

**ANALYSIS OF PHOSPHORUS, CALCIUM AND VITAMIN D IN
CONVENTIONAL AND NON-CONVENTIONAL COMMERCIALIZED
PET FOODS IN MALAYSIA**

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(D18B0027)

A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY
MEDICINE, UNIVERSITI MALAYSIA KELANTAN IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

JANUARY 2023

UNIVERSITI MALAYSIA KELANTAN

CERTIFICATION

This is to certify that we have read this research paper entitled 'Analysis of Phosphorus, Calcium and Vitamin D in Conventional versus Non-Conventional Commercialized Pet Foods in Malaysia by

Uma Devi Rajeswaran It is satisfactory in scope, quality, and presentation as partial fulfillment of the course DVT 55204—Research Project requirement.



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ACKNOWLEDGEMENT

Special thanks to those who have given their support, guidance, advice, and aid in the completion of this project paper:

All the Cats and Dogs

Dr. Choong Siew Shean

Dr. Kavindaran Krisnasamy

Dr. Goh Soon Heng

Royal Canin Malaysia

Family and Friends

DVM 5 class of 2018/2023

Thank You

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KELANTAN

DEDICATIONS

I dedicate this thesis especially to all the cats and dogs for always inspiring me.

I dedicate this thesis to my beloved parents, Rajeswaran Ratnasingam and Rasalechumi Kanagaratnam, who have been there for me throughout my time starting this project and until the completion of this thesis. I want to thank them for their support and encouragement, which have enabled me to focus on this project. My brother, Sivanesan Rajeswaran, for giving me extra boosters.

I dedicate this thesis to my lecturers, my supervisor, Dr. Choong Siew Shean, and my co-supervisors, Dr. Goh Soon Heng and Dr. Kavindaran Krisnasamy from Royal Canin Malaysia, for their valuable knowledge, input, and precious time. Their guidance and support will always be appreciated.

I also dedicate this thesis to my best friends, Subeinthiran Rinagasamy, Nur Athirah Nabilah Binti Suhaimi, and Hari Haraprien K. Kesavan, for giving me ideas and confidence, which helped me complete this thesis.

I would also like to thank my rotation mates and classmates for being there for me throughout the entire program of Doctor Veterinary Medicine. Also, all of them have been my best supporters.

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 55204 – Research Project.

The proper balance of nutrients is essential when feeding dogs and cats. Like in humans, animals cannot make nutrients all by themselves, so a balanced diet of high nutritional value is needed to meet their dietary requirements, giving pet animals energy, and keeping them healthy. The essential minerals and vitamins keep the pet's metabolism and immune system functioning normally. In both dogs and cats, calcium, phosphorus, and vitamin D are crucially necessary nutrients that must be incorporated in a well-balanced diet. The classic functions of vitamin D are to stimulate intestinal calcium and phosphate absorption, renal calcium and phosphate reabsorption, and regulate bone mineral metabolism, making it vital for calcium and phosphorus homeostasis. However, it is unknown if the amount of these minerals in the pet's dietary are sufficient to meet the nutritional requirements. The purpose of this study is to determine if there is any difference in calcium, phosphorus, and vitamin D levels between conventional and non-conventional pet diets, and if both meet the international nutritional standards. The results from this study may assist pet owners in making an informed decision when choosing diets for their pets. The outcomes of this research found that the non-conventional (Biologically Appropriate Raw Food, BARF) diet samples had a higher number (37.5%, n=12/32) of parameters not meeting the nutritional standards as compared to the conventional diets, which only recorded (12.5%, n=4/32), parameters not meeting the standards. Thus, our results indicated that there are potentially more risks of malnutrition in non-conventional (BARF) diets and they may not be ideal pet diets for cats and dogs without further supplements or changes in composition. It is also worth to mention that all premium diets tested in the conventional category met the nutritional standards.

Keywords: BARF diet, biologically Appropriate Raw Food, Bones and Raw Food, pet nutrition

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Keseimbangan nutrien yang betul adalah penting semasa memberi makan kepada anjing dan kucing. Seperti manusia, haiwan tidak boleh menjana nutrien sendiri, jadi diet yang seimbang dengan nilai pemakanan yang tinggi akan memenuhi keperluan pemakanan, memberikan tenaga dan memastikan mereka sihat. Mineral dan vitamin adalah penting bagi memastikan metabolisme dan sistem imun haiwan kesayangan berfungsi dengan normal. Bagi kedua-dua anjing dan kucing, kalsium, fosforus, dan vitamin D adalah nutrien yang sangat diperlukan dan mesti dikandungi oleh diet yang seimbang. Fungsi klasik vitamin D adalah untuk merangsang penyerapan kalsium dan fosfat usus, penyerapan semula kalsium dan fosfat di buah pinggang, dan mengawal metabolisms mineral tulang. Oleh itu, ia adalah nutrien penting untuk homeostasis kalsium dan fosforus. Tujuan kajian ini adalah untuk mengesan samada terdapat perbezaan paras kalsium, fosforus, dan vitamin D antara makanan haiwan peliharaan bukan konvensional (*Biologically Appropriate Raw Food*, BARF) dengan makanan konvensional, serta jika kedua-duanya memenuhi piawaian pemakanan antarabangsa. Keputusan penyelidikan ini boleh membantu pemilik haiwan peliharaan membuat keputusan termaklum tentang makanan terbaik untuk haiwan kesayangan mereka. Keputusan daripada kajian ini menunjukkan bahawa sampel diet bukan konvensional (BARF) mempunyai lebih banyak parameter (37.5%, n=12/32) yang tidak menepati piawaian pemakanan berbanding

dengan diet konvensional, yang hanya mencatatkan (12.5%, n=4/32), parameter tidak menepati piawaian. Maka, hasil dari kajian ini menunjukkan risiko malnutrisi yang lebih tinggi pada diet bukan konvensional (BARF) dan ia bukannya makanan haiwan yang sesuai untuk kucing dan anjing tanpa penambahan zat makanan atau pertukaran komposisi. Juga dimaklumkan, kesemua diet premium dalam kategori konvensional yang dikaji memenuhi piawaian pemakanan.

Kata Kunci: BARF diet, Biologically Appropriate Raw Food, Bones and Raw Food, pemakanan haiwan kesayangan

1.0 INTRODUCTION

Nutrition is the process of providing and acquiring the diets necessary for the health and growth of animals (Wortinger & Burns, 2015). Nutrients are dietary components that serve certain bodily activities and contribute to growth, tissue maintenance, and optimal health (Arai, 2014).

Important micronutrients include calcium, phosphorus, and vitamin D. Through continual resorption and deposition, bone calcium contributes to the maintenance of adequate blood calcium levels, hence ensuring the structural integrity of the skeleton. Calcium levels in the blood are closely regulated by homeostatic mechanisms and have little to do with the amount of calcium an animal consumes. The chemical hydroxyapatite contains phosphorus largely in conjunction with calcium (Case & Daristotle, 2011). Vitamin D stimulates intestinal calcium and phosphate absorption, renal calcium and phosphate reabsorption, and bone mineral metabolism. Therefore, it is essential for calcium and phosphorus homeostasis (Zafalon et al., 2020). There are numerous types of pet diets to accommodate the preferences of pet owners and their pets. Dry, wet, and semi-moist diets are the primary varieties available on the neighborhood market (PFMA, 2020).

Whether dry or wet, commercial pet foods can be further classified as premium and superpremium, regular, and economical brands. On the other side, the raw diets trend is increasing due to the belief that pets should be fed diets similar to how feline and canine in the wild would have ingested the prey in the natural habitat. Non-conventional pet diets, which are part of a new trend for domestic cats and dogs known as BARF (Biologically Appropriate Raw Food) diets and "Bones and Raw Food," are gaining popularity locally. This study aims to see if there is a difference between conventional and non-conventional (BARF) calcium, phosphorus, and vitamin D levels, and if they meet international nutritional criteria. The

findings of this study may aid pet owners in choosing the best diets for their animal companions.

Premium pet foods are those that suit the nutritional needs of dogs and cats at various life stages. These meals are formulated for pet owners, enthusiasts, and professionals concerned with the health and nutrition of their pet. In general, these products are manufactured with high-quality, easily digestible components that have good to excellent nutritional availability. The majority of companies that manufacture and sell premium pet foods produce and advertise items for various dog breed sizes and phases of life (Case & Daristotle, 2011). In order to not only meet but also maintain the Association for American Feed Control's (AAFCO) high criteria, manufacturers of premium pet foods dedicate substantial resources to testing. They begin by analyzing the diets in a laboratory to ensure that the proportions of nutrients to energy and to one another are optimal. This guarantees that the meals are appropriate for the pet's life stage and lifestyle, whether the animal is a puppy, kitten, adult, or senior (Better Pets & Gardens, 2014).

Basic nutrition brands are non-premium (regular food) diets advertised nationally or regionally and sold in grocery store chains and mass merchant outlets. These companies spend a substantial amount of time and money on advertising, resulting in high customer brand recognition. These products are marketed mostly on the basis of the diet's palatability and appeal to pet owners (basic nutrition brands) or its price (high-end nutrition brands or economy brands). Several of these items are manufactured with recipes that vary based on ingredient availability and production costs. This means that the components of a given brand may vary from one batch to the next based on the availability of materials and the cost of production (Case & Daristotle, 2011).

Veterinarian and nutritionist Dr. Ian Billinghurst developed the BARF diet, which is based on feeding dogs the diet they evolved to eat: raw, uncooked, and wild meats and vegetables (Raw Bistro, 2019). Proponents of raw diets assert that because dogs and cats are carnivores, they learn to eat raw food instinctively. There are numerous health benefits associated with this diet. However, there is no credible evidence or statistics to indicate that this feeding strategy has any long-term health benefits when compared to other pet food options. Raw diets, on the other hand, can lead to nutritional imbalances, broken or cracked teeth, gastrointestinal blockages and holes, and bacterial infections. While the nutrition and metabolism of these nutrients have a direct effect on bone health, they also influence animal physiology in numerous other ways and have health effects that extend beyond skeletal health (Stockman et al., 2021).

The manufacturing of pet food adheres to and uses a number of international nutritional standards and guidelines. For instance, the National Research Council (NRC) establishes the minimum nutrients required for healthy dogs and cats to develop and remain healthy. The European Pet Food Industry Federation (FEDIAF) represents the European pet food industry. One of FEDIAF's primary objectives is to ensure the health of pets by ensuring that its member companies provide balanced and nutritious pet food. The Association of American Feed Control (AAFCO) is a private organization that creates non-binding standards for the production of pet food and animal feeds in general. AAFCO is concerned with ensuring the nutritional safety of diets (Savannah, 2019).

1.1 RESEARCH PROBLEM STATEMENT

The knowledge of the nutritional content of commercial pet diets on the diets label allows consumers to choose the best diet for their pets. However, data on certain non-conventional pet diets is still lacking, thus consumers may not have enough information to make the best decision. The current study aims to determine the calcium, phosphorus, and vitamin D content in selected conventional and non-conventional pet foods. For conventional pet food, we have selected for premium and non-premium pet food, and for non-conventional we selected specifically for BARF. The results of this study may be able to elucidate the nutritional value of these diets, which would help pet owners make better decisions for their pets.

1.2 RESEARCH QUESTIONS

Do the content of calcium, phosphorus, and vitamin D in conventional and non-conventional pet food tested fall within the minimum recommended allowance and safe upper limit defined by nutritional standards?

1.3 RESEARCH HYPOTHESIS

Vitamin D, calcium, and phosphorus in non-conventional does not fall within the recommended allowance and/or not within the safe upper limit defined by nutritional standards.

1.4 RESEARCH OBJECTIVES

1. To determine the level of vitamin D, calcium, phosphorus in conventional and non-conventional petfood.
2. To determine whether the level of vitamin D, calcium, phosphorus in conventional and non-conventional petfood fall within the recommended allowance and/or within the safe upper limit defined by nutritional standards.

2.0 LITERATURE REVIEW

2.1. Pet nutrition and its consequences for inadequate

All life stages require a nourishing diet for the maintenance of normal structure and function in pets. A satisfying diet is one that is adequate, balanced, palatable, digestible, and safe (Buffington, 2011). A complete diet refers to a diet that provides appropriate levels of all essential elements. A balanced diet refers to the presence of nutrients in the correct proportions. Because surpluses of some nutrients can lead to shortages of others, balance is essential. In addition, the food must be appealing enough to be consumed in adequate quantities to sustain normal function, and the nutrients must be sufficiently digested to become available to the animal. Even though a diet is complete, balanced, palatable, and easily digestible, it must not contain any dangerous ingredients and be safe. In general, the non-conventional pet meals have a single formulation for all life stages, and it is questionable whether this formulation is adequate for all life stages. The nutrient requirements of dogs and cats can change with age; therefore, veterinarians should review established criteria and provide clients with clear guidance for choosing the appropriate pet food at each life stage (Linder, 2018).

2.2 Effects of nutrients on health and well-being

There are six major groups of nutrients: water, carbs, proteins, fats, vitamins and minerals (Wortinger & Burns, 2015). Essential nutrients are those that cannot be manufactured by the body at a sufficient pace to meet its demands; therefore, they must be consumed in the diet. These nutrients are employed as structural components in bone and muscle, improving or being involved in metabolism, transporting chemicals like oxygen and electrolytes, maintaining appropriate body temperature, and providing energy. Non-essential nutrients are generated by the body and can be ingested or produced internally. Various animal tissues and organs are related with the regulation of animal feeding (Arai, 2014). Energy for animals is

derived from their diet via nutrients. Dietary nutrients are necessary for maintaining health and providing the building blocks for cells in the body. They can be either macro- or micronutrients and come in a variety of forms. Micronutrients, such as vitamins and minerals, are required in lesser quantities but are still necessary to the organism (Bonnie, 2015).

2.3 Essential nutrients - Calcium, Phosphorus and Vitamin D

Calcium and phosphorus are typically discussed together due to the strong relationship between their metabolism and the homeostatic mechanisms that regulate their levels in the body. As bone growth and maintenance occur and the body's plasma calcium requirements fluctuate, the calcium in bone tissue is continuously mobilized and deposited. Plasma calcium levels are carefully regulated by homeostatic mechanisms and are independent of an animal's calcium intake from food. Calcium in circulation is required for nerve impulse transmission, muscular contraction, blood coagulation, activation of specific enzyme systems, maintenance of normal cell membrane permeability and transport, and cardiac function. Similar to calcium, this phosphorus provides structural support for the skeleton and is also released into the bloodstream in response to homeostatic mechanisms, according to Case & Daristotle (2011). The phosphorus found in the body's soft tissues serves a variety of roles and is involved in nearly all of the metabolic processes. Vitamin D consists of sterol molecules that govern the body's calcium and phosphorus metabolism. Vitamin D is essential for the growth and maintenance of healthy bone tissue, as well as the maintenance of the body's calcium and phosphorus pools. These benefits are mediated by vitamin D's effect on the absorption of calcium and phosphorus from the gastrointestinal system and their deposition in bone tissue (Case & Daristotle, 2011).

2.4 The forms of pet diets

There are numerous types of pet diets to accommodate the preferences of pet owners and their pets. Dry, wet, and semi-moist diets are the primary varieties available on the neighborhood market (PFMA, 2020). Where dry pet food may be extruded or baked (kibbles). They have a higher moisture content than wet diets and are consequently offered in lower amounts (PFMA, 2020). Then, the wet pet foods have a high moisture content (between 70 and 80 percent). For semi-moist when moisture content (about 30-35%). They are vacuum-sealed and then roasted at a predetermined temperature to sterilize them, ensuring their safety and preserving their quality throughout their shelf life (PFMA, 2020).

2.5 Conventional vs non-conventional

Since the introduction of conventional dry (kibble) and wet (canned, pouches, and rolls) pet foods to the market, the majority of dogs and cats in industrialized countries have been fed these diets, which are considered by many to be the conventional manner of feeding dogs and cats. It has been argued that the formulation of nutritional targets and the provision of full and balanced commercial products may contribute to the longevity of companion animals. It has been suggested that the formulation of nutritional goals and the availability of full and balanced commercial diets may have contributed to the increased longevity of pets. The AAFCO and the FEDIAF produce regulatory standards and recommendations in North America and Europe, respectively, based mostly on pet nutrition research data compiled by the National Research Council.

Veterinarians are concerned about the nutrient shortages that may emerge if homemade pet diet or raw diets are not properly designed. The most prevalent involve imbalances in the amounts and proportions of calcium, phosphorus, and vitamin D, which can cause obvious consequences

in puppies and kittens, or growth retardation or serious skeletal abnormalities that occur rapidly since these creatures grow so rapidly (Staff, 2020)

On the other side, raw/BARF diets are intended to mimic what cats and dogs consume "in the wild." Variable mixtures of raw meats, cereals, vegetables, and bones make up these diets. As with grain-free diets, there is no scientific proof that a raw diet is more advantageous to an animal's health than a conventional diet. However, there is abundant evidence to suggest that this is not the case. In addition, these diets have led to a debate due to their elevated potential of microbial contamination. The exposure of dogs and their owners to harmful microorganisms can cause severe sickness. To prevent microorganisms from compromising human and animal diets, all surfaces used to handle food must be meticulously cleaned. There is also a risk of gastrointestinal issues and/or injury from bones in the food, as well as the possibility of an unbalanced raw diet producing nutritional deficiencies and disease (OSU Veterinary Medical Center, 2022).

2.6 Premium versus Non-Premium Food

A truly premium diet is a nutritionally complete and well-balanced for its intended purpose, taking into account the varied nutritional needs of dogs at every life stage. The formulation of premium diets adheres to the nutritional profiles outlined in internationally recognized nutritional publications, such as the AAFCO's official publication in the United States and the FEDIAF Nutritional Guidelines in Europe. The formulas of premium diets are constant from batch to batch, the quality of their ingredients is higher and more consistent, and their digestibility is greater, resulting in decreased stool volume. The low ash level of premium diets (mineral content from bone in animal meals) prevents overfeeding of minerals like as calcium and phosphorus. These diets contain safe, regulated amounts of minerals such as salt, a flavor enhancer, to promote kidney and heart health (Ultimatevet, 2019). In contrast,

numerous low-cost diets feature variable formulas as opposed to fixed ones. This means that each batch of diets may contain different ingredients according on what is available on the market or how much the ingredients cost. A premium diet's set formulation is more expensive to maintain than a variable formulation. Non-premium pet diet often fulfill nutritional guidelines, although ingredient and manufacturer quality can vary (Ardente, 2020).

2.7 BARF Food as an Alternative to Conventional Commercial Pet Food

The "BARF" diet is a low-carbohydrate, high-protein, low-fat diet designed to emulate or come as near as possible to the ancestral diet of wolves. The majority of the diet consists of raw meats, bones, organs, certain fruits and vegetables, and vitamins. By analyzing the faeces microbial habitat after each diet, researchers were able to compare the nutritional value of the BARF diet to the commercial diet. PCR was utilized to quantify nutritional parameters such as crude protein and microbiota (Schmidt et al., 2018) discovered that the microbiological composition of the two meals differs significantly, with BARF diets including a larger proportion of *Clostridium perfringens*. A rigorous analysis was conducted to determine the positives and cons of feeding companion animals uncooked beef. The tested raw beef diets included too much vitamin D and not enough calcium and phosphorus. The diets were also deficient in potassium, magnesium, and zinc (Freeman and Michel, 2001). Another investigation was undertaken to determine the prevalence of *Salmonella* species in the raw chicken fed to dogs. This was performed to determine if the many types of *Salmonella* detected in dog feces posed a risk of zoonotic illnesses spreading. Eighty percent of *Salmonella* isolated from BARF meals tested as pathogenic, but none of the commercial diets did. In addition, 30% of BARF-fed test subjects tested positive for *Salmonella* species, while none of the commercial-fed test subjects did (Joffe &Schlesinger, 2002).

2.8 Experts endorse the advantages of conventional pet diet over raw diets.

In this review, unconventional diets are defined broadly to encompass alternatives to what are thought to be normal commercial pet foods, such as commercially available "natural" diets, raw food diets, and vegetarian diets, as well as a range of home-prepared diets. Concerns about raw food diets can be divided into two categories: nutritional adequacy and food safety issues, including public health concerns. Feeding their dogs raw food supplied dietary samples for study of critical elements. All of the meals were found to have nutritional excesses and deficiencies when compared to the AAFCO nutrient profiles for canines. The implications of providing nutritionally inadequate diets are most likely to be severe in animals with elevated nutrient requirements, as indicated by a case of two litters of puppies fed raw food diets; by 6 weeks of age, all of the puppies had developed severe nutritional osteodystrophy. Even raw food diets that include bones might be insufficient in calcium, especially when poultry bones are used. In addition, newly weaned puppies have difficulty consuming bones unless they have been finely pulverized. Regarding the second worry, food safety, home-prepared and commercial raw food diets have been assessed for bacterial contamination, in addition to the risk of gastrointestinal obstruction caused by feeding bones (Michel, 2006).

2.9 Calcium, Phosphorus, and Vitamin D Imbalance: A Case Study

In addition, a puppy fed an organic premix and raw ground beef was diagnosed with Rickets type I, an osteodystrophy caused by a lack of vitamin D or, less commonly, phosphorus (Taylor et al., 2009). Nutritional secondary hyperparathyroidism (NSHPT) is caused mainly by calcium deficiency. Vitamin D-dependent rickets type I and NSHPT are typically accompanied by bone pain, stiff gait, metaphyseal enlargement, bowed limbs, pathologic fractures, and low serum vitamin D and calcium concentrations in growing animals. Most osteodystrophies appear to be caused by inadequate or excessive dietary calcium, phosphorus, or vitamin D.

Because absorption, activation, and utilization of calcium, phosphorus, and vitamin D are all tied to Parathyroid Hormone (PTH), detecting the main shortage is difficult (Taylor et al., 2009).

Since the health of the majority of the world's pets depends on the quality of these diets, it is essential to examine the nutritional basis on which commercially produced diets are formulated. Despite the absence of accurate information on the nutrient needs of cats and dogs, several commercially produced pet diets promote healthy development, reproduction, and maintenance. However, these diets are frequently based on empirical data that cannot be utilized directly to create new diets with various elements. Progress in the nutrition of companion animals necessitates more detailed information on the nutritional needs of cats and dogs at various physiological interactions between nutrients and the role of specific nutrients in disease prevention (Morris & Rogers, 1994).

2.10 NRC, FEDIAF, and AAFCO

National Research Council National Research Council is the operating arm of the National Academies of Sciences, Engineering, and Medicine (commonly known as "NASEM" or "the National Academies") with the aim of giving objective policy advice. The NRC is a non-profit, private organization that gathers and assesses the research of others. The NRC determines minimal nutrient requirements for dogs' growth and maintenance (Savannah, 2019).

The European Pet Food Industry Federation (FEDIAF) represents the European pet food industry. They have members from 18 nations and five companies as members: Affinity Petcare, Hill's Pet Nutrition, Mars PetCare, Nestlé Purina Petcare, and Wellpet. The FEDIAF Dietary Guidelines for Complete and Complementary Pet Food for Cats and Dogs are a nutritional guideline that members must adhere to. This practical guidance for manufacturers includes a comprehensive analysis of NRC data and other available science. Europe-wide

independent veterinary nutritionists re-evaluate the recommendations. One of FEDIAF's primary goals is to ensure the health of pets by ensuring that its member companies provide balanced and nutritious pet food (Savannah, 2019).

The AAFCO is a private organization that creates non-binding standards for the production of pet food and animal feeds in general. AAFCO is not a government organization, yet membership is restricted to government officials. Industry and private groups can participate in AAFCO meetings to provide input. They do not have voting rights. However, industry and private organizations could possess persuasive power. Individual states in the United States have embraced AAFCO's rules, which are not enforceable. States are responsible for enacting AAFCO directives into legislation. AAFCO is concerned with ensuring the nutritional safety of diets. AAFCO neither inspects nor regulates pet diets (Savannah, 2019).

The NRC Guidelines were produced using components that have been purified. This is because digestibility and availability are not changed. This does not apply to pet diets, as unrestricted or near-perfect availability or digestibility cannot be guaranteed for the substances found in regular pet diets. It should not be assumed either for raw or lightly cooked items that are safe for human consumption. AAFCO and FEDIAF are both based on the NRC Guidelines. To account for complicated interactions and reduced bioavailability of specific nutrients, the AAFCO or FEDIAF standards are generally higher than the NRC (Friends, 2021).

The FEDIAF defines guidelines for 95 kcal ME/kg^{0.75} and 110 kcal ME/kg^{0.75} (Table 2.1) for dogs and 75 kcal ME/kg^{0.67} and 100 kcal ME/kg^{0.67} (Table 2.2) for cats, especially when certain breeds require fewer calories to maintain a healthy body weight. All three recommendations are intended to suit the needs of the vast majority of dogs and cats. Theoretically, it may be hard to meet the needs of any individual dog or cat because to their varying energy requirements, habits, and levels of activity (Friends, 2021). In developing these

recommendations, the organization acknowledged that the nutritional needs of dogs and cats alter throughout time (Lifestages, 2018). In addition, Europe acknowledges that a pet's diet should be proportionate to its activity level and caloric needs. The regulatory system of Europe permits both high-calorie and low-calorie pet foods that provide the same amount of vitamins and minerals essential for the pet's body size. Where the United States does not participate in AAFCO (Thixton, 2018). The standard needs for cats and dogs are detailed in Tables 2.3 and 2.4 of the AAFCO's recommendations. whereas Table 2.5 following represents the NRC's standard requirements for cats.

Table 2.1: Recommended nutrient levels for complete dog diets unit per 1000 kcal of metabolizable energy (ME) based on Nutritional Guidelines of FEDIAF (2021)

Nutrient	Unit	Minimum recommended level			Maximum (L) = EU legal limit (given only on a DM basis, see table III-3a) (N) = nutritional
		Adult based on MER of 110kcal/kg ^{0.75}	Early Growth (< 14 weeks) & Reproduction	Late Growth (≥ 14 weeks)	
Minerals					
Calcium	G	1.25	2.50	2.00 2.50	Adult: 6.25 (N) Early growth: 4.00 (N) Late growth: 4.50 (N)
Phosphorus	G	1.00	2.25	1.75	Adult: 4.00 (N)
Ca/P ratio		1/1			Adult: 2/1 (N) Early growth & reprod.: 1.6/1 (N) Late growth: 1.8/1a (N) or 1.6/1b (N)
Vitamins					
Vitamin D	IU	138.00	1250	1250	(L) 800.00 (N)

Table 2.2: Recommended nutrient levels for complete cat diets unit per 1000 kcal of metabolizable energy (ME) based on Nutritional Guidelines from FEDIAF (2021)

Nutrient	Unit	Minimum recommended level		Maximum (L) = EU legal limit (given only on DM basis, see table III-3) N = Nutritional
		Adult based on MER of 100kcal/kg ^{0.67}	Growth & Reproduction	
Minerals				
Calcium	g	0.40	1.00	
Phosphorus	g	0.26	0.84	
Ca/P ratio				Growth: 1.5/1 (N) Adult: 2/1 (N)
Vitamins				
Vitamin D	IU	62.50	70.0	227 (L) 3 000 (N)

Table 2.3: Recommended nutrient levels for complete cat diets unit per 1000 kcal of metabolizable energy (ME) (AAFCO, 2014)

Nutrients	Units per 1000 kcal ME	Growth & Reproduction Minimum	Adult Maintenance Minimum	Maximum
Calcium	g	2.5	1.5	
Phosphorus	g	2.0	1.25	
Calcium:Phosphorus ratio	g			
Vitamin D	IU	70	70	7520

Table 2.4: Recommended nutrient levels for complete dog diets unit per 1000 kcal of metabolizable energy (ME) (AAFCO, 2014).

Nutrients	Units per 1000 kcal ME	Growth & Reproduction Minimum	Adult Maintenance Minimum	Maximum
Calcium	g	3.0	1.25	4.5
Phosphorus	g	2.5	1.0	4.0
Calcium:Phosphorus ratio	g	1:1	1:1	2:1
Vitamin D	IU	125	125	750

Table 2.5: Recommended nutrient levels (safe upper limit) for complete cat diets Unit per 1000 kcal of metabolizable energy (ME) (NRC, 2006)

Based on NRC the cat diets nutrient profiles based on calorie content for calcium	Based on NRC the cat diets nutrient profiles based on calorie content for phosphorus
Safe Upper Limit of Calcium in Cats 2.6 and 4.6 g Ca per 1,000 kcal ME	Safe Upper Limit of Phosphorus for Cats 2.3 g P per 1,000 kcal ME

3.0 MATERIALS AND METHODOLOGY

3.1 Study area

The samples were bought from local marketplace. The conventional pet food, and premium diet were purchased from the pet stores, non-premium pet diets from supermarket/grocery stores, and non-conventional pet food, while the BARF diets from authorized distributors. All samples were packed appropriately and delivered to Bio Synergy Laboratories Sdn Bhd in Seberang Jaya, Penang for analysis.

3.2 Study Design

The study design selected for the current project is a cross sectional study, as the samples were collected and tested once to determine the levels of calcium, phosphorus, and vitamin D content in selected conventional (premium and non-premium) and non-conventional (BARF) pet diets.

3.3 Source population

Selected commercial feed that is available in the local marketplace, including conventional (premium and non-premium) and non-conventional (BARF) pet diets.

3.4 Study Population

There were 12 samples, comprised of eight conventional pet diets (premium and non-premium diets), and four non-conventional pet diets (BARF diets). Each type of diet tested comprised of dog and cat food of two life stages (adult and puppies/kittens).

3.5 Selection criteria

3.5.1 Inclusion criteria

The pet diets selected for this study includes conventional pet food, which are publicly recognized premium pet diets exclusively sold in pet supply stores or other specialty shops. While the non-premium pet diets are readily available at grocery stores, shopping malls, sundry shops, and others. On the other hand, for the non-conventional which is the BARF food, are usually sold by authorized distributors. The samples are randomly chosen from different parent company that are readily available in the market.

3.5.2 Exclusion criteria

There are no exclusion criteria because the samples were specifically selected.

3.6 Sampling Technique

For determination of phosphorus, was by using the method of analysis from nutrition labelling, 1993. For determination of calcium, was by in house method based on Association of Official Agricultural Chemists (AOAC) is used. Vitamin D determination was done by high performance liquid chromatography (HPLC).

3.7 Sampling Procedure

The analytic procedures conducted by the laboratory to determine the calcium, phosphorus, and vitamin D levels are described below.

3.7.1 Determination of phosphorus

Based on the laboratory protocol, the samples were turned to ash before being

heated. The sample of 2 g of ash was heated for four hours at 600 degrees. After cooling the sample, 40ml of hydrochloric acid (HCL) and several drops of nitric acid (HNO₃) are added. Transferring the material to a 200 ml volumetric flask and diluting it with water. A 10-ml aliquot of the filtrated sample was placed in a 100-ml volumetric flask. Then, incorporate 20 ml of the molybdovanadate reagent, dilute and mix thoroughly, and allow to stand for 10 minutes. Afterward, the material was analyzed with a spectrophotometer.

3.7.2 Determination of calcium

According to the laboratory, a 2-10g sample was placed in a crucible and weighed. Then, ash for four hours at 450 degrees. After cooling, 5 mL of 6 M HCL should be added to ensure that all of the ash were in touch with the acid. The residue is then dissolved using HNO₃. The material should then be filtered into a 50ml volumetric flask and diluted with water. Microwave Plasma Atomic Emission Spectroscopy (MP-AES) was used to analyze the samples,

3.7.3 Vitamin D determination

According to laboratory protocol, the sample should be weighed and then transferred to a conical flask. Then, add water and ethanoic potassium hydroxide to the conical flask. Incubate and seal in a water bath. Transfer the sample to a separating funnel after incubation. Add hexane and water, then shake vigorously. Repeat this process multiple times and add phenolphthalein and acetic acid to modify the color. Ultrasonicate the solution after filtration. Finally, add ethanol and continue with high performance liquid chromatography (HPLC). The Laboratory did not mention of the quantity of chemicals, temperature, duration, and color determined during adjustment.

4.0 RESULTS

The level of calcium, phosphorus and vitamin D in conventional and non-conventional pet food are presented in Table 4.1 and 4.2.

Table 4.1: Calcium, phosphorus and vitamin D levels for adult dogs and puppy diets based on FEDIAF & AAFCO nutritional guidelines. (*An absolute value cannot be determined due to the limitation of the analysis method where values below 50mcg/1000g of dry matter).

Parameters (Adult Dog)	Conventional Petfood		Non-Conventional Petfood		REFERENCE FEDIAF	REFERENCE AAFCO
	Premium	Non-Premium	BARF 1	BARF 2		
Kcal/1000g	3843.0	3800.0	1300.0	1490.0	Not Available	Not Available
Ca(g)/1000kcal (ME)	2.9	1.8	6.2	4.0	1.25	Min = 1.5, Max = 4.5
Ph(g)/1000kcal (ME)	1.8	1.3	3.8	2.7	1	Min = 1, Max = 4
Ca:Ph	1.6	1.4	1.6	1.5	Min = 1, Max = 2	Min = 1, Max = 2
Vit D mcg/1000kcal	<13.0*	<13.2*	<38.5*	<33.6*	Not Available	Not Available
Vit D IU/1000kcal (ME)	<520.4*	<526.3*	<1538.5*	<1342.3*	Min = 138.0, Max = 800.0	Min = 125, Max = 750
Parameters (Puppy)	Conventional Petfood		Non-Conventional Petfood		REFERENCE FEDIAF	REFERENCE AAFCO
	Premium	Non-Premium	BARF 1	BARF 2		
Kcal/1000g	4109.0	3400.0	1300.0	1490.0	Not Available	Not Available
Ca(g)/1000kcal (ME)	3.4	5.3	6.2	4.0	2.5	Min = 3.0, Max = 4.5
Ph(g)/1000kcal (ME)	2.4	3.2	3.8	2.7	2.25	Min = 2.5, Max = 4
Ca:Ph (Calc)	1.4	1.6	1.6	1.5	Min = 1, Max = 1.6	Min = 1, Max = 1.6
Vit D mcg/1000kcal	<12.2*	<14.7*	<38.5*	<33.6*	Not Available	Not Available
Vit D IU/1000kcal (ME)	<486.7*	<588.2*	<1538.5*	<1342.3*	Min = 138.0, Max = 800.0	Min = 125, Max = 750

Table 4.2: Calcium, phosphorus and vitamin D levels for adult cats and kitten based on FEDIAF & AAFCO & NRC (*An absolute value cannot be determined due to the limitation of the analysis method used where values below 50mcg/1000g of dry matter was undetectable).

Parameters (Adult Cat)	Conventional Petfood		Non-Conventional Petfood		REFERENC E FEDIAF	REFERENC E AAFCO	REFERENCE NRC
	Premium	Non-Premium	BARF 1	BARF 2			
Kcal/1000g	3839	3260	1300	1320	Not Available	Not Available	Not Available
Ca(g)/1000kcal (ME)	2	4	6	10	1	1.5	Safe Upper Limit of Calcium in Cats 2.6 and 4.6 g Ca per 1,000 kcal ME
Ph(g)/1000kcal (ME)	2	3	3	5	0.64	1.25	Safe Upper Limit of Phosphorus for Cats 2.3 g P per 1,000 kcal ME
Ca:Ph	1	1	2	2	Min = 1, Max = 2	Min = 1, Max = 2	Not Available
Vit D mcg/1000kcal	<13.0*	<15.3*	<38.5*	<37.9*	Not Available	Not Available	Not Available
Vit D IU/1000kcal (ME)	<521.0*	<613.5*	<1538.5*	<1515.2*	Min = 62.50, Max = 7500	Min = 70, Max = 7520	Not Available
Parameters (Kitten)	Conventional Petfood		Non-Conventional Petfood		REFERENC E FEDIAF	REFERENC E AAFCO	REFERENCE NRC
	Premium	Non-Premium	BARF 1	BARF 2			
Kcal/1000g	4087	3255	1300	1320	Not Available	Not Available	Not Available
Ca(g)/1000kcal (ME)	3	5	6	10	2.5	2.5	Safe Upper Limit of Calcium in Cats 2.6 and 4.6 g Ca per 1,000 kcal ME
Ph(g)/1000kcal (ME)	2	3	3	5	2.1	2	Safe Upper Limit of Phosphorus for Cats 2.3 g P per 1,000 kcal ME
Ca:Ph	1	1	2	2	Min = 1, Max = 1.5	Min = 1, Max = 1.5	Not Available
Vit D mcg/1000kcal	<12.2*	<15.4*	<38.5*	<37.9*	Not Available	Not Available	Not Available
Vit D IU/1000kcal (ME)	<489*	<614*	<1538*	<1515*	Min = 70.00, Max = 7500	Min = 70, Max = 7520	Not Available

4.1 The level of calcium in conventional and non- conventional pet diets

The AAFCO dietary recommendation for calcium for adult dogs and puppies fulfilled the nutritional criteria when compared to the standard nutritional requirements of Min = 1.5, Max = 4.5 for adult dogs, and Min = 3.0, Max = 4.5 for puppies. With the exception of BARF 1, both adult dogs and puppies have high calcium levels (6.2g). In addition, one of the conventional pet diets (non-premium feed) for puppies has a greater calcium content at 5.3g (nutritional requirement; Minimum = 3.0, Maximum = 4.5). Although the calcium levels in both of these diets are above the acceptable upper limit, the conventional diet's calcium level is approximately 17% higher than advised, and the calcium level in BARF is 37% higher than the safe upper limit.

Calcium levels in BARF 1 and 2 pet foods are greater for adult cats and kittens, at 6g and 10g, respectively. The safe upper range of calcium for cats, according to NRC standards, is between 2.6 and 4.6 g Ca per 1,000 kcal ME. Similar to puppy diets, one of the non-premium kitten diets was also found to contain a greater calcium concentration of 5g (recommended requirement; 2.6 and 4.6 g Ca per 1,000 kcal ME based on NRC requirements). Again, the Ca for the non-premium conventional kitten food does not satisfy the criteria, but it exceeds the recommended amount by only 9% compared to the non-conventional (BARF) diets, which exceed the recommended intake by 30% and 117%, respectively.

All of the conventional (premium) diet samples for both species (cats and dogs) contained Ca levels that were well within the recommended amount.

4.2 The level of phosphorus in conventional and non- conventional pet diets

Phosphorus for adult dogs and puppies meets the nutritional requirements outlined in the AAFCO dietary recommendation, as shown in Table 4.1. In contrast, for adult cats and

kittens, the conventional BARF 1 and BARF 2 diets had higher phosphorus levels than the non-premium and non-conventional BARF 1 and BARF 2 diets (3 g, 3 g, and 5 g, respectively). The recommended upper limit of phosphorus for cats, according to the NRC, is 2.3 g per 1,000 kcal ME.

4.3 The ratio of calcium to phosphorus in conventional and non- conventional pet diets

According to FEDIAF and AAFCO nutritional requirements, the ratio of calcium to phosphorus in all samples was within the set limits for adult dog, adult puppy, and adult cat (see table 4.1 and 4.2) Except for both non-conventional (BARF) diets for kittens, the ratio of calcium to phosphorus (1:2) was greater than the recommended range of 1:1 to 1:1.5.

4.4 The level of vitamin D in conventional and non- conventional pet diets

Regarding vitamin D, it is not applicable to this study because the laboratory equipment was unable to detect minimum amounts of vitamin D, and all the samples tested showed results with less than 50mcg/1000g. Since all the results were below 50mcg/1000g, there were no absolute value results for vitamin D. According to AAFCO and FEDIAF nutritional standards, the minimum and maximum recommended vitamin D intakes for cats and kittens are 70.00 and 7500, respectively. Then, according to the AAFCO, the Vitamin D levels of puppies and adult dogs should be between 125 and 750, while those of FEDIAF should be between 138.0 and 800.0.

4.5 Summary of analysis

Among the diet tested, the non-conventional diets (BARF) had more parameters not meeting the recommended allowance of nutritional standards, compared to the conventional diets samples. In the conventional group, the non-premium diets showed evidence of not meeting the nutritional standards but not as high as the non-conventional diets.

Table 4.3 Summary of analysed parameters for conventional and non-conventional pet diets.

Species	Life Stage	Parameters	Conventional Diet		Non-conventional Diet	
			Premium	Non-premium	BARF 1	BARF 2
Dog	Adult	Ca(g)/1000kcal (ME)	Green	Green	Red	Green
		Ph(g)/1000kcal (ME)	Green	Green	Green	Green
		Ca: Ph (Ratio)	Green	Green	Green	Green
		Vitamin D (mcg/1000kcal - ME)	Grey	Grey	Grey	Grey
	Puppy	Ca(g)/1000kcal (ME)	Green	Red	Red	Green
		Ph(g)/1000kcal (ME)	Green	Green	Green	Green
		Ca: Ph (Ratio)	Green	Green	Green	Green
		Vitamin D (mcg/1000kcal - ME)	Grey	Grey	Grey	Grey
Cat	Adult	Ca(g)/1000kcal (ME)	Green	Green	Red	Red
		Ph(g)/1000kcal (ME)	Green	Red	Red	Red
		Ca: Ph (Ratio)	Green	Green	Green	Green
		Vitamin D (mcg/1000kcal - ME)	Grey	Grey	Grey	Grey
	Kitten	Ca(g)/1000kcal (ME)	Green	Red	Red	Red
		Ph(g)/1000kcal (ME)	Green	Red	Red	Red
		Ca: Ph (Ratio)	Green	Green	Red	Red
		Vitamin D (mcg/1000kcal - ME)	Grey	Grey	Grey	Grey

Legend:

	Parameters within recommended allowance
	Parameters not within recommended allowance
	Invalid parameters

Table 4.4: Number and percentages of analysed parameters compared to international nutritional standards.

Parameters	Conventional Diet	Non-conventional Diet
Total Parameters Assessed	32	32
Parameters within recommended allowance	20 (62.5%)	12 (37.5%)
Parameters not within recommended allowance	4 (12.5%)	12 (37.5%)
Invalid parameters	8 (25%)	8 (25%)

From Table 4.3 and 4.4, the non-conventional diet samples had a higher number (37.5%, n=12/32) of parameters not meeting the nutritional standards as compared to the conventional diets, where only four parameters (12.5%, n=4/32) did not meet the standards. It should be mentioned that all tested premium diets in the conventional category met the nutritional standards.

5.0 DISCUSSION

According to the results in tables 4.1 and 4.2 for both species and life stages, typical premium diets contain the recommended amount of the nutrient. Different animals require varying amounts of micronutrients, such as calcium, phosphorus, and vitamin D, based on their growth rate. Potentially beneficial effects of micronutrients on bone health include minimizing bone loss and fractures, lowering bone resorption, and promoting bone development (Upadhaya & Kim, 2020).

Developmental orthopedic disorders (DODs) such as osteochondrosis, joint dysplasia, osteodystrophy, and bone abnormalities are frequently observed in dogs with high calcium levels. Calcium and phosphorus, the two primary elements of bone, are linked by a functional and regulatory relationship and should be considered jointly. The absolute requirements of both minerals, as well as the ratio (the suggested range is between 1:1 and 2:1), should be meticulously determined, since an excess or deficit of one or both minerals during the embryonic phase can be a significant influence in terms of nutritionally caused DODs. In retrospective analyses of cases observed by the authors at Ludwig-Maximilian University, the majority of growing dogs with symptoms of DOD were found to have either an excessive or insufficient calcium and/or phosphorus intake (Boswald & Dobenecker, 2022).

In addition to their main diet, calcium supplementation has been recommended for puppies for many years. In contrast, today's commercial dog food is carefully produced and provides the appropriate quantity of calcium for an adult dog. It has been demonstrated that an excessive calcium intake predisposes an animal to bone and joint issues by inhibiting the necessary remodelling of bones during growth. In addition to calcium, phosphorous and vitamin D are required for optimal bone production in a balanced diet (Orto Canis.com, 2020).

In fact, a diet excessive in calcium may inhibit the normal, healthy mineralization and growth of bones. This is especially true for huge and giant-breed puppies (less than one year old) (Scott, 2019). Large breeds that are fed excessive calcium are more likely to develop osteochondrosis (abnormal bone growth) than smaller breeds, as well as hypertrophic osteodystrophy (Grünberg, 2018). Some dogs have developed symptoms resembling rickets due to an excess of calcium. As is the case with the majority of diets that result in poor bone development, the cause is frequently an imbalance in the calcium-to-phosphorus ratio in the diet (osteodystrophies). Rickets is frequent in animals that consume only meat (Grünberg, 2018).

In this study, the phosphorus levels in the conventional diet samples (premium) for all species were well within the acceptable range. In contrast, the non-conventional (BARF) and conventional non-premium pet diets did not meet the recommended allowance for phosphorus and the potential cause. Although phosphorus is an essential nutrient that is required for many bodily functions, new research indicates that consuming too much of it may be harmful to pets' health. In multiple animal models, Chang & Anderson (2017) discovered that a high phosphorus intake can cause calcification of the arteries and kidneys, damage to the renal tubules, and early death. In addition, a phosphorus intake comparable to that of freshly available cat diet can be detrimental to the kidney function of healthy cats (Ludwig-Maximilians-Universität München (LMU), 2018).

High dietary phosphorus (P) intake forces the body to deal with the P burden created by either calcification or renal elimination of excess P. By producing Ca-P-complexes, calcification removes soluble P from the circulatory system, which includes soft tissue calcification, e.g., by osteochondrogenic differentiation of vascular smooth muscle cells (Jono et al., 2000). It is believed that a high urine P content also affects glomerular endothelial cells (Nadkarni & Uribarri, 2014).

In addition, a direct causative involvement of phosphorus in generating and increasing vascular calcification suggests that calcification of vascular cells may occur early in a phosphate-rich environment in healthy persons (Giachelli, 2003). In addition, the literature indicates that a disturbed phosphate balance due to excessive consumption might cause irreversible organ damage and other health issues (Calvo & Uribarri, 2013).

The calcium to phosphorus ratio in this study for adult dog, puppy, adult cat and kitten for conventional pet diets were well within the recommended allowance. Whereas for non-conventional pet diets the adult dog, puppy, adult cat for were well within the recommended allowance, except for kitten non-conventional BARF diet has higher ratio of calcium to phosphorus. The health and development of kittens are highly dependent on receiving sufficient amounts of these two vital nutrients in the correct proportions. Calcium deficiency in the diet can cause the body to lose calcium from the bones, which can lead to fractures, weakness, convulsions, and even death. However, much calcium can inhibit growth or increase bone density, which can lead to joint problems. Calcium excess or shortage can induce constipation. Although exceedingly uncommon, phosphorous deficiency can cause acid-base imbalance and hemolytic anemia (Katz, 2014). The calcium to phosphorus ratio in the non-conventional (BARF) meals examined was appropriate for adult cats, but when the same diet is provided to kittens (as recommended by the manufacturer), it could be deleterious.

Due to vitamin D analysis limitations, accurate values could not be obtained. Where less than 50mcg/1000g ND was indicated in every vitamin D result. Vitamin D, like calcium and phosphorus, is a necessary nutrient that, if given in excess, can be detrimental to cats. Significant is the ratio of calcium to phosphorus in the cat's diet. A nutrient with an unusually high concentration can alter the appropriate ratio and injure bones. Cats' kidneys, gastrointestinal tract, brain, and cardiovascular systems might be adversely affected by elevated calcium levels brought on by excessive vitamin D supplementation (Huston, 2014).

Rickets is a metabolic bone disease that is often caused by a deficit in vitamin D, calcium, or phosphorus in the diet, or by genetic abnormalities that influence vitamin D or phosphorus metabolism. Widening of the physical growth plates of rapidly growing bones, such as the radius and ulna, is the most prevalent clinical anomaly. Histological accumulation of hypertrophic chondrocytes results in thicker, uneven growth plates. Due to nutritional hyperparathyroidism, animals fed imbalanced meat-based diets without vitamin D supplementation are more prone to develop fibrous osteodystrophy than rickets. Transitioning an animal with dietary-induced rickets to a complete and balanced diet is the treatment for rickets (Parker, 2018).

According to table 4.3 and table 4.4, more parameters of non-conventional diets did not meet the nutritional standards as compared to conventional diets. This becomes an indication that there is a potentially higher risk for non-conventional (BARF) diets to cause malnutrition and not ideal pet diets for cats and dogs. In addition to that, all the nutritional standards were met by the tested conventional premium diets. This is a reflection of the commitment premium pet diet companies have toward research and development by devoting enormous resources in order to not only meet but also maintain the exacting international nutritional guideline standards. This ensures that the diet meets the pet's life stage and lifestyle requirements, whether puppy or kitten, adult or senior (Better Pets & Gardens, 2014).

6.0 CONCLUSION

The analysis of calcium, phosphorus, and vitamin D levels in conventional and non-conventional pet diets partially meets the objectives of this study. Due to the limitations of vitamin D analysis, accurate values could not be obtained. Both conventional (premium and non-premium) and non-conventional (BARF) pet feeds fell short of the recommended allowance and/or the safe upper limit established by nutritional standards. However, there were three times as many deviations from nutritional norms in non-conventional diets as in conventional diets. In addition, all conventional premium pet foods evaluated correspond to the standard requirements and safety standards.

Different life stages (young versus adult) of animals regulate their vitamin and mineral requirements, which are governed by nutritional requirements. Growing animals accumulate tissues, necessitating higher nutritional levels for optimal performance. However, excessive supplementation is still contraindicated because these animals are more prone to toxicity. As animals age, their metabolism and body may undergo changes that increase their requirement for certain vitamins and minerals. The results of the current study could offer pet owners with a better understanding of their pets' nutrient requirements, allowing them to make more educated decisions that will result in healthier cats and dogs.

MALAYSIA

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7.0 RECOMMENDATION AND FUTURE WORK

In this study, some limitations were noted. For future research, it is suggested that the sample size be increased, as there were only 12 samples in this study, and only two BARF samples were evaluated. The subsequent phase would involve testing for additional macro and micronutrients, such as protein, magnesium, and vitamin A. Experiments on pigs and observations of various dietary imbalances in other domestic animals suggest that osteochondrosis may be caused by hyper- or hypovitaminosis A or high protein intake, in addition to hypovitaminosis D and low calcium levels (Reiland, 2022). More samples and parameters will result in more valid and trustworthy results. To prevent undetectable value results, the laboratory's deficiencies in vitamin D analysis should be corrected. Lastly, this study focused on detecting nutritional levels in pet food. Blood serum taken from pets given various pet foods could provide a more precise estimate of the nutritional availability from various diets, notably the non-conventional diet (BARF).

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