# The distribution of biogenic amines and polyamines in pheasant meat

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**Abstract**

Biogenic amines are a natural component of living organisms, though in foodstuffs they are primarily indicators of the degradation of proteins. Their formation in meat is frequently seen in connection with the action of contaminating microorganisms during food storage. This study focuses on the influence of various conditions, intravital factors (slaughtered or hunted pheasants) and post-mortal factors (the period and temperature of storage, type of muscle) on the formation of biogenic amines in pheasant meat. The content of biogenic amines in stored meat was evaluated in relation to its microbial status. Selected biogenic amines were discussed as possible indicators of the degradation of meat proteins, and the health risk when the meat is consumed was assessed.

*Pheasant, biogenic amines, E. coli, Enterobacteriaceae, storage*

## Introduction

In the past, game was associated largely with the performance of hunting. Today, however, it is a freely available commodity of increasing popularity that represents added diversity to people's diet. It has a high protein content and low fat content, a favourable proportion of essential and unsaturated fatty acids, and a high content of certain B-group vitamins. It is, however, a foodstuff that goes off extremely easily.

The commonest way of obtaining game is hunting in the autumn months. Feathered game, including pheasants, is hunted with shotguns. Individual birds obtained by hunting are distributed as shot, i.e. not drawn or plucked. Massive contamination of the muscle tissue may, however, occur during hunting and may represent a hygiene problem. According to European Parliament and Council (ES) Regulation No. 853/2004 (EC 2004), the meat of wild animals must, for this reason, be refrigerated in such a way that all parts of the body attain a temperature not exceeding 4 °C within a reasonable period of time after death. On the basis of further legislation, ungutted small wild animals may be stored at temperatures of –1 to 4 °C for a period of 15 days. In this country, however, hunters tend to leave game, including feathered game, hanging freely outside for several days or even weeks. Practically the only factor influencing the period for which game remains fresh during the period for which it is left hanging is the outdoor temperature.

Biogenic amines are substances that are a natural component of foodstuffs in low concentrations. The majority of biogenic amines are formed by the decarboxylation of free amino acids as a result of the action of bacterial decarboxylases (Karovičová and Kohajdová 2005). They are formed during fermentation or decomposition processes in foods (Suhaj and Kováč 1996). At higher concentrations, amines may have a negative impact on human health. Vasoactive biogenic amines (histamine, tyramine and 2-phenylethylamine) and substances associated with a possible influence on the growth of tumours (polyamines – spermine, spermidine and putrescine) are significant from the toxicological viewpoint. Amine determination may serve as one of possible indicators of the quality or level of deterioration of foodstuffs. Their formation is often monitored

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in connection with the action of contaminating microorganisms during the storage of foodstuffs, particularly the meat of slaughter animals and game.

The aim of this work was to study the formation of biogenic amines in connection with the action of microorganisms during the storage of foodstuffs, the possibility of their use as an indicator of freshness, and their assessment from the point of view of food safety. The work also aimed to determine the influence of various intravital factors (farm breeding as opposed to free-living animals) and post-mortal factors (the period and temperature of storage, type of muscle) on the formation of biogenic amines in the breast and leg muscle of the common pheasant and, thereby, their influence on the quality of pheasant meat.

### Materials and methods

Samples of pheasant muscle were taken from birds bred on a farm (n = 100) and birds shot on the Židlochovice Forest Enterprise of the state corporation Lesy ČR, s.p. (Czech Forests) (n = 100). The farm-reared birds were killed at an age of 5 months by pithing, the hunted birds were hunted 1 or 2 days before being supplied to the University of Veterinary and Pharmaceutical Sciences Brno. The slaughtered and hunted pheasants were stored ungutted at refrigerated temperatures (0 to 2 °C) or outdoors (6 to 15 °C) for a period of 4 weeks. Following storage, the pheasants were skinned (with feathers) and drawn, and samples of muscle tissue taken. Samples of breast and leg muscle were taken from each group at weekly intervals for a period of 4 weeks.

Biogenic amines (BA), i.e. histamine, tyramine, tryptamine, 2-phenylethylamine and cadaverine, and polyamines (PA), i.e. putrescine, spermine and spermidine, were extracted from a matrix of pheasant muscle with 10% trichloracetic acid. The BA and PA were subsequently analysed as dansyl derivatives by quantitative RP-HPLC with gradient elution and fluorescence detection (Paulsen et al. 1997). The measurements were evaluated by Empower fluorescence software (Waters, USA).

Microbiological analysis was performed on freshly killed birds and subsequently at regular weekly intervals in each group. Sampling and preparation for microbiological tests were performed in accordance with the requirements of the standard ČSN ISO 7218. The total number of psychrotrophic microorganisms was determined in accordance with the standard ČSN ISO 17410:2003; the number of bacteria of the family *Enterobacteriaceae* in accordance with ČSN 21528-2, and *Escherichia coli* by means of detection on COLIFORM Agar (enhanced selectivity) (Merck KgaA, Germany).

An average of two measurements was used for the purposes of statistical evaluation. The results were statistically evaluated by the program STATISTICA Cz (Statsoft, Czech Republic). A Student's t-test was applied to determine differences between days of storage.

## Results and discussion

The total numbers of microorganisms were not significantly different in relation to origin or storage temperatures. Their content did, however, increase with the period of storage in both breast and leg muscle. The absolute values in both cases were, however, relatively low ($3.2 \times 10^2$ CFU·g$^{-1}$ to $1 \times 10^4$ CFU·g$^{-1}$). Levels of microbial contamination around one order of magnitude lower were discovered in all samples of breast muscle. The increase after 2 weeks of storage was significantly ($P \leq 0.01$) higher, particularly in birds stored outdoors.

The presence of *Escherichia coli* bacteria was detected in hunted pheasants after a week of storage, though not until after 2 weeks in farm-reared pheasants. Their numbers increased significantly ($P \leq 0.01$) with the period of storage, with values of around one order of magnitude higher in hunted pheasants amounting to $1.5 \times 10^4$ CFU·g$^{-1}$. Similar values of *E. coli* in pheasants were reported by Paulsen et al. (2008).

In view of the fact that pheasants belong among gallinaceous birds, their microbial spectrum can be expected to be similar to that of poultry. That will include in particular representatives of the family *Enterobacteriaceae*. During the initial test for microorganisms, bacteria of the *Enterobacteriaceae* family were found only sporadically in the samples; after 2 weeks of storage, however, a value of $3.0 \times 10^3$ CFU·g$^{-1}$ of leg muscle was found in refrigerated storage, and a value almost one order of magnitude higher recorded for outdoor storage.

Petkov et al. (1984) believe that the level of microbial contamination of pheasant meat depends on the time that has elapsed between killing and cooking. If massive tissue

bruising should occur or if the content of the bowels is incorporated into the body cavity, then a suitable environment is created for the growth and reproduction of microorganisms. This means that the period of meat freshness depends on the site of shot impact in hunted pheasants, and on the period and temperature of storage in both groups of birds.

BA in meat are generally formed by the decarboxylation of amino acids as a result of the action of enzymes from contaminating bacteria. The growth in the concentration of putrescine, cadaverine, tyramine and tryptamine is connected to spoilage, while histamine is associated with a health risk (Karovičová and Kohajdová 2005).

The highest concentrations of the BA tryptamine (18.8 mg $kg^{-1}$) and the PA spermidine (7.3 $mg \cdot kg^{-1}$) and spermine (31.0 $mg \cdot kg^{-1}$) were found at the beginning of storage in the muscle of a hunted pheasant; the values found in farm-reared pheasants were insignificantly lower. The concentrations of the polyamines spermidine and spermine, which occur naturally in the organism, fell during storage as polyamines serve as a source of nitrogen for the microflora present. Reporting on the breast muscle of chickens, Balamatsia et al. (2006) mentioned the same initial values of spermidine (7.9 $mg \cdot kg^{-1}$), but higher values of spermine (53.3 $mg \cdot kg^{-1}$). The increased value of cadaverine (14.7 $mg \cdot kg^{-1}$) and tyramine (62.8 $mg \cdot kg^{-1}$) measured in week 4 of storage in the muscle of hunted pheasants stored outdoors signals the development of a microflora with decarboxylation activity at this time (Figure 1).

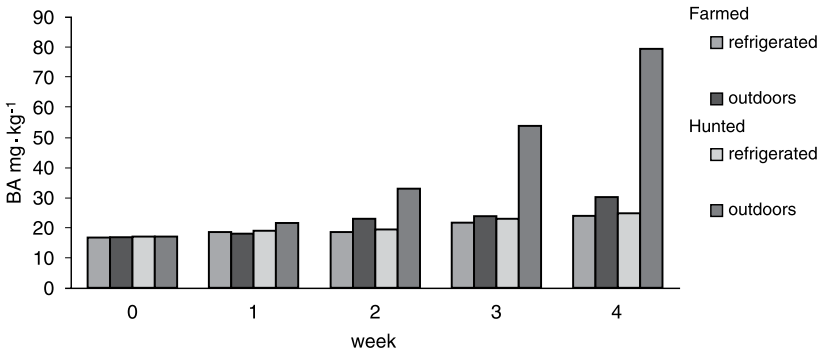


Fig. 1. The effect of the period and temperature of storage on the formation of BA in the breast muscle of hunted and farm-reared pheasants

The given values indicate the initiation of the spoilage process (Patsias et al. 2006). Silva and Glória (2002) also detected tyramine in chicken only at the end of storage in a quantity of 17.4 $mg \cdot kg^{-1}$. In view of the fact that tyramine is produced by coliform microflora and lactic acid bacteria, traces of tyramine (cca 2 $mg \cdot kg^{-1}$) are evidence of a good initial microbiological situation. Histamine being detected only towards the end of storage is also in agreement with the findings of Balamatsia et al. (2006). In view of the fact that significant quantities of histamine are produced by certain representatives of *Enterobacteriaceae,* the absence of histamine during the course of storage and a low level at the end of storage reflects a low number of contaminating bacteria.

It follows from studies into various factors affecting the content of BA that storage at a refrigerated temperature is more suitable for pheasants from the BA growth point of view. Samples stored at a refrigerated temperature showed lower concentrations of BA than samples stored outdoors, and this was true of both farmed-reared pheasants and hunted pheasants. A higher quantity of BA is formed in hunted pheasants regardless of the method of storage or the type of muscle. The reason for this may be damage to the muscle tissue

and the body cavity caused by shot, resulting in greater propagation of contaminating microorganisms. There were significantly more BA ($P \leq 0.01$) in the samples of breast muscle of hunted pheasants stored outdoors. Differences in the content of BA in breast and leg muscle were also discovered. A high quantity of BA was formed in the breast muscle of farm-reared pheasants, and this was true for both methods of storage (Figure 2). The higher content of BA in pheasant breast muscle may be the result of varying proportions of protein in the leg and breast muscle.

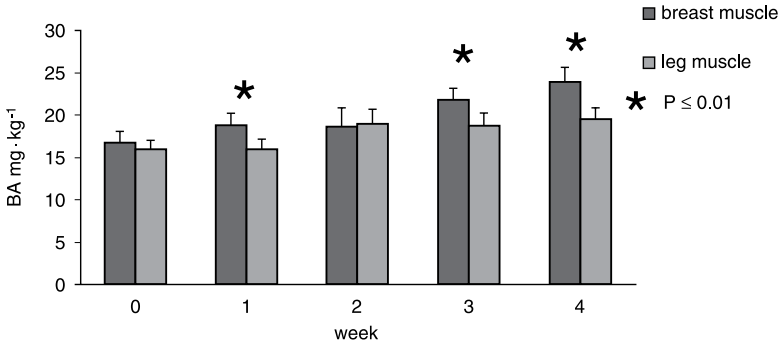


Fig. 2. The effect of the type of muscle tissue on the BA content in farm-reared pheasants stored in the refrigerator

## Conclusions

The total content of BA and their profile depends on a large number of factors. The basic factors are the availability of substrate, the presence of decarboxylase-positive microorganisms, and conditions that allow the bacteria to grow and produce enzymes. The amino acid decarboxylase ability is extremely variable, for example *Enterobacteriaceae* are capable of producing putrescine and cadaverine, while lactobacilli can produce histamine and tyramine. In order to create significant quantities of tyramine, for example, a high content of bacteria is required in matrices, higher than, e.g. $10^6$ CFU·g$^{-1}$. A large number of other factors have an effect on the formation of BA. These may include the intravital and post-mortal factors observed in this study.

The risk of increased formation of BA was demonstrated in our experiment, particularly in hunted pheasants stored outdoors. Even if conditions corresponding to the legislation are maintained, the massive propagation of contaminating microflora and the decaying of the game may occur after two weeks. It is, then, possible that an increased content of BA significant from the viewpoint of hygiene may have a negative effect on the organism of risk groups of people.

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**References**

Balamatsia CC, Paleologos EK, Kontominas MG, Savvaidis IN 2006: Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4 °C: possible role of biogenic amines as spoilage indicators. Antonie van Leeuwenhoek Journal of Microbiology **89**: 9–17

Karovičová J, Kohajdová Z 2005: Review. Biogenic amines in food. Chem Pap **59**: 70–79

Nařízení EP a Rady (ES) č. 853/2004 ze dne 29.dubna 2004, kterým se stanoví zvláštní hygienická pravidla pro potraviny živočišného původu. Ústřední věstník Evropské unie ze dne 30.4.2004, L 139/55, 14–74

Patsias A, Chouliara I, Paleologos, EK, Savvaidis I, Kontominas MG 2006: Relation of biogenic amines to microbial and sensory ganges of precooked chicken meat stored aerobically and under modified atmosphere packaging at 4 °C. European Food Research and Technology **233**: 683–689

Paulsen P, Bauer F, Vali S 1997: Biogene amine in Rohwuersten. 1. Methodische Aspekte zur Bestimmung biogener Amine. Fleischwirtschaft **77**: 450–452

Paulsen P, Nagy J, Popelka P, Ledecký V, Macinčák S, Pipová M, Smulders FJM, Hofbauer P, Lazar P, Dičákova Z 2008: Influence of storage conditions and shotshell wounding on the hygienic condition of hunted, uneviscerated pheasant (*Phasianus colchicus).* Poultry Science **87**: 191–195

Petkov R, Gogov I, Lozenski V 1984: Microbial studies of pheasant meat. Vet Med Sci **21**: 100–105

Silva CMG, Glória MBA 2002: Bioactive amines in chicken breast and thigh after slaughter and during storage at 4 - 1 °C and in chicken – based meat products. Food Chem **78**: 241–248

Suhaj M, Kováč M 1996: Prírodné toxikanty a antinutričné látky v potravinách. Guidebook, Food research institute, SPPK Bratislava, SR, 140s