Microbial Protein as a Novel Ingredient in Pet Food A Sensory Acceptance Study in Dogs and Cats

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Animal Ethics Statement: This study involved a non-invasive, in-home palatability assessment conducted with client-owned dogs and cats in their habitual domestic environments. All procedures adhered to the principles of animal welfare outlined in Directive 2010/63/EU. No experimental interventions, stress-inducing procedures, or confinement were applied. Participation was voluntary, and informed consent was obtained from all pet owners prior to the study. As such, approval from an institutional animal care and use committee was not required under national and EU regulations.

Microbial Protein as a Novel Ingredient in Pet Food: A Sensory Acceptance Study in Dogs and Cats

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Abstract

The increasing demand for protein sources in pet nutrition has driven interest in microbial proteins (MP) as viable alternatives to traditional animal proteins. This study evaluates the palatability of MP in canine and feline diets, focusing on its sensory acceptance. A two-bowl preference test was conducted with 30 dogs and 30 cats to compare a control diet with a test diet containing 10% MP. Both diets were nutritionally equivalent, differing only in protein sources. Trials were performed in-home to ensure natural feeding behavior, and data on food consumption, first approach, and primary consumption were collected over four meals. Results demonstrated a statistically significant preference for the MP-containing diet in both species. Dogs consumed 72% of the test diet compared to 50% of the control ($p < 0.05$), while cats exhibited a similar preference for the MP diet ($p < 0.05$). Behavioral observations indicated that both dogs and cats approached and consumed the MP diet first, suggesting its enhanced sensory appeal. These findings, aligned with prior research highlighting the high digestibility and balanced amino acid profile of MP, further support its suitability as a sustainable protein source. The study underscores the potential of MP to meet nutritional requirements while enhancing palatability, addressing key challenges in sustainable pet food development. However, limitations such as sample size and trial duration warrant further research, including long-term feeding studies and detailed sensory analyses. This study represents an exploratory investigation into the palatability of microbial protein in pet diets, using validated in-home preference testing as a practical first step in assessing sensory acceptance. While the scope was intentionally limited, the findings offer meaningful initial evidence supporting the feasibility of MP incorporation into pet food. As such, the study lays the groundwork for expanded future research focused on long-term feeding outcomes and optimization of palatability.

Keywords

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1 Introduction

The increasing trend of pet humanization has shifted the focus towards providing optimal nutrition to companion animals, which are now often regarded as family members [1]. This shift is accompanied by rising concerns about the availability and environmental impact of traditional protein sources used in pet food. Global meat demand is projected to increase by 75 % by 2050 due to population growth [2]. Notably, this projection does not account for the concurrent increase in the global pet population, which further amplifies the demand for protein-rich diets to meet animals' nutritional requirements. Insufficient intake of essential amino acids may lead to nutritional deficiencies and associated health complications [3]. As global protein demand continues to rise amidst stagnant production volumes of traditional sources, alternative proteins with lower environmental footprints have gained attention as viable solutions [4]. Microbial Proteins (MP) have emerged as promising alternatives to traditional animal proteins in both human and animal nutrition [5]. Produced through efficient and scalable fermentation processes using microorganisms like fungi, yeast, and bacteria, these proteins offer a unique combination of nutritional and environmental benefits. Prior studies have demonstrated that MP exhibit high digestibility and an excellent amino acid profile, making them nutritionally comparable or even superior to conventional protein sources [6, 7]. These characteristics are particularly advantageous for meeting the dietary requirements of companion animals, which depend on balanced amino acid intake for optimal health. Additionally, MP production requires significantly less agricultural land and water while emitting fewer greenhouse gases compared to conventional protein sources, making them an attractive solution for addressing the challenges of sustainable protein production [5]. In the context of pet food, however, the success of such alternatives depends on more than just sustainability and availability. A viable protein source must meet nutritional standards while also being acceptable to pets from a sensory perspective [4]. This integration of environmental, nutritional, and sensory criteria is critical for ensuring the market success of alternative proteins in pet food formulations. The acceptance of alternative proteins is heavily influenced by sensory characteristics such as taste, texture, and aroma [4]. As a result, palatability is a critical determinant in ensuring that pets readily accept these novel protein sources. Consequently, palatability trials are indispensable for evaluating how pets perceive these proteins within their diets and for facilitating their seamless incorporation into pet foods without compromising sensory appeal or overall acceptance [8]. Evaluating the palatability of pet foods typically involves standardized "consumption" tests conducted in controlled environments like kennels or in a natural home setting via in-home use tests (IHUT). These tests offer flexibility in terms of the breeds and sizes of animals used, allowing researchers to gather data across diverse pet populations [4, 8]. Common methods include single-bowl acceptance tests and two-pan preference tests, which assess food appeal and preferences. Acceptability is defined as the extent to which an animal consumes sufficient calories from a food item to maintain health and performance, independent of sensory characteristics such as taste or smell [4, 8]. Preference, by contrast, reflects a comparative choice for one food over another, influenced by sensory factors like aroma and texture [9] . Aroma plays a particularly significant role, as dogs and cats rely heavily on their olfactory senses, enhanced by the vomeronasal organ (Jacobson' s organ), which amplifies scent perception and is influenced by environmental factors like moisture and temperature [4]The "first-choice" response and animal's initial approach to and consumption of a food is often used to assess the impact of aroma. Occasionally, animals reject foods accepted by others due to "conditioned aversion" resulting from prior negative experiences [10]. Behavioral responses like "neophobia" (fear of new foods) and "neophilia" (preference for novel foods) are frequently observed, particularly in cats but also in dogs [4]. Developing alternative protein sources for pet food requires balancing sustainability, nutrition, and palatability. Addressing these factors is essential to reduce the environmental impact of traditional meat production while ensuring that pet food remains both desirable and nutritious for companion animals [3, 4, 8]. The aim of this paper is to explore the potential of alternative protein sources for pet food with a particular focus on their palatability to companion animals. While nutritional adequacy and production efficiency are critical factors, this paper emphasizes the importance of sensory acceptance such as taste, aroma, and texture in determining the success of these novel proteins. This study investigates the impact of replacing traditional animal protein sources with microbial protein (MP) on the palatability of dry extruded diets for dogs and cats. By applying established palatability testing methods and evaluating behavioral responses to new foods, we aim to determine whether this substitution decreases, maintains, or enhances sensory acceptance. We further explore whether incorporating MP may improve the palatability of existing formulations. This work builds on previous findings regarding MPX high digestibility and balanced amino acid profile, while emphasizing its potential contribution to more sustainable pet nutrition. Our central hypothesis is that microbial protein can be incorporated into pet food formulations without compromising palatability and may even enhance acceptance in some cases.

2 Materials and Methods

2.1 Study Design

For the palatability trial, In-Home Use Testing (IHUT) was used to allow for more representative outcomes of the pet preferences [11] . A total of 30 dogs (9 males and 21 females) and 30 cats (14 males and 16 females) participated in the study. All animals were registered in a database that included detailed characteristics, such as breed and weight. The dogs had a minimum weight of 3 kg and a maximum weight of 10 kg, with an average weight of 6 kg. For the cats, the weight ranged from 2 kg to 10 kg, with an average weight of 5 kg. The canine population had representatives from 11 breeds, while the feline one contained individuals from 6 different breeds. All animals were familiarized with the type of food being tested, specifically industrially produced dry food that is commercially available. All animals were described by the owners as being healthy, without history of chronical diseases or allergies. During the trial, each animal received the same amount of food they were accustomed to consuming in their daily routine. This feeding amount was carefully adjusted based on the age and weight of each animal to ensure consistency with their normal dietary intake patterns. This approach aimed to minimize variability due to differences in feeding habits while maintaining the nutritional needs of the animals.

2.2 Control and Test Formulas

The palatability of two formulas was evaluated in the trial. The control food was formulated to mimic a commercially available, complete, and balanced formula marketed with a 'Fish and Rice' flavor. Its composition included Cereals (12% rice), Meat and Animal Derivatives (34%), Poultry Fat, Krill (3%), Beet Pulp (1.6%), Hydrolyzed Animal Protein, Yeast, Carob, Minerals, Vitamins, and Yucca schidigera (0.001%). The test formula was identical to the control formula in nutritional composition but differed in its ingredient profile by including 10% Microbial Protein (MPX-Care from MicroHarvest GmbH). This substitution completely replaced Fish Meal and partially replaced Poultry Meal. Due to the inherently low-fat content of Microbial Protein, adjustments were made to the level of Poultry Fat in the test formula to ensure that the final nutritional analysis of the kibbles remained consistent. These modifications were necessary to avoid variations that could compromise the reliability of the palatability trial results. The composition of both formulas are in **Table 1** and nutritional analysis are described in **Table 2** . Both experimental diets were manufactured under controlled, identical conditions at Ovargado S.A. (São João de Ovar, Portugal), using an industrial-scale single-screw extrusion system (Andritz) with a minimum batch capacity of approximately 3,000 kg. The base mixture was preconditioned with direct steam injection and water addition to facilitate starch gelatinization and initial thermal processing. Extrusion was performed under controlled thermal and mechanical parameters, with barrel temperaturesranging from 120 ℃ to 140 ℃. The extrudate was cut at the die face to form uniform kibbles, which were subsequently dried to a final moisture content below 10 %. Post-extrusion, a portion of the total poultry fat (included at 7–7.5% in the formulation) was applied to the dried kibble using a rotary drum coater at ambient temperature and atmospheric pressure. The coating step included the sequential addition of fat and powdered palatability enhancers, including hydrolyzed animal protein and antioxidant compounds. All processing parameters - including preconditioning, extrusion, drying, and coating - were standardized in the control and test diets, ensuring that the inclusion of microbial proteins was the sole experimental variable.

2.3 Testing Locations and Product Delivery

The experimental trials were conducted in the animals' habitual feeding environments, predominantly in their domestic settings (Portuguese households). Test products were delivered to participants by Sense Test, Lda. (Vila Nova de Gaia, Portugal) technicians or representatives. During individual meetings, the trial conditions, protocols, and requirements were explained, and participants were provided with written instructions, which they were required to acknowledge before beginning the trial. Pet owners were instructed to feed the diets as part of their pets' routine feeding practices to ensure consistency. The products were delivered in appropriate containers, each clearly labeled with a code to identify the product and a generic description of the sample. All samples were coded to maintain blinding and minimize bias. For canine diets, the control formula was coded as 289, and test formula with MP 623. For canine formulas, sample 337 and 065 were, respectively, the control and test formula containing MP. The coding system ensured that participants remained unaware of the specific formulation of the diets, thus mitigating any potential subjective influence on the trial results.

Composition (weight percentage)		
	Control Formula (Fish and Rice)	Test Formula (containing MP)
Cereals	51%	51%
Poultry Meal	28%	22%
Microbial Protein	-	10%
Fish Meal	3.5%	-
Meat and Bone Meal	2%	2%
Poultry Fat	7%	7.5%
Krill	3%	3%
Beet Pulp	2%	2%
Hydrolyzed Animal Protein	2%	2%
Carob	0.2%	0.2%
Yeast	0.3%	0.3%
Premix	0.5%	0.5%
Yucca schidigera	0.001%	0.001%

Table 1: Composition of experimental pet food formulations used in palatability assessment.

Analytical constituents (weight percentage)		
	Control Formula (Fish and Rice)	Test Formula (containing MP)
Crude Protein	30%	30%
Crude Fat	13%	13%
Crude Fiber	2.5%	2.5%
Raw Ash	8.5%	9%
Moisture	8%	8%

Table 2: Analytical constituents of experimental pet food formulations used in palatability assessment.

2.4 Two-Bowl Preference Test

The two-bowl preference test was used to assess palatability and was carried out as follows: each animal was presented two bowls of food, one containing the test product formulated with MP and the other containing the control diet [4, 8] . To minimize potential bias, the positions of the bowls were alternated during each of the four meals. This systematic rotation was implemented to eliminate any potential side bias that could influence the animals' feeding behavior. Both bowls were placed in the animals' feeding area with food portions standardized to identical amounts for both samples, corresponding to the quantities typically provided to the animals in their daily meals. No other meals were provided to the pets during the duration of this study.

2.5 Data Collection and Analysis

The amount of food consumed from each bowl was recorded, and a preference score was calculated based on the proportion of food consumed from the test bowl compared to the control. Statistical analysis, including a paired t-test or Wilcoxon signed-rank test, was performed to determine whether there was a significant difference in food preference between the test and control products [4]. Participants also registered to which bowl their pet oriented first, from which one it ate first, and if the amount consumed in total was higher, lower or the same as in routine meals. These data were collected over two consecutive days, in a total of four meals per animal.

2.6 Ethical Considerations

The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. No animals were harmed during this study, as palatability tests were conducted in the animals' own residences under normal living conditions.

3 Results

3.1 Canine Diets

An IHUT trial was done with 30 dogs from different breeds (average weight 5.9 ±2.4 kg) to assess the palatability of formulations with or without Microbial Protein. Both formulations were equivalent from a nutritional perspective. In average, over the four meals provided, 91% ±3% of the participants reported that their dog ate the usual or more than the normal amount of food. Only 4 participants (13%) reported some alterations in the feces, with two reporting harder and the other two softer stool. This means that both formulations proposed were well adopted by the pet and did not represent a major deviation from their daily routine. Regarding palatability, the two-bowl preference test revealed that dogs exhibited a statistically significant preference for the test diet containing 10% Microbial Protein (designated as 623) over the control diet (designated as 289). Dogs approached first the bowls containing MP **(Figure 1)**, and these were the first being tasted **(Figure 2)**. The average consumption from the test bowl with Microbial Protein (72% of the total offered meal) was recorded to be significantly higher than that of the control (50%) ($p = 0.003$), indicating that dogs found the MP-enriched formulation more palatable **(Figure 1)**.

3.2 Feline Diets

A similar IHUT trial was performed, but this time with 30 cats (average weight 5.3 ±2.1 kg). Three out of four cats (75% ±2%) were reported to have maintained or increased their food consumption during the trial compared to their regular meals. Although this percentage was lower than observed in dogs, it remains significant. The high percentage (75%) of pets maintaining or increasing their food intake suggests strong market potential for the tested product, as it appears to be at least as appealing as the animals' habitual diets for a significant majority of the test subjects [12]. Similarly, for feline participants, the control diet (designated as 337) was compared with the MP-containing test diet (designated as 065). The results indicated a notable preference and acceptability for the test diet, with statistical analysis confirming a significant difference in consumption rates ($p < 0.05$). On average, during the four meals offered, 62% of the cats first oriented themselves to the bowl containing the food containing MP **(Figure 4)**, leading to 68% tasting it before the control formula **(Figure 5)**. On average, cats ate 51% of the sample containing MP compared to 34% of the control formula **(Figure 6)**. This difference was statistically significant ($p=0.009$), suggesting that cats also accepted MP inclusion favorably.

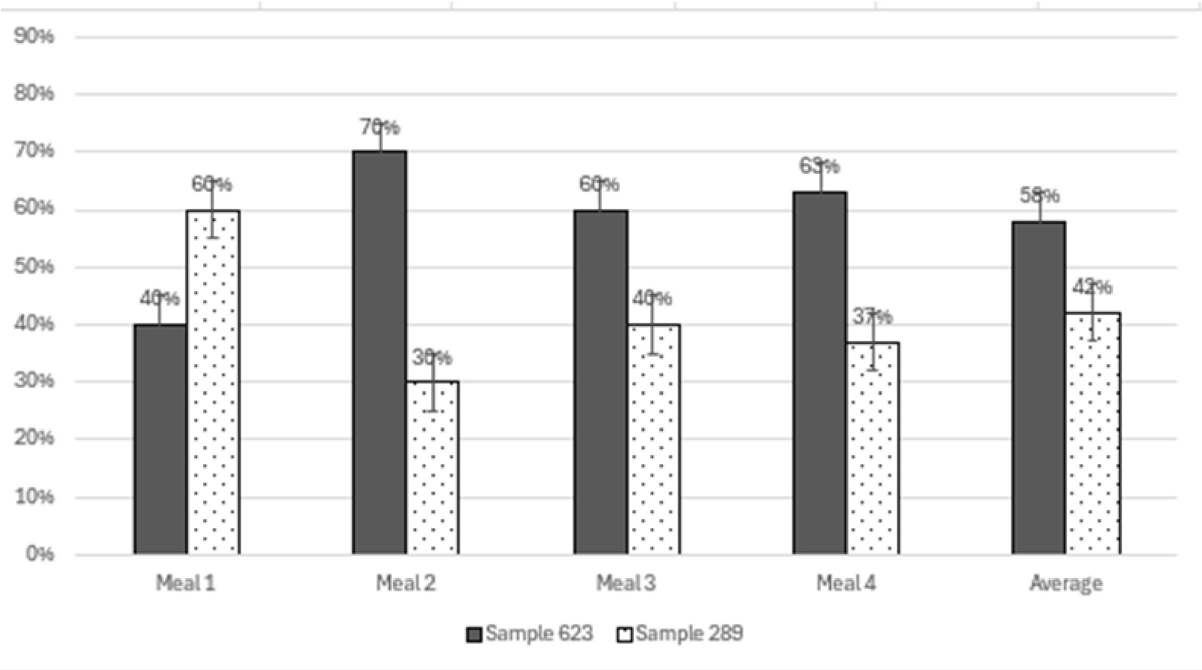

Figure 1: First approach orientation of dogs towards experimental food formulations. Meaning, sample to which the dogs initially oriented themselves, prior to intake. Sample 623: Test formula with Microbial Protein; Sample 289: Control formula.

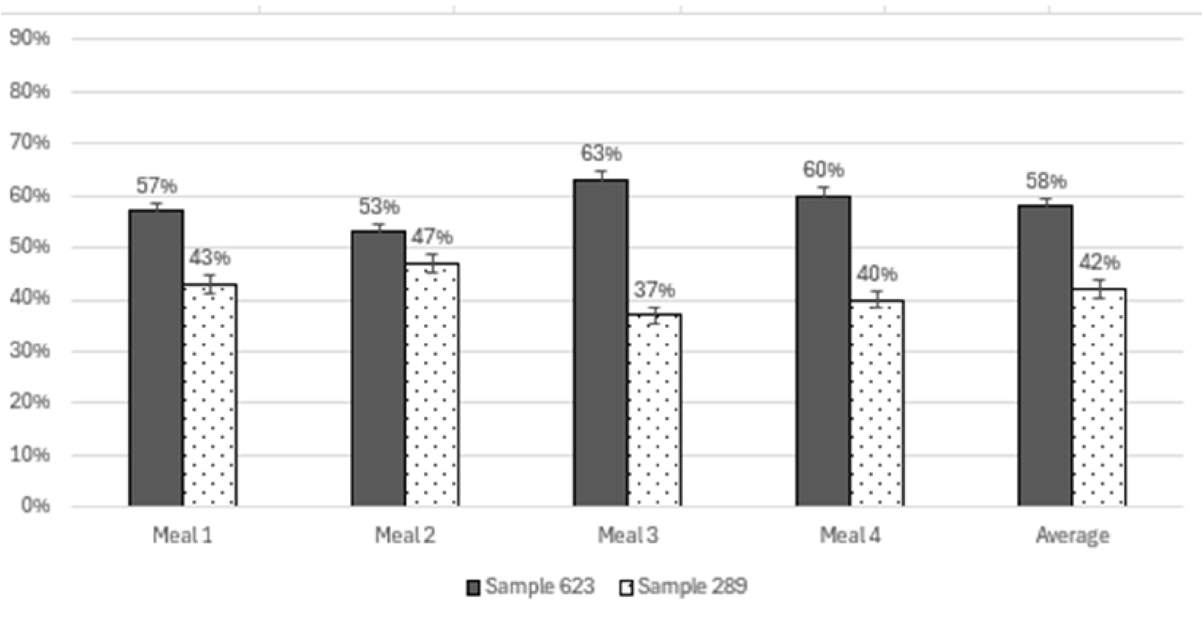

Figure 2: Primary consumption preferences of dogs between experimental food formulations. Sample 623: Test formula with Microbial Protein; Sample 289: Control formula.

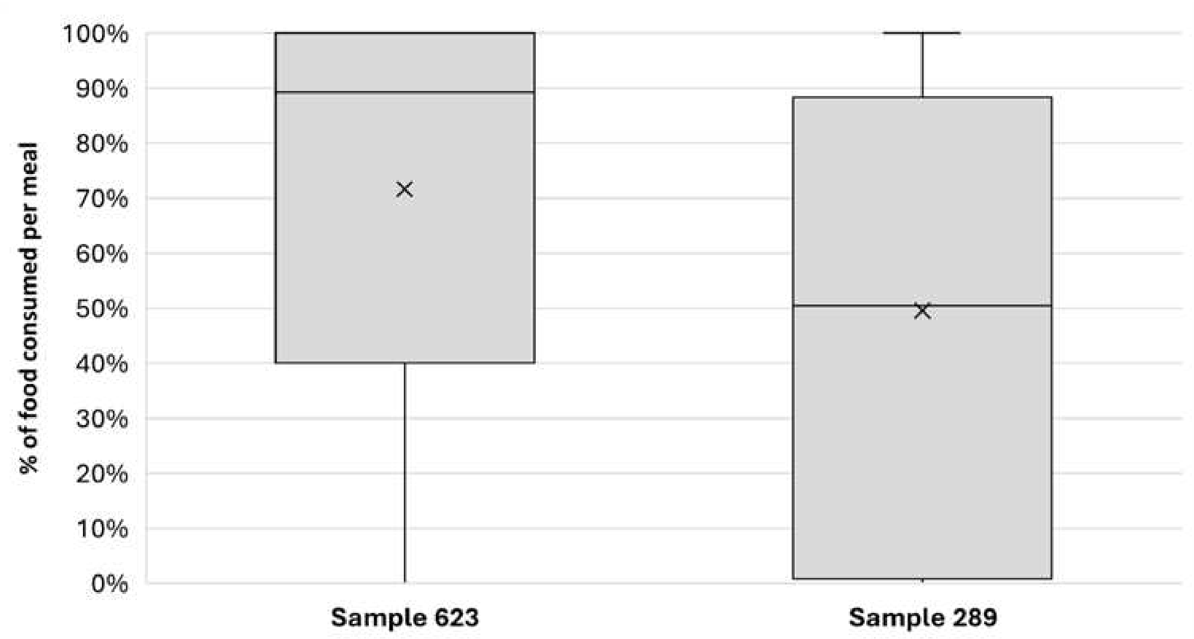

Figure 3: Comparative food intake quantities between experimental dog formulations. Sample 623: Test formula with Microbial Protein; Sample 289: Control formula.

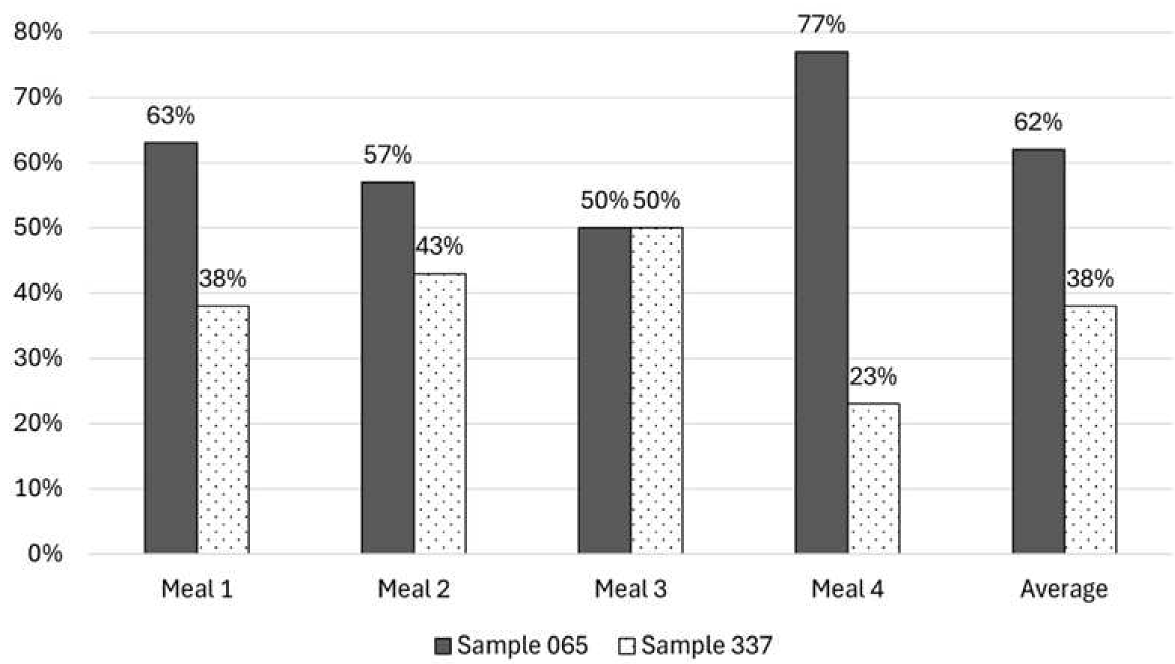

Figure 4: First approach orientation of cats towards experimental food formulations. Sample 065: Test formula with Microbial Protein; Sample 337: Control formula.

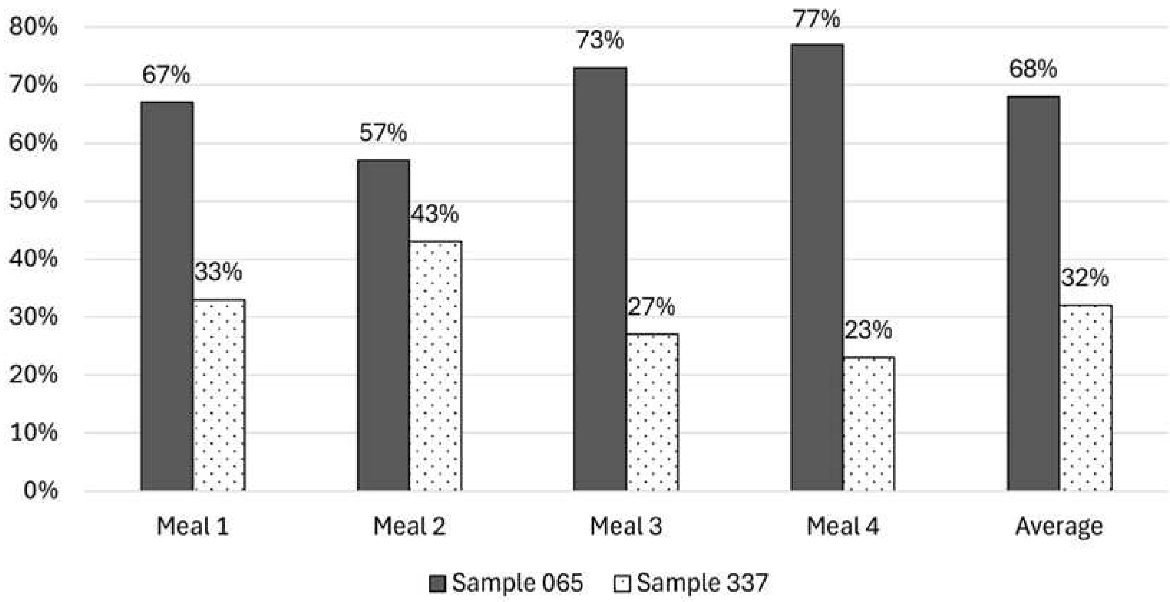

Figure 5: Primary consumption preferences of cats between experimental food formulations. Sample 065: Test formula with Microbial Protein; Sample 337: Control formula.

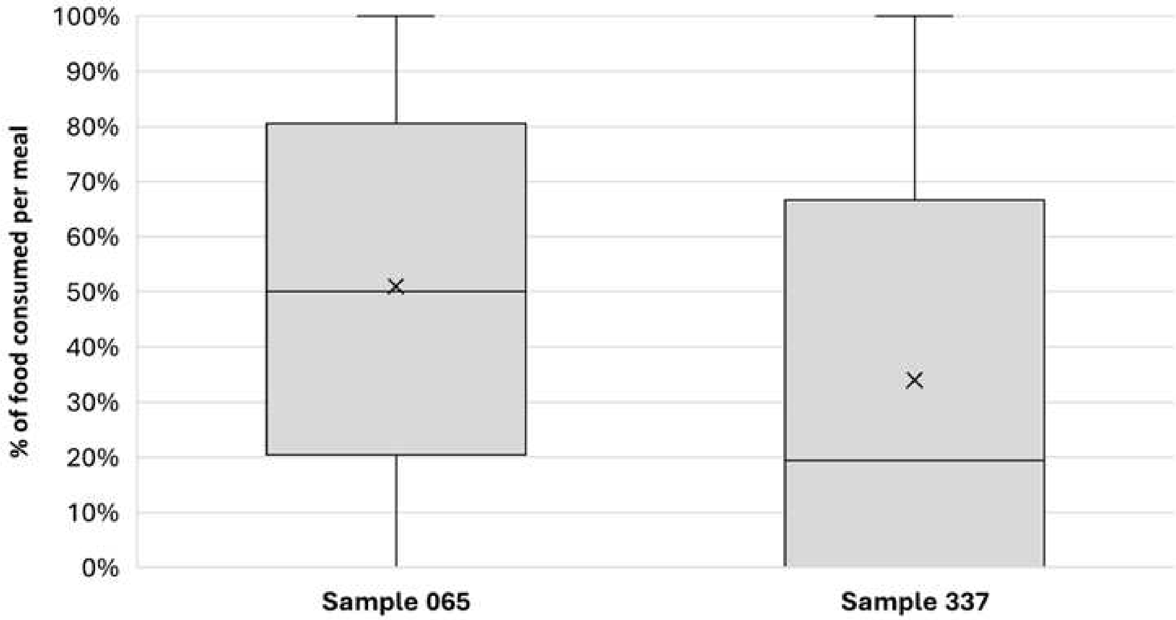

Figure 6: Comparative food intake quantities between experimental cat formulations. Sample 065: Test formula with Microbial Protein; Sample 337: Control formula.

4 Discussion

With this study, the palatability of kibbles containing microbial protein (MP) against a control formula with standard protein sources was assessed using a two-bowl system method within In-Home Use Testing (IHUT) protocol. This two-bowl system is a standard methodology in palatability trials, recognized for its ability to deliver accurate and reliable results [4]. Animals may develop a preference for a specific side of their feeding area due to habit, spatial comfort, or other environmental factors, which could skew the results of the palatability test. By alternating bowl placement, researchers ensure that any observed preference is attributable to the sensory attributes of the food itself, such as taste, aroma, or texture rather than external factors like bowl positioning [4]. The majority of participants of the study reported a total food intake similar or even higher than the amounts normally consumed by their pet (both cats and dogs). The significance of owner-reported observations in these trials is underscored by research indicating that pet owners can accurately identify and interpret their animals' feeding behaviours, which correlates strongly with palatability levels measured through quantitative food consumption [13]. The results suggests that this trial did not cause a disruption on the feeding habits of the individual and that the palatability results are likely to be representative. This study's findings demonstrate that MP can serve as a viable alternative to traditional animal proteins without compromising sensory acceptance, a crucial factor in pet food development [14]. The comparable macronutrient profiles observed between MP-containing and control formulations underscore the potential of Microbial Proteins to meet the nutritional requirements of companion animals. The marked preference exhibited by both dogs and cats for diets containing MP aligns with previous research indicating that pets can adapt to novel protein sources when properly formulated [15]. This acceptance is particularly noteworthy given the inherent neophobia often observed in domestic animals, especially cats [16]. Cats are obligate carnivores with specific nutritional requirements and a more limited taste perception compared to dogs. For instance, cats lack the ability to taste sweetness, which is often used to mask bitterness in human and dog foods [17]. They also have a stronger aversion to bitter tastes, which can be problematic when formulating therapeutic diets that may contain compounds with unpleasant flavors [18]. Furthermore, cats are generally more neophobic (resistant to trying new foods) than dogs, making it more difficult to introduce novel ingredients or formulations. These factors combined make the task of creating palatable cat food, more challenging for pet food manufacturers and formulation scientists [8]. The sensory attributes of MP, including its aroma and texture, likely play a crucial role in its palatability. Olfactory cues are known to be primary drivers of food selection in both canines and felines, with volatile compounds significantly influencing feeding behavior [19]. The formulation tested in this study was developed simply to assess preference of the MP as an ingredient in standard formulation against standard protein sources such as fish and poultry meal. No attempts were done to enhance palatability specifically for the canine or feline population, like by developing separate formulas. Further investigation into the specific flavor profiles and textural characteristics of MP could provide valuable insights for optimizing pet food formulations and potentially enhance even further the acceptance of MP as an alternative protein source. The successful incorporation of MP into pet diets addresses not only palatability concerns but also aligns with the growing demand for pet food options. As the global pet food market continestimated to reach estimated to reach $113.08 billion by 2025 [20] the environmental impact and sustained production of pet food has come under increasing scrutiny. Microbial Proteins offer a potential solution to mitigate the ecological footprint associated with traditional meat-based pet foods, which has been estimated to contribute significantly to greenhouse gas emissions and land use [21]. While this study provides compelling evidence for the viability of MP in pet food formulations, one should also acknowledge its limitations. The sample size and duration of exposure to test diets may limit the generalizability of these findings. Despite two days might be representative enough to demonstrate preference and adaptation for a formulation [22], longer-term feeding trials with larger cohorts are necessary to assess the sustained acceptance or dependency on the source of Microbial Protein (bacterial, yeast, fungi, or species within them). Additionally, comprehensive metabolomics and microbiome analyses could elucidate the physiological effects of MP-based diets on pet health and well-being [23]. Future research directions should include detailed sensory evaluations to identify specific compounds responsible for the enhanced palatability of MP-containing diets. Such investigations could employ techniques like gas chromatography-mass spectrometry (GC-MS) to analyze volatile organic compounds and their impact on pet food acceptance [19]. Moreover, exploring the potential synergistic effects of combining MP with other novel ingredients or protein sources could lead to further improvements in both nutritional value and palatability. But this study offers another compelling demonstration that the integration of Microbial Proteins into pet food formulations represents a significant advancement in addressing both nutritional adequacy and palatability concerns.

5 Conclusion

In conclusion, this study demonstrates the successful incorporation of Microbial Protein, into pet food formulations, showcasing its potential as a palatable and nutritionally valuable alternative to conventional protein sources. While prior research has extensively highlighted the nutritional profile and digestibility of microbial proteins, including their balanced amino acid composition and suitability as a highly digestible, high quality protein source for dogs and cats [6, 7] this study uniquely contributes new insights into their palatability. The results reveal that diets containing 10% MP were preferred by both dogs and cats over a commercial control diet, indicating that microbial proteins can enhance sensory appeal in addition to meeting nutritional requirements. A key distinction of this study lies in its inclusion of both canine and feline data, addressing the gap in previous research that primarily focused on dogs. This broader scope underscores the versatility of MP as a protein source suitable for multiple species. Furthermore, microbial proteins offer consistent availability due to their production in controlled bioreactor systems, independent of agricultural variability, making them a reliable ingredient for pet food formulations [6]. While environmental benefits remain an important consideration, this study emphasizes that microbial proteins are not only sustainable but also capable of enhancing palatability and nutritional adequacy in pet diets. These findings position MP as a promising innovation for the pet food industry, offering a practical solution to meet both consumer demands and the dietary needs of companion animals. Future research should explore long-term feeding trials across diverse breeds and life stages to further validate these findings and expand the applicability of microbial proteins in companion animal nutrition.

Conflict of Interest

The authors are employees of MicroHarvest Unipessoal Lda., which funded this study. MicroHarvest is also the producer of the microbial protein evaluated in this research. The authors were involved in the study conception and manuscript preparation, but did not influence the study's design, execution, data analysis, or interpretation of results, which were conducted independently by Sense Test Lda. The authors declare no other conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

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