WHITE PAPER

Letter A Heat-Stable Lactobacillus

INTRODUCTION

Direct-Fed Microbials (DFM) are gaining popularity in today's animal feed market. DFM are designed to improve nutrient availability from feed sources that result in decreased production costs. According to the FDA, a DFM is a product that is purported to contain live (viable) microorganisms that are said to provide benefits to the animal.

Lactobacillus species are a common lactic acid bacteria (LAB) that is found in the gastrointestinal tract (GIT) and works antagonistically toward pathogens. Supplementation in animal diets is not common since the instability of this bacterium poses a problem to its use as a component of pelleted feed. LAB are typically heat sensitive and when pelleted this can result in a significant loss in viability. LAB normally have a poor shelf life that results in lower cell counts as well. The challenge is finding an LAB that is stable enough to reach the GIT and benefit the animal.



BENEFITS OF LACTIC-ACID BACTERIA

Lactic acid bacteria serve a variety of purposes when used as a component in animal feeds. LAB have been shown to colonize in the mucous membranes within the gut. This restricts the ability of some pathogens to bond with that membrane. Live (viable) LAB also provide the benefits of lowered pH from increased lactic acid content in both the feed product and the animal's GIT. Lowered pH helps to create an environment that promotes a healthy balance of microflora. The supplementation of a Lactobacillus DFM has been proven to have an immunostimulatory effect and thus creates an enhanced resistance to enteric pathogens in broiler chickens (Rami A. Dalloul, 2003).

Since young animals have impaired gastric HCl secretion, a high stomach pH and increased quantity of undigested feed entering the lower GIT may cause a microbial imbalance in weanling pigs (Smith and Jones, 1963; Partanen and Mroz, 1999), resulting in overgrowth of pathogenic bacteria (e.g., coliforms) and a reduced population of favorable bacteria (e.g., lactobacilli and bifidobacteria; Barrow et al., 1977). Research has also shown that decreasing the pH in the stomach plays an important role in preventing undesirable bacteria from colonizing along the GIT (Maxwell and Stewart, 1995) while creating preferential conditions for the growth of beneficial bacteria such as lactobacilli (Fuller, 1977). Several studies have shown that lactic acid can assist with nutrient utilization in young animals by assisting in lowering the pH in the GIT (Jongbloed et al. 2000 and Tsiloyiannis et al. 2001a,b).

In a 2004 study, L. plantarum was shown to inhibit the growth of Escherichia coli and Salmonella typhimurium. Murry concluded that the high concentrations of lactic acid and low pH levels produced by the L. plantarum were the cause of the inhibition of the pathogenic bacteria on the poultry feed. (Murry et. al, 2004)

LACTOPLAN SOLVES OLD PROBLEMS WITH NEW TECHNOLOGIES

LactoPlan is a direct-fed microbial product that provides the benefits of an LAB, without the fragility of traditional Lactobacillus strains. LactoPlan contains a patented strain of *Lactobacillus plantarum* GB-LP1 that is heat stable and capable of maintaining its high cell count after extended periods of storage. LactoPlan is manufactured by solid-state fermentation, which provides several advantages when compared to other manufacturing techniques of Lactobacillus species.

LactoPlan is not grown and then concentrated like other direct-fed microbial products on the market. LactoPlan is fermented on a feed grain substrate; therefore, it tends to be heartier than those grown on artificial substrates. The microbe is acclimated to utilize nutrients that are commonly found in diets, giving it higher activities, heat stability, and it retains the metabolites created during fermentation.

Lactic acid is one of the valuable metabolites created during fermentation of LactoPlan. Lactic acid is beneficial to the animal since it can improve nutrient availability by preserving labile proteins and amino acids.



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Typically, storage of lactobacillus species has been of concern as microbial counts can be significantly reduced after storage for 2 weeks at 25°C. Research by Abd-Talib and co-workers (Abd-Talib, Mohd-Setapar, Khamis, Nian-Yian, & Aziz, 2013) has shown that storage of lactobacillus species resulted in a 5 log decrease in cell count when stored at room temperature for two weeks (3.25×10^7 cfu/ml to 4×10^2 cfu/ml). The unique fermentation process of LactoPlan allows it to maintain its microbial guarantee of 1.2×10^8 cfu/g when stored under normal conditions for 6 months. There are very few lactobacillus direct-fed microbial products that are as stable as this.

Heat sensitivity is a concern with lactobacillus species as typically a significant log count loss can be observed when pelleted. A study by Angel (Angel, Dalloul, & Doerr, 2005) has shown a 3 log reduction (1,145 times less) in lactobacillus count when the product was pelleted, reporting a change from 7.1 x 108 to 6.2×105 cfu/g. LactoPlan retains a microbial count of 93% of its guarantee (from 1.2×108 to 1.1×108 cfu/g) at pelleting temperatures from 85-95°C, thus making it a heartier lactobacillus species. The chart below represents the heat stability of LactoPlan as it remains live (viable) after pelleting.

| Items | Replica | Before Pellet | After Pellet | Recovery |
|--|---------|---------------|--------------|----------|
| Feed 1 | 1 | 4.66 | 4.54 | 97% |
| | 2 | 4.81 | 4.46 | 93% |
| Feed 2 | 1 | 4.70 | 4.52 | 96% |
| | 2 | 4.40 | 4.28 | 97% |
| Feed 3 | 1 | 4.46 | 3.95 | 89% |
| | 2 | 4.65 | 4.00 | 86% |
| *Feed 1: Broiler (85°C) Feed 2: Broiler (90°C) Feed 3: Duck (95°C) | | | | |

SUMMARY

LactoPlan is an innovative direct-fed microbial product containing a patented strain of Lactobacillus plantarum. LactoPlan is a heat stable product that can deliver the benefits of LAB in animal feed without losing viability during pelleting.



ABOUT NUTRAFERMA

Nutraferma® is an innovative biotech solutions company that utilizes a cutting edge solid-state fermentation process to produce high-value proteins, microbials, and enzymes. Nutraferma's extensive research work has also produced state-of-the-art yeast products, bioactive peptides and phytogenic additives. Headquartered in South Dakota, Nutraferma has manufacturing and R&D facilities in South Dakota (USA) and Korea.

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