Timisoara, 25-26 May

IDENTIFYING ANIMAL AND VEGETAL SPECIES AND INCORRECT LABELLING IN PET FOODS

BOLDURA O.M.*[1], TUDOR B.A.M.[1], MARC S. [1], SAVICI J. [1], TULCAN C. [2],POPESCU S. [2], HUȚU I. [1], MIRCU C. [1]

1 University of Life Sciences, Faculty of Veterinary Medicine, 300645, Calea Aradului, 119, Timișoara, România

2 University of Life Sciences, Faculty of Engineering and Applied Technologies

**e-mail: oanaboldura@usvt.ro*

Abstract: *Mislabelling, falsifying and substituting food products is a growing problem in highly processed foods, including pet foods. The study aimed to conduct market surveys of pet foods sold by specialised pet stores to identify the present animal and vegetal species and any instances of incorrect labelling. Twenty commercial dry products were collected from specialised pet stores. DNA was extracted from each product in duplicates, and the component species were identified using polymerase chain reactions (PCR). Species-specific analyses were used to verify the existence of meat species (bovine, pigeon, pig, chicken, turkey, pork, fish and horse). Plant species were present in all the products analysed, a large share, in this case, corn and soybeans. Poultry meat products are the most used in the composition of the analysed products. They are detected in 80% of the samples and are correctly labelled. Fish meat products are also widely used, especially in cat food. The percentage of 75% is also reached by using fish oils in the composition of these products. Products from ruminants are present in a high percentage, both as the main component and as animal protein or fat sources. Pig and horse products were detected in a reasonably low percentage and level. They are not mentioned on the labels and can be classified as animal protein and fat used in the composition of the analysed pet food.*

• Introduction

Products intended for feeding animals encompass a wide range of options, including different types of feed, nutritional supplements, vitamins, minerals, and food additives. These products are designed to provide the necessary nutrients for animal growth, development, and overall health, considering species, age, weight, and physiological stage. They can be made from plant, animal, or synthetic ingredients, with some specifically formulated to meet the unique nutritional needs of certain animals.

The composition of animal food plays a critical role in maintaining the health, energy, longevity, and behaviour of pets like dogs and cats. Consumer trends are leaning towards healthier and more natural food options, prompting regulatory agencies to enforce compliance with food labelling, nutritional quality, and food origin. This helps prevent false claims from food manufacturers and ensures the safety and well-being of animals. The legislation and standards regarding pet food quality and adulteration vary from country to country. Regulatory authorities establish and regulate minimum quality standards for pet food. These standards typically cover nutritional composition, safety, and labelling requirements. They may specify the proportions of protein, fat, and carbohydrates, as well as the types of ingredients used. Economically motivated adulteration poses a risk in the pet food industry, where fraudulent substitution or addition of ingredients may occur to increase product value or reduce production costs. Protein detection in animal feed is conducted through various methods, such as enzyme-linked immunosorbent tests (ELISAs) and DNA analysis. ELISA-based methods have been used to detect residues of herbicides like glyphosate in commercial animal feed, but they have shown less sensitivity and precision compared to PCR-based methods specifically designed for canine food analysis. DNA analysis can be employed to identify the presence or absence of specific animal species, such as pork, chicken, or beef, to detect adulteration in feed. Overall, ensuring the quality, authenticity, and nutritional value of animal feed products is crucial for maintaining animal health, meeting consumer demands, and safeguarding public safety.

• Material and method

The reference material in this project used, as a positive reaction control DNA isolated from various matrices. The plant reference material was represented by DNA solutions isolated from corn and soybean flours. The reference material of animal origin was represented by DNA solutions isolated from dried and ground muscle tissue from the species for which the detection experiments were performed: chickens, fish, ruminants, pigs and horses. The biological material is represented by 20 samples of dry food intended for dogs and cats, purchased from speciality stores on the domestic market.

Isolation and purification of DNA from the samples taken in the study was carried out with the help of the kit "NucleoSpin DNA Forensic" (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. The DNA solution was qualitatively and quantitatively evaluated by the spectrophotometric method using the NanoDrop 8000 equipment. PCR reactions were performed using the Surecycler Thermocycler, Agilent Technologies, Santa Clara, CA, US. The reagents that made up the amplification mixture were as follows: PCR kit: GoTaq Green Master Mix (Promega, Oregon, US) - 12.5 µl, 20 pmol of each primer, template DNA – 1 µl, adjusted with distilled water up to 25 µl. PCR products were migrated in agarose gel in the presence of ethidium bromide, and visualized under UV light. The gel image was captured and analyzed using Vision Works software, UVP, Analytik Jena, Germany.

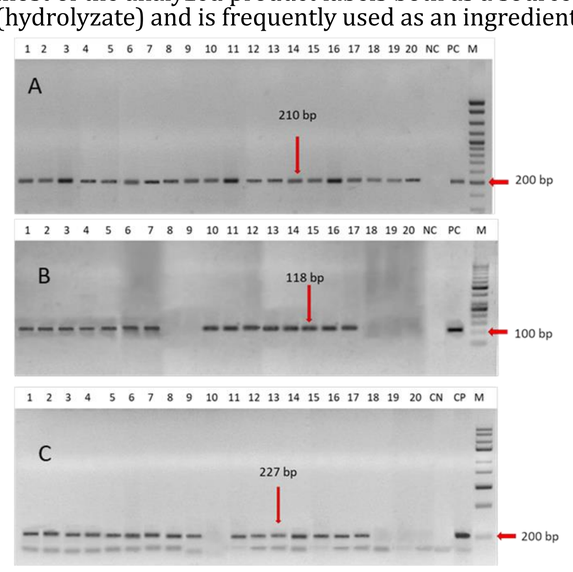
• Results and discussions

Protein-based methods for species identification can fail after excessive proteolysis or heat-induced denaturation of indicator proteins. It is suggested that genomic DNA from milk somatic cells persists in the processed material and can be amplified and analysed for species identification. Also, many of these methods are based on the properties of the proteins found in the meat of the species from which the raw material was taken. These are just some of the methods used to detect the composition of pet food. Other methods can be used, depending on the needs and resources of each animal food production company, and these techniques based on DNA analysis appear to be the most accurate and rapid. The combination of materials of vegetable and animal origin in the composition of pet food refers to the use of plant-based and animal-based ingredients in the formulation of pet food products. Plant-based ingredients commonly used in pet food include grains, vegetables, fruits, and various plant proteins. These ingredients can provide a source of carbohydrates, fiber, vitamins, and minerals. Animal-based ingredients used in pet food typically include meat, poultry, fish, and their by-products.

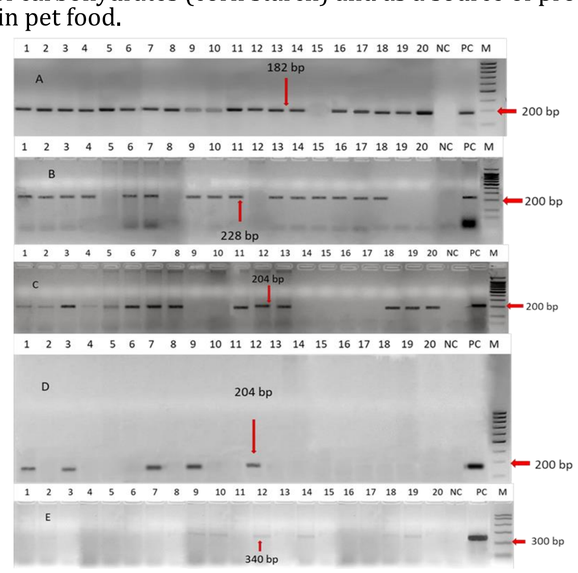
In this context, the first stage of the presented experiment was represented by a screening that allowed the identification of the material of plant origin by using its specific markers, namely for the ribulose-1,5-bisphosphate-carboxylase (RuBisCo) gene.

• Results and discussions

By identifying this gene, it was possible to certify the presence of material of plant origin as well as certify the amplifiable quality of the obtained DNA. The most frequently encountered plant species in the composition of pet food are soy and corn. Thus the first screening analyses had in mind the highlighting of the presence of plant material originating from soy and of the plant material of corn, the presence of the latter being noted on most of the analyzed product labels both as a source of carbohydrates (corn starch) and as a source of protein (hydrolyzate) and is frequently used as an ingredient in pet food.



The results of the PCR analysis using gene-specific primers from the plant material: Panel A - RuBisCo gene; Panel B – lectin gene; Panel C – zein gene. Lanes 1 – 20 Amplification products for the biological samples analyzed in this experiment; NC – negative reaction control (DNA isolated from material of animal origin); PC – positive reaction control – DNA solution isolated from material of vegetable origin (soy flour or corn) M – molecular weight marker for nucleic acids – PCR marker (Promega).



The results of the PCR analysis using gene-specific primers from the animal material: Panel A - chicken; Panel B – fish; Panel C – ruminants; Panel D – pork; panel C - horses. Lanes 1 – 20 Amplification products for the biological samples analyzed in this experiment; NC – negative reaction control (DNA isolated from plant material); PC – positive reaction control – DNA solution isolated from material of animal origin (specific to each analyzed species – reference materials) M – molecular weight marker for nucleic acids – PCR marker (Promega).

In our study we considered the detection of the most common animal species in pet food, namely: bird, ruminant, fish, pig and horse. the results of the analyzes are presented in the images and systematized in the table below.

Results from the screening study for species identification

Sample	Tagged species	Species identified
1	poultry, corn, other plant species, animal fats, animal proteins, yeast, fish oil, soybean oil.	Poultry, fish, ruminants, pigs, soybeans, corn
2	poultry, corn, other plant species, animal fats, animal protein, fish oil, soybean oil.	Bird, fish, ruminants, soybeans, corn.
3	poultry, corn, other plant species, animal fats, animal protein, fish oil, soybean oil.	Bird, fish, ruminants, pork, soybean, corn.
4	poultry, corn eggs, other vegetable species, animal fats, animal proteins, fish oil, soybean oil.	Bird, fish, ruminants, soybeans, corn.
5	poultry, corn, other plant species, animal fats, animal proteins, yeasts, soybean oil.	Bird, ruminants, soybeans, corn.
6	poultry, corn, other vegetable species, animal fats, animal proteins, fish oil,yeasts, soybean oil.	Bird, fish, ruminants, soybeans, corn.
7	fish, poultry, corn, other vegetable species, animal fats, eggs, fish oil, soybean oil.	Bird, fish, ruminants, pork, soybean, corn.
8	poultry, other plant species, animal fats, animal proteins, yeasts.	Bird, ruminants, corn.
9	poultry, corn, other vegetable species, animal fats, animal proteins, fish oil,yeasts.	Bird, fish, pig, horse, corn.
10	fish, other vegetable species, animal fats, animal proteins, fish oil,yeasts, soybean oil.	Bird, fish, horse, soybean.
11	poultry, fish, whole grains, other vegetable species, animal fats, animal proteins, fish oil, soybean oil.	Bird, fish, ruminants, soybeans, corn.
12	poultry, corn, other plant species, animal fats, animal protein, soybean oil.	Bird, ruminants, pig, horse, soybean, corn.
13	poultry, corn, other vegetable species, animal fats, animal proteins, fish oil,yeasts, soybean oil.	Bird, fish, ruminants, soybeans, corn.
14	poultry, corn, other vegetable species, animal fats, animal proteins, fish oil,yeasts, soybean oil.	Bird, fish, horse, soybean, corn.
15	corn, other vegetable species, animal fats, animal proteins, fish oil, soybean oil.	Fish, soybeans, corn.
16	poultry, corn, other vegetable species, animal fats, animal proteins, fish oil,yeasts, soybean oil.	Fish, soybeans, corn.
17	poultry, corn, other vegetable species, animal fats, animal proteins, fish oil,yeasts, soybean oil, algae.	Bird, fish, soybeans, corn.
18	lamb, bird, other vegetable species, bird fat, fish oil,yeasts, crustaceans.	Bird, fish, ruminants, horse.
19	lamb, bird, other vegetable species, bird fat, fish oil,yeasts, crustaceans.	Bird, ruminants, horse.
20	lamb, bird, other vegetable species, bird fat, fish oil,yeasts, crustaceans.	Bird, ruminants.

• Conclusions

Molecular analyses based on the detection of nucleic acids can be successfully used in the identification of the species that enter the composition of pet food, allowing the correctness of the label to be accurately established. The standardization of these methods, to be used in the authentication of commercial products, is necessary, as these methods are also applicable to highly processed products.

Plant species are present in all the analysed products, a large share, in this case, corn and soy. Poultry meat products are the most used in the composition of the analyzed products; they are detected in 80% of the samples and are correctly labelled. Fish meat products are also widely used, especially in cat food. The percentage of 75% is also reached by the use of fish oils in the composition of these products. Products from ruminants are present in a high percentage, both as the main component and as sources of animal protein or fat. Pig and horse products were detected in a fairly low percentage and level; they are not mentioned on the labels and can be classified as animal protein and fat used in the composition of the analysed pet food.