Effects of storage temperature on biogenic amine concentrations in meat of uneviscerated pheasants (*Phasianus colchicus*)

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Abstract

The aim of this study was to determine the hygienic quality of the pheasants reared for high-quality meat production by the biogenic amine concentrations in their meat. The content of biogenic amines was measured in the meat of sixty male pheasants killed by pithing and stored uneviscerated for 21 days under different storage temperatures (0 °C, 7 °C and 15 °C). The samples of breast and thigh muscles of pheasant were tested at weekly intervals. Biogenic amines were analysed by reverse phase liquid chromatography and detected by tandem mass spectrometry. Concentrations of biogenic amines (except spermin and spermidin) in thigh muscle were higher than in breast muscle. Highly significant difference ($P < 0.01$) was found in tyramine (5.80 mg/kg and 1.38 mg/kg for thigh and breast muscle, respectively), cadaverine (40.80 mg/kg and 14.43 mg/kg for thigh and breast muscle, respectively), putrescine (13.42 mg/kg and 3.16 mg/kg for thigh and breast muscle, respectively) and histamine (5.51 mg/kg and 1.70 mg/kg for thigh and breast muscle, respectively) concentrations after 21 days of storage at 15 °C. This study provides information on the dynamics of biogenic amine formation in pheasant meat during 21 days of storage at different temperatures. Based on our results, we can recommend storing pithed uneviscerated pheasants at 0–7°C for up to 21 days, or at 15 °C for up to 7 days. Concentrations of biogenic amines gained in our study can be helpful in evaluating freshness and hygienic quality of the pheasant game meat.

Putrescine, cadaverine, tyramine, game meat, hygienic quality, pithed pheasant

Biogenic amines are low-molecular weight nitrogen compounds formed mainly by decarboxylation of amino acids or amination and transamination of aldehydes and ketones (Santos 1996). Biogenic amines in food are formed as a result of the growth of decarboxylase-positive microorganisms under conditions propitious for that enzyme activity. Production of biogenic amines in foods is associated with the presence of microorganisms and their activity in food (Halász et al. 1994). The monitoring of biogenic amine concentrations is very important because, from the human health point of view, their presence in food at elevated concentrations has been involved in various cases of food-borne diseases and cases of human poisoning (Shalaby 1996), and has been associated with negative effects on human heath (Balamatsia et al. 2006; Gallas et al. 2010; Naila et al. 2010; Standarová et al. 2012) and various other toxicological effects (Hernández-Jover et al. 1997).

Biogenic amine concentrations in meat and meat products have been studied by many authors. It has been established that high biogenic amine concentrations in meat and meat products are generated as a result of microbial contamination and improper storage conditions (Santos 1996). In terms of human health, the most important biogenic amines are putrescine, cadaverine, histamine, tyramine, spermine, spermidine and beta-phenylethylamine (Sahalaby 1996). While spermine and spermidine are always present in meat and meat products, concentrations of the other biogenic amines vary (Hernández-Jover et al. 1996, 1997). Biogenic amines serve as indicators of poor quality of meat

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and meat products (Durlu-Ozkaya et al. 2001). Putrescine, cadaverine, histamine and tyramine are considered freshness markers and can be used as indicators of microbial spoilage (Bóka et al. 2012). Biogenic amines in poultry have been studied by Silva and Gloria (2002), Balamatsia et al. (2006) and Tamim and Doerr (2003).

In feathered game, biogenic amines may be highly significant as indicators of hygienic quality. This is because of the customary handling of game birds, which are hung in feathers for a period of several days to improve sensory quality of their meat. In view of game bird microbial contamination and temperature variables during hanging, we might assume a deterioration of hygienic quality of the game and an associated marked increase in biogenic amine content in the meat of game birds. Paulsen et al. (2008) looked into the issue of biogenic amines in uneviscerated pheasants. Raising pheasants for slaughter and the subsequent meat production has recently acquired great importance (Hofbauer et al. 2010). Consequently, the question of biogenic amine concentrations in the meat of slaughtered or pithed pheasants has also become very important. The issue of biogenic amines in the meat of pithed pheasants has been dealt with by Standarová et al. (2012).

The aim of our experiment was to study how long uneviscerated pheasant carcasses may be stored at different temperatures (0 °C, 7 °C or 15 °C) by the content of biogenic amines in the meat of common pheasant slaughtered (pithed) for meat production.

Materials and Methods

In our experiment we used 60 male common pheasants of a mean weight of 1 250 g ± 183 g. The pheasants were killed by pithing which means interrupting their spinal cord and destroying the brain. Pheasant carcasses were not eviscerated (the body cavity was not opened), which is a standard procedure for handling feathered game. Then, carcasses were divided into three groups of 20 birds each and placed into three different coolers each set at a different temperature (0 °C, 7 °C and 15 °C). We chose the storage temperature of 0 °C as a temperature at which no major changes in meat are expected due to low microbial activity at this temperature and hence no major changes in biogenic amine concentrations. The temperature of 7 °C was chosen in order to obtain data on changes in biogenic amine concentrations in meat at a temperature when some changes due to microbial contamination can already occur. The temperature of 15 °C was chosen as a temperature at which pheasant may be stored when hung in the open air, or in the case of e.g. some cooling system failure when pheasants are stored in coolers, and at which the development of microbial activity in pheasant meat is assumed.

The storage period was 21 days. During this period at regular weekly intervals on days 1, 7, 14 and 21 after, they were killed, samples of 5 pheasant carcasses from each group stored at a specific temperature were used for examination. To determine the concentration of biogenic amine (putrescine, cadaverine, histamine, spermidine, spermine, tyramine, phenylethylamine and tryptamine), breast and thigh muscles (m = 0.5g) were collected from each of the carcases. To ensure the representativeness of samples the collected breast and thigh muscles were pureed. From this homogenised material the amount of 0.5 g was consequently withdrawn for biogenic amines assessment. One-step extraction using mixtures of trichloracetic acid in water (5%) followed by clean-up step using 0.45 μm syringe filter was employed for sample preparation. Biogenic amines were subsequently separated by reverse phase liquid chromatography using C_{18} (2.1 mm × 50 mm, 1.9 μm; Thermo, San Jose, CA, USA) column and detected by tandem mass spectrometry using a heated electro spray-ionization in positive ion mode. Thermo Scientific UHPLC Accela 1250 system was connected to a Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo, San Jose, CA, USA).

The results were processed by the Unistat 5.6 statistical software package and evaluated using the Kruskal-Wallis test. Statistical difference was presented as $P < 0.05$ and $P < 0.01$.

Results

We found that no major changes in biogenic amine concentrations occurred in breast muscles of pithed, uneviscerated pheasants stored at 0 °C and 7 °C for 21 days. Variations in the concentrations of individual biogenic amines, with the exception of spermine and spermidine, did not exceed 0.2 mg/kg.

Changes in biogenic amine concentrations recorded in breast muscle of uneviscerated pheasant stored at 15 °C are shown in Table 1. The highly significant ($P < 0.01$) changes

Table 1. Biogenic amine concentrations (mg/kg) in breast muscle of uneviscerated pheasants stored at the temperature of 15 °C.

	Days of storage			
Biogenic amines	1	7	14	21
Putrescine	0.01 ± 0.00	0.07 ± 0.13	1.64 ± 2.61[b]	3.16 ± 2.75**[b]
Cadaverine	0.03 ± 0.00	0.03 ± 0.00	4.72 ± 6.07[b]	14.43 ± 12.51**[b]
Histamine	0.01 ± 0.00	0.10 ± 0.07	0.58 ± 0.47[b]	1.70 ± 1.68**[b]
Spermidine	2.11 ± 0.61	1.74 ± 0.24	2.24 ± 0.68	2.87 ± 0.45
Spermine	59.40 ± 5.09	38.49 ± 1.10	52.91 ± 2.17	55.13 ± 2.67
Tyramine	0.00 ± 0.00	0.00 ± 0.00	1.17 ± 1.02	1.38 ± 0.98**[a]
Phenylethylamine	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.04	0.05 ± 0.05
Tryptamine	0.00 ± 0.00	0.00 ± 0.01	0.13 ± 0.21	0.24 ± 0.06

Data presented as mean ± standard deviation; in each term 5 samples were assessed
**Highly significant change ($P < 0.01$) compared to the biogenic amine concentration assessed on the 1st day of storage, [a]Highly significant difference ($P < 0.01$) between breast and thigh muscle, [b]Significant difference ($P < 0.05$) between breast and thigh muscle

in biogenic amine concentrations (in excess of 0.5 mg/kg) during the whole storage period occurred in cadaverine, tyramine, putrescine and histamine. Changes in phenylethylamine and tryptamine concentrations were non-significant (less than 0.5 mg/kg). Elevated initial concentrations were recorded in spermidine and spermine and no major increases in their concentrations occurred during the rest of the storage period. Changes in biogenic amine concentrations in the breast muscle of pheasants were found already on storage day 7 in cadaverine, tyramine, histamine and putrescine, however, these changes were not significant ($P > 0.05$).

We found no marked changes in biogenic amine concentrations during the entire period of 21 days in the thigh muscle meat of uneviscerated pheasants at storage temperatures of 0 °C and 7 °C (changes in the concentration of individual biogenic amines did not exceed 1.0 mg/kg).

Table 2. Biogenic amine concentrations (mg/kg) in thigh muscle of uneviscerated pheasants stored at the temperature of 15 °C.

	Days of storage			
Biogenic amines	1	7	14	21
Putrescine	1.74 ± 0.80	1.59 ± 0.87	14.13 ± 9.06*[b]	13.42 ± 6.80[b]
Cadaverine	0.05 ± 0.05	0.26 ± 0.16	20.91 ± 10.85[b]	40.80 ± 17.35**[b]
Histamine	0.57 ± 0.20	0.49 ± 0.35	2.15 ± 0.82[b]	5.51 ± 3.77*[b]
Spermidine	1.87 ± 0.68	1.57 ± 0.19	2.72 ± 0.21	2.76 ± 0.29
Spermine	39.14 ± 3.51	26.29 ± 2.26	35.07 ± 2.03	36.71 ± 2.84
Tyramine	0.00 ± 0.00	0.35 ± 0.65	2.94 ± 1.65	5.80 ± 3.68**[a]
Phenylethylamine	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.06	0.12 ± 0.10
Tryptamine	0.00 ± 0.00	0.01 ± 0.01	0.17 ± 0.14	0.31 ± 0.08

Data presented as mean ± standard deviation; in each term 5 samples were assessed
**Highly significant difference ($P < 0.01$) compared to the biogenic amine concentration assessed the 1st day of storage, *Significant difference ($P < 0.05$) compared to the biogenic amine concentration assessed on the 1st day of storage, [a]Highly significant difference ($P < 0.01$) between breast and thigh muscle, [b]Significant difference ($P < 0.05$) between breast and thigh muscle

Changes in biogenic amine concentrations found in the thigh muscle of uneviscerated pheasants stored at 15 °C are shown in Table 2. The highly significant ($P < 0.01$) changes in biogenic amine concentrations (changes in excess of 0.5 mg/kg) occurred in cadaverine and tyramine. There was a significant ($P < 0.05$) increase in histamine concentrations. Putrescine concentrations increased from 1.74 mg/kg to 13.42 mg/kg during the storage period but the increase was not significant ($P > 0.05$). Changes in phenylethylamine and tryptamine concentrations were non-significant (less than 0.5 mg/kg). Initial spermidine concentrations were relatively high, and concentrations of that biogenic amine increased only slightly during storage. Spermine concentrations were already high after the first day of storage, and no major increases in its concentrations occurred during the rest of the storage period. Concentration of biogenic amine increased in the thigh muscle of pheasants already after storage day 7. The increase in putrescine concentrations was significant ($P < 0.05$), whereas concentration increases of other biogenic amines, i.e. of cadaverine, tyramine and histamine were not significant.

It follows from the comparison of results shown in Tables 1 and 2 that the increase in cadaverine, tyramine, histamine and putrescine concentrations in the thigh muscle meat of uneviscerated pheasants stored at 15 °C is greater than that in the breast muscle meat. After 14 days of storage, a significant difference ($P < 0.05$) between the breast and thigh muscles was found in cadaverine, putrescine and histamine. After 21 days of storage, a highly significant difference ($P < 0.01$) was found in tyramine, and significant differences ($P < 0.05$) were found in cadaverine, putrescine and histamine.

Discussion

Our results show that no changes in biogenic amine concentrations occurred in the meat of breast and thigh muscles of uneviscerated pheasants killed by pithing and stored at temperatures of 0 °C or 7 °C. This is in agreement with data reported by Paulsen et al. (2008), who also found very low concentrations of biogenic amines in pheasants stored for 14 days at temperatures of 0 °C and 4 °C (in 90% of samples, biogenic amine concentrations did not exceed the 1 mg/kg limit). Low concentrations of biogenic amines were ascribed to low incidence of contaminating microorganisms (Paulsen et al. 2008). Based on our results, we may conclude that uneviscerated pheasants killed by pithing can be stored, as far as biogenic amines are concerned, for up to 21 days at temperatures from 0 to 7 °C.

Storage at 15 °C led to changes in cadaverine, putrescine, tyramine and histamine. Our results confirmed the findings of Bóka et al. (2012) that cadaverine, putrescine, tyramine and histamine can be considered markers of meat freshness and indicators of microbial spoilage. We found the biggest changes in concentration of cadaverine and putrescine, and also in tyramine. These findings confirm the conclusion of Balamatsia et al. (2006), who studied the relationship between sensory changes, microflora and biogenic amines in chicken meat stored at 4 °C. They found that tyramine concentrations remained low; putrescine and cadaverine concentrations grew linearly over the period of storage. We did not find any major changes in tryptamine or phenylethylamine concentrations, which is in agreement with results obtained in poultry meat (Silva and Gloria 2002; Balamatsia et al. 2006). Tamim and Doerr (2003) found that concentrations of those biogenic amines did not increase markedly before the onset of meat decomposition during the process of putrefaction. In our study significant changes occurred in cadaverine, putrescine, tyramine and histamine mainly after seven days of storage. It follows from our results that when temperatures at which pheasants are stored rise above 15 °C (e.g. due to fluctuating autumn temperatures during outside storage or due to a failure of the cooler) the storage period should not exceed 7 days.

In our study, we found higher increases in concentrations of cadaverine, putrescine, tyramine and histamine in the thigh muscle compared to the breast muscle. These results differ from those reported by Standarová et al. (2012) for pithed uneviscerated pheasants. The authors of this study found a higher increase in biogenic amine concentrations in the breast muscle compared to the thigh muscle. We ascribe our results (elevated concentrations of biogenic amines in thigh muscle) to the fact that uneviscerated pheasants were hung by the neck during their storage. When birds are hung in this way, the content of their intestinal tract and thus also its microbial contamination may penetrate more easily from the body cavity to thigh muscles where, as a result of microbial activity, elevated levels of biogenic amines are found.

In all meat samples rather high initial values of spermine and spermidine were found, which confirms the finding that elevated concentrations of these biogenic amines are always present in meat (Hernández-Jover et al. 1996, 1997; Silva and Gloria 2002) and that spermine and spermidine concentrations cannot be considered meat quality indicators (Balamatsia et al. 2006). The sum of cadaverine, putrescine and tyramine concentrations was also compared to the value of 5 mg/ kg considered by Hernández-Jover et al. (1997) as a marker of high hygienic quality, and of 101 mg/kg considered by Balamatsia et al. (2006) as a marker of meat spoilage. In our study, even when the storage of pheasant carcasses at temperatures above 15 °C led to a loss of high hygiene quality, signs of meat spoilage were not found.

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