www.foodandfeed.fins.uns.ac.rs
FOOD AND FEED RESEARCH
Journal of the Institute of Food Technology – FINS
University of Novi Sad
ISSN
2217-5369
(print version ceased in 2023)
2217-5660 (online)

UDK 577.175.8:636.087.6]:636.045
https://doi.org/10.5937/ffr0-61243

Original research paper

HISTAMINE IN PET FOOD

Sandra M. Jakšić[*1], Tamara M. Galić[2], Ema Z. Gardić[1], Tomislav I. Kačarević[1], Ksenija D. Nešić[3], Nenad S. Popov[1], Milica M. Živkov Baloš[1]

[1]Scientific Veterinary Institute "Novi Sad", 21000 Novi Sad, Rumenački put 20, Serbia
[2]University of Novi Sad, Faculty of Sciences, Department of Chemistry, Biochemistry and Environmental Protection, 21000 Novi Sad, Trg Dositeja Obradovića 3, Serbia
[3]Institute of Veterinary Medicine of Serbia, Food and Feed Department, 11070 Belgrade, Smolućska 11, Serbia

Abstract: Histamine is a biogenic amine formed by microbial decarboxylation of histidine and serves as a significant indicator of protein degradation in animal-based raw materials. Although traditionally associated with fish and fishery products, recent studies have confirmed its presence in pet foods containing fish and meat meals. Factors such as improper storage, elevated temperatures, and prolonged pre-processing intervals significantly contribute to the accumulation of histamine. Regulatory limits for histamine exist in foods intended for human consumption; however, comparable legal thresholds for pet food are currently lacking. Nevertheless, elevated histamine levels may negatively impact palatability, nutrient absorption, and animal health, particularly in sensitive populations such as juveniles and immunocompromised pets. This study examines histamine levels in various animal-derived raw materials and commercial pet foods (both dry and canned), with a particular focus on chicken, game, and fish meals. An HPLC-UV method was used to analyse 13 protein-rich raw materials for pet food samples and 14 pet food samples. Histamine was not detected in shrimp, lamb, quail or pork meals. In chicken and fish meals, histamine content ranged from 23.86±2.60 to 256.98±14.69 mg/kg. In 8 out of 10 dry pet food samples, histamine levels ranged from 9.18±0.56 to 54.24±4.12 mg/kg. Canned pet food samples were free of detectable histamine.

Key words: *histamine, meat meal, fish meal, pet food*

INTRODUCTION

Biogenic amines are nitrogen-containing compounds formed through the microbial decarboxylation of amino acids or transamination of aldehydes and ketones (Fig. 1.). Their presence has been documented in a wide range of food products, particularly those rich in proteins, such as meat, fish, cheese, and fermented products (Petrović et al., 2016; Torović, Gusman & Kvrgić, 2023). Although biogenic amines play a physiological role in various cellular processes, their accumulation in food can lead to serious health concerns. Among them, histamine is considered the most toxic, and its ingestion can

Corresponding author: Phone: +381214895363 Fax: +381214895344
E-mail address: sandra@niv.ns.ac.rs

cause so-called "scombroid poisoning," characterized by symptoms such as skin flushing, headache, diarrhea, vomiting, and, in severe cases, respiratory distress (Petrović et al., 2016; Durak-Dados, Michalski & Osek, 2020). The toxic effects of histamine can be further exacerbated by secondary amines such as putrescine and cadaverine, which exert a synergistic effect (Durak-Dados et al., 2020).

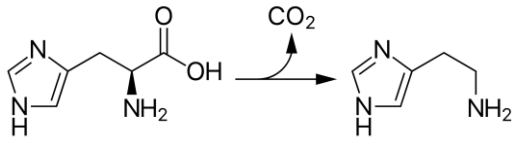

Figure 1. Conversion of histidine to histamine by histidine decarboxylase

Regulations concerning the presence of biogenic amines in food are not fully harmonised (DeBeer, Bell, Nolte, Arcieri & Correa, 2021), particularly with respect to pet food. The EU limit of 100 mg/kg was established in food, only for fishery products (EC, 2005). Literature reports suggest that histamine concentrations in food above 100 mg/kg may be potentially toxic, while some studies have observed adverse effects at levels as low as 50 mg/kg, which is often cited as a recommended upper intake limit (Montegiove et al., 2023). The European Pet Food Industry Federation has recognised histamine as a relevant hazard in fish and meat meals, and has established good manufacturing practice guidelines to ensure product safety (FEDIAF, 2025).

The safety of pet food largely depends on the quality of the raw materials used in its production. Fresh meat and meat meals are most commonly used, with evidence indicating that products containing meat meal generally have higher levels of biogenic amines due to the lower quality of input materials and inadequate storage conditions (Montegiove et al., 2020). Although some studies have shown an increased risk of histamine contamination in feed material of fish origin (Paulsen, Bauer, Kukleci, Smulders & Dicakova, 2021a), the results of pet food studies have not confirmed a higher risk compared to pet foods containing beef protein (Paulsen, Bauer, Bauer & Dicakova, 2021b). Regarding animal toxicity, dogs and cats have exhibited histamine intolerance symptoms similar to those observed in humans, including gastrointestinal disturbances, respiratory issues, and allergic reactions (Blonz & Olcott, 1978; Verlinden, Hesta, Millet & Janssens, 2006; Craig, 2019).

A variety of analytical methods are employed to determine histamine in food. The most commonly used techniques include enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), gas chromatography, capillary electrophoresis, and thin-layer chromatography (Bassanese et al., 2012; Jakšić, Živkov Baloš, Mihaljev, Prodanov Radulović & Nešić, 2017; Learey, Crawford-Clark, Bowen, Barrow & Adcock, 2018). The standard method for determining histamine in fish and fishery products involves pre-column derivatisation with dansyl chloride and UV detection after separation by liquid chromatography (ISO 19343:2017). Recently, there has been a growing adoption of high-resolution mass spectrometry-based techniques, which allow accurate quantification of histamine in complex matrices (Montegiove et al., 2020). Monitoring histamine concentrations is of critical importance, not only as an indicator of potential health risks for animals but also as a marker of microbial contamination and raw material quality (Learey et al., 2018).

Given the increasing consumption of pet food and the importance pet owners place on animal health, understanding the factors influencing histamine formation, along with the implementation of proper control measures during production, represents a key component in ensuring the safety and quality of these products.

The occurrence of histamine may be attributable to contamination introduced during either the manufacturing process or storage of the final product. Accordingly, this investigation encompassed both the raw materials employed in pet food production and the finished products. The aim of this study was to determine which raw material used in pet food production is most susceptible to histamine contamination, as well as to assess the safety and freshness of pet food available on the Serbian market.

MATERIALS AND METHODS

This research investigates the amount of histamine present in various feed materials (protein-rich raw materials for pet food) used in the production of pet food (13 samples), as well as in commercial cat and dog foods, including

both dry (10 samples) and canned (4 samples) products. The pet food used in this study was produced by Serbian pet food manufacturers. Samples were collected directly at the production site and included products formulated with chicken- and fish-based ingredients. Particular attention was given to meals derived from chicken, game, and fish. A detailed overview of the sample types is provided in Table 1.

Sample preparation

Histamine levels were measured using the HPLC-UV technique following the ISO 19343:2017 standard method. The extraction process involved mixing the sample with perchloric acid. Samples of pet food and meal were prepared for analysis by weighing 5 g of the sample into a 50 mL cuvette, adding 0.1 M perchloric acid (10 mL or 20 mL, depending on the sample), mixing the solution thoroughly until homogenized, and then centrifuging at 4000 rpm for 5 minutes. Pre-column derivatization was carried out using dansyl chloride (A13828.06, Thermo Scientific, Germany). Further sample preparation was carried out entirely in accordance with the ISO standard method. All samples were prepared and analysed in triplicate.

HPLC-UV determination

Separation of histamine from other biogenic amines was achieved through an HPLC Dionex UltiMate 3000 Series system (Thermo Scientific, Germany), using a SupelCosil™ LC-18-DB column (250 x 4.6 mm ID, 5 µm particle size). A gradient elution method was employed with acetonitrile and water as the mobile phase, and detection was done via UV at 254 nm. Chromeleon®7 software (Thermo Scientific, Germany) was used to operate the system. The histamine concentration (mg/kg) was determined by comparing the area ratio of histamine (L09198.06, Thermo Scientific, Germany) to that of the internal standard (1,7-diaminoheptane; L02853.06, Thermo Scientific, Germany), using a matrix-matched calibration curve ranging from 0 to 500 mg/kg.

Method performance

The standard method used was validated in the laboratory for the determination of histamine in fish and fish products, and its reliability was further confirmed through participation in the FAPAS (2024) proficiency testing scheme. For this study, a matrix-matched calibration curve was constructed based on the analysis of spiked pork meal sample, demonstrating linearity ($R^2 > 0.997$). The recovery rate of the method was also confirmed through spiking experiments, yielding 98%. The limit of detection was 2.5 mg/kg for raw materials and 5 mg/kg for extracted finished pet food products.

RESULTS AND DISCUSSION

The results of histamine analysis by HPLC-UV are shown in Table 1. Based on the presented results, a considerable variability in histamine content can be observed across different types of raw materials and pet food samples. The highest histamine levels were detected in fish meal, with concentrations reaching up to 256.98 ± 14.69 mg/kg (Fig. 2). This finding indicates that fish is particularly prone to histamine formation, consistent with the well-documented activity of histidine decarboxylase in bacteria commonly found in fish tissue. In comparison, chicken meal contained much lower, though still significant, amounts of histamine (25.97 ± 0.52 and 56.84 ± 3.25 mg/kg), while shrimp, lamb, quail, and pork meals showed no detectable histamine. These results suggest that the risk of histamine accumulation depends on the type of raw material. While Altafini et al. (2022) showed that storage conditions influence histamine accumulation, with increases at elevated temperatures, our study did not evaluate storage dynamics, but the high variability observed among meals suggests that pre-processing handling of raw materials likely plays a similar role.

With regard to finished pet food, the results show that histamine was not detected in two of six analysed chicken-based dry food, while in several samples low to moderate concentrations were present (9.18–15.78 mg/kg, Fig. 3). In fish-based dry food (Fig. 4), the histamine levels ranged from 13.01 to 54.24 mg/kg, with one sample showing a notably elevated value. In contrast, canned fish-based pet food contained no detectable histamine, most likely due to thermal processing and sterilization procedures that reduce microbial activity and inhibit the formation of biogenic amines.

Overall, the findings indicate that the greatest risk for histamine presence lies in animal-derived meals, particularly those of fish and chicken origin, whereas finished pet foods based on this type of meal generally appear safer, with lower or non-detectable concentrations.

Nevertheless, the occurrence of elevated values in certain fish-based dry food samples highlights the importance of continuous monitoring and the implementation of stricter control measures throughout the production chain. This is essential to ensure the safety of animal feed and to protect the health of pets. The study by Altafini et al. (2022) reported histamine levels in commercial fish-based cat foods ranging from 1.5 to 30.1 mg/kg, with the majority of samples below the EU safety limit of 100 mg/kg for fishery products.

Table 1.
Histamine content (mg/kg) in raw materials serving as protein sources in pet food and pet food samples

Sample	Origin	No samples	Histamine (mg/kg)
Protein-rich raw materials	Chicken	2	25.97 ± 0.52
			56.84 ± 3.25
	Fish	7	256.98 ± 14.69
			23.86 ± 2.63
			27.93 ± 3.29
			35.89 ± 1.95
			118.82 ± 1.38
			31.78 ± 2.13
			65.74 ± 2.98
	Shrimp	1	ND
	Lamb	1	ND
	Quail	1	ND
	Pork	1	ND
Pet dry food	Chicken	6	ND
			ND
			9.18 ± 0.56
			15.26 ± 1.21
			15.78 ± 2.80
			12.03 ± 0.56
	Fish	4	14.75 ± 0.77
			13.84 ± 2.12
			13.01 ± 1.23
			54.24 ± 4.12
Pet canned food	Fish	4	ND

Results are given as mean ± standard deviation (n = 3); ND – not detected

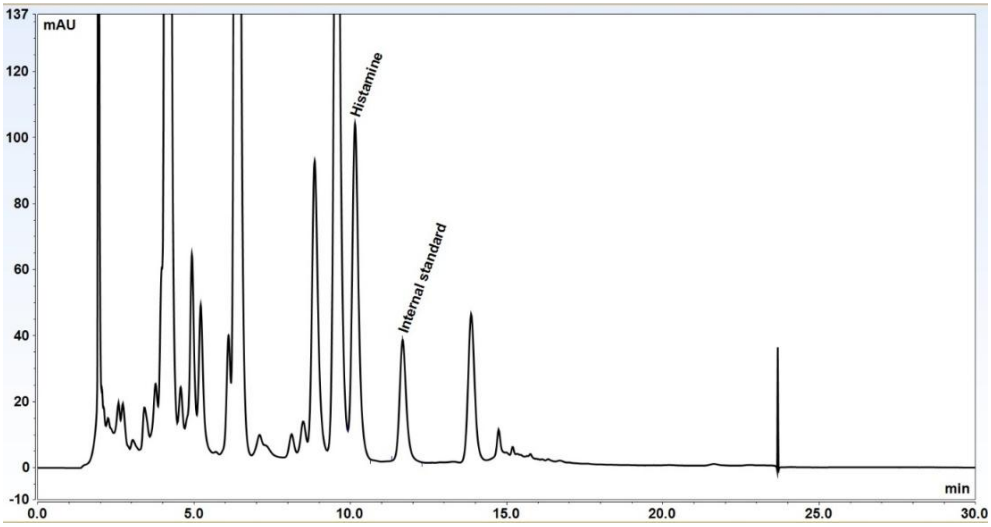

Figure 2. Chromatogram of fish meal sample containing 256.98 mg/kg of histamine

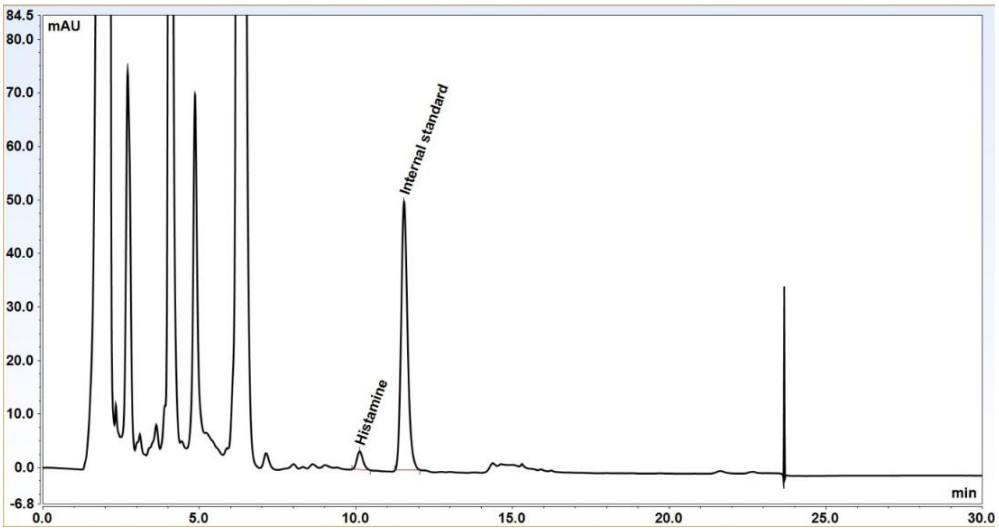

Figure 3. Chromatogram of cat dry food sample with chicken protein containing 15.78 mg/kg of histamine

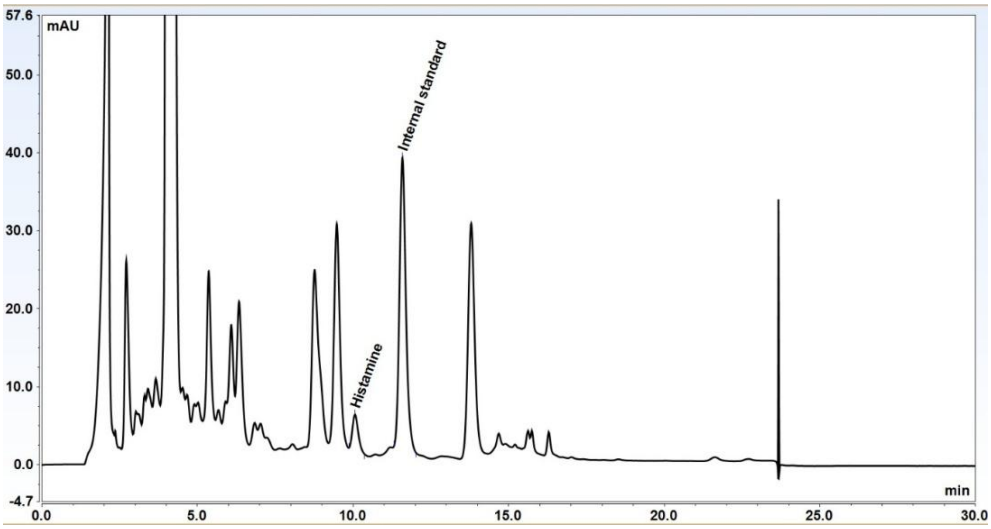

Figure 4. Chromatogram of dog dry food sample with fish protein containing 14.75 mg/kg of histamine

This range is consistent with the results presented in this study for dry fish-based foods. Importantly, both studies confirm that canned foods tend to be safer, with no significant histamine detected after processing.

Montegiove et al. (2020) focused on fresh meats and meat meals as raw materials for dry pet food production and demonstrated that meat meals contained significantly higher histamine concentrations than fresh meats. For example, salmon meat meal contained up to twenty times more histamine than fresh salmon. This aligns with our finding that fish meals carried the highest histamine burden, while finished dry foods contained lower amounts, reflecting both dilution with other ingredients and the partial inactivation of microbial decarboxylases during extrusion. Montegiove et al. (2020) also emphasized that biogenic amines are stable to heat and therefore persist in the final product, underscoring the need for high-quality raw material selection.

In the study by Paulsen et al. (2021b), while more cat food samples contained significantly higher amounts of tyramine, dog food samples contained significantly higher amounts of histamine and spermine. However, in our study, sample with highest histamine content was cat food, and pet food samples without histamine were dog food. These results are consistent with the fact that the finished product, pet food, used in cat nutrition is based on a higher percentage of animal nutrients compared to dog food. In the aforementioned study median contents of his-

tamine in a variety of canned food for dogs and cats on the Austrian market were 14.5 mg/kg, and maximum values were 61.6 mg/kg wet weight. This is in agreement with our results, although our research included a significantly smaller number of samples. Contrary to our results, these authors state a lower histamine content in fish-based pet food compared to fish-free pet foods. However, they analysed significantly more fish-free samples

CONCLUSIONS

This study provides relevant evidence on the occurrence of histamine in raw materials and pet foods, clearly showing that raw materials, particularly fish and chicken meals, could be major critical point for contamination. The detection of concentration above 250 mg/kg in fish meal demonstrates the high susceptibility of these raw materials to microbial activity and emphasizes the need for rigorous monitoring. In contrast, other protein sources such as shrimp, lamb, quail, and pork showed no detectable histamine, suggesting that the risk is species-dependent.

An important contribution of this work is the comparison between raw materials serving as protein sources in pet food and finished products. While extrusion and sterilization markedly suppress histamine production histamine levels, especially in canned foods, our results confirm that biogenic amines persist in dry products, with concentrations up to 54.24 mg/kg. This finding underlines that technological processing cannot fully compensate for poor raw material quality, emphasizing the need for strict quality control at the earliest stages of production.

From a practical perspective, these findings point to the importance of sourcing high-quality raw materials, improving storage and transport conditions, and introducing systematic monitoring of histamine in both ingredients and final products. From a regulatory perspective, the results support the need for establishing specific threshold values for histamine in pet food, analogous to those already defined for human consumption. Future research should expand to other biogenic amines, assess synergistic toxic effects, and evaluate the impact on animal health under real feeding conditions. Overall, this study contributes valuable data for science community, industry, and regulators, representing an important step toward ensuring the safety and nutritional quality of pet food.

AUTHOR CONTRIBUTIONS

Conceptualization S.M.J.; Methodology, S.M.J.; T.I.K. and E.Z.G.; Investigation, formal analysis, validation, writing-original draft preparation, S.M.J. and T.M.G.; Writing-review and editing, S.M.J.; N.S.P. and K.D.N.; Supervision, M.M.Ž.B.

DATA AVAILABILITY STATEMENT

Data contained within the article.

ACKNOWLEDGEMENTS

This research was funded by Ministry of Science, Technological Development and Innovation of Republic of Serbia by the Contract of implementation and funding of research work of NIV-NS in 2025, Contract No: 451-03-136/2025-03/200031.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Altafini, A., Roncada, P., Sonfack, G. M., Guerrini, A., Romeo, G. A., Fedrizzi, G., & Caprai, E. (2022). Occurrence of histamine in commercial cat foods under different storage conditions. *Veterinary Sciences*, *9*, 270. https://doi.org/10.3390/vetsci9060270

Bassanese, D. N., Crawford-Clark, S., Bowen, B. J., Henderson, L. C., Barnett, N. W., & Conlan, X. A. (2012). The determination and characterisation of 2-naphthyloxycarbonyl chloride derivatised biogenic amines in pet foods. *Journal of Separation Science*, *35*, 1110–1117. https://doi.org/10.1002/jssc.201200012

Blonz, E. R., & Olcott, H. S. (1978). Effects of orally ingested histamine and/or commercially canned spoiled skipjack tuna on pigs, cats, dogs and rabbits. *Comparative Biochemistry and Physiology*, *61C*(1), 161–163. https://doi.org/10.1016/0306-4492(78)90127-2

Commission Regulation (EC). (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of European Union*, *L338*, 1–26.

Craig, J. M. (2019). Food intolerance in dogs and cats. *The Journal of Small Animal Practice*, *60*, 77–85. https://doi.org/10.1111/jsap.12959

DeBeer, J., Bell, J.W., Nolte, F., Arcieri, J., & Correa, G. (2021). Histamine limits by country: A survey and review. *Journal of Food Protection, 84,* 1610-1628. https://doi.org/10.4315/JFP-21-129

Durak-Dados, A., Michalski, M., & Osek, J. (2020). Histamine and other biogenic amines in food. *Journal of Veterinary Research, 64,* 281-288. https://doi.org/10.2478/jvetres-2020-0029.

FEDIAF. (2025). Nutritional Guidelines for complete and complementary pet food for cats and dogs; FEDIAF: Brussels, Belgium, September 2025. Retrieved 12.01.2026. from https://europeanpetfood.org/wp-content/uploads/2025/09/FEDIAF-Nutritional-Guidelines_2025-ONLINE.pdf

Ferrante, M.C., & Mercogliano, R. (2023). Focus on histamine production during cheese manufacture and processing: A review. *Food Chemistry, 419,* 136046. https://doi.org/10.1016/j.foodchem.2023.136046.

ISO 19343:2017 Microbiology of the food chain -- Detection and quantification of histamine in fish and fishery products -- HPLC method.

Jakšić, S., Živkov Baloš, M., Mihaljev, Ž., Prodanov Radulović, J., & Nešić, K. (2017). Comparison of analytical methods for the determination of histamine in reference canned fish samples. In *IOP Conf. Series: Earth and Environmental Science of the* 59th *International Meat Industry Conference* MEATCON 2017, *85*, 012066. Zlatibor, Serbia. https://doi.org/10.1088/1755-1315/85/1/012066

Learey, J. J., Crawford-Clark, S., Bowen, B. J., Barrow, C. J., & Adcock, J. L. (2018). Detection of biogenic amines in pet food ingredients by RP-HPLC with automated dansyl chloride derivatization. *Journal of Separation Science*, *41*, 4430–4436. https://doi.org/10.1002/jssc.201800455

Montegiove, N., Calzoni, E., Cesaretti, A., Alabed, H., Pellegrino, R. M., Emiliani, C., Pellegrino, A., & Leonardi, L. (2020). Biogenic amine analysis in fresh meats and meat meals used as raw materials for dry pet food production. *Scientific Bulletin. Series F. Biotechnologies*, *24*, 33–41. https://www.researchgate.net/publication/349412287

Montegiove, N., Leonardi, L., Cesaretti, A., Pellegrino, R.M., Pellegrino, A., Emiliani, C., & Calzoni, E. (2023). Biogenic amine content analysis of three chicken-based dry pet food formulations. *Animals, 13,* 1945. https://doi.org/10.3390/ani13121945

Paulsen, P., Bauer, S., Bauer, F. & Dicakova, Z. (2021b). Contents of polyamines and biogenic amines in canned pet (dogs and cats) food on the Austrian market. *Foods*, *10,* 2365. https://doi.org/10.3390/foods10102365

Paulsen, P., Bauer, S., Kukleci, E., Smulders, F.J.M., & Dicakova, Z. (2021a). Gehalte an biogenen Aminen in Tierfutterkonserven und in zur Rohverfütterung bestimmter Rindermuskulatur (BARF). *Journal of Food Safety and Food Quality, 72,* 89–93.

Petrović, J, Babić, J, Jakšić, S, Kartalović, B, Ljubojević, D. & Ćirković, M. (2016). Fish product-borne histamine intoxication outbreak and survey of imported fish and fish products in Serbia. *Journal of Food Protection, 79,* 90-94. https://pubmed.ncbi.nlm.nih.gov/26735034/

Torović, Lj., Gusman, V., & Kvrgić, S. (2019). Biogenic amine and microbiological profile of Serbian dry fermented sausages of artisanal and industrial origin and associated health risk. *Food Additives & Contaminants: Part B, 13,* 64–71. https://doi.org/10.1080/19393210.2019.1697380

Verlinden, A., Hesta, M., Millet, S. & Janssens, G.P. (2006). Food allergy in dogs and cats: a review. *Critical Reviews in Food Science and Nutrition*, *46,* 259-273. https://www.tandfonline.com/doi/abs/10.1080/10408390591001117

HISTAMIN U HRANI ZA KUĆNE LJUBIMCE

Sandra M. Jakšić[*1], Tamara M. Galić[2], Ema Z. Gardić[1], Tomislav I. Kačarević[1], Ksenija D. Nešić[3], Nenad S. Popov[1], Milica M. Živkov Baloš[1]

[1]Naučni institut za veterinarstvo "Novi Sad", 21000 Novi Sad, Rumenački put 20, Srbija
[2]Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Departman za hemiju, biohemiju i zaštitu životne sredine, 21000 Novi Sad, Trg Dositeja Obradovića 3, Srbija
[3]Naučni instiitut za veterinarstvo Srbije, Odeljenje za ispitivanje hrane i hrane za životinje, 11070 Beograd, Smolućska 11, Srbija

Sažetak: Histamin je biogeni amin koji nastaje mikrobnom dekarboksilacijom histidina i predstavlja značajan pokazatelj razgradnje proteina u sirovinama životinjskog porekla. Iako se tradicionalno povezuje sa ribom i ribljim proizvodima, nedavne studije su potvrdile njegovo prisustvo u hrani za kućne ljubimce koja sadrži riblja i mesna brašna. Faktori kao što su nepravilno skladištenje, povišena temperatura i produžen period skladišenja pre prerade značajno doprinose akumulaciji histamina. Zakonske granice za maksimalno dozvoljeni sadržaj histamina u hrani namenjenoj ljudskoj ishrani postoje; međutim, odgovarajući limiti za hranu za kućne ljubimce trenutno ne postoje. Ipak, povišeni nivoi histamina mogu negativno uticati na ukus hrane, apsorpciju hranljivih materija i zdravlje životinja, naročito kod osetljivih populacija kao što su mladunci i imunokompromitovani ljubimci. Ova studija ispituje nivoe histamina u različitim sirovinama životinjskog porekla i komercijalnoj hrani za kućne ljubimce (suvoj i konzervisanoj), sa posebnim fokusom na pileće i brašna divljači i ribe. Za analizu 13 uzoraka sirovina za hranu za kućne ljubimce i 14 uzoraka hrane za kućne ljubimce korišćena je HPLC-UV metoda. Histamin nije detektovan u uzorcima proteinskog brašna od škampa, jagnjetine, prepelice ili svinjetine. U pilećem i ribljem brašnu, sadržaj histamina se kretao od 23,86±2,60 do 256,98±14,69 mg/kg. U 8 od 10 uzoraka suve hrane za kućne ljubimce, nivoi histamina su se kretali od 9,18±0,56 do 54,24±4,12 mg/kg. U uzorcima konzervisane hrane za kućne ljubimce histamin nije detektovan.

Ključne reči: *histamin, mesno brašno, riblje brašno, hrana za kućne ljubimce*

Received: *02 September 2025/* ***Received in revised form:*** *01 December 2025/* ***Accepted:*** *01 December 2025*

Available online: *February 2026*