Assessment of commercial insect-based pet food products: nutrients' concentration, labelling adequacy and determination of animal deoxyribonucleic acid (DNA)

Short title: **Assessment of commercial insect-based pet food products**

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Abstract

Over recent years, the availability of insect-based pet food products has increased. However, concerns have arisen regarding potential nutritional challenges associated with using edible insects in pet food, and no studies have yet evaluated the adequacy of these products. This study assessed the nutrient composition and labelling accuracy of twenty-nine commercially available insect-based pet foods. Of these, twenty-four were dog products (four for growth, seventeen for adults, three for all life stages) and five were cat products (two for adults, three for all life stages), all labelled as complete and balanced. The products were analysed for proximate composition, essential amino acids, and mineral content (calcium, phosphorus, potassium, magnesium, copper, iron, zinc, selenium, mercury, and molybdenum) according to AOAC guidelines. The results were compared with label declarations, considering nutritional and legal tolerances, as well as recommendations from FEDIAF and NRC for the intended species and life stages (g/1000 kcal ME). Heavy metals were compared to maximum tolerable limits from the FDA. The twenty products labelled as 'hypoallergenic' were assessed for animal DNA using next-generation sequencing. The analysis revealed that 22 products (76%) did not comply with declared nutritional values and tolerances for at least one nutrient, with nine products (31%) showing discrepancies in two or more; key issues were in crude fibre and metabolizable energy. Three products (10%) met FEDIAF's recommendations, and seventeen (59%) met NRC's recommendations. Only one (3%) adhered to both label and FEDIAF's recommendations. Most nutritional inadequacies were seen in selenium, calcium, phosphorus, Ca/P ratios, and taurine, potentially posing health risks to pets. Furthermore, fifteen out of twenty (75%) hypoallergenic-labelled products complied with the labelled species. Despite the potential benefits of insect-based pet foods, this study underscores the need for further research and stricter quality control to ensure safety and efficacy, ultimately improving pet nutrition and consumers' trust.

Keywords

insect protein; pet food quality, nutrient analysis, DNA analysis, pet food labelling

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Introduction

The use of insects as a sustainable, novel protein source is gaining popularity for both ecological and nutritional reasons. Edible insects offer a high nutritional value, with protein and fat levels comparable to traditional animal meats (Rumpold and Schï, 2013), and studies indicate a lower environmental impact than conventional sources like beef, pork, and chicken (Miglietta, *et al.*, 2015; Oonincx and de Boer, 2012).

In 2018, the European Union approved the use of edible insects, resulting in the production of various insect-based products, including pet food (Siddiqui *et al.*, 2023). Insects fall under the 'novel food' legislation in Europe (Council Regulation (EC) 2015/2283), and due to that, several insect-based pet foods are labelled as 'hypoallergenic', although there are no regulatory guidelines for this claim.

Despite the enthusiasm, concerns remain about the variability in insects' nutritional composition, influenced by factors like diet, gender, life stage and environment (Makkar, *et al.*, 2014; Oonincx and Van Der Poel, 2011; Rumpold and Schï, 2013; Sönmez and Gülel, 2008). Some insect species may lack essential nutrients for pets: Black Soldier Fly (BSF), for instance, may not meet dogs' needs for methionine and threonine or cats' requirements for methionine, arginine, and leucine (Bosch and Swanson, 2021). Similarly, yellow mealworms (YMW) may lack methionine for dogs and methionine, leucine and taurine for cats (Bosch and Swanson, 2021; Mccusker, *et al.*, 2014).

Although insect-based pet foods are now widely available, their nutritional compliance remains largely unexamined. This study addresses this gap, hypothesizing that deficiencies may arise from insect ingredients or production factors.

Additionally, while insects are considered a novel protein source potentially suitable for managing adverse food reactions, concerns about their allergenicity remain (EFSA Scientific Committee, 2015). Cross-reactivity between YMW and mites (Verhoeckx *et al.*, 2014), is particularly concerning for atopic dogs sensitive to dust mites (Nuttall, *et al.*, 2006). Furthermore, cross-contamination during production also poses risks. Past studies have documented instances of pet foods containing undeclared animal DNA (Fossati, *et al.*, 2018; Kanakubo, *et al.*, 2017; Pagani *et al.*, 2018; Ricci *et al.*, 2018), raising questions about labelling accuracy, especially for products labelled as 'hypoallergenic'. Given the lack of studies examining DNA content in insect-based pet food, this study also aims to screen for animal DNA, ensuring transparency and reliability in hypoallergenic labelling.

Materials and methods

Samples

Insect-based pet food products were sourced online in December 2022. Eligible products contained insects, were labelled as complete and balanced, and were available in Europe. Both dog and cat products in dry or wet format were included. If multiple products were from the same manufacturer, only those with distinct nutritional composition were selected. Each product's smallest available package was purchased, and nutritional label information (ingredients, macronutrients, origin, batch number) was recorded. Products were classified as veterinary if formulated for managing clinical diseases (gastrointestinal or adverse food reactions) or as over-the-counter (OTC) if intended for general wellness. See Table 1.

Table 1. Summary of the products

Product ID	Country of origin	Form	Species	Life stage	Classification	Insect species declared	Claims
1	Germany	Extruded	Dogs	Adult	OTC	-	Grain-free
2	The Netherlands	Extruded	Dogs	All life stages	OTC	BSF	Hypoallergenic
3	Germany	Extruded	Dogs	Adult	OTC	-	Hypoallergenic; Gluten-free
4	Germany	Extruded	Dogs	Adult	OTC	-	Hypoallergenic; Gluten-free
5	Germany	Extruded	Cats	Adult	OTC	-	Gluten-free
6	United Kingdom	Extruded	Dogs	Adult	OTC	BSF	Grain-free
7	United Kingdom	Canned	Dogs	Adult	OTC	BSF	Grain-free
8	United Kingdom	Extruded	Dogs	Adult	OTC	BSF	Hypoallergenic
9	United Kingdom	Extruded	Dogs	Growth	OTC	BSF	Hypoallergenic
10	United Kingdom	Extruded	Dogs	Adult	OTC	BSF	Hypoallergenic
11	United Kingdom	Canned	Dogs	Adult	OTC	BSF	Hypoallergenic
12	United Kingdom	Extruded	Cats	All life stages	OTC	BSF	Hypoallergenic
13	The Netherlands	Extruded	Dogs	Adult	Veterinary	BSF	Hypoallergenic
14	The Netherlands	Canned	Dogs	All life stages	Veterinary	BSF	Hypoallergenic
15	The Netherlands	Extruded	Cats	All life stages	Veterinary	BSF	Hypoallergenic
16	The Netherlands	Extruded	Dogs	Adult	OTC	BSF	Hypoallergenic; Grain-free
17	The Netherlands	Extruded	Dogs	Adult	OTC	BSF	Hypoallergenic
18	The Netherlands	Pressed	Dogs	Adult	OTC	BSF	Hypoallergenic
19	The Netherlands	Canned	Dogs	Adult	OTC	YMW	Hypoallergenic; Gluten-free
20	Belgium	Extruded	Dogs	Growth	OTC	YMW	Hypoallergenic
21	Belgium	Extruded	Dogs	Adult	OTC	YMW	Hypoallergenic
22	Belgium	Extruded	Cats	Adult	OTC	YMW	Hypoallergenic
23	The Netherlands	Extruded	Dogs	Growth (>14 weeks)	Veterinary	-	Hypoallergenic
24	The Netherlands	Extruded	Cats	All life stages	Veterinary	-	Hypoallergenic
25	Czech Republic	Extruded	Dogs	Adult	Veterinary	-	Hypoallergenic
26	The Netherlands	Extruded	Dogs	Growth	OTC	BSF	Hypoallergenic
27	Belgium	Extruded	Dogs	Adult	OTC	-	-
28	Belgium	Extruded	Dogs	Growth	OTC	-	-
29	The Netherlands	Extruded	Dogs	Adult	OTC	-	Hypoallergenic; Grain-free

BSF= Black soldier fly; YMW = Yellow mealworm; OTC = Over-the-counter diet

Comparison with guaranteed analyses

Four samples were collected per product. Dry products were homogenized before sampling. Packaging was sanitized with alcohol to prevent contamination, and samples were handled with sterile gloves before being stored in sterile containers for DNA and chemical analysis, at room temperature and in the dark. The wet samples were shipped to the laboratory in sealed cans. For chemical analysis, wet samples were dried at 60°C for 72 hours, and all samples were milled to 1mm. One set was stored at room temperature for proximate and amino acid analyses; the other at -20°C for mineral analyses.

Proximate composition and mineral analyses

Proximate composition analyses were performed according to AOAC, 2006: dry matter (DM) (ISO 6496), crude ash (ISO 5984), acid hydrolyzed ether extract (EE) (ISO 6492), crude fibre (CF) (ISO 6865), and crude protein (CP; nitrogen × 6.25) (ISO 16634-1). Nitrogen-free extract was calculated by subtracting CP, EE, CF, and crude ash from the total DM. CF was analyzed in triplicate with <5% variance. Metabolizable energy (ME) was calculated following FEDIAF, 2021, thus, the NRC 2006 calculation was used for dry products and the modified Atwater calculation for wet products.

Calcium (Ca), phosphorous (P), potassium (K), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn) and molybdenum (Mo) were determined by ICP-OES (Thermo Fisher Scientific, iCAP 7400), ISO 11885. Selenium (Se) and mercury (Hg) were determined by ICP-MS, ISO 11885. All products were measured as single samples. Dry and wet reference materials with verified analysis from an external lab were used as controls, with <15% variance deemed adequate.

Amino acids analyses

Amino acids (AAs) were quantified using reversed-phase high-performance liquid chromatography (RP-HPLC) with diode array detection (DAD). Acid hydrolysis preceded chromatographic analysis to release AAs from their protein matrix. Cyst(e)ine and methionine were oxidized to cysteic acid and methionine sulfone, respectively, before hydrolysis. For tryptophan determination, alkaline hydrolysis was applied. Hydrolysis methods were based on ISO 13903:2005 and AOAC Method 988.15.

Following hydrolysis, samples were filtered through a 0.45-µm syringe filter (25 mm, PTFE, VWR, Belgium) and transferred to 2-mL glass vials for HPLC. Analysis followed Agilent Technologies' SOP using ortho-phthalaldehyde (OPA) and 9-fluorenyl methyl chloroformate (FMOC) derivatization on an Agilent 1290 Infinity II LC system. The AAs were first converted into OPA and FMOC derivatives using the 1260 Infinity II Vialsampler (Agilent, USA), after which separation was performed on an InfinityLab Poroshell 120 HPH-C18 column (4.6 mm × 100 mm × 2.7 µm; Agilent, USA). The mobile phases, at a flow rate of 2 mL/min, consisted of 10 mM Na2HPO4, 10 mM Na2B4O7, 0.5 mM NaN3 at pH 8.2 (eluent A) and acetonitrile/methanol/mQ in a ratio of 45/45/10 (v/v/v) (eluent B), and followed the gradient in the SOP. Absorbance was measured at 262 nm for FMOC-AAs (Pro, Hyp) and 338 nm for OPA-AAs. Calibration FAA standards (22.5–900 µM) and internal standards (0.5 mM) were used. Norvaline and sarcosine served as internal standards for OPA and FMOC-AAs, respectively.

Next generation sequencing

Next-generation sequencing (NGS) was used to confirm the declared animal species in 'hypoallergenic-labelled' products and to detect any additional animal species. Analyses were conducted externally, with DNA extraction, amplification, and sequencing on an Illumina MiSeq platform (San Diego, CA, USA) using the 16S Metagenomics protocol (Illumina, 2013). Species were identified using in-house, Barcode of Life and/or NCBI databases, covering mammals, poultry, fish, and insects (*Hermetia illucens, Tenebrio molitor, Acheta domesticus, Alphitobius diaperinus, Musca domestica, Gryllus assimilis*). Samples were tested in duplicate with a detection limit of 0.5% of total reads.

Evaluation of label and nutritional adequacy

Label-guaranteed analyses were compared to analytical results and the tolerances permitted by Regulation (EC) No 767/2009 (Annex IV, part A). Minimum and maximum guaranteed nutrient contents were calculated, and analytical results were expressed as a percentage of the guaranteed values.

Nutritional adequacy was assessed by comparing the analytical results (g/1000kcal) to NRC (minimal requirements [MR] or adequate intake [AI] [using MR levels when specified and AI when MR was not available] and safe upper limit [SUP]) and FEDIAF (recommended and maximum) guidelines for the intended species and life stage. All life stages products were compared to both maintenance and growth recommendations. Levels of Fe, Cu, Zn and Se were compared to FEDIAF's legal SUP (100 g of DM), and Hg and Mo to FDA maximum tolerable levels (MTL) (FDA, 2011). Products failing any nutrient recommendations were considered unsuitable for the intended species and life stage; all life stage products failing to meet any life stage recommendations were considered non-compliant.

Statistics

Descriptive statistics were reported as the median and range of each dietary nutrient per product category. Data analysis was conducted using SPSS Statistics 29.0 (2022).

Results

Description of the insect-based products

A total of 44 insect-based pet foods from 16 brands were identified online in December 2022. From those, 29 products from 14 brands met the inclusion criteria and were purchased; exclusions were due to similar compositions within brands or shipping restrictions to Belgium. Twenty-three products were OTC, and six were veterinary diets; five were for cats and 24 for dogs. Among the cat products, two were formulated for adults and three for all life stages. For dogs, four products were designed for puppies (one specifically for late growth), 17 for adult maintenance, and three for all life stages. Nineteen dog products and four cat products were labelled 'hypoallergenic' (six veterinary, 17 OTC diets). Ingredient-wise, four products contained YMW, 15 BSF, and 10 did not specify the insect species. Additionally, five products included an animal protein source alongside insects, four were gluten-free and five were grain-free (Table 1).

CP, EE, crude ash, and CF were declared on all product labels. Ca was labelled on 24 products, P on 20, K on four, Mg on three, moisture on 15, and ME on 16 (six additional products listed ME on their website). Overall, 22 products (76%) did not meet the declared values for at least one nutrient on the label, and 9 products (31%) were non-compliant for two or more nutrients.

Six products had nutrient levels below the guaranteed minimums: three for EE (10%), one for crude ash (3%), two for CF (7%), and one for Ca (4%). Conversely, 20 products exceeded the maximum tolerance levels: 12 for ME (54%), one for CP (3%), one for EE (3%), four for crude ash (14%), five for CF (17%), two for Ca (8%), one for K (25%), and two for Mg (67%). More details in Table 2.

Table 2. Comparison of declared vs. measured analytical constituents in insect-based pet food products as % of aberrancy beyond legally tolerated concentrations (EC No 767/2009; Annex IV, part A). Values are represented as median (range).

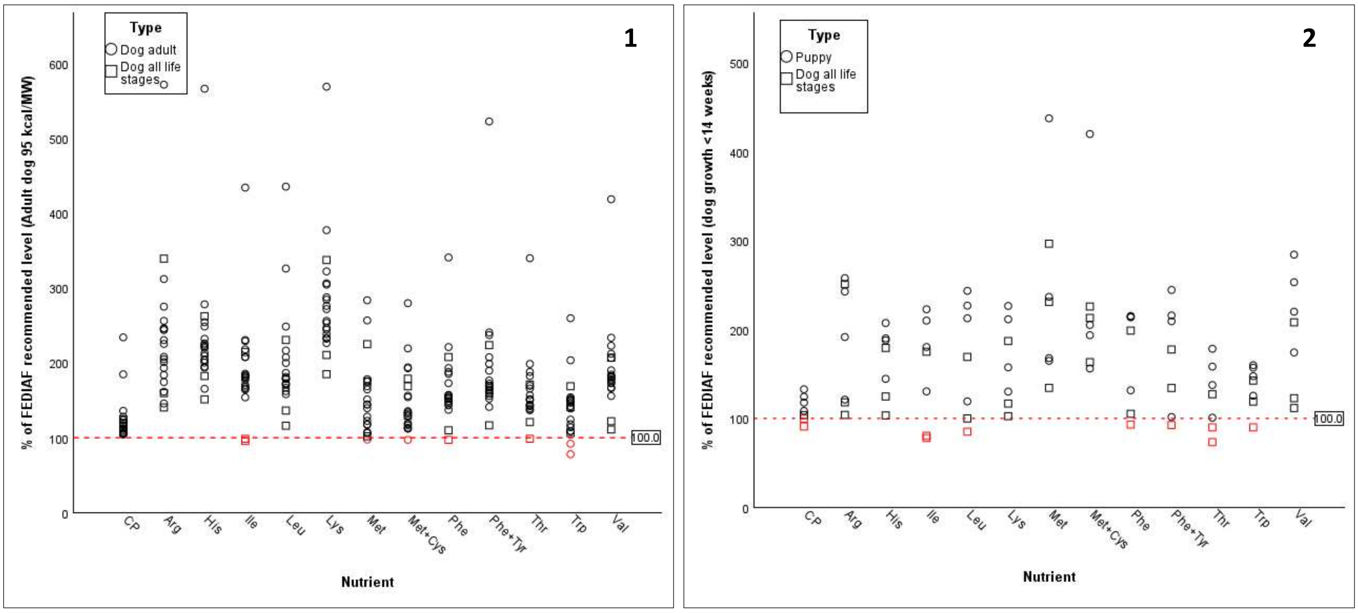
Constituent	Number of analysed products	Number of products compliant with EU Regulation	Discrepancies detected	
			Below	Above
Moisture	15	15	No limits set	0
aberrancy [%] median (range)				
Metabolized energy	22	10	0	12
aberrancy [%] median (range)				4.2 (0.2-12.4)
Crude protein	29	28	0	1
aberrancy [%] median (range)				5
Crude fat	29	25	3	1
aberrancy [%] median (range)			6.9 (4.6-8.6)	2.5
Crude ash	29	24	1	4
aberrancy [%] median (range)			15.9	2.7 (1.5-3.7)
Crude fibre	29	22	2	5
aberrancy [%] median (range)			11.5 (10.5-12.6)	14.2 (5.1-28.8)
Calcium	24	21	1	2
aberrancy [%] median (range)			3.0	10.8 (1.9-19.6)
Phosphorous	19	19	0	0
aberrancy [%] median (range)				
Potassium	4	3	0	1
aberrancy [%] median (range)				20.6
Magnesium	3	1	0	2
aberrancy [%] median (range)				58.1 (30.0-86.2)

Comparison with FEDIAF

Only three products (10%) fully complied with FEDIAF guidelines for all nutrients for the intended species and life stages (three canine diets: two for adult and one for growth).

For adult canine diets (n=20), three products (15%) met the recommendations for 95 kcal/MW, while 10 (50%) met the recommendations for 110 kcal/MW. Deficiencies were found in Se (n=4; 20%), P (n=1; 5%), Cu (n=1; 5%), isoleucine (n=2; 10%), methionine (n=1; 5%), methionine + cysteine (n=1; 5%), phenylalanine (n=1; 5%), threonine (n=1; 5%), and tryptophan (n=2; 10%). Additionally, excesses were found in Zn (n=2; 10%), Se (n=6; 30%), Cu (n=1; 5%) and Ca/P ratio (n=2; 10%) (Figure 1, Supplementary Table S1).

Among the seven puppy diets, one product (14%) met the recommendations for early growth (Figure 2, Supplementary Table S3), and two (29%) met the recommendations for late growth. Deficiencies were observed in CP (n=2; 29%), isoleucine (n=2; 29%), leucine (n=2; 29%), phenylalanine (n=1; 14%), phenylalanine + tyrosine (n=1; 14%), threonine (n=2; 29%), tryptophane (n=1; 14%), Ca (n=2; 29%), P (n=2; 29%) and Se (n=4; 57%). One product (14%) exceeded the legal SUP for Zn.



Figures 1 and 2. Comparison between the nutrient analysis of 20 complete and balanced insect-based adult dog foods against FEDIAF minimum recommendations for adult dogs (95kcal/MW) unit/1000 kcal of ME **(Figure 1)**. Comparison between the nutrient analysis of seven complete and balanced insect-based dog foods for growing dogs against FEDIAF minimum recommendations for early growth (<14 weeks) unit/1000 kcal of ME **(Figure 2)**. Results are shown as percentages of FEDIAF fulfilment. The red dotted line marks 100% of the FEDIAF minimum recommendation, with red icons indicating products exceeding maximum limits or falling below minimum requirements.

For feline adult maintenance, none of the five diets met FEDIAF recommendations for 75 kcal/MW (Figure 3 and Supplementary Table S5), but two (40%) complied with the 100 kcal/MW standard. Inadequacies were seen in CP (n=3; 60%), taurine (n=3; 60%), EE (n=1; 20%); K (n=2; 40%), Se (n=2; 40%), and Ca/P ratio (n=2; 40%).

Among the three diets intended for kittens, none met the growth and reproduction recommendations (Figure 4 and Supplementary Table S7). Deficiencies were seen in CP (n=1; 33%), taurine (n=2; 66%), EE (n=1; 33%), Ca (n=3; 100%), P (n=2; 66%), Ca/P ratio (n=2; 66%), and Se (n=1; 33%).

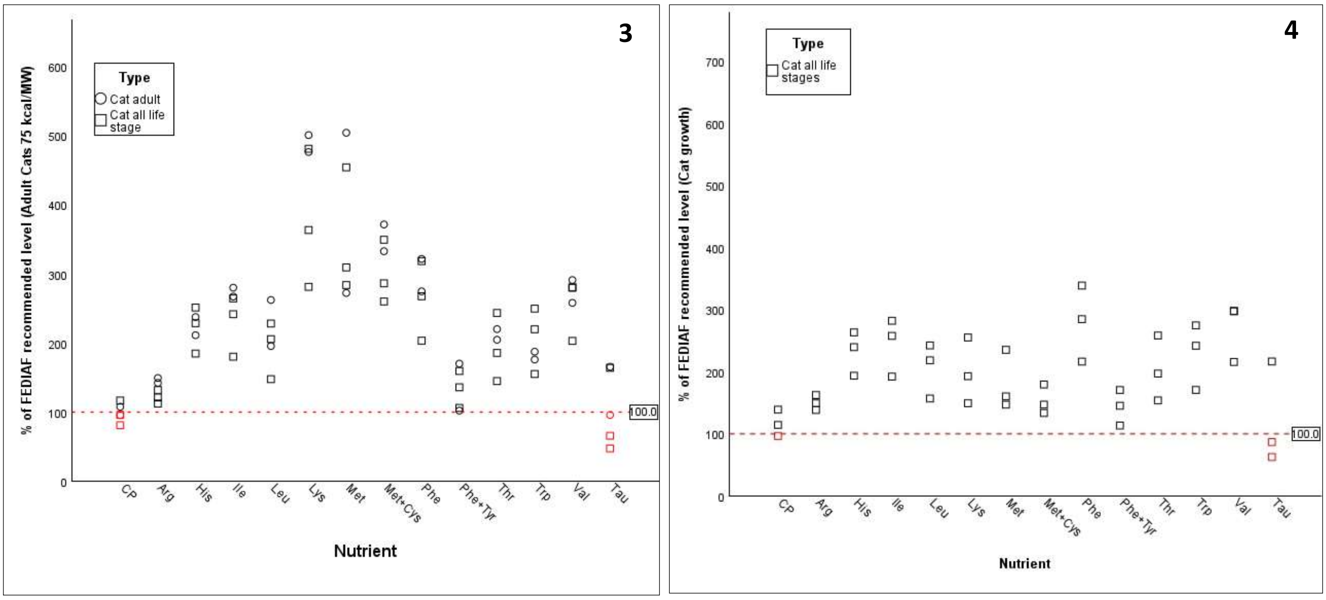


Figure 3 and 4. Comparison between the nutrient analysis of five complete and balanced insect-based adult cat foods against FEDIAF minimum recommendations for adult cats (75kcal/MW) unit/1000 kcal of ME **(Figure 3)**. Comparison between the nutrient analysis of three complete and balanced insect-based cat foods for growing cats against FEDIAF minimum recommendations for cats under growth unit/1000 kcal of ME **(Figure 4)**. Results are shown as percentages of FEDIAF fulfilment. The red dotted line marks 100% of the FEDIAF minimum recommendation, with red icons indicating products exceeding maximum limits or falling below minimum requirements.

Comparison with NRC recommendations

Seventeen products (59%) met NRC standards. Specifically, 11/20 (55%) of the adult canine diets, and 4/7 (57%) of the growth-phase canine diets were compliant. For cats, 3/5 (60%) adult maintenance products and 1/3 (33%) growth products were compliant.

In the adult canine products, eight (40%) had Se below AI (71% of average supply; range: 36.8-99.7). Among the canine growth products, one (14%) had isoleucine below MR (97% of supply), and two (29%) had P below AI (70% of average supply; range: 63-77%). In adult feline diets, one product (20%) supplied 98% of EE, and one (20%) supplied 75% of Se compared to AI recommendations. One (33%) all life stage product for cats provided 98% of AI recommendations for EE in kittens. No product exceeded NRC SUP.

Comparison with FDA recommendations

All 29 products met the FDA, 2011, with Mo and Hg levels below MTL (see Supplementary material: Supplementary Tables S2, S4 and S6).

Comparison between declared protein source with DNA analyses

Twenty hypoallergenic-labelled products (canine: 15 dry and one wet; feline: four dry) were analysed for DNA content. Five samples (25%) did not comply with their declared animal sources, lacking any or containing undeclared animal DNA; three of these were from the same brand.

Of the products tested, 14 specified the insect species, and 12 (86%) matched their labels. However, two samples deviated from their claims: one wet product contained no detectable animal

DNA, and another had a different insect species (BSF instead of YMW). All five samples that did not specify an insect species contained BSF DNA. Additionally, 15% (3/20) of the samples contained DNA from non-declared, non-insect sources (chicken, turkey, and salmon) (Table 3).

Table 3. Labelled animal protein and fat sources compared to Next Generation Sequencing (NGS) DNA results from twenty products labelled as 'hypoallergenic'.

Product ID	**Insect species declared**	**Labelled animal protein source**	**Labelled animal fat source**	**Identified species DNA by NGS**
3	-	Insect protein	N/A	BSF (*Hermetia illucens*)
4	-	Insects	N/A	BSF (*Hermetia illucens*)
8	BSF	Insects	Insect oil	BSF (*Hermetia illucens*)
9	BSF	Insects	Insect oil	BSF (*Hermetia illucens*)
10	BSF	Insects	Insect oil	BSF (*Hermetia illucens*)
12	BSF	Insects	Insect oil	BSF (*Hermetia illucens*)
13	BSF	Insect meal	Poultry fat, insect oil	BSF (*Hermetia illucens*)
14	BSF	Insect meal	Salmon oil	No animal species detected
15	BSF	Insect meal and poultry liver	Poultry fat, fish oil	BSF (*Hermetia illucens*)
16	BSF	Insect meal	Animal fat, salmon oil	BSF (*Hermetia illucens*)
17	BSF	Dried insects	Animal fat, salmon oil	BSF (*Hermetia illucens*)
18	BSF	Insect meal	N/A	BSF (*Hermetia illucens*)
20	YMW	Insect meal	Insect oil	YMW (*Tenebrio molitor*); **Chicken (*Gallus gallus*); Turkey (*Meleagris gallopavo*)**
21	YMW	Insect meal	Insect oil	**BSF (*Hermetia illucens*); chicken (*Gallus gallus*);** YMW (*Tenebrio molitor*)
22	YMW	Dried insects, hydrolysed animal protein	Animal fat	**BSF (Hermetia illucens)**
23	-	Dried insects	Poultry fat, fish oil	BSF (*Hermetia illucens*)
24	-	Dried insects	Poultry fat, fish oil	BSF (*Hermetia illucens*); **Atlantic Salmon**
25	-	Insect protein and krill protein	Salmon oil	BSF (*Hermetia illucens*)
26	BSF	Dried insects	Poultry fat, salmon oil	BSF (*Hermetia illucens*)
29	-	Dried insects	Purified animal fat	BSF (*Hermetia illucens*)

BSF= Black soldier fly; YMW = Yellow mealworm; OTC = Over-the-counter diet; N/A – not applicable; NGS = Next Generation Sequencing; DNA = Deoxyribonucleic acid

Compliance with all the analyses

In total, only one product (3%) met label declarations and complied with NRC and FEDIAF. This compliant product was a wet, BSF-based OTC product for adult dogs. Although labelled as hypoallergenic, no DNA analysis was conducted due to limited sample availability.

Discussion

Despite the rising popularity of insect-based pet foods, no prior studies have evaluated their compliance with nutritional guidelines or screened for cross-contamination. This study addresses these gaps by analyzing the nutritional adequacy of these products and testing for undeclared animal DNA in hypoallergenic-labelled items, offering new insights into their quality and reliability.

Label adequacy

Overall, the products showed poor compliance with declared values and tolerances, raising concerns regarding labelling accuracy in the pet food industry. Prior research has found similar issues across both niche (e.g., raw, vegetarian diets, or those from smaller companies) (Burdett *et al.*, 2018; Kanakubo *et al.*, 2015; Vecchiato *et al.*, 2022), and conventional brands (Burdett, *et al.*, 2018; Hill, *et al.*, 2009; Jacuńska, *et al.*, 2023; Kanakubo, *et al.*, 2015; Vecchiato, *et al.*, 2022).

Nearly half of the products underestimated energy content, potentially contributing to obesity (Speakman, 2004), with a mean ME of +4.2% (range: +0.2% to +12.4%). Although this mean may seem modest, even a small increase in energy content can significantly affect an animal's total energy intake, resulting in unintended weight gain (German, 2006; Hill *et al.*, 2009). While the inclusion of ME on labels is not legally required (Regulation (EC) No 767/2009), it is a valuable addition, especially for animals with specific energy needs. Importantly, although in this study ME was estimated using predictive equations, which generally align well with *in vivo* studies (Calvez *et al.*, 2019), there are limitations. Some manufacturers may have obtained more accurate ME values by using feeding trials—a gold standard in ME assessment (FEDIAF, 2024)—providing a potentially more reliable representation of true energy content. Differences in methods and variations in the ingredients used could account for the observed discrepancies, suggesting that findings should be interpreted with caution.

Our results showed a wide range of CF content in the products (1.6% to 10% DM), with many underestimating fibre levels by up to -29%, raising concerns about labelling accuracy in insect-based pet foods. Since excessive fibre can impair nutrient digestibility (Kröger *et al.*, 2017; Marx *et al.*, 2022) inaccurate labelling may have health implications, though it remains unclear if certain fibre types, such as chitin, have this effect (Kröger *et al.*, 2020; Penazzi *et al.*, 2021). In general, CF analysis tends to underestimate total fibre, while total dietary fibre (TDF) gives a more comprehensive view of fibre content (de-Oliveira *et al.*, 2012). For insect-based products, which contain chitin, there may be additional variability in fibre and chitin content based on the type and inclusion levels of insect ingredients. Although it is uncertain how much of an impact the CF and TDF methods have on chitin readings specifically, adding chitin measurements as a separate label item could improve transparency. This would help consumers better understand fibre content and its potential effects on health and digestibility as insect-based ingredients become more common.

Nutritional analyses vs Guidelines

Most products failed to meet FEDIAF's recommendations for lower energy intake but aligned better with higher energy intake values and NRC. While NRC MR or AI levels are often deemed adequate, they may not fully meet the needs of pets with specific energy and nutrient demands. FEDIAF standards, which account for digestibility, energy requirements, and nutrient bioavailability, are often more appropriate for pets with lower energy needs (FEDIAF, 2021). For example, FEDIAF guidelines raise NRC recommendations by 20% to account for housed dogs' typically lower energy needs. NRC recommendations, based on moderate activity, may overestimate energy requirements by 10–60% (Männer, 1991), as most pets do not exercise as much (Slater *et al.*, 1995).

Nutrient inadequacies were most common in Se, Ca, P, Ca/P ratios, and taurine. Two-thirds of the products had inappropriate Se levels, either too low or too high. Previous researchers have also identified suboptimal Se levels in pet food (Davies *et al.*, 2017; Paulelli *et al.*, 2018; Pereira *et al.*, 2018). Notably, FEDIAF only provides a maximum legal tolerance for Se. The nutritional effects of high Se levels are unclear, but diets with 5.0 mg/kg of Se have been linked to severe liver damage in dogs (Levander, 1986), while low levels, can lead to muscular weakness, depression, subcutaneous oedema, dyspnoea and coma (Vleet, 1975). However, Se concentrations in this study ranged from 0.08 to 1.4 mg/kg, well below the critical 5.0 mg/kg threshold.

Insects are considered a great protein and fat source alternative (Rumpold and Schï, 2013), generally meeting most AA and CP requirements for dogs and cats (Mccusker *et al.*, 2014). While some insect species may lack certain AA like methionine and leucine (Bosch and Swanson, 2021), most dog products in this study met most EAA levels, but some feline products were low in taurine. While BSF contains taurine (0.19 mg/g DM) (Mccusker *et al.*, 2014), levels may not always meet feline needs (NRC: 0.32 mg/g DM; FEDIAF: 1.0-2.7 mg/g DM). Comparatively, taurine levels in other commonly used animal protein sources in pet foods range widely from 0.2 to 22.0 mg/g DM. In contrast, plant-based products have either very low or undetectable amounts of taurine (Spitze *et al.*, 2003), reinforcing the importance of taurine supplementation when formulating feline diets based on alternative protein sources. Therefore, if manufacturers replace traditional animal ingredients with insects and plant sources in pet food, it is advisable to supplement taurine in feline diets. Taurine deficiency can lead to serious health issues in cats such as central retinal degeneration (Hayes *et al.*, 1975), and dilated cardiomyopathy (Pion *et al.*, 1987). In the current study, four out of five cat products were supplemented with taurine, yet two fell below FEDIAF standards, with the lowest level in a product that did not disclose any taurine addition. Nonetheless, all cat products exceeded NRC's MR for taurine.

Several products exhibited inadequate Ca, P, and Ca/P ratios, potentially affecting bone, muscle, and metabolic health (Calvo, 1993). Inadequacies were particularly concerning in diets for growing pets, which may impair skeletal development, and lead to orthopaedic or metabolic issues. In adult diets, some cat and dog products showed low and high Ca/P ratios respectively, which could pose risks to bone and kidney health (Calvo, 1993; Dobenecker *et al.*, 2017). Although the use of insect-based protein, like many common protein sources used in pet foods, naturally contains more phosphor and less calcium (Spranghers *et al.*, 2017), these imbalances are not exclusive to insect-based products. Other researchers have observed similar issues in traditional pet foods; for instance, Summers *et al.* (2020) found inverted Ca/P ratios in 13 out of 82 cat products, while Pereira *et al.* (2018) reported suboptimal Ca/P ratios in dog diets.

The variability in insect nutrient composition, due to factors like developmental stage and environment (Makkar *et al.*, 2014; Oonincx and Van Der Poel, 2011; Rumpold and Schï, 2013; Sönmez and Gülel, 2008), may explain some of the discrepancies found in this study. For instance, insects can differ greatly in Ca, Se and other nutrients depending on their feed (Ferrari *et al.*, 2022; Proc *et al.*, 2020; Spranghers *et al.*, 2017). This variability emphasizes the need for manufacturers to analyze insect meal nutrient content independently rather than relying solely on supplier data. However, nutrient inconsistencies are not exclusive to insect-based pet foods and have also been observed across various pet food categories (from traditional to alternative diets from different market segments) (Burdett *et al.*, 2018; Davies *et al.*, 2017; Dodd *et al.*, 2021; Hill *et al.*, 2009; Jacuńska *et al.*, 2023; Kazimierska *et al.*, 2020; Kępińska-Pacelik *et al.*, 2023; Paulelli *et al.*, 2018;

Pereira *et al.*, 2018; Summers *et al.*, 2020; Vecchiato *et al.*, 2022), emphasizing the broader need for improvements in formulation and production practices across the industry.

DNA analyses

Despite the lack of regulatory guidelines for ‘hypoallergenic’ claims in pet food, these products are often used to manage adverse food reactions. Our study, like previous research, suggests that cross-contamination may occur in single-protein pet foods (Fossati *et al.*, 2018; Kanakubo *et al.*, 2015; Pagani *et al.*, 2018). Quantifying animal DNA was beyond the scope of this study, preventing a clear distinction between cross-contamination and fraud. Other studies also did not perform quantification (Fossati *et al.*, 2018; Kanakubo *et al.*, 2015; Pagani *et al.*, 2018), raising concerns about detecting extremely low levels (<0.1%) of animal DNA through PCR analyses (Yancy *et al.*, 2009) which may have no significance. Human allergy studies show even trace amounts —far less than 0.1%— can trigger allergic reactions (Hourihane *et al.*, 2017; Taylor *et al.*, 2014). Conversely, others may require higher doses to elicit a response, highlighting the variability in individuals (Perkin *et al.*, 2016; EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2014). Unlike humans, however, the study of allergen thresholds in dogs and cats remains limited, necessitating more research to establish allergen thresholds in pets. To further ensure transparency and consumer trust, producers should consider labelling their pet food products with 'may contain traces of...' when there is a risk of cross-contamination, distinguishing these from products that are rigorously controlled to avoid any potential allergens.

Notably, no animal DNA was detected in one wet food, raising concerns about potential fraud or inaccuracies in labelling. Although processing might affect DNA detectability, past studies have shown that animal DNA can still be detected in wet pet foods (Pagani *et al.*, 2018), suggesting either no animal DNA was present, or levels were below the detection limit.

Limitations

This study has some limitations that should be noted. Achieving sample homogeneity is challenging in pet food; however, a thorough mixing of small packages was performed to obtain representative samples. Due to budget constraints, most nutrient analyses were conducted on single samples, which might reduce precision. This was mitigated by using control products with known profiles, yielding high concordance with lab results (CI < 15%). Lastly, while the study's sample size of 29 products may seem limited, it represents a substantial portion of the available insect-based pet foods, suggesting that the findings provide a reasonably accurate snapshot. Nonetheless, further research with larger samples and a broader nutrient range (e.g. vitamins, and fatty acids) would offer a more comprehensive view of these products' nutrient compositions.

Conclusion

In conclusion, the findings reveal that almost all products had at least one issue, whether related to label inadequacy (76%), non-compliance with FEDIAF (90%), NRC (41%) nutritional recommendations or declared animal DNA (25%). Ensuring accurate nutrient information and addressing these discrepancies through quality controls is essential for product reliability and pet nutrition. While insects show promise as a sustainable protein source, further research and standardized protocols are needed to optimize their integration into pet diets.

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Conflict of interest

Authors have no conflict of interest to declare.

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