The effect of treating method of pithed pheasant on the content of biogenic amines in the meat during the course of storage

Z. Hutarova,*1  V. Vecerek,*  I. Steinhauserova,†  P. Marsalek,*  G. Borilova,‡ and  P. Forejtek*  
*Department of Veterinary Public Health and Toxicology, Faculty of Veterinary Hygiene and Ecology; †CEITEC—Central European Institute of Technology, and ‡Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, 612 42, Brno, Czech Republic

ABSTRACT The study monitored the effect of various methods of treating pheasant carcasses after killing on the hygienic quality of the venison. Pithed pheasants treated by evisceration (n = 60), drawing (n = 60), or left untreated (n = 60) were stored for a period of 21 d at temperatures of 0, 7, and 15°C. For determination of biogenic amines, samples of breast and thigh muscles were taken on d 1, 7, 14, and 21 after killing of the pheasants. Biogenic amines were separated by reverse-phase liquid chromatography and consequently detected by tandem mass spectrometry. The sum of determined biogenic amine concentrations (cadaverine, putrescine, histamine, tyramine, tryptamine, phenylethylamine) was compared with the value of the index for meat of high hygienic quality (5 mg/kg). At a storage temperature of 0°C, the sum of biogenic amine concentrations did not exceed the value of 5 mg/kg in either breast or thigh muscle at any time during the storage period in untreated and drawn pheasants, and for a period of 14 d in eviscerated pheasants. At a storage temperature of 7°C, values lower than the limit of 5 mg/kg were recorded throughout the storage period in untreated pheasants, for a period of 14 d of storage in drawn pheasants, and for a period of just 7 d of storage in eviscerated birds. At the highest storage temperature (15°C), a value of 5 mg/kg was exceeded in eviscerated and untreated pheasants during the course of the first week of storage, and in drawn pheasants after the first week of storage. Our results indicate that the most suitable method of treatment to ensure high hygienic quality of the meat (assessed according to concentration of biogenic amines) for the longest period during the storage of pithed pheasants is to leave the pheasant carcasses untreated, followed by the drawing, with the least suitable method being the widely recommended method of evisceration.

Key words: biogenic amine, hygienic quality, storage temperature, treatment method, pheasant

INTRODUCTION

Pheasant farms are currently operated both for the purpose of supplying birds for hunting and for slaughtering and subsequent production of meat (Golze, 2010; Kuzniacka and Adamski, 2010; Kokoszyński et al., 2012). One way of killing pheasants is to cut the spinal cord (“pithing”). After killing, the birds’ carcasses may be treated in 3 various ways. The first method is evisceration (removal of digestive tract through an opening created by a short cut leading from cloaca in the direction of the sternum; Winkelmayer et al., 2004). The principal advantage of this method of treatment is the fact that the digestive tract may be removed completely without violating the integrity of the guts, thereby reducing the risk of contamination of the body cavity by microorganisms penetrating through the wall of digestive tract during the course of storage of the venison. The disadvantage, however, is that evisceration performed a short time after the bird is killed may lead to greater bacterial contamination of the surfaces of the muscles (El-Ghareeb et al., 2009). Another possible way of treating feathered game after killing is “drawing” (removing the coils of the intestines through the cloacal opening without violating the integrity of the body cavity). This is a traditional method used by hunters, during which the coils of the intestines are wound on a special hook introduced into the body cavity through the cloacal opening and subsequently pulled out of the body. However, this method is considered unsuitable in terms of hygiene because of the possible tearing of intestinal coils and the resulting release of digestive tract contents into the body cavity (Winkelmayer et
Birds and Their Treatment

Common pheasants (n = 180, male, at the age of 1 yr), slaughtered on the farm, were used to monitor the effect of treating method on the content of biogenic amines in meat (breast and thigh muscles) during the course of storage. The pheasants were killed by pithing (breaking the spinal cord and subsequent destruction of the brain).

The carcasses of pitted pheasants were divided into 3 groups of 60 individuals in each according to the method of treatment. The carcasses in the first group were treated in traditional way used by hunters, with drawing the digestive tract (i.e., by catching the digestive tract in the body cavity and pulling it out using a special hook introduced into the body cavity through the cloacal opening). The recommended method for treatment of feathered game carcasses (i.e., making a cut in the body cavity and extracting the organs) was used in the second group. The pheasant carcasses in the third group were left intact without further treatment (a common method of treating pheasants in hunting practice). Each group was further divided into 3 subgroups of 20 individual pheasants in each according to storage temperature. The first subgroup of pheasants was placed in a refrigerator commonly used to chill venison set to a temperature of 0°C. The second subgroup was stored at a temperature of 7°C, and the third one at a temperature of 15°C.

Samples of breast and thigh muscles were taken from 5 pheasants from each subgroup on d 1, 7, 14, and 21 of storage for determination of biogenic amine concentrations.

In each monitored group and subgroup of pheasant carcasses, average concentrations of individual biogenic amines determined in the breast and thigh muscles were added together, and the resulting concentration compared with the value of 5 mg/kg was considered as the critical concentration of biogenic amines for meat of high hygienic quality.

Biogenic Amine Assessment

Measurement of 8 underivatized biogenic amines was based on high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry. The modified method of Sagratini et al. (2012) was used for the determination. Homogenized tissue (0.5 g) was weighed in a 10-mL glass tube. The samples were extracted for 1 min with 4 mL of trichloracetic acid solution (5%) in water with an IKA Genius 3 mixer (IKA GmbH, Königswinter, Germany) and then in ultrasonic bath (Bandelin Electronic GmbH & Co., Berlin, Germany) for 20 min. The samples were centrifuged at 800 × g for 10 min at 20°C. Supernatant was filtered through 0.45-μm nylon filter (Millipore, Billerica, MA) and used for liquid chromatography-electrospray ionization tandem mass spectrometry analysis. A Thermo Scientific Hypersil C18 (2.1 mm × 50 mm, 1.9 μm) column was used at a constant flow rate of 300 μL/min. The mobile phase consisted of water containing 0.5% formic acid (vol/vol; solvent A) and acetonitrile containing 0.5% formic acid (solvent B). The gradient used was a 0 to 2 min linear gradient from 10 to 30% B, 2 to 2.5 min held at 30% B, 2.5 to 3 min from 30 to 10% B, and 3 to 3.1 min held at 10% B in order for the column to reequilibrate before the next injection. The full loop injection volume of the tissue extract was set at 10 μL. A Thermo Scientific UHPLC Accela 1250 system was connected to a Thermo Scientific TSQ Quantum Access MAX Triple Quadrupole Instrument (Thermo
Scientific, San Jose, CA) equipped with heated electrospray ionization (HESI-II) probe. The heated electrospray ionization was operated in the positive-ion mode under the following conditions: capillary temperature, 325.0°C; vaporizer temperature, 300.0°C; sheath gas pressure, 35.0 psi (241.3 kPa); auxiliary (drying) gas, 10 arbitrary units (arbitrary unit is a relative unit to show the ratio of amount of gas used by manufacturer/instrument); and spray voltage, 3,300 V. The optimal parent/product ion transitions and detection limits of biogenic amines are shown in Table 1. Standards of cadaverine, histamine, phenylethylamine, putrescine, tryptamine, and tyramine as well as trichloroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO). All solvents were of residual analysis purity (Chromservis, S.R.O., Praha, Czech Republic).

**Statistical Analysis**

Results were analyzed using the statistical package Unistat 5.6. (Unistat Ltd., London, UK). For all variables tested, normality was checked using a Shapiro-Wilk test (Zar, 1999). Data, because of their heterogeneous variances, were subjected to a Kruskal-Wallis ANOVA and subsequently to nonparametric Tukey-type multiple comparison tests with ranked sums to assess the differences between all possible pairs of groups (Zar, 1999). A $P$-value less than 0.05 was considered as significant, and a $P$-value less than 0.01 was considered as highly significant.

**RESULTS**

The overall concentrations of biogenic amines in the breast and thigh muscles of the common pheasants stored at a temperature of 0°C for the individual methods of treating are shown in Figure 1.

It is clear from Figure 1 that at a temperature of 0°C, the concentrations of biogenic amines in the breast and thigh muscles of the common pheasant carcasses left without treatment after hunting and those treated by drawing did not exceed the value considered as the critical concentration for meat of high hygienic quality (5 mg/kg) at any time during the storage period (21 d). In the carcasses of pheasants treated by evisceration, the limit value of 5 mg/kg was exceeded after d 14 of

![Figure 1. Storage at 0°C: the overall concentrations of biogenic amines in the breast and thigh muscles of pheasants treated by evisceration, drawing, or without treatment. **Statistically highly significant increase ($P < 0.01$).](https://academic.oup.com/ps/article-abstract/92/8/2182/1524083)

<table>
<thead>
<tr>
<th>Biogenic amine</th>
<th>Parent ion $^2$ (m/z)</th>
<th>Product ion $^2$ (m/z)</th>
<th>Detection limit $^2$ (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine</td>
<td>89</td>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>103</td>
<td>86</td>
<td>52</td>
</tr>
<tr>
<td>Histamine</td>
<td>112</td>
<td>95</td>
<td>27</td>
</tr>
<tr>
<td>Spermidine</td>
<td>146</td>
<td>112</td>
<td>0.71</td>
</tr>
<tr>
<td>Spermine</td>
<td>203</td>
<td>112</td>
<td>0.67</td>
</tr>
<tr>
<td>Tyramine</td>
<td>138</td>
<td>121</td>
<td>35</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>122</td>
<td>105</td>
<td>3.4</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>161</td>
<td>144</td>
<td>6.2</td>
</tr>
</tbody>
</table>

$^1$Detection limits were calculated as the concentration of the analyte that gives a signal equal to average background plus 3 times the SD of the blank.

$^2$m/z = the mass-to-charge ratio.
storage in thigh muscle. This increase in concentration of biogenic amines (mainly cadaverine and tyramine) was statistically significant.

When comparing breast and thigh muscles, the higher values of biogenic amines were found in thigh muscle in all groups of pheasant carcasses. This difference was statistically significant ($P < 0.01$) for values exceeding the limit of 5 mg/kg (eviscerated pheasants, after 21 d of storage).

A comparison of the overall concentrations of biogenic amines in the breast and thigh muscles of common pheasants treated in the 3 different ways and stored at a temperature of 7°C is shown in Figure 2.

Differences in intensity and speed of increasing in concentrations of biogenic amines are evident in all pheasants carcasses stored at a temperature of 7°C. The overall concentrations of biogenic amines did not exceed a value of 5 mg/kg at any time during the storage period (21 d) in the case of pheasant carcasses left untreated following killing. The limit value of 5 mg/kg was exceeded after 14 d of storage in the thigh muscle of pheasants treated by drawing (the main increase was observed in cadaverine and tyramine concentrations). This increase in overall concentrations of biogenic amines was statistically significant ($P < 0.01$). In the case of pheasant carcasses treated by evisceration, the limit value was exceeded in the thigh muscle after 7 d of storage (the main increase was observed in cadaverine and tyramine concentrations). The increase in overall concentrations of biogenic amines was statistically significant ($P < 0.01$).

When comparing breast and thigh muscles, the higher values of biogenic amines were detected in thigh muscle for all methods of treating pheasants, and this was statistically different for values exceeding the limit of 5 mg/kg in untreated pheasants on the third date of sampling ($P < 0.01$) and in drawn pheasants on the fourth date of sampling ($P < 0.05$).

The speed and degree of biogenic amines formation in all the monitored groups at a storage temperature of 15°C are shown in Figure 3.

A considerable increase in the overall concentrations of biogenic amines is evident in all the monitored groups at a storage temperature of 15°C. In the case of pheasant carcasses left without removal of the digestive tract from the body cavity, the overall concentrations of biogenic amines exceeded the limit value after 7 d of storage in both breast and thigh muscles. The increase in these concentrations was significant ($P < 0.01$) in both breast and thigh muscles (the main increase was observed in cadaverine and putrescine concentrations in thigh muscle and cadaverine and putrescine concentrations in breast muscle). In the group of pheasants treated by drawing, the limit value was exceeded by d 7 of storage in both breast and thigh muscles. Increasing these concentrations was significant ($P < 0.01$) in both breast and thigh muscles (in both cases the main increase was observed in cadaverine and tyramine concentrations). In the group of pheasant carcasses treated by evisceration, the limit value of 5 mg/kg was exceeded by d 7 of storage in thigh muscle. The increase was statistically significant ($P < 0.01$; main increase was observed in cadaverine and putrescine concentrations).

When comparing breast and thigh muscles, the higher concentrations of biogenic amines were found in thigh muscle for all methods of treating. A statistically significant difference was found for values exceeding the limit of 5 mg/kg in untreated pheasant carcasses on the third date of sampling ($P < 0.05$), in eviscerated pheasants on the second date of sampling ($P < 0.01$) and in drawn pheasants on the third date of sampling ($P < 0.05$).
DISCUSSION

The effect of storage temperature and method of treating pheasants killed by pithing on the increase in overall concentrations of biogenic amines is clear from the results obtained by our study. From the viewpoint of overall concentrations of biogenic amines, the carcases of common pheasants may be stored at a temperature of 0°C while retaining a high hygienic quality of meat (when the overall concentrations of biogenic amines did not exceed the value of 5 mg/kg) for 21 d in the case of untreated pheasants and pheasants treated by drawing, and for 14 d in the case of pheasants treated by evisceration.

Pheasant carcases may be stored at a temperature of 7°C for a period of 21 d untreated, for 14 d when treated by drawing, and for 7 d when treated by evisceration.

Pheasant carcases may be stored at a temperature of 15°C for a period of 7 d untreated, and just for an extremely short period of 1 to 2 d when treated by drawing or evisceration.

The increased concentrations of biogenic amines found in the meat of pheasants stored at higher temperatures is in accordance with the study previously described on dependency of storage temperature with formation of biogenic amines (Shalaby, 1996). Lower temperatures inhibit the growth of microorganisms and also reduce their enzymatic activity and, thereby, the concentration of biogenic amines (Bremer et al., 1998; Du et al., 2002). When comparing the overall concentrations of biogenic amines in breast and thigh muscles, we can classify thigh muscle as having a higher risk from the viewpoint of biogenic amines formation on the basis of the results obtained. The higher overall concentrations of biogenic amines in thigh muscle in comparison with breast muscle were found in all 3 studied groups of pheasants treated in various ways. The same results were found in meat of wild duck which their carcases left intact and treated by drawing after killing (our unpublished data). Different results were presented by Standarova et al. (2012), who found the higher concentrations of biogenic amines in the breast muscle of shot and pithed pheasants left untreated after killing. The higher concentrations of biogenic amines were described in the breast muscle of chickens in comparison with thigh muscle by Silva and Gloria (2002) and also in breast muscle of wild ducks treated by evisceration, stored at 7 and 15°C (our unpublished data).

The generally recommended method for treating pheasants is evisceration (Winkelmayer et al., 2004). Our results indicate, however, that from the viewpoint of the speed and intensity of formation of biogenic amines, the best procedure during the storage of slaughtered pheasants is to leave the pheasant carcases untreated. The second best procedure is the drawing method traditionally used by hunters, while the least suitable is the generally recommended method of evisceration. The reason for this may be the higher contamination of the surfaces of muscles occurring during evisceration which is done in a short period of time after killing (El-Ghareeb et al., 2009). The evisceration of pheasants after killing may minimize the risk of penetration and multiplication of microorganisms of the digestive system, though on the other hand the opening of the body cavity associated with this method of treatment may represent a pathway for secondary contamination. When unplucked pheasants are stored for a certain time, this pathway for contamination of the muscle tissue may be highly significant, and according to the results of this study, the content of biogenic amines in pheasant breast and thigh muscles represent a greater
risk than possible contamination by microorganisms of the digestive tract during treatment by drawing or leaving the carcasses of pheasants untreated.

ACKNOWLEDGMENTS

This work was supported by the project CEIT-EC–Central European Institute of Technology (CZ.1.05/1.1.00/02.0068) from the European Regional Development Fund and the project IGA 93/2011/FVHE.

REFERENCES


