Quality and safety of meat from wild boar hunted in Molise region

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ABSTRACT
A study was carried out to evaluate meat quality traits and heavy metals content of longissimus thoracis muscle from wild boar of different estimated live weight (50, 70, 100 kg; n = 25, 24, 18, respectively), sex and hunting area of Molise region. Meat quality data were analysed by GLM, live weight and sex were the main factors, for heavy metals the hunting area was also included. Neither live weight nor sex affected pH, colour, vitamin E and total lipid. Cholesterol was tendentially affected by live weight and sex. Collagen content was affected only by sex. Lighter boars showed a higher content (p < .05) of polyunsaturated fatty acid (PUFA), n-6 and n-3 PUFA, and PUFA/SFA ratio (p < .05) compared to other weight classes. Lighter boars had a better atherogenic index compared to boars of intermediate weight. Compared to females, males (M) had higher n-3 PUFA and a lower n-6/n-3 ratio (p < .05). Weight, sex and hunting area did not affect cadmium (0.001 mg/kg), lead (0.011–0.026 mg/kg), copper (0.696–1.151 mg/kg) and manganese (0.083–0.130 mg/kg) levels. Chromium was affected by sex (M: 0.072 mg/kg; p = .012). Nickel (Ni) was higher (p < .01) in the heaviest boars (0.035 mg/kg) than the other weight classes (0.017 mg/kg). Significant differences between the two areas were found for Ni content, interactions (p < .01) were detected among all factors. In conclusion, lighter boars showed a better meat nutritional quality. The low content of heavy metals in the meat indicates a low level of anthropogenic pollution of the areas under study.

HIGHLIGHTS
• Meat from younger boars showed a better meat nutritional quality.
• Low levels of heavy metals in meat indicates a low level of anthropogenic pollution of the studied areas.

Introduction
The wild boar (Sus scrofa, L. 1758) is one of the widely distributed mammals in Eurasia, where it inhabits different habitat types, from semi-arid environments to marshes, forests and alpine grasslands. The wild boar population, from the 1960s, has continuously increased in number in Europe, as a consequence of the combination of various factors: biological factors (i.e. species ecological plasticity, high reproduction rate), environmental changes, depopulation of rural areas, artificial stocking, decrease of predators and modification in breeding practices (Pittiglio et al. 2018). In particular, in Italy the wild boar population has expanded its range and increased dramatically in abundance, originating conflicts with human activities and biodiversity. In this context, harvest of wild boar has shown a significant increase leading to a major interest towards this species as a meat producer, consisting in an alternative to meat obtained from domestic animals (Sales and Kotrba 2013). Moreover, wild boar meat is considered to be an “organic” alternative to commercially produced pork (Malmsten et al. 2021). Game meat can satisfy the requests of today’s consumers for healthier and safe meat because it is characterised by a good chemical composition, with low fat and high protein content (Tomasevic et al. 2018), greater mineral content (Strazdina et al. 2014) and a desirable fatty acid composition compared to pork (Quaresma et al. 2011; Dannenberger et al. 2013). Factors as live weight of animals, sex and hunting season could have a great influence on meat quality of wild boar, in particular on fatty acid profile (Razmaite et al. 2012; Russo et al. 2017). Moreover, it has been
found that also the hunting region could influence the fatty acid and micronutrient levels in muscles of wild boar (Dannenberger et al. 2013).

Regarding the safety of game meat, due to their large geographical distribution, residential way of life, feeding habits and relatively long life-span, wild animals, especially wild boar, are good bioindicators of heavy metal pollution and exposure in the terrestrial food chain in diverse geographical areas (Bilandžić et al. 2010; Prevendar Crnić et al. 2015; Malmsten et al. 2021). In general, liver and kidneys represent the principal bioaccumulation site of heavy metals, followed by muscle and fat (Medvedev 1999; Falandysz et al. 2005), and their content in tissues and organs can present significant regional differences (Aastrup et al. 2000). Safety requirements of game meats have been addressed recently by Regulations (EC) No.853/2004. However, there are no specific limits regarding the concentration of heavy metals such as for meat and offal of farm animals, as stated by the Regulation (EC) 1881/2006 and lately amended. Therefore, it is relevant to assess the risk for the population that can be more exposed to toxic elements for different reasons, in particular hunters and their families, which are the major consumers of wild boar meat. Local-scale exposure studies are thus appropriate for wild boar meat that is consumed mainly within the harvesting area. The Molise region, situated in south Italy, is known as a hunting area for wild boar. An estimation of regional wild boar harvesting reports a number of head harvested during the hunting season 2019–2020 of 2,734 heads (personal communication, Ambito Territoriale di Caccia, Molise region). No studies are available on the quality and the heavy metals content of wild boar meat hunted in this region. In addition, there are few information in literature regarding the physico-chemical and nutritional characteristics of meat from wild boar of different body weight and sex. In the light of this, the aim of this study was to evaluate meat quality traits and heavy metals content of meat from wild boar of different live weight and sex, hunted in two different areas of Molise region.

Materials and methods

Animals and sampling

The study area was represented by two wild boar hunting districts, within the boundaries of the Molise Region: Bagnoli del Trigno – Poggio Sannita (A1), situated in Province of Isernia and Roccavivara – Civitacampomarano (A2), situated in Province of Campobasso. The choice of these two areas was dictated by the considerable increase of the wild boar population in recent years. The area A1 ranges from 300 to 767 m above sea level. The average annual temperature varies between 13.5 and 14.9 °C, the average minimum temperatures of the coldest month between 0.4–2.1 °C. The average annual rainfall is between 750 and 840 mm. The area A2 ranges from 520 to 652 m above sea level; the average annual temperature varies between 14.0 and 16.0 °C, and the average minimum temperatures of the coldest month between 2.7–5.3 °C. The annual rainfall is about 674 mm. The natural landscape of these two areas is characterised by large forest areas of oaks and beeches, alternated to uncultivated pastures and mountain meadows in the highest altitudes. The research was carried out on 67 wild boar carcases obtained from animals hunted in wild conditions in accordance with the provision of national laws on game and hunting (National Law 157/1992 and subsequent amendments). The hunting activities were carried out by professional game hunters; the method used to hunt animals was that in which hunters waited for the quarry. The longissimus thoracis (LT) muscle samples were removed from the left side of the carcases between the 12th–14th rib, for a total of 67 wild boar (A1: n = 34; A2: n = 33) of different estimated live weight (approx. 50, 70 and 100 kg; n = 25, 24 and 18, respectively), and sex (M = male: n = 29; F = female: n = 38). Muscles were individually packed in polyethylene bags, and transported in a refrigerated container within one hour to the laboratory of the Department of Agricultural, Environmental and Food Sciences of University of Molise in Campobasso.

Physico-chemical analyses

On the muscle samples, pH and colour were recorded after 24 h (pH24) post mortem between 12th and 14th rib. The pH was measured using a portable pH metre (FiveGo™, Mettler-Toledo, Switzerland) equipped with a penetrating glass electrode in order to evaluate the presence of meat anomalies. Tri-stimulus colour coordinates (lightness L°; redness a° and yellowness b°) were detected on the muscle samples using a Chroma Metre CR-300 (Italia s.r.l., Milano). Reflectance measurements were performed after the samples had been oxygenated in air for at least 30 min by which time measurements were stable, taking three readings for each sample. A portion of the LT muscle samples was vacuum packaged and stored frozen (−20 °C) for analyses.
Muscle cholesterol, collagen and α-tocopherol content

Cholesterol was extracted using the method of Maraschiello et al. (1996) and then quantified by HPLC. A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a Kinetex 5 μ C18 reverse-phase column (150 cm x 4.6 mm x 5 μm; Phenomenex, Torrance, CA), was used. The HPLC mobile phase consisted of acetonitrile: 2-propanol (55:45, vol/vol) at a flow rate of 1.0 mL/min. The detection wavelength was 210 nm. The quantitation of muscle cholesterol content was based on the external standard method using a pure cholesterol standard (Sigma, St. Louis, MO).

The intramuscular collagen determination was performed using the method described by Tavaniello et al. (2014). Briefly, 100 g of LT muscle (wet weight) were thawed at room temperature, trimmed of fat and epimysium, lyophilised for 48 h, and hydrolysed in Duran tubes in 5 mL of 6 N HCl at 110 °C for 18 to 20 h for determination of hydroxyproline. Intramuscular collagen concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight and expressed as micrograms of hydroxyproline per milligram of lyophilised tissue.

The levels of vitamin E (α-tocopherol) in the LT muscle were determined and quantified as described by Zaspel and Csallany (1983) and then quantified by HPLC (Kontron Instruments, Milan, Italy) model 535 equipped with a C18 reverse-phase column (150 cm x 4.6 mm x 5 μm) (Phenomenex, Torrance, CA). The mobile phase was 100% methanol at a flow rate of 1.5 mL/min and the detection wavelength was 292 nm.

Muscle lipid and fatty acid composition

Lipid extraction from LT muscle was performed using the method described by Folch et al. (1957). Fatty acids (FA) were quantified as methyl esters (FAME) using a gas chromatograph GC Trace 2000 (ThermoQuest EC Instruments) equipped with a flame ionisation detector (260 °C) and a fused silica capillary column (SGE Forte BP × 90, Phenomenex, Torrance, CA, USA) 100 m × 0.25 mm × 0.25 μm film thickness. Helium was used as a carrier gas. The column temperature was held at 100 °C for 5 min, then raised 4 °C/min up to 240 °C and maintained for 20 min. The individual fatty acids peaks were identified by comparison of retention times with those of known mixtures of standard fatty acids (37 Component FAME MIX and Docosapentaenoic acid (cis-7,10,13,16,19), Supelco, Bellofonte, PA, USA) run under the same operating conditions. Results were expressed as percentage of the total FA identified. To assess the nutritional implications, the ratio of n-6 PUFA to n-3 PUFA (n-6/n-3) and the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) (P/S) were calculated. Moreover, to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, the atherogenic index (AI) and the thrombogenic index (TI) were calculated according to the formulas suggested by Ulbricht and Southgate (1991).

Heavy metals

The determination of the contents of heavy metals, including manganese, cadmium, lead, copper, chromium and nickel (Mn, Cd, Pb, Cu, Cr, and Ni, respectively), in muscle tissues was performed using an inductively coupled plasma mass spectrometer (ICP-MS 5110, Agilent Technologies, USA). Meat samples (3 g) were oven-dried at 100 °C to a constant weight. One gram of each dried sample was weighted into a porcelain crucible and dry-ashed in a muffle furnace by stepwise increase of temperature up to 450 °C within 1 h and then leaving to ash at this temperature for additional 20 h. The ash was dissolved in concentrated nitric acid (1 mL) and the sample volume was adjusted (to 25 mL) with deionised water. Trace elements were analysed twice for each sample and expressed as mg/kg of wet weight.

Statistical analysis

Data on meat quality traits were analysed by GLM procedure using the SPSS statistical package (SPSS 2010, PC+ Statistics. 18.0, SPSS Inc., Chicago, IL). The model included live weight (50, 70, 100 Kg) and sex (M, F) and their interactions. Data on heavy metals content were analysed by GLM procedure including live weight, sex and the hunting area (A1 and A2) and their interactions. Differences among the means were determined with Scheffe’s test.

Results and discussion

Physico-chemical characteristics

Neither live weight nor sex affected pH and colour of LT muscle (Table 1). The post-mortem pH decline, caused by lactic acid accumulation, is of crucial importance for muscle transformation and resulting quality of meat. Meat acidity, determined at 24 h post-mortem, (ranging from 5.73 to 5.81) was in the...
acceptable range. These values are similar to those reported by Avagnina et al. (2012) in wild boar hunted in the Upper Susa Valley (5.77) and by Ludwickczak et al. (2020) in wild boar hunted in the Northwest Poland (ranging from 5.45 to 5.88 in m. semimembranosus). Differently, lower values were found in wild boar (5.46 in males and 5.47 in females), 4 years old, hunted in Frasin forest of Romania (Postolache et al. 2011), and in wild boar hunted in Germany (5.45; Müller et al. 2000). These differences in pH values could be due to both different glycogen content and different fibre types (Ruusunen and Puolanne 2004) but also to differences in hunting practices (Ramanzin et al. 2010). Viganò et al. (2019) reported that animals culled with a nonfatal shot and/or not properly bled showed a slower pH decrease in the first hours after death. Muscle colour is an important criterion in consumer perception of meat quality and acceptability. It is a result of the myoglobin concentration and meat’s chemical state, surface structure and intramuscular fat. Also, diet, age, weight of animals and exercise may have an effect on meat colour. Wild boar meat is darker than that from domestic species and consumers consider it as a typical feature of game meat. Analysing the colour values of all parameters (L*, a*, b*), it was found that they are typical for game meat (Pedrazzoli et al. 2017; Kasprzyk et al. 2019). In our study, a very limited effect on colour parameters was observed. In fact, only meat from heavier animals had a tendentially lower L* (p = .062). Similar findings were reported by Stanisz et al. (2019). Usually, with the increase in age and weight of the animals increases the concentration of haem pigment. This could be the reason for the increase in meat redness found in heavier carcasses. On the other hand, Skewes et al. (2014) suggested that a higher haem pigment content in the heavier wild boar carcasses is associated with the higher activity and increased oxidative metabolism in adults and older animals.

**Total collagen, vitamin E and cholesterol**

Generally, the most important factors that affect meat quality from wild boar are the season, feed resources, sexual activity and living conditions. Table 2 shows the cholesterol, collagen and vitamin E content of LT muscle of wild boar. Live weight did not affect (p>.05) collagen content, while it was higher (p<.05) in females (+21.1%) than in males. This finding contrasts the results of Postolache et al. (2011); they did not find statistical evidence regarding the sex effect on collagen content of different muscles in adult wild boar (4 years old). Intramuscular collagen is a contributing factor to variation in meat tenderness and texture. However, the amount of intramuscular connective tissue, structure and composition vary widely between muscles and different domestic animals (McCormick 2009; Tavaniello et al. 2014). Differences in collagen content between the sexes of animals also are found in pork (Nold et al. 1999) attributed to hormonal effect. Of particular interest is the comparison with pork, which contains less amount of collagen compared with wild boar meat, approximately 27% (Maiorano et al. 2013; Rossi et al. 2013). This could be due to the physical exercise of the animals, which may have an effect on the increase of intramuscular collagen content, responsible for the background toughness of meat (McCormick 2009; Maiorano et al. 2013).

Vitamin E content (ranging from 5.87 to 6.05 μg/g of α-tocopherol) did not differ (p>.05) between the live weight classes and sex (Table 2). Our results are partially in line with those of Quaresma et al. (2011), who found higher values of α-tocopherol content in psoas major of adult animals than youngsters’ wild boar, but no significant difference between sex. The difference between age groups may be due to differences in feeding habits and metabolic rates (Sales and Kotrba 2013). Alpha-tocopherol concentration in wild boar meat described by Quaresma et al. (2011) is two-fold higher than those in the present study. These differences could be attributed mainly to genotype and type of muscle, resulting in different vitamin requirements in the relation of functional and metabolic differences of fibre, and different fat contents (Maiorano et al. 2007). Vitamin E is a powerful lipid-soluble antioxidant in biological systems, due to its capacity to break the chain of lipid oxidation in the cell.
membranes protects fresh meat from lipid oxidation, promotes and extended stability of meat, preventing the formation of rancid flavour during storage (Buckley et al. 1995).

An important factor for consumers is the link between diet and health. Cardiovascular diseases and arteriosclerosis are among the most important causes of human mortality and are strongly associated with dietary intake of cholesterol and saturated fatty acids (Shramko et al. 2020). Overall, total cholesterol content (ranging from 64.60 to 74.47 mg/100 g) was tendentially affected by live weight (p<.05); in particular, lighter boars had higher cholesterol compared with other weight classes. In contrast, Quaresma et al. (2011) did not find any significant effect of live weight on cholesterol content in psoas major muscle (57.13 mg/100 g) from wild boar hunted in Portugal. Differently, cholesterol content was tendentially lower in females (−8.0%) than in males (p=.073), similar to the results of Quaresma et al. (2011) who found 3% less in females (p>.05). The cholesterol content detected in the present study is quite similar with that reported by Maiorano et al. (2013) in the longissimus lumborum of pigs from different genotypes (from 61.03 to 66.24 mg/100 g) and by Auqui et al. (2019) in longissimus dorsi of Chato Murciano pig (light weight: 50.74 mg/100 g and heavy weight: 70.65 mg/100 g).

**Lipids and fatty acid profile**

Total lipids and fatty acid composition in LT muscle are shown in Table 3. Lipid content, ranging from 5.27% to 7.70%, appeared to be higher in the heaviest animals compared to the lighter two groups; however, this difference was not statistically significant. This difference is due to an increase in the size of the adipocytes with age, which increases the lipid content as the animal grows (Urrutia et al. 2018). However, in the present study, the middle group showed the lowest lipid values (p>.05), suggesting that the lipid content could also be influenced by the feed. On the other hand, the wild boar meat lipid content and mostly FA composition depends primarily on diet (Pedrazzoli et al. 2017). No sex effect (p>.05) was evidenced on lipid amount, which was similar to another study of wild boar muscle lipids (Dannenberger et al. 2013). Lipid content could be an interesting characteristic since it contributes to the juiciness of meat products encouraging consumer acceptability.

The fatty acid composition of meat is considered an important index for meat quality and it is extensively studied due to its implications for human health. Overall, the fatty acid composition of the LT muscle observed in the present study fell within the range of values shown in other studies (Quaresma et al. 2011; Dannenberger et al. 2013; Pedrazzoli et al. 2017; Russo et al. 2017). The total SFA, ranging from 38.22% to 39.67%, was not affected by live weight classes. Among SFA, palmitic acid (C16:0; average: 22.27%) was detected in the highest proportion followed in descending order by stearic acid (C18:0; average: 14.15%) and myristic acid (C14:0; average: 0.95%). Myristic acid content was lower in lighter boars compared to the other weight classes (p<.05); while, palmitic acid was lower in lighter boars compared to boars of intermediate weight (p<.05). These results are consistent with Chen and Sui (2018) who found a rising trend of myristic and palmitic acids with the increase of live body weight in Ziwuling wild cross-bred pigs. The results can be attributed to the higher fat content in muscle because in most individuals SFA are mainly related to stored fat, not to the structural lipid of muscle; in fact, with increasing age, more fat is absorbed in the muscles of animals (Albrecht et al. 2006).

In the present study, the total MUFA content varied widely from 34.27% to 43.19%, the differences were not significant (p>.05). MUFA were mainly in the form of oleic acid (18:1 cis 9), which increased with the live weight, even if not significant, due to the high variability of the data. However, palmitoleic acid (16:1) was lower (p<.05) in lighter boars compared to the other two weight classes. These results are in accordance with many research findings (Quaresma et al. 2011; Razmaite et al. 2012; Pedrazzoli et al. 2017; Gálık et al. 2018), which showed an increase of the MUFA

| Table 2. Intramuscular collagen, α-tocopherol and cholesterol content of longissimus thoracis muscle in wild boar of different live weight and sex. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Traits          | Live weight, kg (W) | Sex (S)          | p Value         |
|                 | 50              | 70              | 100             | M              | F              | SEM W | S      | WxS |
| Total collagen, μg/mg* | 25.57           | 23.95           | 26.87           | 22.46          | 28.47          | 1.407 | .058  | .073 | .565 |
| α-tocopherol, μg/g     | 5.90            | 5.87            | 5.98            | 6.05           | 5.79           | 0.107 | .913  | .238 | .844 |
| Cholesterol, mg/100g   | 74.47           | 69.07           | 64.60           | 72.29          | 66.47          | 1.530 | .058  | .073 | .565 |

*Liophylized muscle tissue. SEM: standard error mean.
and oleic acid when the body weight increases. Similarly, in domestic pigs, as the live weight increases, the intramuscular fatty acid composition changes with an increase in oleic acid (Wood et al., 2008; Pedrazzoli et al., 2017). The high amount of oleic acid could be related to the availability of acorns, mainly in the forests, as well as to the crop residues present in agricultural land (e.g. sunflowers seeds, corn). From a nutritional point of view, oleic acid has a relevant importance in the human diet, reducing both LDL cholesterol and triglycerides (Shramko et al., 2020).

Live weight of the wild boar affected \( p < .05 \) the total PUFA content (ranging from 18.53% to 27.51%), which was higher (about +8%; \( p < .05 \)) in the meat of the youngest animals compared to the other ones. The same trend was found for \( n = 6 \) PUFA and \( n = 3 \) PUFA \( p < .05 \). These findings are partially in line with the results of Quaresma et al. (2011), who found higher \( n = 3 \) PUFA in the younger animals, and Pedrazzoli et al. (2017), who observed higher \( n = 6 \) PUFA in the younger animals. The \( n = 3 \) PUFA content in the present study was higher than the values reported by Quaresma et al. (2011) and Pedrazzoli et al. (2017), but slightly lower than the contents observed by Dannenberger et al. (2013). The precursor of the \( n = 6 \) family, the linoleic acid (C18:2 \( \text{n} = 6 \); ranging from 13.6% to 19.7%), quantitatively the most concentrated \( n = 6 \) PUFA, was higher \( p < .05 \) in younger animals compared to the other ones. Pedrazzoli et al. (2017) found linoleic acid more abundant in young animals with respect to adults. Regarding the individual \( n = 3 \) PUFA, C18:3 \( \text{n} = 3 \) (\( \alpha \)-linolenic acid, ALA) was higher \( p < .05 \) in lighter boars compared to the intermediate weight class; while eicosapentaenoic acid (EPA, 20:5 \( \text{n} = 3 \) ) was higher \( p < .05 \) in lighter boars compared to the heaviest boars. No significant differences were found for other \( n = 3 \) PUFA (C22:5, DPA and C22:6, DHA). Similarly, Razmaite et al. (2012) found ALA, DPA and DHA higher in lighter animals (47.7 Kg) compared to heavier ones (50.6 Kg).

### Table 3. Total lipids (g/100g) and fatty acid composition (% of total fatty acid) and nutritional indices of longissimus thoracis muscle in wild boar of different weight and sex.

| Traits            | Live weight, kg (W) | Sex (S) | p Value | SEM |
|-------------------|---------------------|---------|---------|-----|
|                   | 50                  | 70      | 100     | M   | F   |
| Total lipids      | 6.17                | 5.27    | 7.70    | 6.3 | 6.5 |
| Fatty acids       |                     |         |         |     | SEM |
| C 14:0            | 0.83b               | 1.00a   | 1.02a   | 0.94| 0.96|
| C 16:0            | 21.33b              | 22.83a  | 22.64ab | 22.04| 22.49|
| C 18:0            | 14.53               | 14.43   | 13.47   | 14.17| 14.13|
| C 20:0            | 0.61                | 0.67    | 0.46    | 0.61| 0.56|
| C 22:0            | 0.48                | 0.43    | 0.41    | 0.44| 0.44|
| C 24:0            | 0.44                | 0.32    | 0.28    | 0.34| 0.34|
| C15:1 c10        | 0.05                | 0.05    | 0.06    | 0.05| 0.05|
| C 16:1 c9         | 1.36b               | 2.56a   | 2.68a   | 2.13| 2.27|
| C17:1 c9          | 0.20                | 0.20    | 0.24    | 0.22| 0.21|
| C 18:1 c9         | 32.16               | 37.25   | 39.66   | 35.87| 36.85|
| C 20:1 c11        | 0.49                | 0.52    | 0.55    | 0.53| 0.51|
| C 18:2 n – 6      | 19.67               | 13.60a  | 13.65b  | 16.30| 14.98|
| C 18:3 n – 3      | 1.29a               | 0.69a   | 0.94ab  | 1.06| 0.89|
| C 20:2 n – 6      | 0.62                | 0.43    | 0.42    | 0.49| 0.49|
| C 20:4 n – 6      | 4.70                | 3.96    | 2.72    | 3.65| 3.93|
| C 20:5 n – 3      | 0.44a               | 0.33a   | 0.23b   | 0.32| 0.34|
| C22:5 n – 3       | 0.70                | 0.63    | 0.49    | 0.75| 0.46|
| C22:6 n – 3       | 0.10                | 0.11    | 0.10    | 0.11| 0.10|
| Total fatty acidsa|                     |         |         |     | SEM |
| \( \Sigma \) SFA | 38.22               | 39.67   | 38.28   | 38.53| 38.92|
| \( \Sigma \) MUFA| 34.27               | 40.58   | 43.19   | 38.80| 39.89|
| \( \Sigma \) PUFA| 27.51               | 19.75a  | 18.53a  | 22.67| 21.19|
| \( \Sigma \) PUFA n – 6| 24.96      | 17.99b  | 16.79b  | 20.44| 19.40|
| \( \Sigma \) PUFA n – 3| 2.53c      | 1.76b   | 1.74b   | 2.23| 1.79|
| \( \Sigma \) PUFA \( \text{n} = 3 \) |                     |         |         |     | SEM |
| \( \Sigma \) \( \text{n} = 6/\text{n} = 3 \) | 0.01      | 0.10    | 0.32    | 0.99| 10.80|
| P/S               | 0.72b              | 0.50a   | 0.48a   | 0.59| 0.54|
| Al                | 0.40b              | 0.45a   | 0.43a   | 0.42| 0.43|
| Ti                | 0.98               | 1.11    | 1.05    | 1.02| 1.07|

\( a \): SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. 
\( b \): P/S: PUFA/SFA ratio; Al: atherogenic index; Ti: thrombogenic index. SEM: standard error mean.

Means within a row lacking a common superscript differ \( p < .01 \).

Means within a row lacking a common superscript differ \( p < .05 \).

Means within a row lacking a common superscript differ \( p < .001 \).
Nutritional indices \((n-6/n-3, P/S, AI, and TI)\) were partially affected by live weight. The \(n-6/n-3\) ratio was not affected by live weight with values above the recommended maximum of 4, related to the diet resources of feral wild boar that are mainly acorns, chestnuts, potatoes or maize, foods rich in \(n-6\) PUFA \((18:2n-6)\). The P/S value was better in the youngest animals compared to the other ones \((p<.01)\). The values found in this work are favourable \((ranging from 0.48 to 0.72)\), considering that the recommended ratio of P/S should be increased to above 0.4 (Wood et al. 2004). The AI and TI indexes, the criteria for evaluating the level and interrelation through which some FA may have, respectively, atherogenic or thrombogenic properties, were more favourable in the youngest wild boar compared to the intermediate weight class, even significant only for AI \((p<.05)\). These values are comparable with those reported in literature for wild boar meat (Razmaite et al. 2012; Razmaitė and Šiukys 2019), pork (Salvatori et al. 2008), poultry meat (Banaszak et al. 2020; Tavaniello et al. 2020) and rainbow trout (Renna et al. 2017), but lower in comparison with lamb and goat (D’Alessandro et al. 2019, Liotta et al. 2020) and rabbit (Dabbou et al. 2017).

Comparison between the sexes revealed no differences \((p>.05)\) in total lipid, SFA, MUFA and PUFA percentages. However, compared with females, males showed a higher \((p<.05)\) content of total \(n-3\) PUFA and consequently a lower \((p<.05)\) content of \(n-6/n-3\) ratio, which could be favourable from nutritional point of view. No significant effect of sex was found for other nutritional indexes \((P/S, AI, and TI)\). Our results partially confirmed the findings by Skewes et al. (2009) and Quaresma et al. (2011), which reported that sex had no influence on the muscle fatty acid composition. Differently, Razmaite et al. (2012) found higher SFA and AI values in longissimus dorsi of males than that of females. No significant interactions were found among factors (weight and sex) for single FA, total FAs and nutritional indices \((n-6/n-3, P/S, AI, TI)\).

**Heavy metals**

Industrial development, technological innovations and the increase of world population have led to the alteration of ecosystems both at a chemical-physical and biological level. Among environmental contaminants, heavy metals play a key role. In fact, heavy metals and metalloids such as cadmium (Cd), mercury (Hg) and lead (Pb) are carcinogenic, nephrotoxic and neurotoxic, and may cause immunosuppression, negatively affect the reproductive system and nervous system, and damage cardiovascular and lung (Satarug et al. 2003). Wild boar meat can represent an important source of heavy metals, following their growing consumption linked to the increase in hunting activities (Ramanzin et al. 2010). Table 4 shows the heavy metal content in the LT muscle of wild boar. Weight, sex and hunting area did not affect Mn \((ranging from 0.083 to 0.130 \text{ mg/kg})\), Cd \((0.001 \text{ mg/kg})\), Cu \((ranging from 0.696 to 1.151 \text{ mg/kg})\) and Pb \((ranging from 0.011 to 0.026 \text{ mg/kg})\) contents. Cd and Pb levels found in the present study are well below the limit reported by the Regulation (EC) 1881/2006 and lately amendment Commission Regulation (EC) 629/2008 for meat of farm animals (bovine animals, sheep, pig and poultry; Cd: 0.050 mg/Kg and Pb: 0.010 mg/Kg).
was no difference between data collected on the presence of Cd in wild boar muscle in the present paper and those reported in the literature (ranging from 0.001 to 0.355 mg/kg), as well as for Pb content (ranging from 0.03 to 0.441 mg/kg) (Bilandžić et al. 2010; Rudy 2010; Taggart et al. 2011; Amici et al. 2012; Gašparik et al. 2017). In particular, it has been reported that differences in Pb levels in muscle could be related to lead dispersion in the animal body due to bullet fragmentation. Recently, Pb contamination due to bullets has received great attention in the literature due to the impact on human health (Amici et al. 2012). Levels of Cu found in the present study are similar with those reported by Gašparik et al. (2017) in wild boar hunted in Slovakia. On the contrary, higher values were found by Amici et al. (2012; 12.20 mg/kg) in wild boar hunted in Italy. The authors stated that a possible explanation for these differences in the mean level of Cu accumulation may be due to the particular geo-environmental context of the Province of Viterbo which is prevalently of volcanic origin. Mn levels found in the present study falls within the range reported by Długaszek and Kopczynski (2013), in wild boar meat from 0.08 to 1.39 mg/kg. Cr was not significantly affected by weight and hunting area, but was higher (p<.05) in males than in females. Cr levels are lower than those found by Amici et al. (2012; 0.139 mg/kg). Ni content was higher (p<.01) in the heaviest boars (+51.4%) than the other weight classes, and in those hunted in the A2 area (+30.8%). Sex did not affect (p>.05) Ni content. Significant interactions among the studied factors were found for muscular Ni content. Heavier wild boar (100 Kg) of both sexes showed a higher Ni content in the A2 area compared to A1 (F: A1 = 0.019, A2 = 0.049; M: A1 = 0.021, A2 = 0.050). The same trend was found only in lighter females (50 Kg) (F: A1 = 0.016, A2 = 0.018; M: A1 = 0.017, A2 = 0.017), while the Ni levels were similar between the two hunting areas for both males and females of intermediate weight (70 Kg) (F: A1 = 0.016, A2 = 0.016; M: A1 = 0.017, A2 = 0.017). Ni concentrations are higher than those found in wild boar meat from Austria (0.005 mg/kg; Ertl et al. 2016). It has been stated that accumulation of heavy metals in muscles and in liver increases with the age of wild boar (Rudy 2010; Gašparik et al. 2017), but the sex factor seems to have no influence on the levels of the metals in the meat and liver (Gašparik et al. 2017). Significant differences in heavy metals among the Croatia regions were also reported (Bilandžić et al. 2010, 2012). It has been demonstrated that accumulation of toxic heavy metals in plants and soil (Nagajyoti et al. 2010) may increase the risk of transfer to wild animals and consequently to the food chain which could represent a risk to human health (Amici et al. 2012). However, we are not able to explain the difference in the cumulative levels of Ni recorded in the two different areas because, as far as we know, there is no available scientific information on the chemical soil composition and pollution level in the areas studied. These first results indicate a very low level of anthropogenic pollution of the areas under study, making wild boar meat safe from a health point of view.

**Conclusions**

The results obtained from this study allow to broaden the knowledge on meat quality characteristics and heavy metals content of wild boar of different live weight and sex hunted in different area of Molise region. The data displayed a high extent of variation among animals, due to the varied environmental factors of free-range animals. Although the individual variation was high, the present study showed live weight and sex differences in fatty acid composition; while intramuscular collagen content was affected only by sex. High content of vitamin E was also detected which could be favourable to extend the shelf life of meat. As wild boar represents a valuable bioindicator of environmental pollution, these first results indicate a very low level of anthropogenic pollution of the area under study, making wild boar meat safe from a health point of view. The results obtained from this study are of fundamental importance as they represent the only data available on the quality of wild boar meat hunted in the Molise region. However, further research is needed by taking into consideration other areas of Molise in order to obtain more information on the situation in the entire region.

**Ethical approval**

This study was performed in compliance with current Italian laws.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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