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Technical Bulletin

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Gut Health Management

The role of Short Chain Fatty Acids and Prebiotics

Gut Health

A healthy Gut is vital for the optimum performance of Poultry. The microbial balance of the gut (Eubiosis) is of importance in maintaining gut health. Intensive rearing of poultry inflicts considerable metabolic stress altering the gut environment and habitat, creating an imbalance in the gut microflora, ultimately affecting flock productivity.

Antibiotics in feed have been used to address this imbalance. Today, world over, there is a move away from the use of antibiotics in feed prompted by legislation and consumer resistance. In the absence of this option, the industry has explored several approaches towards gut modulation and optimization including the use of probiotics, prebiotics, short chain fatty acids, herbs and spices, essential oils, enzymes etc.

This paper explores the option of combining select Short Chain Fatty Acids (SCFA) with a Prebiotic, an approach that has shown very promising results in optimizing gut health and consequently improving growth and economic performance of poultry.

Gut Microflora

Gut is a complex ecosystem maintaining a fine tune between the host intestinal cells, producing and secreting enzymes for digestion and subsequent absorption of food material, and its microbial habitat that includes a diversified group of microbes (Gabriel *et al.*, 2006). It is difficult to define the exact composition of intestinal microflora, but it is assumed that hundreds of species colonise the intestine. There are three types of bacteria: the dominant (< 10^6 CFU/g content), the sub-dominant (10^6 to 10^3 CFU/g) and the residual bacteria (<10^3 CFU/g). In chicken the main sites of bacterial activity are the crop, caeca, and, to a lesser extent the small intestine (Gabriel *et al.*, 2006). Anaerobic bacteria are found to be in a large number as compared to the other bacteria present in the intestine (Zhu *et al.*, 2002).

Extensive studies on the culturable bacterial flora of chickens have been conducted (Jiangrang *et al.*, 2003). Lactobacilli are the predominant bacteria in the crop along with enterococci, coliforms and yeast. Gizzard and proventriculus have a fewer bacteria because of the lower pH, and duodenum too does not have much microbial population due to the presence of numerous enzymes and high oxygen pressure. Ileum contains 10^9 bacteria/g content with the predominance of lactobacilli along with enterococci and coliforms. 10^{11} bacteria are found per gram of ceca with *Clostridiaceae* comprising the majority along with other strict and facultative anaerobes (Gabriel *et al.*, 2006).

Role of Gut Microflora

Gut microflora plays a vital role in the health and performance of chicken through its effect on gut microbiology, nutrition and pathogenesis of enteric disease and immune response. The intestinal microflora may be health promoting or potentially pathogenic, depending on the physiological status of the bird.

The health-promoting microflora (eg: Lactobacilli, Bifidobacteria) promote flock health by inhibition of growth and establishment of harmful microbial species by competitive exclusion, stimulation of the immune

system through non-pathogenic means, vitamin production etc.

The pathogenic intestinal microflora induce pathogenesis through the onset of localised or systemic infections, intestinal putrefaction, toxin formation, production of growth depressing, mutagenic and carcinogenic substances, ammonia formation, bile acid biotransformation etc.

The pathogenic and health-promoting microflora are involved in at least two sub-ecosystems: The Luminal microflora and the Mucosal microflora (Suzen *et al.*, 2002). The composition of the Luminal microflora is mainly determined by the nutrients available, the rate of passage and the effects of antimicrobial substances. The composition of Mucosal microflora is mainly determined by the host's expression of specific adhesion sites on the Enterocyte membrane, the rate of mucus production, the production of secretary immunoglobulin and the extrusion of cellular material from the membrane into the mucus. Mucosal microflora thus interacts intimately with the intestinal wall of the host. Gut health is determined by the mutual interaction of luminal and mucosal microflora.

Gut wall

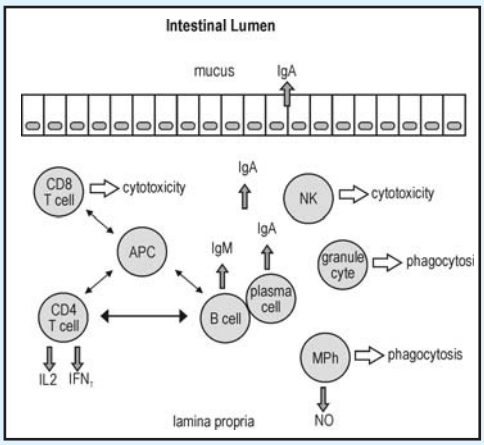
Apart from gut microflora, the immunity, integrity and functionality of the intestinal wall determine the gut health and thereby the bird's health (Suzen *et al.*, 2002).

Gut Immunity

It is defined as the cells and products belonging to the immune system in the gut

Immunity of the intestinal wall may be innate or specific in offering protection against the foreign antigen/pathogen (Figure 1)

Figure 1
A schematic representation of the innate and specific immunity in the intestine.



(Suzen *et al.*, 2002)

Note: APC: antigen-presenting cell; CD4: helper phenotype; CD8: cytotoxic phenotype; IFNγ: interferon gamma; IgA: immunoglobulin A; IgM: immunoglobulin M; IL2: interleukin 2; MPh: macrophage; NK: natural killer cell; NO: nitric oxide

Innate immunity acts as a first line of defense by restricting the growth and spread of pathogens and operates through the natural killer cells, Granulocytes and Macrophages and their secreted products, such as Nitric Oxide and various Cytokines.

Specific immune response, has two aspects:

- Humoral immunity (antibodies produced by B lymphocytes and Plasma Cells)
- Cellular immunity (helper and Cytotoxic T lymphocytes).

Humoral Immunity or Cellular Immunity, or a combination of both is induced, depending on the type of pathogen and the type of cell that it infects. Both types of immunity are characterised by high specificity for the pathogen and by memory formation.

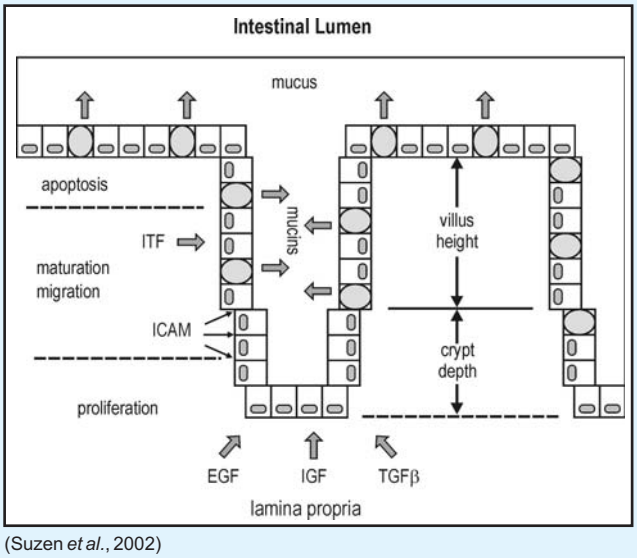
The cells that constitute the innate and specific defenses are located in three distinct areas of the intestinal wall:

- Organised lymphoid tissues, such as caecal tonsils, Meckel's Diverticulum and Peyer's Patches (Jeurissen *et al.*, 1994)
- The Lamina Propria of the intestine provides local defense.
- Intestinal epithelium with, T and B lymphocytes in the small intestine, and macrophages in the esophagus (Vervelde and Jeurissen, 1993)

Gut Integrity

Gut Integrity is defined as the cells and products

Figure 2
A schematic representation of intestinal integrity.



(Suzen *et al.*, 2002)

EGF: Epidermal Growth Factor; ICAM: Intercellular Adhesion Molecule; IGF: Insulin-like Growth Factor; ITF: Intestinal Trefoil Factor; TGFß: Transforming Growth Factor Beta.

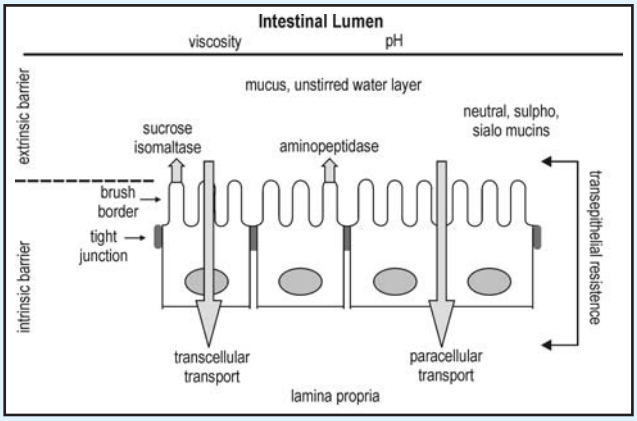
constituting the barrier against leakage or translocation of feed components, microbial toxins and microorganisms from the lumen to the body. This is of crucial importance in inferring protection against the leakage of unwanted substances from the intestinal lumen into the submucosal tissue.

Integrity of the intestinal wall is primarily formed by the continuous layer of epithelial cells and the mucus that act as a barrier. Epithelial integrity is vital for the resistance of enteric diseases (Mantle and Allen, 1989). Intestinal health requires a balance in proliferation, maturation and apoptosis of the epithelial cells.

Gut Functionality

Gut Functionality is determined by physicochemical parameters such as gut pH and viscosity which affect the microbial activity in the intestinal tract.

Figure 3
A schematic representation of functionality of the intestine



(Suzen *et al.*, 2002)

Factors Influencing Gut Functionality

Digestion and absorption of nutrients within the gut are affected by the viscosity, pH and osmolality of the chyme.

• Viscosity

Increased viscosity reduces the rate of nutrient absorption, by increasing the thickness of the unstirred water layer covering the mucosa cells (Johnson and Gee, 1981). Increased digesta viscosity also limits the mixing of nutrients with pancreatic enzymes and bile acids within the gastrointestinal tract (Edwards *et al.*, 1988) and the movement of nutrients from the lumen to the mucosal surface (Fengler and Marqurdt, 1988), which further limits the nutrient digestion and absorption. Since increased viscosity reduces mixing and passage rate, it decreases luminal oxygenation thereby allowing increased microbial production due to increased residence time (Bedford, 1996).

• pH

The gastrointestinal pH decreases as the chyme passes from the crop into the proventriculus and gizzard and then becomes progressively less acidic along the length of the small intestine. Lowered pH is conducive for the growth of favourable bacteria simultaneously hampering the pathogenic bacterial growth.

• Osmolality of the chyme

In all small intestinal segments, the chyme supernatant is hypotonic to blood plasma. In case of increased intestinal viscosity, the osmolarity of the chyme is increased, which further limits the sodium and water absorption from intestinal lumen, leading to increased litter moisture
(Klis and Lensing, 2007).

Strategies to improve nutrient efficiency involve modifications of the gut development, maintenance and turn-over and health and immunity. Thus, maintaining a structurally sound gut should be the prime strategy for enhancing the production performance of poultry, by increasing the commensal population and decreasing the pathogenic bacteria. In this context, a combination of specific short chain fatty acids and specific prebiotic sugars act as ideal candidates to replace in-feed antibiotics.

Short Chain Fatty Acids

Organic acids have been used in feed preservation, protecting feed from microbial and fungal contamination. The organic acids associated with specific antimicrobial activity are short-chain acids (C1–C7) and are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing an hydroxyl group (usually on the α carbon) such as lactic, malic, tartaric, and citric acids. They are also formed through microbial fermentation of carbohydrates in the large intestine (Partanen and Mroz, 1999). Other acids, such as sorbic and fumaric acids have some antifungal activity and are short chain-carboxylic acids containing double bonds. Organic acids are weak acids and are only partly dissociated. Most organic acids with antimicrobial activity have a pKa (the pH at which the acid is half dissociated) between 3 and 5.

The organic acids produced within the body are particularly short-chain fatty acids (Carbon chain length1- 7) like Acetic Acid, Propionic Acid and Butyric Acid (volatile fatty acids) in millimolar quantities (20 to 131 millimol/L). These characteristically occur in high concentrations in regions where strictly anaerobic microflora are predominant.

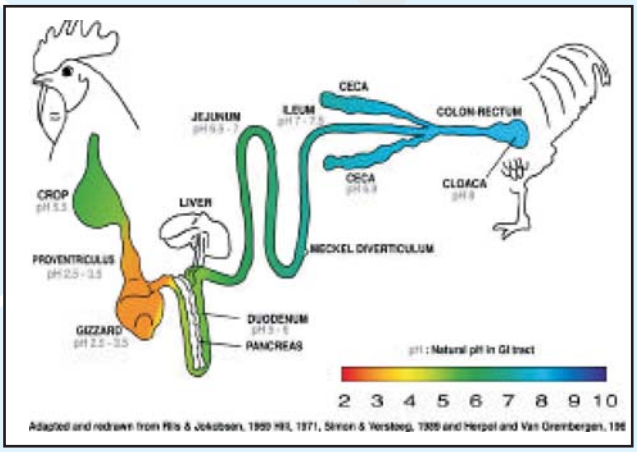
The average feed transit time and pH of the different parts of chicken GI tract is given in Table 1.

Table 1

GIT compartment	Duration of transit time (min.)	pH
Crop	50	5.5
Proventriculus & gizzard	90	2.5-3.5
Duodenum	5-8	5-6
Jeunum	20-30	6.5-7
Ileum	50-70	7-7.5
Rectum	25	8

Simon & Versteeg 1989 in Vanbelle M.

Figure 4
The pH of different parts of GI tract of poultry



Short Chain fatty acids have also being used for decontamination and prevention of recontamination of feed. The objective of feed acidification is the inhibition of intestinal bacteria competing with the host for available nutrients and a reduction of possibly toxic bacterial metabolites, e.g. Ammonia and Amines. In poultry, organic acids have mainly been used to sanitise the feed as well as the gut (Thompson and Hinton, 1997).

The physico-chemical characters of various organic acids are given in Table 2.

Mode of Action of Organic Acids

1. Antibacterial effect

The mode of action of organic acids has been reviewed by Cherrington *et al.* (1991) and Russell (1992). The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, solubility in water and lipids (kp value) and the pH of the surrounding media. The antibacterial activity increases with decreasing pH-value (Fig 5). The undissociated acids are lypophilic and easily enter the bacterial cell. Once in the cell, the acid releases the proton in the more alkaline environment, resulting in the decrease of intracellular pH. This influences the microbial metabolism, inhibiting the action of important microbial enzymes and forcing the bacterial cell to use energy to release protons, leading to an intracellular accumulation of acid anions. This toxic anion accumulation ultimately leads to bacterial cell death (Brul and Coote, 1999 and Ricke, 2003). Organic acids serve as uncouplers that generally dissipate pH and

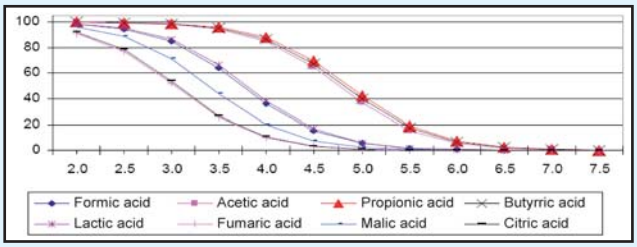
Table 2

Acid	Formula	MM (g/mol)	Density (g/m)	State	pKa	Solubility in water	Corro sivity	MEn MJ/kg	Taste
Formic	HCOOH	46.03	1.220	Liquid	3.75	=	+++	11.34	-
Acetic	CH3COOH	60.05	1.049	Liquid	4.76	=	++	12.19	-0
Propionic	CH3CH2COOH	74.08	0.993	Liquid	4.88	=	++	17.78	-0
Butyric	CH3CH2CH2COOH	88.12	0.958	Liquid	4.82	=	+	22.43	+
Lactic	CH3CH(OH)COOH	90.08	1.206	Liquid	3.83	V	+	14.53	++
Sorbic	CH3CH : CHCH : CHCOOH	112.14	1.204	Solid	4.76	S	+		0
Fumaric	COOHCH : CHCOOH	116.07	1.635	Solid	3.02 4.38	S	+		0
Malic	COOHCH2CH(OH)COOH	134.09	1.601	Liquid	3.40 5.10	=	0	9.79	
Tartaric	COOHCH(OH) CH(OH)COOH	150.09	1.760	Liquid	2.93 4.23	V	+		
Citric	COOHCH2C(OH) (COOH)CH2COOH	192.14	1.665	Solid	3.13 4.76 6.40	V	0	10.29	++
Phosphoric	H2PO4	-	-	-	2.0, 7.0 12.0	=	++–+	-	-

Note: MM- molecular mass in grams.
Solubility in water: = soluble in all proportions; V = very soluble; S = slightly soluble
Corrosive: 0 = no corrosive; + to ++++ = slightly to highly corrosive
Taste: - = negative; 0 = neutral; + to ++ + favorable (Source: Gauthier, 2002)

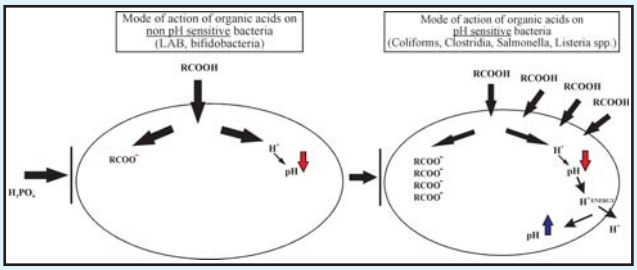
electrical gradients across cell membrane (Russell, 1992).

Figure 5
Percentage of Non-Dissociated Acid in Relation to Gut pH.



(Piva A., Univ. of Bologna, Italy, personal communication)

Figure 6
Mode of action of organic acid



Generally lactic acid bacteria are able to grow at relatively low pH, which means that they are more resistant to organic acids than other pathogenic bacterial species, e. g. E.coli. An explanation for this may be that gram-positive bacteria have a high intracellular potassium concentration, which provides a counteraction for the acid anions (Russell and Diez-Gonzalez, 1998). Generally the antimicrobial effect of organic acids increases with increasing concentration and increasing length of the carbon chain. However, gram-negative bacteria are able to uptake and metabolise long and medium-chained organic acids. Vegetative cells are more sensitive to organic acids than the corresponding spore forms.

2. Regeneration of Intestinal Villi

Intestinal Villi are the absorptive surface of the intestine. The mean life span of epithelial cells of Villi is 48-72 hours. Obviously intestinal health requires a balance in proliferation, maturation and apoptosis of the epithelial cells of the Villi. Many different factors are involved in this process. Among the SCFA, Butyric acid is a critical nutrient for the epithelial cells and is a regulator of cellular growth and differentiation through stimulating the secretion of Insulin like Growth Factor (IGF) from pancreas. SCFA is an instant source of energy for Villi regeneration and an increase in the aspect ratio of the Mucosal Villi and the depth of the crypts. This, in turn, increases the surface area of the Mucosa and hence the ability of the mucosa to absorb nutrients from within the contents of the intestine.

Site of action of Organic Acids

Organic acids exert their antimicrobial action both in the feed and in the GI-tract of the animal. Often, organic acids are recovered only from the fore-gut of poultry. The strongest effect of organic acids with respect to digesta pH and antimicrobial activity are found in the crop and the small intestine and only little acid reaches the lower digestive tract and the caeca.

Benefits of Organic Acids

Short Chain fatty acids in general and Butyrate in particular promote the growth of lactobacilli and bifidobacteria and play a crucial role in the colon physiology and metabolism (Roy *et al.*, 2006). Acidification of diet causes an improvement in digestive enzyme activity (Nitsan *et al.*, 1991), microbial phytase activity and increased pancreatic secretion (Thaela *et al.*, 1998) and decreases pathogenic bacterial (*E. coli* or *Salmonella*) colonization in crop and caeca (Maheswari *et al.*, 2001; Tarazi and Alshawabkeh, 2003 and Rama Rao *et al.*, 2004).

Limitations of Organic acids

- The pKa value, solubility of the organic acid, dose available at the site (in undissociated form), the contact time with microbes and the prevailing pH of gut determine the efficacy of organic acid.
- The caecum is the site of highest colonisation by pathogens. This is related to the presence of specific receptors in the organ, the physiology of caecal peristalsis, pH etc. Even organic acids with higher pKa value do not travel down to the hind-gut and are thus ineffective in offering protection against harmful bacteria in caecum.
- Response to organic acid also varies according to pH, buffering capacity and water activity of the feed, water and gut content (Chung and Goeffert, 1970), cleanliness of the production environment and heterogeneity of gut microbiota.

Prebiotics

Prebiotics are defined as non-digestible food ingredient that are potentially beneficial to the health of the host, due to their fermentable properties which may stimulate the growth and/or activity of one or limited number of bacteria in the colon or caecum (Crittenden and Playne, 1996).

According to this definition, Prebiotics includes a very variable and wide range of chemical substances. These are carbohydrates which are not hydrolysed by the host endogenous enzymes and are thus available

for the microbiological fermentation in lower parts of non-ruminant gastrointestinal tract. The dominant Prebiotics are Fructooligosaccharide products (FOS, Oligofructose, and inulin). Trans-Galactooligosaccharides, Glucooligosaccharides, Glycooligosacchriades, Lactose, Lactulose, Lactitol, Maltooligosaccharides, Xylo-Oligosaccharides, Stachyose, Raffinose and Sucrose Thermal Oligosaccharides have also been investigated. Although Mannan Oligosaccharides (MOS) have been used in the same manner as the Prebiotics listed above, they do not selectively enrich beneficial bacterial populations. They are thought to act by binding and removing pathogens from the intestinal tract and stimulation of the immune system. To a certain extent, they may be hydrolysed by the exogenous enzymes

Mode of Action of Prebiotics

Prebiotics act by supplying nutrients to beneficial microbes favouring their growth and activity which in turn cause pathogen inhibition by competition for nutrients, production of toxic conditions and compounds (volatile fatty acids, low pH, and Bacteriocins), competition for binding sites on the intestinal epithelium and stimulation of the immune system

(Gibson *et al.*, 2005). These are not mutually exclusive mechanisms; hence, some microorganisms may act via a single mechanism, whereas others may use several mechanisms.

Tricking pathogenic bacteria to attach to the oligosaccharide rather than to the intestinal mucosa reduces the intestinal colonisation and the microbes that are attached to the prebiotic will be excreted from the bird along with other undigested food.

Benefits of Prebiotics

Modify Intestinal Microbiota	Enhance Animal Performance
Prevent Pathogen Colonisation	Decrease Carcass Contamination
Stimulate Immune System	Reduce Inflammatory Reactions
Increase Production of VFA	Decrease Ammonia and Urea Excretion
Increase B Vitamin Synthesis	Lower Skatol, Indole, Phenol, etc.
Improve Mineral Absorption	Prevent Bile Salt Bile Deconjucation

Dietary prebiotic inclusion improved the gastrointestinal microflora, improved feed efficiency, reduced mortality and reduced colonization by enteropathogens and the total viable count (Ammerman *et al.*, 1988; Patterson *et al.*, 1997; Marioka *et al.*, 2000 and Elangovan *et al.*, 2005)

Synergism between SCFA and Prebiotics

The synergistic effect of Prebiotics and Organic Acids on the control of colonisation and infection of pathogens in chicken depends on the type of sugar or SCFA, dose, route of administration etc.

Synergistic composition comprising, SCFA (e.g. Butyric Acid) and a prebiotic compound has been found to act throughtout the entire gastrointestinal tract by causing a reduction in entire gut pH, pathogenic bacterial count and improvement in beneficial microflora.

The combination of SCFA (Select salt of Butyrate) and a Prebiotic (Select Technical grade of Aletobiose) is synergistic in that the two ingredients are believed to generate Butyric Acid and other VFA in the lower intestine of a chicken, which complements the Organic Acid, simultaneously encouraging the growth of beneficial bacteria in the lower intestine. This thereby synergistically increases the rate of weight gain and/or feed utilisation of the chicken (Table3).

Table 3
Effect of Acidifier and Prebiotic Combination on Broiler Performance

Treatment	*42 day BW*	*F/G*	*Mortality %*	*EPEF*
Control	2470	1.794	0.741	325
Control + Sodium Butyrate	2620	1.725	1.481	356
Control + Prebiotic	2633	1.724	2.222	356
Control + Sodium Butyrate and Prebiotic	2668	1.709	0.741	369

BW - Body Weight; F/G - Feed Gain ratio; EPEF - European Performance Efficiency Factor
(Source - Nutrisys Centre for Animal Nutrition)

While not being bound to any particular mechanism, it is believed that SCFA (Select salt of Butyrate) is degraded, presumably by acid hydrolysis and/or lipase activity, in the Proventriculus and upper small intestine to yield butyric acid. The released butyric acid is partially used as an energy yielding nutrient with the rest being utilized in the metabolism of enterocytes as a trophic, restorative and invigorating element.

Select grade of Aletobiose passes through the fore-gut essentially unchanged and ultimately enters the large intestine, where they are preferentially utilised by the lactic acid group of bacteria, including Lactobacilli and Bifidobacteria. These beneficial bacteria produce mainly lactic acid as a fermentation end product which lowers the pH of the lower gut disabling the growth of undesirable micro-organisms. This encourages a more favourable microbial balance in the GI tract; improving gut health, consequently enhancing the bird health and its productivity.

Table 4
Effect of Acidifier and Prebiotic on Intestinal Bacterial Count (CFU/g - colony forming unit per gram)

Organ	*Treatments*	*E.coli Count (CFU/g)*	*Lactobacilli Count (CFU/g)*
Duodenum	Control	4.4×10^6	3.0×10^3
	Acidifier	8.0×10^5	1.4×10^5
	Acidifier + Prebiotic	5.0×10^5	1.5×10^7
Jejunum	Control	2.6×10^{10}	2.1×10^9
	Acidifier	2.0×10^9	1.2×10^7
	Acidifier + Prebiotic	3.6×10^8	7.2×10^5
Caecum	Control	4.2×10^6	1.4×10^5
	Acidifier	9.0×10^8	1.8×10^8
	Acidifier + Prebiotic	7.0×10^5	1.6×10^7

According to Table 4, the beneficial bacteria thrive in the entire gut due to the presence of a ready energy source such as a Prebiotic, which produce Lactic Acid (which is also antimicrobial), thereby competitively exclude colonisation of other potentially detrimental organisms. These beneficial bacteria may also produce antimicrobial compounds, including Bacteriocins, Hydrogen Peroxide, etc., which also inhibit the growth of undesirable and/or pathogenic organisms.

Another advantage of the combination is that they may prevent conversion of Pre-Oncogenic compounds into Oncogenic compounds by enzymatic hydrolysis.

Added advantage of the composition is the intrinsic sweetness of prebiotic coupled with the taste of SCFA (Select salt of Butyrate), which is far less pungent than Butyric Acid itself. This composition is, therefore, more palatable especially in swine which are more sensitive to taste. The synergistic combination is particularly useful when used as a feed additive for poultry and swine to improve the gut health and thereby production performance.

Summary

The experiment led to the following conclusions

- Pathogens have to overcome numerous obstacles in order to colonise the intestinal tract and cause an infection. In addition to the physical restraints of low gastric pH and rapid transit time in the small intestine, pathogens have to overcome the inhibitory effects of the intestinal Microbiota, the physical barrier of the epithelium and the response of host immune tissues.
- The concept of cross-talk between these systems, pathogens and the epithelium is well established. Some species of non-pathogenic intestinal microflora communicate with the epithelium and immune system, modulating tissue physiology and ability to respond to infection.
- Short chain Fatty Acids and Prebiotics alter the intestinal microflora and immune system to reduce colonisation by Pathogens and enhance the intestinal villi height and width and also rejuvenate the damaged Intestinal Villi thereby improving the production performance of birds and is a viable alternative for antibiotics.

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AVITECH ANIMAL HEALTH PVT LTD
GP-51, Udyog Vihar, Sector-18, Gurgaon-122001, Haryana, India
Tel.: +91-124-4011147, 4278511/12 Fax: +91-124-4013620
Email: info@avitechnutrition.com Website: www.avitechnutrition.com