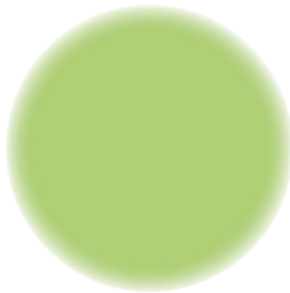


SCIENTIFIC RELEASE //
ENSURING RELIABLE
PALATABILITY MEASUREMENT



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INTRODUCTION

Palatability is a critical attribute that could transform a petfood product launch from top... to flop! That's why petfood manufacturers truly need to have a specific and objective evaluation of their products' palatability. When conducted properly, palatability testing is a useful scientific and marketing tool they can rely on to make strategic decisions related to product development, formula optimization and positioning versus competition.

Two-bowl testing and monadic feeding are the main palatability measurement methodologies commonly used by pet food manufacturers. While these methodologies are quite different, they both provide valuable information when using specific and controlled parameters to ensure maximum testing reliability. For instance, environmental control and stability, combined with the protocol's accuracy, are indispensable in attaining consistent palatability results. However, even with the best management of these critical points, some biases can occur when working with animal panels. These biases, that can appear both in in-home and expert panels, can significantly distort test results.

One of the responsibilities of palatability measurement experts and animal behaviorists is to continuously define preventive and corrective actions to guarantee reliable answers. PANELIS has long-term experience in the two-bowl testing methodology and has recently expanded its expertise in the monadic feedings method. Thanks to this strong know-how in palatability measurement, PANELIS established several good practices to avoid measurement biases. The main criteria for ensuring reliable palatability measurement are presented below.

A LARGE PANEL SIZE

The fewer animals in a panel, the higher the risk of unreliability! Results of two-bowl palatability tests are usually analyzed using Student test. However, literature about statistical analysis states that this type of parametric tests can only be used when mean distribution is normal. In order to obtain a mean's normal distribution, expert statisticians recommend always working with a minimum population of 30.

In the particular case of palatability testing where individual variability is significant, data generally show bimodal distribution. Figure 1 confirms that for this type of data, normal hypothesis is verified when the sample size is above 30.

PANELIS systematically conduct its palatability tests with 36 dogs or 40 cats. In order to confirm panel size's impact, the results of 3 two-bowl tests conducted with 36 dogs during two days, and 3 two-bowl tests conducted with 40 cats during two days were statistically re-analysed considering only 20 individual animals randomly selected from the initial full panel.

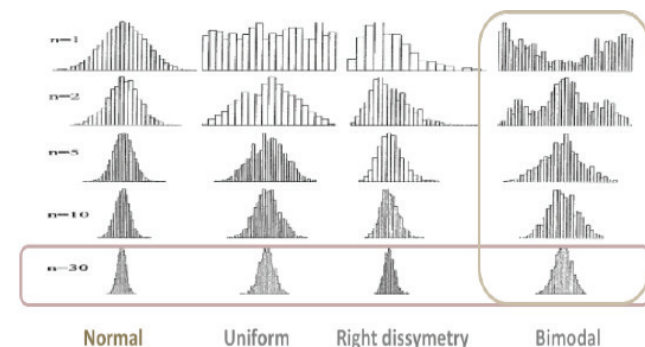


Figure 1 : Impact of sample size (n) on distribution
 (Source : Pagès J. (2005), Statistiques générales pour utilisateurs - tome 1, méthodologie)

3 categories of tests were chosen for statistical analysis:

- **Tests A = B:**
same kibbles - no significant difference (NS) expected
- **Tests A > B:**
same kibbles with different palatability enhancer dosages or nature - significant difference (S) expected
- **Tests A >> B:**
coated kibble versus uncoated kibble - highly significant (HS) to very highly significant (VHS) difference expected

For each type of test, 10 000 random samples of 20 individual animals were used to evaluate the answers that would have been obtained with smaller sample.

For both cats and dogs, in more than 98% of cases, the results obtained for A=B and A>>B trials on small samples were equivalent to those obtained on larger samples.

For A > B trials, 30% of the simulations with 20 cats led to non significant differences between A and B, while the difference was significant with 40 cats. For dogs, the same discrepancy was observed although less pronounced: 15% of the simulations with 20 dogs showed non significant differences between A and B, where the result with 40 dogs was significant.

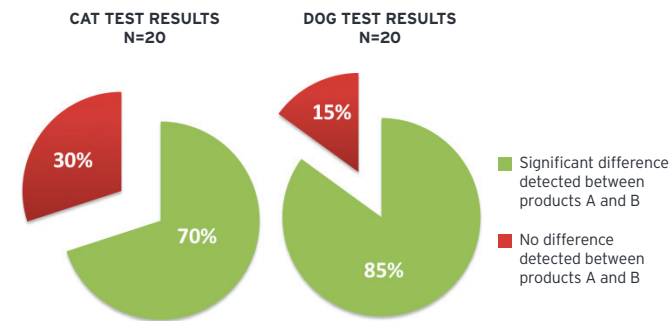


Figure 2 : Percentage of test results showing significant or non significant difference between A and B when running simulations with n = 20 animals. Expected result is a significant difference between A and B (A>B).

These results confirmed the need to work with the highest number of pets to get an accurate palatability measurement, especially when difference between products is small. This panel-size effect is observed in both two-bowl and monadic feeding methodologies and is even stronger for trials performed with in-home panels due to the owner's interaction with animal and other environmental effects.

According to PANELIS' experience, palatability tests conducted in expert panels should include a minimum of 30 individual animals while palatability tests conducted in home should include around 100 animals to avoid biases.

The more pets, the better!

- Expert panels: 30 individuals minimum
- In home panels: 100 individuals minimum
- Individual variability counts: when assessing palatability difference between 2 products, 2-day testing with 40 pets is much more powerful and representative than 4-day testing with 20 pets!

A REPRESENTATIVE POPULATION

Pet food market segmentation according to size and breed is a global and lasting trend, especially in dog food.

With 600 cats and 250 dogs worldwide representing more than 60 breeds, PANELIS is a unique expert "collection" of pet food's final consumers. Conscious of the importance of preparing well-balanced dog-panels in terms of size category, PANELIS created panels that are most representative of targeted populations.

In particular, toy dogs have recently been introduced into PANELIS' existing multibreed dog-panels in order to follow the growing importance of the small dogs segment in petfood market. Small and toy breeds are known as fussy dogs. This may be partly explained by their living environment and their "education", however, even in expert panels, they express specific feeding behaviors.

In order to objectively evaluate this difference in feeding behavior, PANELIS decided to analyse the results of 1 329 two-bowl tests conducted between January and June 2012 in its multibreed dog panels. Results of the study are presented in figure 3.

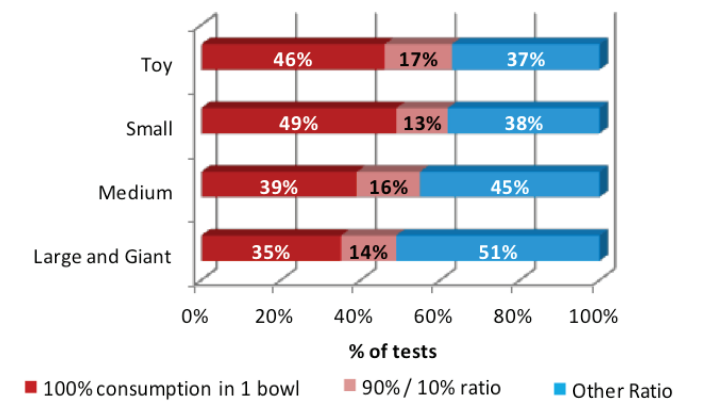


Figure 3 : Distribution of high ratios according to dog size
 PANELIS data - 1329 Tests January-June 2012

Results showed that small and toy dogs make more marked choices compared to bigger dogs; when they prefer a product, they tend to show higher ratio scores. In this study, around 63% of toy and small dogs' consumption ratio was between 90/10 and 100/0 (1 bowl) while it was 55% for medium and 49% for large and giant dogs' ones.

However, it is important to note that no specific preference according to size was identified during this study; the only difference according to size was the degree of discrimination.

- Small and toy dogs make more discriminate food choices
- Unbalanced panels in terms of dog-size categories can lead to misinterpretation (skewed or less discerning differences)
- Panel's population should be as varied as possible: males/females, intact/neutered pets, pure breed/mixed breeds...



DIETARY PAST

When facing a new food, dogs and cats express a more neophilic behavior than a neophobic one; they often prefer novelty, at least as a first spontaneous reaction. Some cats may even show a clear preference for change or variation from familiar food: this phenomenon is called metaphilia.

Preferences are rarely definitively fixed and behavior can evolve depending on feeding experiences:

- Preference can be reinforced by a long exposition to the product (habituation) (Bradshaw et al., 2000)
- Some animals develop weariness towards the usual diet, inducing palatability decrease (O'Malley, 1998)
- Other animals continue to show a stable preference for the usual diet (Bradshaw, 2000, Larose 2004)

PANELIS in partnership with SPF have conducted several studies to evaluate the impact of dietary past on animal feeding preferences. In 2004, they pointed out that strong food habits play an important role in animals' choice (Larose, 2004). In 2010, a new study confirmed the impact of pre and postnatal olfactory-gustatory exposure via maternal ingestion on kittens (Becques et al., 2010).

In 2012 PANELIS and SPF assessed the link between cat's level of exposure to a product variable and its individual preference for this variable (Forges et al., 2012). An extensive statistical analysis was made on 350 000 data collected by PANELIS during 28 months of tests conducted on 8 panels of 40 cats. Figure 4 shows an example of results obtained during this study.

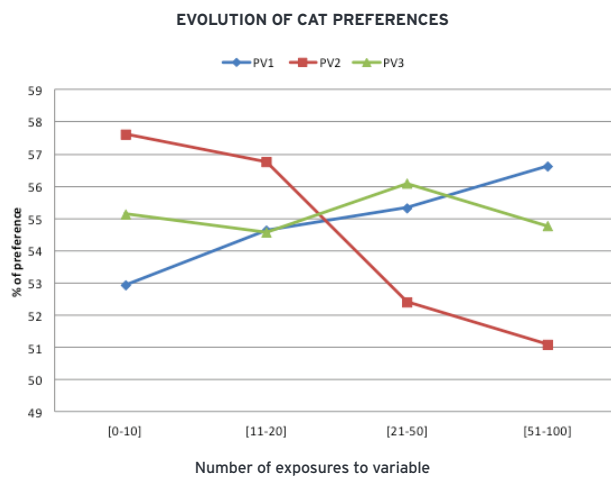


Figure 4 : Individual preference evolution in cats according to their exposition degree toward 3 products variables

In the above example:

- Preference for product variable 1 (PV1) increased through repeated exposure
- Preference for product variable 2 (PV2) decreased through repeated exposure
- Product variable 3 (PV3) was preferred from the first exposure and remains stable throughout time

This study pointed out how complex the development of feeding preferences can be. It confirmed that cats' preferences evolve in relation with their feeding experience and that some precautionary measures need to be taken to limit panels' answer bias induced by feeding past.

- Recruit pets as young as possible and feed them a high diversity of foods = food training
- Perform tests on at least 2 meals for strategic decisions
- Follow-up on quality indicators such as pets' results on qualification trials and on tests performed in external panels for comparison, levels of repeatability...
- Schedule product tests series on different panels
- Regularly reorganize panels by combining sub-groups differently ("standardization")

BOWL POSITION EATERS BIAS

During a versus palatability trial, dogs and cats are offered the choice between two products in two bowls. Whatever the products tested, some pets systematically eat from the right or left-hand bowl. They are called "position eater".

Different scientific studies showed that dogs and cats as other species can be lateralized (Pike & Maitland, 1997). In dogs, Wells' findings revealed that lateralized behavior was highly gender-related (Wells, 2003). Wells established the same link in cats and also pointed out a possible relationship between lateralized behavior and task complexity (Wells and Millsopp, 2009). This lateralized behavior has been examined as a manifestation of cerebral functional asymmetry.

No literature can be found on position eaters in palatability testing centers. However, behavior observed during palatability testing is probably not linked to cerebral functions but might be attributed to several other factors such as the degree of difference between the compared products, product's nature, test system, type of protocol, health pattern, etc.

Since this bias can strongly affect palatability results coming from two-bowl testing, it is important to clearly identify the "true side position eaters" within panels. These "critical" pets, more often cats than dogs, are the ones showing persistent bowl-side bias regardless of the products tested or the environmental conditions.



Ensuring reliable palatability measurement

In order to identify critical cats and dogs within a population, PANELIS has set up a specific indicator: the Lateralization Index (IL). Calculated every three months, this index considers the frequency of meals during which a significantly higher intake is observed on one side. PANELIS then defined acceptability margins based on knowledge of its pets' usual feeding behavior (intake levels, reaction to minor palatability differences...) in order to exclude the critical pets' answers from the data analysis.

Several good practices can be followed to avoid bowl position eaters bias. In addition to defining a relevant lateralization index, the regular renewal of pets in panels is an essential preventive action to limit the impact of true side position eaters on palatability results.

- Identify true side position eaters by setting and following a Lateralization Index
- Exclude true side position eaters only
- Renew panels regularly (at least 10% of the panel/year)

CONSUMPTION VARIABILITY

Consumption variability has been observed in both PANELIS and other dog panels worldwide. PANELIS researchers have clearly noted that consumption variability was sometimes stronger due to parameters such as:

- **Meal time:** morning meals showing lower intake levels than afternoon meals
- **Season:** lower intake ratios in the summertime, possibly linked with temperature, photoperiod or metabolism regulation

Figure 5 illustrates food intake variation observed in PANELIS dog panels according to the meal time and the season. The consumption of three dog panels on a three years period were monitored and analysed.

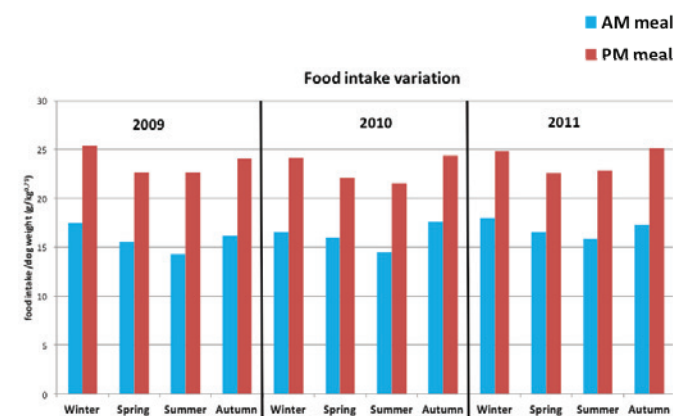


Figure 5 : Dog feed intake variation according to meal time and season (PANELIS data)

These results also explain why it may sometimes be more difficult to get accurate conclusions with monadic feeding trials than with two-bowl tests. Indeed in versus tests, pets are asked to make a choice between two products offered simultaneously. Whatever the consumption level, if there is a difference between products, versus test will be more consistent in detecting it.

Preventive actions to counterbalance consumption variability in monadic feeding trials:

- The more pets the better... again!
- Repeat test on at least 2 meals
- Randomize presentations for multi-product trials



WELL-BEING

Last but not least, PANELIS has always been strongly committed to pets' well-being within expert testing centers, from first an ethical point of view, but also from a scientific one. Indeed, no reliable information can be expected if animals are stressed. Representative answers can only be obtained with healthy, happy and unstressed pets.

In "Pets' Resort by PANELIS®", cats and dogs enjoy living conditions modelled after-real a life home environment. They are actively stimulated and socialized through varied activities such as education, grooming, running and also canine and feline show with skilled and devoted animal technicians.

Representative and reliable answers can only be obtained with healthy, happy and unstressed pets!



● CONCLUSION: BUILDING EXCELLENCE IN PALATABILITY MEASUREMENT

The risk of generating false conclusions from palatability trials exists due to biases that can appear if protocols and panels are not properly controlled. However, various solutions exist to minimize this risk of unreliability.

PANELIS continues developing its expertise and understanding of dog and cat feeding behaviors by implementing additional methodologies such as monadic trials, shorter screening trials, and video observations. The combination of all these complementary methodologies provides us with a deeper understanding of biases and their possible control and allows us to measure new “petcentric” criteria reflecting behaviors identified as meaningful for the pet owners. PANELIS expert panels are now measuring pet foods’ palatability not only with intake ratios, but also with innovative criteria identified at home to be significant signs of pets’ satisfaction.



**Multiple eyes, one vision:
excellence in palatability measurement.**

● IF YOU NEED FURTHER INFORMATION, DO NOT HESITATE TO CONTACT THE AUTHORS



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