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Nutritional Profile and Determination of Aflatoxins in Puppy Food Available in the Local Market

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Abstract

The journey of dog domestication has been extensive and is still progressing, especially in their dietary practices. In the beginning, wild dogs relied solely on hunting for sustenance, but with domestication, they began to eat meals provided by humans. As obligate carnivores, dogs require animal protein in their diets, which should be complemented with easily digestible components to enhance nutrient absorption. This research included an examination of dry puppy food to assess its nutritional value and aflatoxin levels. The puppy food that was collected randomly from the local market showed a protein content of 22.80 ±0.50% and a calculated energy value of 377.00 ± 0.50 Kcal/100g. The findings were compared against the nutrient standards established by the Association of American Feed Control Officials (AAFCO). According to AAFCO guidelines, the minimum protein content in puppy food, based on a dry matter basis, should be no less than 22-32%. Importantly, the analysis indicated that aflatoxins (B1, B2, G1, G2) were absent in the puppy food, confirming its safety for canine consumption based on the aflatoxin evaluation. Therefore, based on the nutritional and aflatoxin analysis, the puppy food acquired from the market is beneficial for the health of puppies.

Keywords

Puppy food; aflatoxins; nutritional aspects; threat

1. Introduction

The pet food sector is anticipated to expand at a yearly growth rate of 2.6% [1]. Pet food may present significant hazards stemming from biological, physical, or chemical agents found in animal feed, potentially resulting in illness or injury to pets if adequate production controls are not enforced [2].

The rising popularity of pets in homes is fueling significant expansion in the pet food industry. Nowadays, many pet owners frequently use pet food because of its convenience, cost-effectiveness, and easy availability, offering a dependable way to nourish pets throughout their lives. With a better comprehension of dogs' nutritional needs, diets can now be tailored according to breed, age, and activity level. As dogs are frequently regarded as essential family members, owners are progressively prioritizing the dietary requirements of both their pets and themselves [3].

The predominant category of pet food available in the market is typically dry, which offers ease of storage and adequately satisfies the nutritional needs of pets. As per EU regulations (EC 767/2009), when complete pet food is supplied as the exclusive source of nutrients for a prolonged duration, it meets all the dietary requirements of the particular animals according to their species and life stages [4].

It is crucial to evaluate the quality of pet food, and many studies have been carried out to assess dog food [5, 6]. Another significant risk factor affecting the safety of animal food is the presence of fungi and mycotoxins [7]. Understanding the relationship between food, feed, and fungi is essential for assessing the risk of mycotoxin contamination. Research has shown that these substances found in pet foods can pose serious risks to pet health, resulting in both acute and chronic poisoning depending on the contamination level and duration of exposure [8]. Dogs, in particular, are especially susceptible to the acute hepatotoxic effects of aflatoxins [9].

Aflatoxins are a group of highly toxic mycotoxins that can contaminate various feed products, including dog food available on the market. This contamination poses a significant health risk to dogs, as aflatoxins are associated with a range of health problems such as liver damage, compromised immune function, and cancer. The research highlights the importance of ongoing monitoring for aflatoxin contamination in commercial dog food, especially in repackaged items. It is crucial to maintain stringent hygiene standards during the storage and repackaging processes to prevent the development of aflatoxins [10].

This study aimed to evaluate the nutritional quality of dry puppy food and its compliance with established nutritional standards.

2. Materials & Methods

This study was carried out at the Food Department of the PCSIR Laboratories Complex in Lahore, Pakistan. Dry pellet puppy food samples were randomly collected from a local market in Lahore, Pakistan, for nutritional analysis. The puppy food sample was processed to a mesh size of 1mm and kept in sealed plastic bags in a cool, dry environment to maintain its nutritional integrity.

2.1 Nutritional Analysis

The analysis involved assessing the moisture content, ash, crude fiber, and crude protein levels of the puppy food using AOAC-approved methods under ISO 17025:2017 accreditation. The nutritional quality of the pet food under evaluation was determined by measuring dry matter (DM), crude protein (CP), crude fiber (CF), and crude ash (CA). All analyses were performed in compliance with ISO 17025 (2017) accredited methods [11], adhering to the guidelines established by AOAC (2023) [12]. A 10 g sample was dried in a hot air oven at 105 °C until a stable weight was reached, with the weight difference recorded as the moisture content. To assess ash content, one gram of the sample was placed in a pre-weighed porcelain crucible and ignited in an ashing furnace at 600 °C until white ash was produced and a constant weight was obtained. The nitrogen content was measured using the micro-Kjeldahl method and then multiplied by 6.25 to calculate the crude protein content. Additionally, the carbohydrate content was determined according to the guidelines set forth by AOAC, 2023. The carbohydrate content (expressed as a percentage on a wet basis) is calculated by subtracting the total percentages of crude protein, crude fat, ash, and moisture content from 100.

Carbohydrate % (on wet basis) = 100- (% of crude protein+% of Crude fat+% ash content+ % content of moisture)

2.2 Aflatoxin Determination

The evaluation of aflatoxins in puppy food was conducted using the thin layer chromatographic method [13]. A 50g powdered sample was mixed with 250ml of chloroform and agitated for 30 minutes. Following this, 50ml of the resultant solution was evaporated in a water bath. The volume was then adjusted (specific final volume not stated), and the sample was applied to a TLC plate according to the prescribed procedure. Different concentrations of aflatoxin standards were used for comparison with the sample extract to quantify aflatoxins (AOAC, 2023) [12]. The total aflatoxins (AFB1+AFB2+AFG1+AFG2) were computed using the designated formula [14]. Below is the formula for detecting aflatoxin B1:

$$\text{Aflatoxin B1 } (\mu g/kg) = \frac{S \times Y \times V}{W \times Z}$$

3. Results & Discussions

Like all living beings, puppies require a balanced diet for proper growth and development and to maintain their health as they reach adulthood. Dry puppy food is the most commonly used type of dog food. Many puppy owners choose dry food because it enables them to leave a bowl of food available for their pet for a longer time without

worrying about spoilage. Having accurate information about the nutrient composition of puppy food is crucial for appropriate canine feeding. In the absence of this information, some puppies may receive excessive or insufficient amounts of specific nutrients, which could lead to various health problems.

According to the nutrient content standards for dry dog food set by Case et al. [15], the guidelines suggest a minimum of 26% crude protein, at least 15% crude fat, a maximum of 5% crude fiber, around 37% carbohydrates, approximately 7% minerals, and no more than 10% moisture. The results are given in Table 1.

Table 1. Nutritional Assessment of Puppy Food

Sr. No.	Parameters (%)	Values
1.	Moisture	6.70 ±0.25
2.	Ash	4.48 ±0.12
3.	Protein	22.80 ±0.50
4.	Fat	8.12 ±0.30
5.	Fiber	4.72 ±0.25
6.	Carbohydrates	53.18 ±0.15
7.	Energy (Kcal/ 100g)	377.00 ± 0.50

The present findings suggested that the average concentrations of crude protein and crude fat are deficient, whereas the levels of crude fiber and ash are in close alignment with the established recommendations. It is important to note, however, that dry dog food has demonstrated lower concentrations of crude protein. The moisture content is below 10%, in accordance with the recommendations. The results of the nutritional analysis indicate that this dog food can be classified as a high-calorie choice, offering 377.00± 0.50 Kcal per 100g, to fulfill the dietary requirements of puppies.

According to the AAFCO (2014) [16], the nutrient profile for dog food designed for growth and reproduction suggests that adult dogs should receive at least 18% protein and 5.5% fat on a dry matter basis (DMB), along with a caloric density ranging from 300 to 400 kilocalories of metabolizable energy (ME) per 100 grams of dry matter (DM). The inadequate fat content in dry dog food presents a considerable risk, as fat is the main energy source and provides essential fatty acids, in addition to being one of the most easily digestible nutrients. Furthermore, fat improves the palatability and texture of pet food. A significant deficiency of fat in dry dog food may lead to energy shortages, especially for working dogs [17, 18]. Aflatoxins are toxic fungal metabolites with carcinogenic potential that can greatly affect food quality. The most common Aflatoxins include Aflatoxin B1, B2, G1, and G2 [10]. The interaction between aflatoxins and dietary lipids may cause digestive problems, as it diminishes the activity of digestive enzymes, leading to a malabsorption syndrome [19]. In this assessment, the product was tested for Aflatoxin contamination and was found to be safe, as no Aflatoxins were identified. This confirms that the puppy food sample is safe for canine health (Table 2).

Table 2. Analysis of Total Aflatoxins in Puppy Food

Sr. No.	Aflatoxin (ppb)	Concentration
1.	B1	Not Detected
2.	B2	Not Detected
3.	G1	Not Detected
4.	G2	Not Detected

Domestic dogs, like other pets, are susceptible to aflatoxin poisoning that can be present in their food, which represents a serious risk to their health [20]. While fatalities from aflatoxicosis are generally associated with very high levels of aflatoxin (between 100 and 6700 ppb), there have been cases of deaths occurring at lower concentrations (ranging from 13 to 91 ppb). Despite the safety protocols and protective measures that have been put in place

to protect dog food ingredients and ensure accurate recipe formulation, instances of food contamination by aflatoxins can lead to health problems and even death in animals. Veterinarians are essential in quickly recognizing the adverse health effects associated with contaminated food products [21]. Prompt communication with manufacturers and regulatory agencies, along with comprehensive documentation, sample collection, and analytical testing, will help confirm or rule out potential toxic exposure to aflatoxins.

4. Conclusion

The objective of this study was to compare the analyzed nutrient composition of puppy food with the nutritional values declared by the manufacturer. The growing dependence on puppies for emotional support and companionship is believed to play a significant role in the rapid growth of the pet food industry, leading to an increased demand for dog food from both pet owners and breeders. Some pet food companies mention that claims regarding nutritional adequacy have not been thoroughly validated. Providing puppy food without clear details on nutrient levels does not adequately meet a puppy's dietary requirements. Thus, it is essential for manufacturers to accurately reveal the nutrient levels in their puppy food to ensure the health of puppies.

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