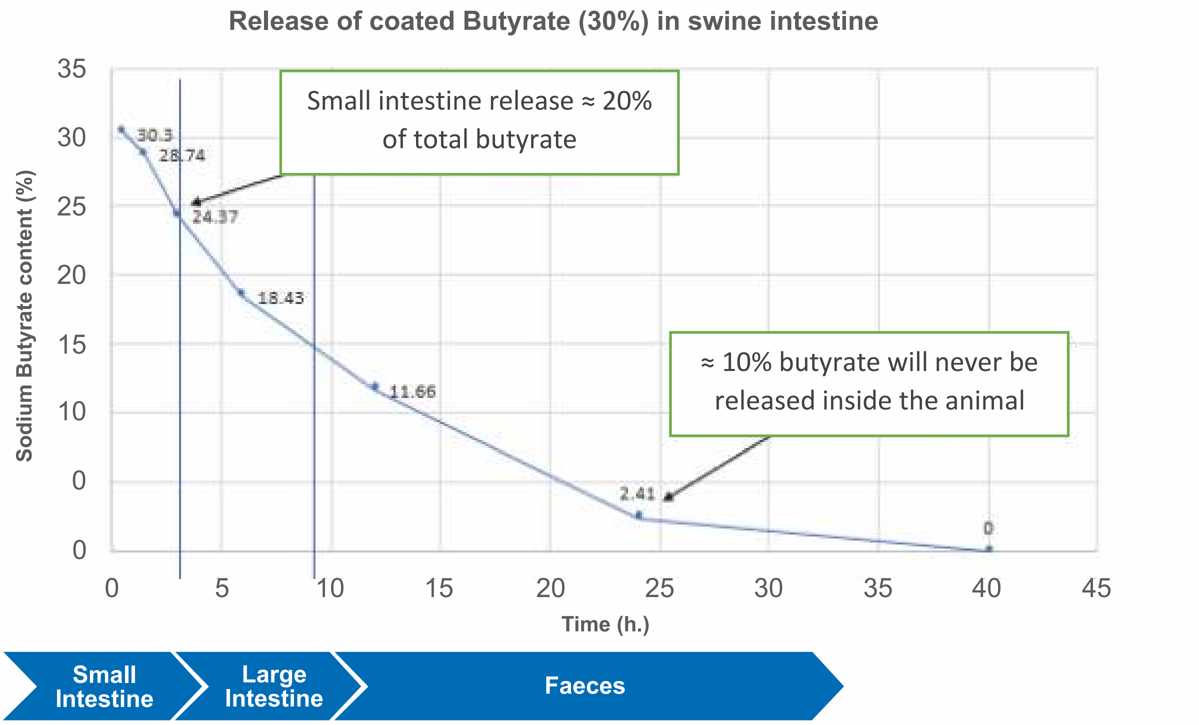
Utilization of Buffer protected Sodium Butyrate along with intestinal tract



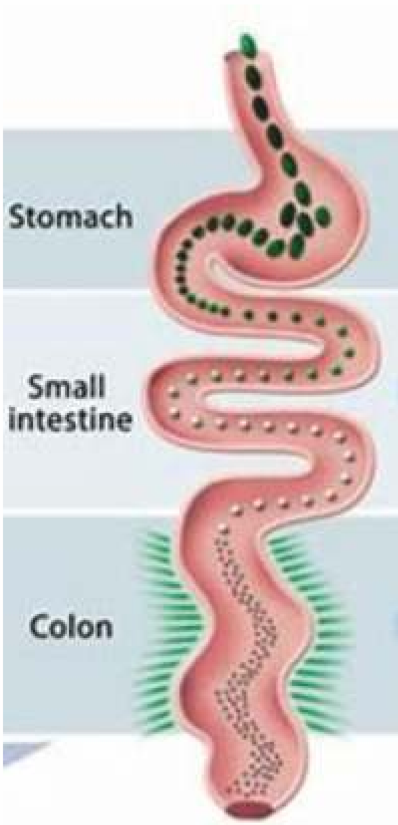
Villi development by using buffer protected Sodium Butyrate



Percentage release of coated Butyrates in different segments of gut



Percentage release of Butyrins and Buffer protected Butyrate in different segments of gut



Butyrins	Buffer protected Butyrate
10 %	0 %
25 %	85 %
65 %	15 %

Comparison of different forms of Butyrates			
Parameters	**Coated Butyrates**	**Glycerol Ester of Butyric Acid**	**Buffer protected sodium Butyrate**
Active content	Sodium or Calcium Butyrate 30-50%	Glycerol Ester of Butyric Acid	Sodium Butyrate 54%
Protection technique	Generally fat or vegetable oil coated	Esterification of Butyrate by Glycerol	Protected by a buffer matrix
Pelleting temp stability	Stable up to the melting point of fat. Most fats melt at temperatures beyond 60^0 C	Stable	Stable under pelleting as well as extrusion conditions.
Stability in the stomach (gizzard)	Stable if coating is intact (after pelleting)	Stable	Stable
Release of Butyric acid in the System	Lipase activity needed for release. This release is inadequate and slow specially in case of young animals (chicks and growers)	Need to be digested like oils and fats, hence Lipase is essential.	Release induced by solubility of salts after stomach, not dependent on Lipase.
Availability in the small intestine	Low release in jejunum and ileum	Subject to enzymatic breakdown of butyrins	Mainly available in the small intestine (85%)
Support to early villi development in chicks	Not ideal	Not ideal	Ideal

Conclusion

Butyric acid is well known for its role in gut health and as a major energy source for epithelial cells of the villi. It helps regulate critical functions of the intestine, maintaining integrity of the epithelial lining of the intestine and enteric wall, promoting growth of villi and microvilli and Improving the digestion and absorption of nutrients. Butyric acid protects intestinal cells against pathogens, improves local immunity and promotes balanced growth of beneficial microflora.

In Butyric Acids commercially available forms, buffer protected Butyrate is found to be the most suitable option for poultry. The release of buffer protected Butyrate is not dependant on Lipase, therefore enabling butyrate release in the intestine through all stages of the life cycle. Buffer protected Butyrates remain stable under pelleting and extrusion processes, making them appropriate for all kind of poultry feeds.

**References available upon request.*

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