Understanding the Drivers for Palatability:

FROM BASIC SCIENCE TO COMPLEX PRODUCT SYSTEMS

To understand the drivers for palatability, we need to understand how animals experience the food they eat – their biology – as well as having a comprehensive knowledge of the stimuli to which they are being exposed.

This requires fundamental research into what animals can perceive, the modulators of that perception, how these perceptions are translated into behaviors, as well as research to identify those characteristics of the palatant in the context of the finished product that determine the animal's acceptance and intake.

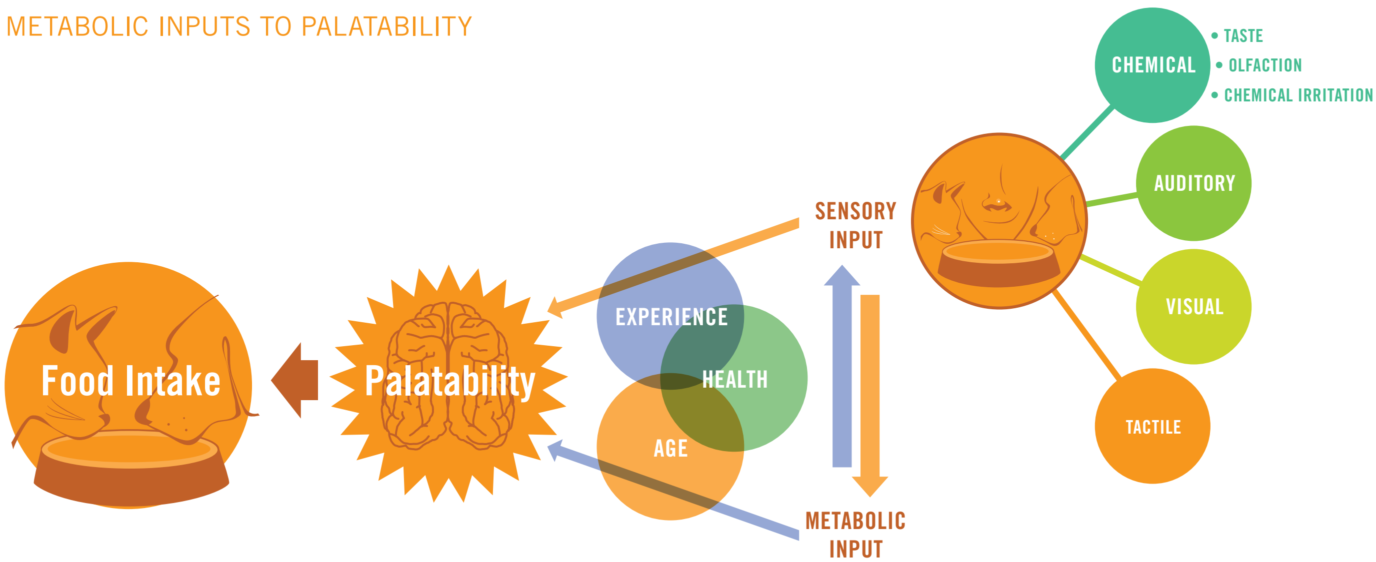
Palatants are complex systems comprised of many different macro and micromolecules (including carbohydrates, fatty acids, proteins, peptides, amino acids, vitamins, etc). These ingredients serve multiple functions, enhancing the sensory experience of the companion animal and its owner, masking unpleasant taste compounds and improving appetite in an animal that is failing to eat due to health issues. The palatant must appeal to all the sensory capacities of the animal – olfactory, chemesthetic (chemical irritation), taste and texture, and may even need to accommodate or enhance the visual or auditory impact of the product. In addition, the palatant must work in combination with the product on which it is applied, which itself is a complex mixture of tastes, textures, and aromas with differing chemical, physical and sensory characteristics. Accordingly, palatants may incorporate diverse constituents designed to enhance, mask, stabilize and/or synergize with the overall sensory impact of the base product.

To design palatants that effectively achieve these objectives, while also working within manufacturing, regulatory, marketing and resource constraints, a research and development process is needed that encompasses both understanding what is driving the animals' feeding behavior and flavor preference as well as what finished product characteristics are meeting these needs.

1. Dissecting palatant drivers: understanding the sensory experience

In view of this complexity, how can we dissect the sensory drivers for flavor preference? Palatability in animal feeding is classically defined in terms of relative preference for one product over another. The metric most often used to determine this preference is amount consumed over a typical feeding period of product A vs. product B, and can also include consideration of the product approached first and/or tasted first. Some research laboratories may further quantify rate of eating, number of eating occasions or amount consumed per eating occasion. Researchers may also consider behaviors other than consumption that may relate to 'enjoyment' or the owner's perceptions of their animal's enjoyment. These latter types of studies are far more technically challenging and resource intensive, but can yield valuable information and insight into consumer behavior and market success.

FIGURE 1: DIAGRAM DEPICTING SENSORY AND METABOLIC INPUTS TO PALATABILITY



Understanding the "why" of palatability is more challenging than determining "what" product is consumed more in a two-pan feeding trial, and requires consideration of both internal and external factors that may influence an animal's eating behavior and flavor preferences (Figure 1). Recent research using neural imaging and recording techniques has clearly demonstrated that "flavor" is a central percept requiring inputs from multiple orosensory modalities. This perception, reflected in the observed preference for, or palatability of, a product, is a function of sensory and metabolic inputs, which are filtered or modulated through the influence of prior experience, age and health factors. Metabolic processes are monitored to direct behaviors to insure that metabolic needs are met. Hormonal pathways involved in this monitoring also modulate sensory pathways to shift sensitivity to the stimuli as well as directing the central pathways involved in regulation of metabolism.

Standard palatability tests are excellent at demonstrating the "what", but are less effective in revealing "why". To design effective palatants in a systematic and directed fashion, it is essential to look beyond "what" works and work towards an understanding of "why" a product works or fails. When we approach this challenge, we quickly discover that there are many basic questions we must first answer. For instance, we need to know what cats can taste or smell, what they can discriminate, what modalities of sensory stimuli are more/less important in their determining their food preferences, and what internal and external factors influence how they perceive and respond to these stimuli. We know a great deal about many of these questions for human and rodent consumers. In contrast, our knowledge of these topics in the companion animal field, while growing rapidly, remains comparatively rudimentary.

To address these kinds of questions, we can draw from methods used in human sensory psychophysics, animal behavior (ethology) and behavioral neuroscience research. These disciplines provide an excellent foundation for approaches and experimental designs, however the tools and techniques must often be adapted to the particular features and challenges of the companion animals we study and the context in which we must study them.

When studying human flavor preference, it is easy to ask individuals to rate detection, similarity ("discrimination"), liking ("hedonics") or intensity. With training, humans can provide accurate and detailed qualitative descriptors for flavors, rate with accuracy the degree of difference in qualities or intensity, or how much they like a product using quantitative, validated psychophysical assessment tools. With nonverbal subjects, this task becomes far more challenging. Methods developed for use with a variety of nonhuman animal models have been developed. Many of these require a protocol designed to equalize or maximize the animal's motivation to exhibit the general behavior. For instance, rodents are deprived of water overnight in order to motivate them to drink immediately in short-term two-bottle tests or to make water a rewarding stimulus in operant conditioning studies. In the companion animal world, the comfort of the animal is paramount and methods that use food or water restriction are not likely to be acceptable. Consequently, we must adapt our methods to work within the animals' normal level of motivation and behavior. This requires adopting a perspective akin to the ethological scientist, whose goal is to observe animals' natural behaviors in order to understand them, rather than controlling or inducing a behavior in order to manipulate one factor at a time with the least potential for noise. While the latter may facilitate mechanistic understanding, the former will provide a closer link to the 'real world' behaviors that we are trying to influence. So how do we approach these questions?

WHAT CAN AN ANIMAL DETECT?

The two-bottle test is the simplest method for determining taste preference or aversion thresholds (the lowest concentration which the animal exhibits a preference for or an avoidance of). Water bottles may be connected to drinkometers ('lickometers') which record the number of licks, and observation periods are minimized to reduce the potential for positive or negative post-ingestive effects to contribute to the response. Automated feeding stations can be used for similar studies using solid matrices. AFB has developed and validated a two-tube method to determine the responses of individual cats to flavored solutions (Figure 2a). Our initial series of tests explored the perception that "cats like sour taste" [1]. A review of the available data failed to produce any studies that specifically addressed this question, and our results to date do not support this simple statement. Our experiments took advantage of the ability to manipulate sourness while controlling pH, as sourness

FIGURE 2: A) Graph showing preference data comparing average intake over 1 hour of a highly palatable flavor vs. water using the two-tube testing method ($n = 12$, $p < 0.001$). **B)** of citric acid or water ($n = 12$, NS). IR: Intake ratio: volume citric acid/total consumed. Average intake (± sd).

FIGURE 2A

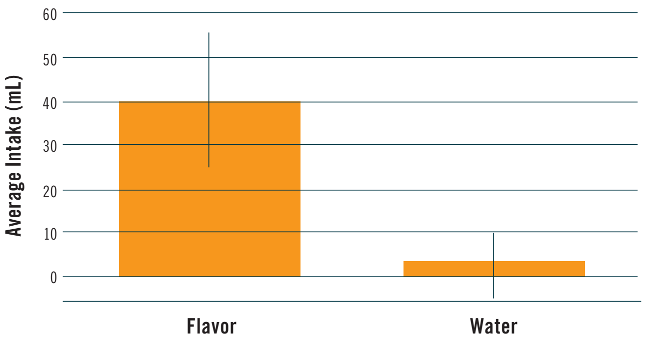
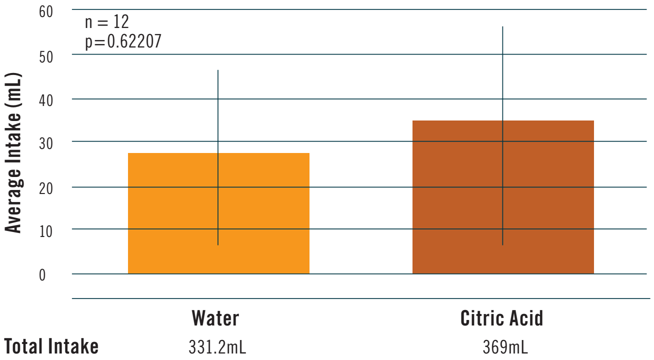


FIGURE 2B



is determined by titratable acidity [2]. Citric acid has a higher titratable acidity than water at the same pH, so we measured intake of citric acid vs. water at pH = 5.7. To humans, these solutions are discriminable based on sourness. Based on a series of experiments like this, our data indicate that the cats' response to sour taste stimuli is far more complex and is not consistently positive (Figure 2b). This may indicate fundamental differences between cats and humans in their ability to detect and discriminate these solutions, as well as differences in their response to other characteristics of these acidic solutions. While research continues, challenging this type of assumption is important to support data-based palatant design, and more quickly and efficiently create palatants that target those sensory modalities with true impact on performance.

The two bottle/pan methods provide preference threshold data, and if a preference is demonstrated, one may conclude that the animal detects the stimulus. This is not necessarily the case however. Preference for one stimulus may reflect true preference for one of two sensory stimuli, or failure to detect one of the stimuli at the concentration presented. In the latter case, a different concentration of the apparently less preferred stimulus might be preferred. Alternatively, an animal may be avoiding a detected stimulus and preferring a non-detected one; this could lead to inclusion of a non-detected ingredient that isn't really contributing to a positive palatability impact. If no preference is demonstrated, the animal may detect both stimuli but fail to discriminate them, in which case they may appropriately be considered comparable. However, the animal may detect both and be capable of discriminating them, but like them equally – which could lead to substitution of an ingredient concluded to be comparable that may not perform comparably in different settings because it actually has a distinct flavor. To understand what animals can detect and discriminate, more elaborate behavioral methods are needed.

Operant testing systems in which animals are taught to associate a taste or odor with a reward (or punishment) are used to determine whether animals can detect or discriminate sensory stimuli such as specific taste or aroma compounds, how similar the stimuli are qualitatively, and to determine detection threshold concentrations. These methods are extremely powerful and also permit studies to better understand factors that promote or impair flavor-based learning, which can markedly influence the response to a particular diet in standard palatability tests. One common paradigm involves water-depriving a rodent and using water as the positive reward. The animal must perform a task (such as a bar press or a nose poke) to obtain the reward, and this reward is only available when a specific stimulus is presented. Multiple stimulus delivery ports and bars can be used to test for detection of a stimulus vs. water or discrimination of two stimuli. When working with companion animals, we must adapt these techniques to avoid adverse treatments and work within the behavioral repertoire and motivation level of the animals. However, the basic principles of conditioning are used extensively in training companion animals, and can readily be applied to the task of understanding flavor perception.

Facial reactivity and behavioral analysis techniques are also being used in palatability research with rodents [3], human infants [4] and cats [5]. Facial reactivity scoring involves quantitative analysis of facial movements that reflect innate reactions to pleasant or unpleasant orosensory stimuli. These prototypical mouth, tongue and facial responses reflect fundamental rejection or acceptance of oral stimulation with taste or flavored solutions. Whole animal behavioral analysis can also lend insight into the basis for owner interpretations of their pet's eating experience to identify specific behavioral responses to target. AFB is adapting these and other methods to fully understand our palatants' performance from the animal's perspective.

2. Dissecting Palatant Drivers: understanding the complex product system

While we work to explore the animal's sensory experience using simple flavor systems and basic research tools, we must also work to understand the major and significant components inherent in the complex palatant system. In order to break down and dissect the specific performance-driving components inherent in a palatant, it is important to evaluate other factors beyond the common proximates which are usually measured in the industry. Therefore, since palatants consists of hydrophilic as well as hydrophobic molecules, some of which drive different chemical aspects (taste, olfaction, chemical irritation) of the sensory input of palatability, other factors including chemical, physical, and textural components will need to be evaluated. As a result, a number of different analytical methods and techniques are usually employed for breaking down the palatant, and characterizing these components. These include chromatography (liquid and/or gas), as well as detection techniques such as mass spectrometry, flame ionization, refractive index, diode array, and ultraviolet methods.

Taste: To the pet owner, there are five different universal taste characteristics namely sweet, salty, sour, bitter, and umami. Therefore, depending on the composition of a palatant relative to its protein, amino acid, fatty acid, and vitamin contents amongst others, it could exhibit one or more of the taste characteristics. Isolation and dissection of the taste active components of palatants employ techniques such as liquid chromatography coupled with mass spectrometry, refractive index, or fluorescence detection methods, and spectroscopic methods. In chromatography, the palatant components are separated based on their interaction with specific packing media in a separation column, while the components are separated according to their interactions with energy in spectroscopy. Other unique techniques which AFB has being using include electronic tongue technology [6], which employs cross selective metal oxide sensors for discriminating and screening the different taste profiles of palatant systems.

In this regard, research work at AFB has shown that the amount of certain taste active, fat-containing chemicals are essential for optimum performance of select palatants. Furthermore, a group of taste active amino acids has shown positive correlations to the performance of these palatants. Therefore, these parameters are now being incorporated for use in monitoring palatant consistency and performance. In addition, this insight is informing our basic research to better understand the sensory mechanisms and behavioral responses to these materials.

Olfaction (Aroma): Upon approaching the diet containing the palatant, the first thing the animal experience is the aroma or smell which is determined by the volatile and semi volatile composition of the palatant system. The overall aroma profile of a palatant has a significant contribution to the animals' overall choice. The most common method used for dissecting this part of the palatant make up is called gas chromatography coupled with detection techniques such as mass spectrometry, flame ionization, and olfactometry. In this case, the palatant can be presented to the instrument for analysis in several ways, depending on the degree of sensitivity desired and nature of the palatant matrix. For instance, certain volatile chemicals such as alkenals, which are products of lipid oxidation, could provide off notes [7] when present or detected in palatants at certain levels. Off notes are detrimental to optimum performance of the palatant because they suggest the possibility of product oxidation. A fresh palatant will have optimal performance as opposed to an oxidized product. Other identified chemicals with positive impact on palatant performance include level and type of esters present in a palatant containing yeast-type materials.

Texture: This is another important parameter with significant contribution to palatant performance. For dry palatants, relevant textural parameters for dissection in the product system include cohesiveness, and caking. Also included are other relevant physical parameters which affect texture indirectly, such as particle size, shape, coarseness, and density [8, 9]. Viscosity is another important parameter for liquid palatants. From AFB's research, the degree of flowability of a dry palatant has direct correlation to its level of performance.

Therefore, AFB has developed and instituted a process called the Product Champion Program. This program provides a comprehensive and extended approach through which palatants are evaluated for critical in-process and finished product factors driving final performance. Once these performance driving factors are identified and validated for direct correlation to palatability, palatants are subsequently monitored using these important factors throughout the product's lifetime. Sensory research methods such as those described above can then be used to help understand how attributes identified through this program impact the animal's eating behavior and food preferences.

Combining research perspectives from the "nose" to "tail" – from the animal to the production floor and back again - enables AFB to manufacture and deliver consistently performing palatants to insure our customers' success in the market.

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