The effect of sex on meat quality of fallow deer (Dama dama) from the farm located in the Middle Bohemia

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**ABSTRACT**

The venison is popular for many properties that make it unique, for example, low intramuscular fat (IMF) content, good nutrition and sensory properties. The aim of this study was to determine the difference between sex in carcase traits, chemical and fatty acid composition in different body parts. The meat analysis was performed in 9 stag and 9 doe individuals from the farm breeding in Mokrovraty in Czech Republic. The analysis was carried out on the basis of samples taken from the carcasses of fallow deer. Chemical analysis of meat from musculus longissimus lumborum et thoracis (MLLT), musculus semimembranosus (MS) and musculus serratus ventralis (MSV) was performed. The fatty acid analysis was measured in MLLT. Most of the measured carcase weight parameters show sexual dimorphism, however no effect on percentage of main meat parts was detected. From chemical meat composition, the dry matter content was higher \((p<.001)\) in stags compared to does as well as crude protein \((p=.017)\). IMF content was not affected by sex. When compare chemical composition of muscles, MLLT and MS had higher dry matter \((p=.004)\), ash \((p<.001)\) and lower protein content \((p=.011)\) compared to MSV. The fatty acid analyses showed, that stags had significantly higher content of myristic \((C14:0; p=.015)\), pentadecanoic \((C15:0; p=.008)\), palmitic \((C16:0; p=.024)\), margaric \((C17:0; p=.009)\) and myristoleic acid \((C14:1c9; p=.001)\) in MLLT than does. Sex had no effect on the sums and ratios of fatty acids and atherogenic and thrombogenic index (TI). In conclusion, sex affected mainly weight of carcase and the chemical meat composition.

**Highlights**

- Stags of fallow deer had higher dry matter and protein content than does.
- Fat content was not affected by sex.
- The fallow deer meat has high nutrition value because of the beneficial fatty acids profile.

**Introduction**

Intensive farming of the fallow deer is important as it significantly contributes to the meat industry (Ward et al. 2014). Deer farming is an alternative to farming other animal species in many countries. The main reason is to expand the range of non-traditional meat products with unique sensory properties. In the Czech Republic, we can talk about the positive development of fallow deer breeding. Nowadays, there are about 188 farms breeding 6176 fallow deer, in the 80 farms are kept 2993 individuals of red deer, another 49 farm records 706 mouflons and at 17 farms live 171 individuals of wild boars. In addition, the number of farms is still increasing. Outside the production of breeding animals is able to secure quality meat, which by its nutritional composition fulfils the requirements of modern nutrition.

Like all wild animals, fallow deer adapts food intake to the possibilities of vegetation that is just available. He likes mostly grass and crops. Do not heat even bushes, such as hawthorn, thorn, blackberry, in the forest environment, leaves and buds of young stands, as well as the bark of older trees, sometimes cause considerable damage in the forest (Gill 1992).

The weight of the fallow deer shows sexual dimorphism as in many ungulates (Jarman 1983; Weckerly 1998; Loison et al. 1999). The live weight of the adult fallow deer individual is from 40 kg in does to 94 kg in stags (Bothma 2014). The weight of the...
fallow deer fed by daily supplement of 500 g concentrate/head is around 50 kg and their carcase has higher carcase yield, while the weight of the normal fed ones is around 45 kg (Volpelli et al. 2002).

From meat quality, for most of the game species the intramuscular fat (IMF) content is lower than 3% (Onyango et al. 1998; Hoffman 2000). Mostly meat contains 70–75% of water, 18–22% of protein, 2–3% of fat, 1–1.5% mineral substances, 0.9–1.0% of extractive non-nitrogen substances and 1.7% extractive nitrogen substances. To achieve the desired flavour properties of the meat, the IMF content is sufficient 1–2%. Venison is requested by consumers because of its specific flavour and favourable nutrient composition (Hoffman and Wiklund 2006). High content of protein, vitamins (Sampels et al. 2006; Purchas et al. 2010) and low content of IMF (Volpelli et al. 2003; Polak et al. 2008) makes venison attractive meat product.

Therefore, the aim of this study was to evaluate the effect of sex on the carcase value, proximal chemical composition in different muscles of fallow deer from the farm located in Middle Bohemia. The next point was to analyse fatty acid composition in meat of stags and does of these fallow deer.

Materials and methods

The experiment was approved by the Ethics Committee of the Central Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (Prague, Czech Republic) and was carried out in accordance with Directive 2010/63/EU for animal experiments.

Animals and experimental design

The animals in this experiment were from the Řípa’s family farm located in the district of Příbram in the village Mokrovraty in Czech Republic. The average annual temperature according to local meteorological station in the Příbram district is 7.3 °C, the average annual precipitation is 623 mm. The municipality of Mokrovraty is located at an altitude of 365 m asl.

The animals have a 60-hectare grazing area, and eight hectares of meadows, corn (oats) serve to provide a part of the feed base. In addition to quality hay, sage, and kernels in the winter, the supplementation of minerals and vitamins and feed water available in tanks is an obvious part of the dosage. For easier manipulation of the herd, contact feeding is practiced on the farm, and a special fixation device is used to capture the animals themselves. The farm is fenced, trees are planted in the middle. Among the trees, pine, beech, oak, maple, chestnut and pear are most represented. There is a shelter used to protect against unfavourable weather.

For the evaluation of carcase characteristics and meat analysis, 18 individuals (1:1 stags and does) of farmed fallow deer at the average age of 2.5 years were used from autumn slaughtering. The slaughter was provided by a shooting to the head or to the neck and each of them was bleed out. The animals were eviscerated 30 min after shooting, carcases were aged for 24 h at 5 °C in an ageing room, suspended from both hind legs.

Carcase value

The carcase weight was measured after removal of the skin and internal organs. The edible and non-edible viscera were weighed separately. The weighing was carried out on the scale 1T6080 A12 (Lesak, Brno, Czech Republic). The dressing percentage on the basis of the hot carcase weight and the bled body weight was calculated. The carcases were cut into half and the right side of each one was split into the following parts according to the methodology of Stanisz et al. (2015): loin, leg, shoulder and neck. Each body parts were weighed on the laboratory digital scale the first and the second class VIBRA AJ (Shinko Denshi Co. Ltd., Tokyo, Japan). After 24 h chilling period at 5 °C, the representative samples of *musculus longissimus lumbarum et thoracis* (MLLT), *musculus semimembranosus* (MS) and *musculus serratus ventralis* (MSV) were taken for chemical analysis.

Analysis of proximal chemical composition and fatty acids profile

The dry matter content was determined from the difference of the sample weight before and after drying with sea sand under the conditions of AOAC (2005). The IMF was set by gravimetric determination after extraction in SER 148–Soxhlet extractor (VELP Scientifica, Usmate, Italy) by non-polar solvent (petrolather). Protein content is based on amino nitrogen content by Kjeldahl method (KjelFlex K-360, Büchi, Flawil, Switzerland), then converted to the (gross) protein content after multiplication by the appropriate factor. Ash content was determined via burning the sample at 550 °C (Ht40AL, LAC, Rajhrad, Czech Republic) until organic substances were burnt.

For fatty acid analysis MLLT was taken 24 h post mortem, frozen and stored at −80 °C (Jouan HX450S, Trigon-plus, Říčany, Czech Republic). The methyl esters
of fatty acids after extraction of total lipids according to the methodology of Folch et al. (1957) were detected. Methanolation was carried out under the catalytic effect of potassium hydroxide and extraction of the acids in the form of methyl esters into the heptane. Isolated methyl esters were detected by the flame ionisation detector (FID) using split regime of the chromatography Master GC (Dani Instruments, S.p.A., Milano, Italy) equipped with the Famewax column with the stationary phase of polyethylene glycol (Famewax; 30 m × 0.32 mm × 0.25 μm). Helium was used as carrier gas at a constant flow of 5 mL/min, split ratio was 1:9. The peaks were identified using Clarity 5.2 and quantified based on known retention times of standards Food Industry FAME Mix (Restek Corporation Company, Bellefonte, PA). Atherogenic index (AI) was calculated from monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) as follows:

$$ AI = \frac{C12:0 + 4 \times C14:0 + C16:0}{MUFA + PUFA}. $$

The thrombogenic index (TI) was calculated as follows:

$$ TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times MUFA + 0.5 \times n-6 PUFA + 3 \times n-3 PUFA + n-3/n-6 PUFA).} $$

### Statistical analysis

The data were evaluated using software SAS 9.4 software (Statistical Analysis System, SAS Institute Inc., Cary, NC) ANOVA methods. The bled body weight, carcass characteristics and fatty acid analysis were assessed by a one-way analysis of variance. Duncan’s multiple range test was used to appraise differences between the groups. The chemical meat composition was evaluated by a two-way analysis of variance. The t-test was used to evaluate differences between values of sex and muscle interactions. All data were expressed as mean ± standard deviation values. Differences of $p \leq 0.05$ between mean values were considered as statistically significant.

### Results

The results of the carcass analysis are provided in Table 1. The bled body weight of fallow deer represents the mean weight of the stags and does at the time of slaughter. There were detected significant differences ($p = .012$) between stags and does in bled body weight with higher value in stags (57.16 kg) than in does (43.13 kg). This difference indicates sexual dimorphism of fallow deer and it is supported by following analysed carcass characteristics. The carcass weight is related to the slaughter weight. Higher carcass weight ($p = .036$) was observed in stags (36.60 kg) compared to the does (27.00 kg), i.e. the differences in carcass weight between sexes were 26.2%. On the other hand, the carcass yield was not significantly affected by sex and it was 63.82% in stags and 62.25% in does. The edible viscera including liver, kidneys and heart were not significantly affected by sex with the average value for stags 3.00 kg and for does 3.45 kg. Likewise, the whole viscera weight including edible and non-edible viscera was non-significantly heavier in stags than in does. Significantly higher weights of leg ($p = .011$), shoulder ($p = .009$) and neck ($p = .017$) were observed in stags of fallow deer compared to does.

The percentages of individual parts are more important for the comparison of main meat parts in carcass. The significant differences were detected only in percentage of edible viscera with higher values ($p = .050$) in does than in stags. The other parts were not significantly affected by sex. The differences in live weight, carcass weight and the most of other analysed carcass characteristics indicate sexual dimorphism of fallow deer.

The results of the chemical composition of meat are shown in Table 2. There were not found significant interaction of sex and muscle in any of chemical characteristics. Dry matter was significantly affected by muscle ($p = .004$) with the higher value in the MLLT and MS and the lowest in MSV. The stags had higher ($p = .001$) dry matter content than does in all of observed muscles.

It is important for the flavour of meat is IMF content, which was not influenced by either sex or muscle type. The non-significantly higher IMF content was observed in MS and MSV contrary to MLLT. Higher protein content was found in stags ($p = .017$) and in MLLT and MS compared to MSV.

### Table 1. Bled body weight and carcass characteristics of fallow deer stags and does.

|                      | Stag       | Doe        | Significance |
|----------------------|------------|------------|--------------|
|                      | Mean ± SD  | Mean ± SD  |              |
| Bled body weight (kg)| 57.16 ± 4.40 | 43.13 ± 8.10 | .012         |
| Carcase weight (kg)  | 36.60 ± 5.03 | 27.00 ± 6.16 | .036         |
| Dressing (%)         | 63.82 ± 5.10 | 62.25 ± 4.94 | ns           |
| Edible viscera (kg)  | 3.00 ± 0.79  | 3.45 ± 1.10  | ns           |
| Nonedible viscera (kg)| 8.80 ± 1.92 | 6.75 ± 1.71  | ns           |
| Loin (kg)            | 6.94 ± 1.23  | 4.91 ± 1.43  | ns           |
| Leg (kg)             | 12.91 ± 1.36 | 9.43 ± 1.72  | .011         |
| Shoulder (kg)        | 5.76 ± 0.51  | 4.20 ± 0.79  | .009         |
| Neck (kg)            | 5.00 ± 0.80  | 3.38 ± 0.75  | .017         |
| Percentage of carcass value |          |            |              |
| Edible viscera (%)   | 5.25 ± 1.32  | 7.92 ± 2.07  | .050         |
| Loin (%)             | 19.12 ± 3.21 | 17.98 ± 1.53 | NS           |
| Leg (%)              | 35.58 ± 4.02 | 35.27 ± 2.47 | NS           |
| Shoulder (%)         | 15.85 ± 1.33 | 15.72 ± 1.29 | NS           |
| Neck (%)             | 13.75 ± 1.85 | 12.63 ± 1.80 | NS           |

SD: standard deviation; ns: non-significant

Numbers with various letters are significantly different ($p \leq .05$).
percentage (p < .001) was detected in MLLT and MS, but with no effect of sex.

Table 3 shows differences due to sex on the fatty acid composition of MLLT. The fatty acid analyses indicate statistically significant difference between stag and doe in the content of the following saturated fatty acids: myristic acid (p = .015), pentadecanoic acid (p = .008), palmitic acid (p = .024) and margaric acid (p = .009). From MUFA fatty acids were significantly affected by sex only myristoleic acid (p = .001). The content of these acids was higher in stags than in does. The relative amount of PUFAs was higher in does than in stags, although these differences were not significant.

Sex had no effect on the content of SFA and MUFA (Table 4). The does had non-significantly higher total PUFAs content and lower n-6/n-3 content than stags. Likewise, other ratios of fatty acids, related to human health, were not significantly affected by sex. On the other hand, it is obvious that meat from fallow deer is a nutritionally valuable meat and beneficial from the point of view of health (e.g. n-6/n-3 PUFAs ratio was 5).

**Table 2.** Chemical composition of fallow deer meat.

| Variable       | MLLT Mean | SD | MS Mean | SD | MSV Mean | SD | Significance |
|----------------|-----------|----|---------|----|---------|----|--------------|
| Dry matter (%) | 26.230    | 0.270 | 23.520  | 1.320 | 25.330  | 0.740 | 24.200       |
| IMF (%)        | 1.850     | 0.280 | 1.650   | 0.240 | 1.760   | 0.480 | 1.990        |
| Crude protein  | 22.400    | 0.920 | 21.860  | 0.240 | 22.370  | 0.490 | 20.980       |
| Ash (%)        | 1.210     | 0.060 | 1.190   | 0.070 | 1.180   | 0.060 | 1.270        |

MLLT: musculus longissimus lumborum et thoracis; MS: musculus semimembranosus; MSV: musculus serratus ventralis; IMF: intra-muscular fat; SD: standard deviation; M: muscle; S: sex; ns: non-significant

Numbers with various letters are significantly different (p < .05).

**Table 3.** Fatty acid composition of MLLT of fallow deer stags and does.

| Fatty acid (% of total fatty acids) | Stag Mean | SD | Doe Mean | SD | Significance |
|------------------------------------|-----------|----|----------|----|--------------|
| Saturated fatty acid               |           |    |          |    |              |
| C4:0 Butyric                        | 0.000     | 0.000 | 0.030    | 0.060 | ns           |
| C6:0 Caproic                        | 0.040     | 0.020 | 0.030    | 0.060 | ns           |
| C8:0 Caprylic                       | 0.010     | 0.010 | 0.000    | 0.000 | ns           |
| C10:0 Capric                        | 0.020     | 0.020 | 0.000    | 0.000 | ns           |
| C12:0 Lauric                        | 0.050     | 0.010 | 0.040    | 0.050 | ns           |
| C13:0 Tridecyclic                   | 0.010     | 0.010 | 0.000    | 0.000 | ns           |
| C14:0 Myristic                      | 2.370a    | 0.460 | 1.170b   | 0.720 | 0.015       |
| C15:0 Pentadecanoic                 | 0.870a    | 0.130 | 0.490b   | 0.180 | 0.008       |
| C16:0 Palmitic                      | 22.210a   | 2.280 | 16.770b  | 3.440 | 0.024       |
| C17:0 Margaric                      | 2.190a    | 0.200 | 0.680b   | 0.310 | 0.009       |
| C18:0 Stearic                       | 29.750    | 1.020 | 31.100   | 3.570 | ns           |
| C20:0 Arachidic                     | 0.250     | 0.050 | 0.270    | 0.130 | ns           |
| C21:0 Heneicosanoic                 | 1.010     | 0.200 | 1.680    | 0.900 | ns           |
| C22:0 Behenic                       | 0.110     | 0.160 | 0.220    | 0.450 | ns           |
| C24:0 Lignoceric                    | 0.140     | 0.270 | 0.500    | 0.900 | ns           |
| Monounsaturated fatty acids         |           |    |          |    |              |
| C14:1c9 Myristoleic                 | 0.140a    | 0.040 | 0.020b   | 0.020 | 0.001       |
| C16:1c9 Palmitoleic                 | 1.320     | 0.300 | 1.080    | 0.160 | ns           |
| C17:1c9 Heptadecenoic               | 0.370     | 0.070 | 0.470    | 0.120 | ns           |
| C18:1c9 Oleic                       | 15.410    | 0.910 | 15.420   | 1.420 | ns           |
| C20:1c11 Eicosanoic                 | 0.250     | 0.080 | 0.330    | 0.110 | ns           |
| C22:1c13 Erucic                     | 0.020     | 0.050 | 0.080    | 0.170 | ns           |
| C24:1c15 Nervonic                   | 0.020     | 0.040 | 0.140    | 0.210 | ns           |
| Polyunsaturated fatty acids         |           |    |          |    |              |
| C18:2c9,12 Linoelic                 | 17.550    | 3.650 | 20.140   | 6.060 | ns           |
| C18:3c9,9,12 γ-Linolenic            | 0.020     | 0.020 | 0.050    | 0.050 | ns           |
| C18:3c9,12,15 α-Linolenic           | 3.130     | 0.490 | 5.050    | 2.730 | ns           |
| C20:2c11,14 Eicosadienoic           | 0.270     | 0.070 | 0.400    | 0.130 | ns           |
| C20:3c11,14 Eicosatrienoic          | 0.180     | 0.050 | 0.190    | 0.050 | ns           |
| C20:4c5,8,11,14 Archidonic          | 2.660     | 0.840 | 3.170    | 1.200 | ns           |
| C22:5c5,8,11,14,17 Eicosapentaenoic | 0.020    | 0.020 | 0.000    | 0.000 | ns           |
| C22:2c13,16 Docosadienoic           | 0.030     | 0.060 | 0.100    | 0.200 | ns           |
| C22:6c4,7,10,13,16,19 Docosahexaenoic| 0.470    | 0.210 | 0.440    | 0.370 | ns           |

SD: standard deviation; ns: non-significant
Numbers with various letters are significantly different (p < .05).
Table 4. Sums of ratios of fatty acids and indexes of MLLT of fallow deer stags and does.

| Variable      | Stag   | Doe    |
|---------------|--------|--------|
|               | Mean   | SD     | Mean  | SD     | Significance |
| SFA           | 58.14  | 3.50   | 52.91 | 7.15   | ns          |
| MUFA          | 17.53  | 1.15   | 12.57 | 1.73   | ns          |
| PUFA          | 24.33  | 4.47   | 29.53 | 7.85   | ns          |
| n-6 PUFA      | 20.22  | 4.30   | 23.36 | 6.37   | ns          |
| n-3 PUFA      | 3.62   | 0.39   | 5.49  | 2.58   | ns          |
| n-6/n-3 PUFA  | 5.62   | 1.22   | 4.70  | 1.42   | ns          |
| n-3/n-6 PUFA  | 0.19   | 0.04   | 0.24  | 0.10   | ns          |
| SFA/MUFA      | 3.32   | 0.14   | 3.02  | 0.42   | ns          |
| SFA/PUFA      | 2.49   | 0.72   | 1.96  | 0.85   | ns          |
| MUFA/PUFA     | 0.75   | 0.21   | 0.64  | 0.20   | ns          |
| AI            | 0.77   | 0.16   | 0.48  | 0.23   | ns          |
| Ti            | 1.83   | 0.26   | 1.42  | 0.59   | ns          |

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; AI: atherogenic index; Ti: thrombogenic index; SD: standard deviation; ns: non-significant Numbers with various letters are significantly different (p ≤ 0.05).

Discussion

The bled body weight of fallow deer stags and does from our experiment was in the range reported by Bothma (2014). Similar body weight found Stanisz et al. (2015). There is a sexual dimorphism between stags and does of fallow deer, where the stags are bigger. Morris et al. (1992) showed the difference between sex of fallow deer around 9.3 kg at yearling weight. However, the live weight may be very variable due to different environmental influences (Volpelli et al. 2002).

The carcase weight depends on live weight and thus is higher in stags, which is in accordance with the studies of Janiszewski et al. (2011). However, these authors detected higher carcase weight in fallow deer than in this study. It could be caused by different specification of the carcase weight, different age of the individual or various live conditions.

Similarly with our results, Stanisz et al. (2015) did not detect the differences between sexes in dressing percentage. The proportion of the main meat parts has higher testifying value than the weight of these parts. The differences between stags and does in percentage contribution of commercial cuts (shoulder, loin, leg and neck) were not significant in present experiment and are in agreement with Stanisz et al. (2015).

Differences between stags and does are connected with the time of sexual maturation, when the does of ruminants matures earlier than stags (Guerrero et al. 2013). Meat quality can be evaluated by the chemical meat composition. The dry matter content of fallow deer from this study was higher in stags than in does, which agrees with the results of Hutchison et al. (2014) and Piaskowska et al. (2015) who reported dry matter content of stag 25.7% and doe 24.9%. On the other hand, Stanisz et al. (2015) did not find the effect of sex on dry matter content in MS. From the analysed muscles, the MLLT had the highest dry matter content corresponding with the study of Bykowska et al. (2018a).

The IMF content is important for sensoric qualities and special flavour of the meat, especially venison. Normal average IMF content without difference of species is 2–3%. Most of the game species has fat content lower than 3%, in fallow deer even lower than 1%. Generally, low IMF content in fallow deer corresponded with the results of Purchas et al. (2010) and Daszkiewicz et al. (2012). The low IMF content of fallow deer meat confirms the high dietetic value of venison. There were not detected differences between sexes and muscles in IMF content. The MLLT had non significantly lower fat content and these results agree with Bykowska et al. (2018a) who showed lower IMF content in MLLT than in MS.

The protein content showed higher values in stags. Daszkiewicz et al. (2012) in their study reported differences between sexes, but differently from our study detected higher protein content in does of roe deer. The results contrasted with Stanisz et al. (2015) and Ludwiczak et al. (2017), who did not find the differences between stags and does in protein content. Similarly with our results, Bykowska et al. (2018b) reported higher protein content in MLLT.

No effect of sex on ash content was detected in this study, while there were detected effects of muscle type on ash percentage. Contrary to our results, when the lower concentration of ash was ascertained in MSV, Dahlan and Norfarizan-Hanoon (2008) reported lower ash content in MLLT. The differences in chemical meat composition of analysed muscles can be explained by different structures associated with their activity and post mortem changes (ZochowskaKujawska et al. 2007).

The fatty acid composition provides a more comprehensive account of the nutritional value of meat. Pasture feeding compared to supplementary feeding gives better meat fatty acid profile, which is richer in PUFA. Similar results of fatty acids composition as in our experiment determined Polak et al. (2008) in red deer meat. On the other hand, Volpelli et al. (2003) found higher fatty acid content in MS of fallow deer than in our results. Different muscles and various pastures could cause the differences in fatty acid composition. In comparison with domestic ruminants, venison exhibits lower amounts of fatty acids and its composition is richer in PUFAs and poorer in MUFA and SFA.
(Velasco et al. 2001; Volpelli et al. 2003). Grazing ruminants have very low n-6/n-3 PUFA ratio in meat, because of higher levels of linolenic acid found in grass (Wood et al. 2004). In this study, n-6/n-3 PUFA ratio was 5. This is in line with recommendation of Department of Health (1994), when the ratio should be 4–5 or less. However, how the IMF content is very low in fallow deer, the fatty acid composition is not of major concern (Volpelli et al. 2003).

**Conclusions**

Summing up, the weight of the animals analysed in this study was on a proper level for 2.5-year-old farmed fallow deer; the stags had higher bled body weight and weight of the main meat parts higher than does. Stags of fallow deer had higher dry matter and protein content than does. The highest dry matter content and protein content was detected in MLLT. The fallow deer meat has high nutrition value, especially because of the beneficial fatty acids profile in relation to human health. The fatty acid analyses indicate higher content of saturated fatty acids (myristic, pentadecanoic, palmitic and margaric acid) and MUFA fatty acid (myristoleic acid) in stags than in does. The relative amount of PUFA was higher in does than in stags, although these differences were not significant.

With the emerging trend of healthy nutrition and the improvement of available food sources, venison becomes a suitable and sought-after product that meets its demanding demands for the quality of the modern user.

**Ethical approval**

The experiment was approved by the Ethics Committee of the Central Commission for Animal Welfare of the Ministry of Agriculture of the Czech Republic (Prague, Czech Republic) and was carried out in accordance with Directive 2010/63/EU for animal experiments.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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