volac

Animal Nutrition

BioSynergy

THE SCIENCE BEHIND BIOSYNERGY

Background

For animal production we tend to concentrate on the nutrients that are absorbed from the digestive tract. In reality, we need to think about the "net" absorption of nutrients and the efficiency of transferring feed nutrients to animal production. The net absorption is affected by the maintenance cost of the GI tract. The value of a healthy 'microbiome', the collection of digestive tract bacteria, has been increasingly identified as a key to maintaining animal health. Fortunately, there are key ingredients/additives that can help keep the mix of bacteria on the positive side.

Seventy percent (70%) of the immune system is found within the gastro-intestinal (GI) tract. Along with the skin, it is the major defense against potential pathogens. The GI tract contains nutrients and provides an environment that supports a diverse and broad spectrum of microbial growth. This microbiome, a mixture of live species, contains beneficial, benign, and pathogenic microbes. Normally, nature provides a homeostatic situation that allows the animal to maintain a healthy functional gut. Unfortunately, there are a myriad of things that can upset this system. Changes in feed, changes in weather, illness and toxins, can all upset the normal balance and allow pathogens to dominate the internal fermentation.

Broad spectrum antibiotics are not the answer. They require a veterinary visit and prescription. Antibiotics can be non-discriminating, killing beneficial bacteria as well, delaying the re-establishment of a normal microbiome and interrupting the production of saleable milk or delaying marketing of meat animals.

Gut Pathogens

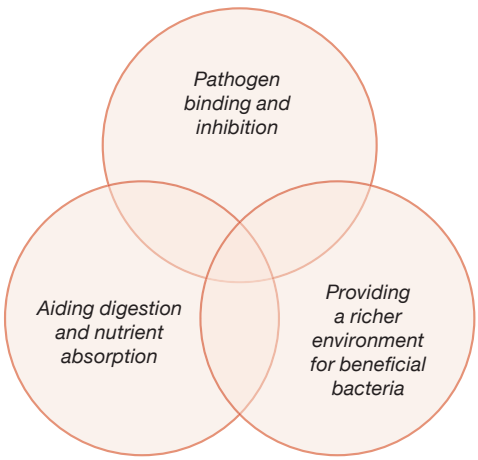
Pathogens are always present in the digestive tract and their numbers can "explode" when conditions allow. Salmonella and *E. coli* are two common (Gram -) gut micro-organisms that can cause illness and reduce available nutrients to the host animal. Perhaps of even greater concern are the multiple species of Clostridia (Gram +) bacteria that can produce exotoxins that can damage the cells of the digestive tract and in severe cases may lead to hemorrhagic bowel syndrome.

Acute problems are easier to see. The greater losses can come from the subclinical conditions that rob us of production through increased cost of the immune system and reductions in nutrient absorption.

Biological Solutions

Fortunately, we now know that there are natural ingredients and beneficial bacteria that can enhance the return to a healthy microbiome status. Lactic acid producing bacteria have often been supplemented in probiotic products to help repopulate with common gut bacteria. This solution has merits but may fall short of success if the gut environment has not been restored to a place where they can flourish. The presence of a large number of pathogens may diminish the effectiveness of typical *lactobacillus* supplementation.

To effectively reduce pathogens natural ingredients and bacteria can be supplied that can "tip" the scales toward reclaiming a healthy positive microbiome.



Benefits of Bacillus spp. as probiotics in animal feed

Bacillus is a genus of an extremely diverse group of gram-positive, rod-shaped bacteria. Many species of *Bacillus* have proven applications in a wide range of industries including the production of antibiotics and enzymes but also as probiotic supplements. Probiotics (also referred to as "Direct-Fed-Microbials" (DFMs)) are defined as live bacterial organisms selected to beneficially modify/stabilize the gut microbiome thereby:

The successful application of *Bacillus* spp. as probiotics in animal feed is dependent on two critical characteristics associated with the organisms.

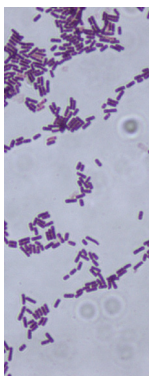
1. Facultative anaerobes

Bacterial organisms described as facultative anaerobes can survive under both aerobic and anaerobic conditions. The GI tract is supposed to be anaerobic, but oxygen can be present at small amounts. Such fluctuations between these two environmental states can influence the balance of the microflora but *Bacillus* organisms are able to survive under both. Other common probiotic organisms such as *Lactobacilli* and *Bifidobacteria* are oxygen sensitive.

2. The production of endospores

The endospores are produced under stressful environmental conditions such as high temperatures during pelleting. The endospores remain in a dormant state until the environment is conductive for growth i.e. the intestinal tract where they can attach to the gut wall and multiply. This gives them a natural advantage over other common probiotic organisms e.g. *Lactobacilli* and *Bifidobacteria* spp. which are not heat tolerant or stable over long periods of time.

The most commonly used species of *Bacillus* for use as probiotics in animal nutrition include:

B. Amyloliquefaciens	*B. Licheniformis*	*B. Pumilus*	*B. Subtilis*
			

When ingested these species of *Bacillus* undertake one or more of these four activities:

1. Scavenge oxygen
2. Produce antimicrobial substances
3. Bind to gut epithelial cells
4. Produce enzymes

Utilization of oxygen, production of antimicrobial substances and binding to gut epithelial cells all have the beneficial effect of inhibiting activity by pathogenic organisms such *Clostridia, Salmonella* and *E. coli*. A consequence of this is improved resistance to disease but also supports animal production by reducing the damage to the gut wall from pathogens and subsequent nutrient absorption.

These same activities will also contribute to increasing the populations of *Lactobacilli* and *Bifidobacteria* spp. which are often described as beneficial bacteria. They produce antimicrobial substances and out compete with pathogens for both nutrients and binding sites and so aid in disease prevention, as well as digestion and nutrient absorption.

With regards to the production of antimicrobial substances the *Bacillus* genus has been associated with 45 molecules. These include a range of bacteriocins, surfactants and bacteriocin-like-inhibitory substances.

Within the bacteriocins group, the most numerous group of substances are described as lantibiotics, of which nisin is the most famous for its activity against *S. aureus*. Amylocyclin is an example of a circular bacteriocin that has been identified as effective against *Listeria* and *Clostridia* organisms. Whilst *Lactobacillus* and *Bifidobacteria* also produce antimicrobial substances the ones produced by *Bacillus* are generally recognized as being active against a wider range of pathogens including both gram negative pathogens like *E. coli* and gram-positive organisms like *S. aureus*.

As indicated in Table 1, some species of *Bacillus* are more effective at inhibiting certain pathogens and it must be emphasized that the exact profile of substances produced by a *Bacillus* organism will vary according to the species and the strain. For this reason, it is best to use a combination of *Bacillus* species to provide the optimal protection.

Table 1: *Species of Bacillus with proven antimicrobial activity against common animal pathogens based on zone of inhibition studies (green indicates strong activity >10mm zone of inhibition, orange indicates moderate activity (0 – 10mm zone of inhibition)*

Pathogen	*B. Licheniformis*	*B. pumilus*	*B. subtilis*
Escherichia coli	√	√	√
Campylobacter jejuni	√	√	√
Clostridium perfringens	√	√	√
Yersinia enterocolitica	-	-	√
Salmonella enterica	√	√	√
Staphylococcus aureus	√	√	√
MRSA	√	√	√
VRSA	√	√	√
Streptococcus uberis	√	√	-
Flavobacterium columnare	√	√	√
Aeromonas salmonicida	√	√	√
Vibrio harveyi	√	√	-

These *Bacillus* spp. are also prolific enzyme producers. The enzymes produced by these species include amylase, protease, lipase, esterase, cellulase and xylanase. The first four complement what is already secreted into the intestine by the host animal for feed digestion thereby reducing the energy required for this process enabling it to be redirected to growth. The latter two are not produced endogenously by the host animal but are widely incorporated into animal feed exogenously to utilize the fibrous component of the feed but also reduce the anti-nutritional effects of dietary components like beta-glucans and xylans. The secretion of these enzymes in the intestinal tract contributes to feed digestion, improving feed efficiency and growth performance.

B. amyloliquefaciens secretes antimicrobial peptides in the presence of *E coli*, against *E. coli*. Additionally strains of *B. amyloliquefaciens* have been shown to produce bactericidal proteins and inhibit *Clostridium difficile*. C. *difficile* can and are developing resistance to commonly used antibiotics in both animal and human medicine resulting to increased pressure not to use these antibiotics until necessary. *Bacillus* species that inhibit *Clostridial* growth may provide a practical solution.

Additionally, B. subtilis has been demonstrated to increase interleukin-10 (IL-10), an anti-inflammatory modulating signalling molecule, which can increase the production of proteins that provide tight junctions between cells of the digestive tract. By reducing inflammation and improving gut integrity, it reduces energy loss in maintenance of the GI tract. *B. pumilus* has been shown to stimulate the growth of the common GI tract bacteria *F. prausnitzii* that has demonstrated an ability to produce anti-inflammatory compounds and provides a positive effect on gut tissue.

Figure 1: *Overview of the activity of Bacillus organism in the GIT and how it results in improved animal performance.*

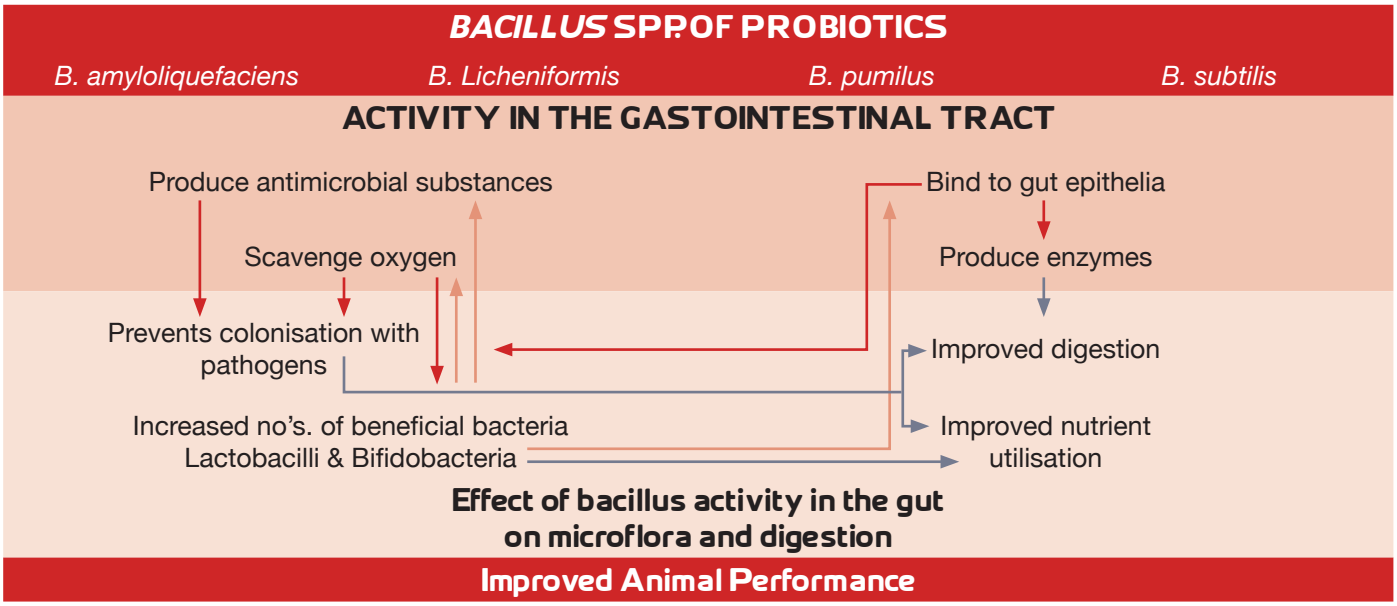
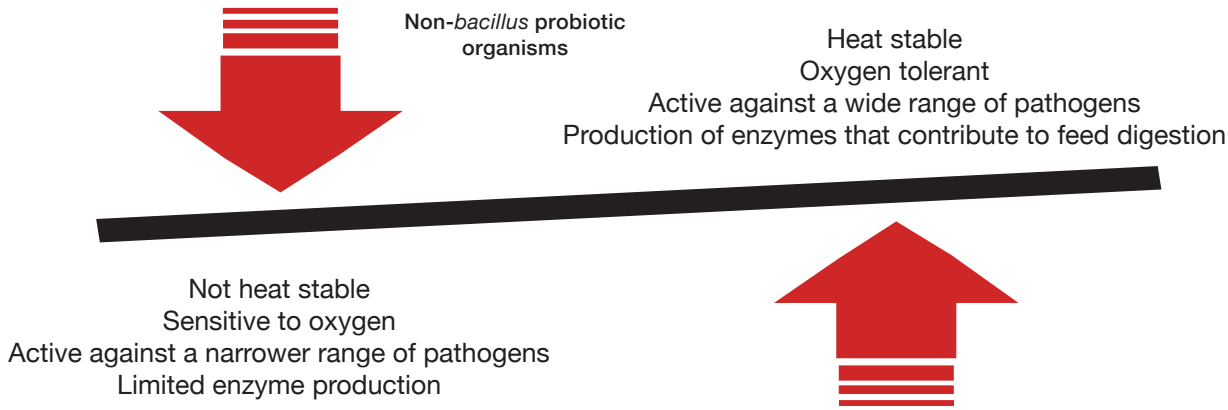


Figure 2: *Key advantages of Bacillus spp verses other organisms (Lactobacilli/Bifidobacteria) as probiotics in animal feed*



In summary, *Bacillus* organisms have many attributes that show they are more suitable for use as probiotics in animal nutrition compared to other bacterial organisms (Figure 2). In particular, it is their ability to withstand extreme processing conditions and supplement endogenous enzyme production in the intestinal tract which gives them the overall advantage.

Through supplementation with *Bacillus* organisms, such as those used in products manufactured by Volac Inc., producers can do more than just balance the microflora to prevent harmful activity from pathogens to actively improve feed digestion creating more value from the diet being fed.

The chosen ones

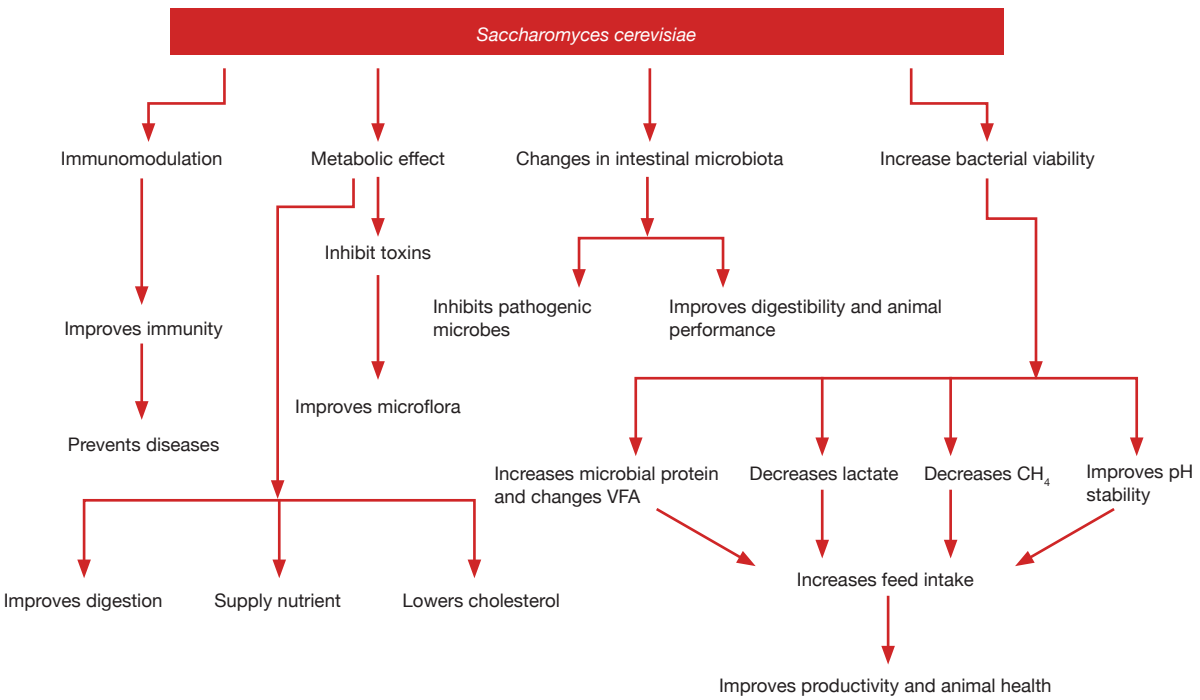
In product design we principally choose from our bank of strains from these five species of Bacillus, chosen based upon their biological characteristics which have been further refined during screening and selection processes to identify strain specific capabilities for both internal and external environment applications.

- ***Bacillus subtilis***
- ***Bacillus pumilus***
- ***Bacillus licheniformis***
- ***Bacillus amyloliquefaciens***
- ***Bacillus stearothermophilus***

Live Yeast contribution

Live yeast use in ruminant nutrition is well established for its role in reducing oxygen content in the rumen and supporting greater fiber digestion. However live yeast can also have positive effect on the biome and health of the intestinal tract. Studies indicate that a supply of yeast can result in greater villi height and inhibition of some pathogens. One of the first shields against oxidation is the superoxide dismutase (SOD) enzyme. *S. cerevisiae* has been shown to increase SOD activity. Studies indicate that *S. cerevisiae* can increase the production of short chain fatty acids in the small intestine and improved gut health.

Figure 3. J of Applied Microbiology, Volume: 128, Issue: 3, Pages: 658-674, First published: 19 August 2019, DOI: (10.1111/jam.14416)

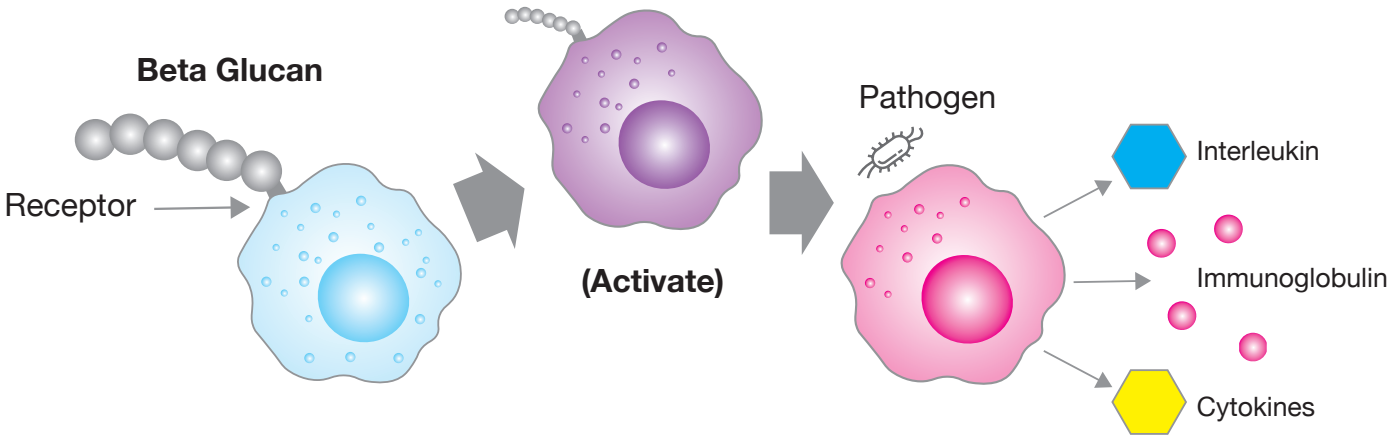


Beta Glucan contributions

β-glucans have been reported to provide anticancer, anti-inflammatory and immune-modulating effects. β-Glucans are a strong immune stimulant and an antagonist against benign and malignant tumors

The growth of *Lactobacilli* and *Bifidobacteria* are supported by the β-glucans in both lab and animal testing. A unique property of β-glucan is that it can bind to a large number of macrophages, which are present as β-glucan receptors on the mucosal lining of the intestinal wall. These macrophages combined with β-glucan are easily activated by pathogens. Once attacked, various immune cells begin to proliferate rapidly. Tight-junction proteins and mucins are important components of the intestinal barrier allowing for homeostasis and helping maintain the health of the intestinal lining.

Macrophage activations via cell surface receptor



BioSynergy **is a natural product that combines multiple products and bacterial strains that have a proven ability to modify and support a strong healthy microbiome and gut integrity. Improving gut health and stasis is important to allow for maximum animal performance and provides supportive activities to animals that have come under stress.**

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