

PROBIOTIC STABILITY MATTERS



TAKE HOME MESSAGES

- The World Health Organization defines probiotics as: *"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"*
- Probiotics need to be alive (viable) to exert beneficial effects
- Spore-forming bacilli are hardier and show longer stability and viability than lactic acid bacteria (LAB)
- In general, the stability and viability of probiotics can be significantly impacted by species, temperature, and water activity
- Chr. Hansen's pet probiotics remain stable in their original packaging for up to 24 months and viable post-application on Dry Pet Food Kibble for up to 18 months
- The viability of probiotics in pet products (food, treats, supplements) depends largely on the specific characteristics of the product. Therefore, using a science-based approach, testing of viability post-application and during the product's shelf-life will help ensure the right probiotic inclusion and data to support label guarantees

INTRODUCTION

The World Health Organization defines probiotics as: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." This means that these microorganisms need to be alive when they are ingested by dogs or cats to provide health benefits. However, some studies report that a significant percentage of probiotic products do not contain the organisms listed on the label in a viable form. Several factors, including species, water activity, temperature, pH, matrix, additives, can play a role in the survival and viability of the beneficial microorganisms before reaching the animal.

FACTORS IMPACTING STABILITY

SPECIES

Lactic acid bacteria (LAB) are among the best known and most commonly used probiotics. The idea of using LAB to alter the intestinal microbiota and cause a beneficial effect came from observations by scientists at the beginning of the 20th century.

These early observations of populations with high consumption of fermented milk drove the research on several species and strains of LAB. All the species included as LAB remain in their vegetative state their entire life, and changes to their environment will therefore impact their viability. In general, LAB have a relatively narrow range of conditions in which they can be stable, survive, and thrive. On the other hand, there is another group of microorganisms that can form spores. Spores are multi-layer structures (a proteinaceous coat, the peptidoglycan cortex, the germ cell wall, and the inner membrane), which surround the spore core containing the DNA and other biomolecules. This particular structure makes spores one of the most resistant forms of life, and they can endure a variety of stress conditions, including desiccation, freezing, elevated temperatures in dry or wet conditions, high pressures, UV, and γ -radiation. When facing unfavorable conditions, spore-forming bacilli will form spores. When the right conditions for thriving are present, these spores, will germinate and become vegetative cells again.

TEMPERATURE

Most vegetative forms of microorganisms are characterized for thriving at a relatively narrow range of temperatures and exhibiting high rates of dying as a result of thermal inactivation at temperatures above 104 °F (40°C). This biological limitation is due to enzymatic and biological processes having limits for optimal temperatures at which they function. Temperatures outside the ideal range can be used in two ways:

- Refrigeration and freezing can slow down biological processes and help preserve the viability of microbes for longer.
- Heat is broadly used to inactivate microorganisms. Common examples can be found in cooking processes such as the ones used in manufacturing pet foods and treats, autoclaving, etc.

The impact of temperature will vary depending on the species and sometimes the strain of the microorganisms. Spore-forming probiotics can withstand harsher conditions such as high temperatures without losing viability (Figure 1). Lactic-acid bacteria, on the other hand, can be inactivated and lose viability more quickly as a function of temperature (Figure 2).

Figure 1. *Bacillus* counts of a probiotic product applied to commercial pet food kibble (Source: US application lab. SF case: 349706)

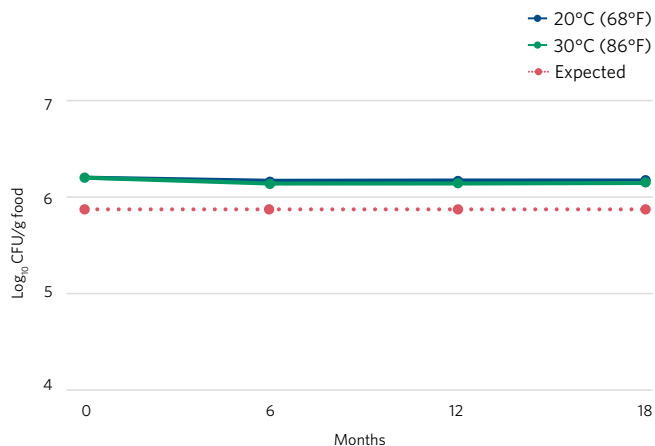
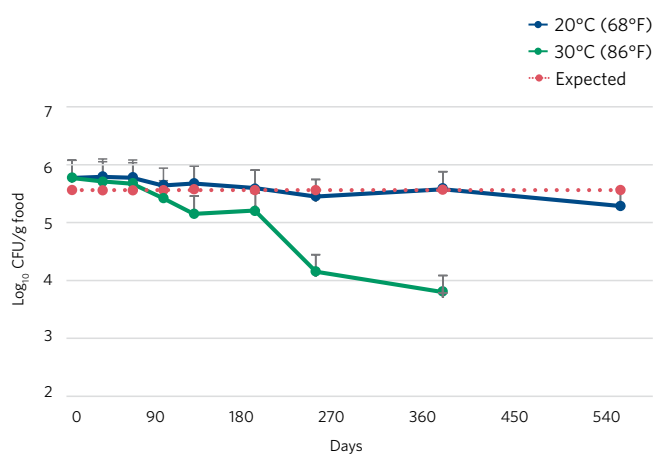


Figure 2. LAB Counts of a probiotic product applied to commercial pet food kibble (Source: US application lab. SF case: 349706)



WATER

Water not only provides an environment for life, but also acts as a substrate for biochemical reactions. Water removal below a certain level will slow down metabolic functions in the microorganisms, making dehydration a method that allows for maintaining the viability of microbes. Water in pet products can be measured in two ways:

- Moisture content: a quantitative measure of water in a product, and is measured as the proportion or percent of water by weight
- Water activity (a_w): a measure of the water that is available for microorganisms to use for their functions. It can be interpreted as the water that is free so microbes can access it

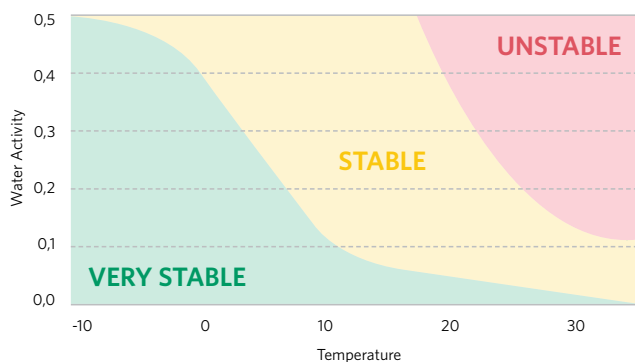
Moisture and water activity are related, but their relationship is very complex and not linear, depending on several factors including temperature and matrix composition. Because water activity represents the available water for microorganisms, it is a more reliable factor for predicting microbial viability (Table 1).

Table 1. Water Activity (a_w) and Microbial Growth (Source Adapted from L.R. Beuchat, Cereal Foods World, 26:345 (1981).)

a_w Range	Example of Organisms Generally Inhibited by the lowest a_w in the range
0.95 - 1.00	<i>Pseudomonas</i> , <i>Escherichia</i> , <i>Bacillus</i> , <i>Clostridium perfringens</i> , some yeasts
0.91 - 0.95	<i>Salmonella</i> , <i>Lactobacillus</i> , some molds and yeasts
0.87 - 0.91	Many yeasts, <i>Micrococcus</i>
0.80 - 0.87	Most molds, <i>Staphylococcus aureus</i>
0.75 - 0.80	Most halophilic bacteria, <i>mycotoxigenic aspergilli</i>
0.65 - 0.75	<i>Xerophilic molds</i> (<i>Aspergillus chevalieri</i> , <i>A. candidus</i> , <i>Wallemia sebi</i>), <i>Saccharomyces bisporus</i>
0.40 - 0.65	<i>Osmophilic yeasts</i> , few molds

In general, probiotics at lower water activity will be more stable, and viable for longer. More importantly, both temperature and water activity interplay in probiotic stability, affecting how microbes respond to different levels of each individual factor. At lower temperatures, stability can be maintained at higher water activity. At the same time, stability can be maintained at higher temperatures when the water activity is lower (Figure 3).

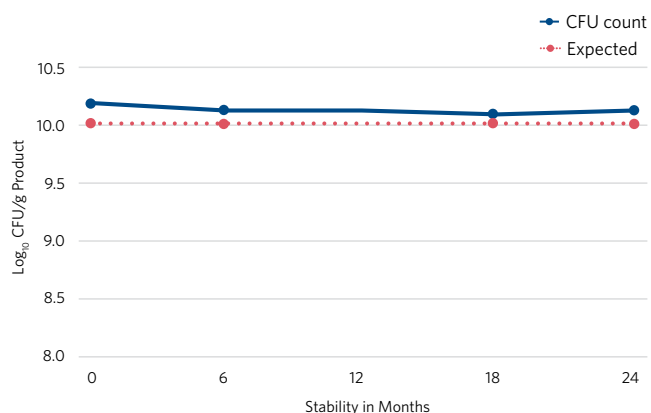
Figure 3. Stability of LAB probiotics as function of temperature and water activity (Source: Chr. Hansen Internal Data)



STABILITY AND VIABILITY OF CHR. HANSEN PET PROBIOTICS

In general, stability can be defined as the capability of remaining stable or unchanged, while enduring challenges. For probiotics, stability refers to the ability of probiotic microorganisms to maintain their viability and functional properties over a period of time under various conditions. In simpler terms, will the probiotic remain alive during storage and application to pet products like foods, treats, and supplements? Viability is defined as the capability of living and functioning adequately. In other words, will the pet ingest live probiotics in the right amounts as guaranteed on the label of the product during its shelf-life? At Chr. Hansen we take the answers to these questions very seriously. To test their stability, our probiotic products undergo an extremely rigorous testing program that includes multiple production lots and several replicates per sample. During this testing program, **CHR. HANSEN PET-PROESSENTIALS™** products have been shown to remain viable for at least 24 months (Figure 4).

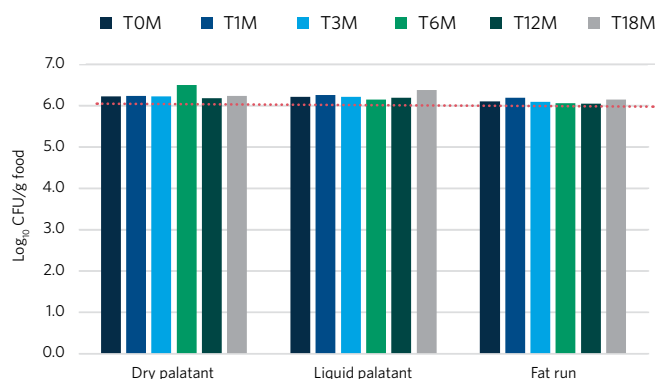
Figure 4. Stability of CHR. HANSEN PET-PROESSENTIALS™ (Source: Shelf-life CHR-HANSEN-PET-PROESSENTIALS_Standard_25C_24M_FormatGlobal_English_2023-04-12_SFL0161-1)



STABILITY AND VIABILITY OF PROBIOTICS IN YOUR PET PRODUCT

Understanding the fundamentals of stability and viability of probiotics is key when planning for probiotic inclusion in pet products and their testing. At the same time, it is paramount to have a clear idea of the manufacturing process of the pet product. Most pet products manufacturing will include steps with high moisture, high temperature, or both. These steps need to be carefully considered when planning the application of probiotics. For example, in the manufacturing of dry extruded pet food adding them to the mix of ingredients that will go through the high-temperature extrusion process will result in a major if not total loss of viability of probiotics. The recommended step to apply probiotics would be during the coating of the kibble. At this step, probiotic products can be applied through the fat that is coated onto the kibble, previously mixed with palatants and then coated, or, depending on the equipment, they can be applied directly on the kibble. With this in mind, a trial was run to evaluate the impact of the application of probiotics during the coating of dry kibble (post extrusion). CHR. HANSEN PET-PROESSENTIALS™ probiotics were shown to survive the application with the three methods, as well as to remain viable for up to 18 months when applied to kibble (Figure 5).

Figure 5. Viability of CHR. HANSEN PET-PROESSENTIALS™ when applied to kibble (Source: Trial 80841)



In general, pet products also have very complex matrices that can impact the viability of probiotics. Stability and viability testing can be performed to better understand the impact of the matrix and particular processes. This testing follows international standards and can be done in real-time or accelerated by using harsher conditions. These conditions are determined depending on the geography and weather in which it will be used.

ZONE	REAL-TIME		ACCELERATED	
I - II	22 - 25°C	40 - 60%	37-40°C	40 - 60%
	72 - 77°F	RH	98-104°F	RH
IV	30°C	65% RH	40°C	75% RH
	86°F		104°F	

On one hand, real-time stability is considered the gold standard. However, in the fast-paced product development cycle, sometimes data is needed in a shorter time. On the other hand, accelerated stability can provide a quick read of the stability of probiotics, but the higher temperatures used for the accelerated testing can exceed the biological capacity of some species. Therefore, the stability testing of probiotics needs to be designed carefully, considering all the factors that could influence it and the market where the products will be commercialized.

SUMMARY

Species, temperature, and water activity can have a significant impact on the stability and viability of probiotics. However, there is a diverse range of matrices and processes in the pet food industry that could also impact the viability of probiotics in specific pet products in ways that cannot always be fully predicted. Using a science-based approach, testing of viability post-application and during the product's shelf-life will help ensure the right probiotic inclusion and data to support label guarantees.

SCAN & LEARN MORE
about our ProStability Program



REFERENCES

Available upon request.

OUR LIVE PROBIOTICS WILL MAKE YOU SMILE

Our LIVE PROBIOTICS are stable and remain viable up to 18 months post-production in pet food, treats and supplements.*

Having that peace of mind about label guarantees will make you smile, but even better - it will make your regulatory team happy.

TRY US IN YOUR PRODUCT.

* Viability results may vary.



CHR HANSEN

Improving food & health