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Hunting area affects chemical and physical characteristics and fatty acid composition of wild boar (*Sus scrofa*) meat

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Received: 30 September 2014/Accepted: 6 March 2015/Published online: 1 April 2015 © Accademia Nazionale dei Lincei 2015

Abstract Wild boar (*Sus scrofa*) populations have increased in the last few decades throughout Europe, and this trend will probably continue in the coming decade. The hunting of *S. scrofa* will increase the availability of meat for both home consumption and market. The aim of the present paper is to investigate how different geo-graphical hunting areas influence chemical composition, quality traits, fatty acid composition and lipid quality indexes of wild boar meat. The geographical hunting area influenced the cooking loss percentage (P < 0.05), dry matter and protein content (P < 0.05). The major fatty acids in *Longissimus thoracis* muscle of wild boar are oleic (18:1*cis*-9), linoleic (18:2*n*-6), palmitic (16:0) and stearic (18:0) acids. Palmitic and stearic acids comprise 20.46 and 14.7 % of the total fatty acids, respectively, in all

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This peer-reviewed article is a result of the multidisciplinary project coordinated by the "Accademia Nazionale delle Scienze detta dei XL", Rome, Italy, in the area of the Presidential Estate of Castelporziano near Rome.

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Department of Animal Sciences and Food Control, University of Naples Federico II, Naples, Italy experimental groups. The polyunsaturated α -linolenic (C18:3*n*-3; *P* < 0.001) content as well as the long-chain *n*-3 polyunsaturated fatty acid content was affected by the hunting area. The geographical hunting area did not affect the pH value at 72 h, the thawing loss or the lipid quality indexes, such as the atherogenic and thrombogenic indexes.

Keywords Wild boar · Meat quality · Lipids · Fatty acids · Sus scrofa

1 Introduction

The wild boar (*Sus scrofa*) population has increased in the last 20 years and has grown in Europe in the incoming decade (Scillitani et al. 2010). This increase has been the consequence of several factors: the absence of natural predators, land cover changes and rural depopulation. The consequence of the increased abundance was a parallel increase of hunting to reduce excessive density, as it represents a threat both to agricultural practices (Amici et al. 2012; Geisser et al. 2004) and to ecosystems (Herrero et al. 2006). Due to the lack of natural predators, hunting is the main method of reducing wild boar density and its consequent negative effects on crops (Geisser et al. 2004).

Game meat can represent an interesting food source for humans in Italy (Ramanzin et al. 2010) and in other countries (Winkelmayer and Paulsen 2008). In addition, game hunting can represent an economic activity and an additional income for farms. In any case, the meat quality of gun-harvested animals should be accurately assessed, as hunting technique affects some quality traits as lipid and colour stability after freeze conservation (Cifuni et al. 2014). The fatty acid composition of meat (muscle and adipose tissue) is important for two main reasons: it determines nutritional value and it affects various traits of meat quality, including shelf life and flavour (Wood et al. 2008).

The most beneficial characteristic of wild boar meat is its low intramuscular fat and cholesterol content (Sales and Kotrba 2013). In addition, the lipid profiles have a desirable low concentration of saturated fatty acids and high concentration of long-chain n-3 and n-6 polyunsaturated fatty acids (Hoffman and Wiklund 2006).

The effect of feeding, environment and genetics on the meat quality of wild boar have been studied in recent decades according to different rearing systems and diets (Tarricone et al. 2010; Skewes et al. 2009), but the variability in natural conditions is not well known. There is considerable variability in the meat quality of wild boar in wild conditions, and it depends not only on many factors including diet, sex, age, fatness, hunting season, hunting method but also on initial processing (exsanguination, evisceration and hanging of the carcass), temperature of carcass storage and meat conservation (Razmaitė et al. 2012). Previous studies have highlighted some differences due to these factors, but few studies were available on this topic (Amici et al. 2011; Marsico et al. 2007).

It should be expected that the nutritional status of wild animals, that being a consequence of the seasonal condition and available vegetation, will influence fat content and quality. Laurent and Roper (2003) demonstrated that wild boar diet is variable according to geographic areas, presumably reflecting local differences in feed availability. Consumption of crops intended for animal feeding (corn plants and natural grassland) is seasonal and dependent on the proximity of cultivated fields. Main crops such as maize, potatoes, oats, wheat and vegetables represent an important dietary component of wild boar, and their presence in the diet significantly influences the fatty acid composition of fat tissues (Laurent and Roper 2003).

The aim of this study was to evaluate the fatty acid composition and the chemical and physical quality of meat from wild boar hunted in natural conditions in three different areas of the Lazio region, characterised by important habitat differences.

2 Materials and methods

2.1 Study areas

The animals were hunted in three areas of central Italy. The first of the three areas is a flat zone of southern Maremma (SM) in the province of Viterbo; the second is a hilly sub-Apennine (SA) area in the province of Viterbo near the borders between Tuscany and Umbria. The third, in the province of Rome, is the National Preserve of Castelporziano, a flat zone close to the Tyrrhenian Sea (TC). The first two hunting areas are separated by a wide urban area and intensively cultivated lands. All three zones match wild boar habitat preferences (Abaigar et al. 1994; Fonseca 2008). Land cover of the three hunting areas is shown in Fig. 1 (CUS 2004).

The areas, of 60 to about 200 km², were characterised by different use of agricultural lands and forest cover type and percentage as reported in Fig. 1. The main differences were represented by the percentage of woods and natural surfaces (28, 47, and 88 % in SM, SA, and TC respectively) and inverse rate of arable surfaces (67, 50 and 11 % in SM, SA and TC respectively). The most prevalent forest cover in the three areas was broadleaf (18, 29 and 48 % respectively for SM, SA and TC). In TC (Table 1), high percentages of thermophilic pinewood (18 %), moors and heathland (12 %) and pasture (8 %) were also reported.

2.2 Animals and hunting techniques

In this study, 48 wild boars with an average age of 17.4 ± 4.51 months were used. The animals were divided by geographical hunting area.

Animals were collected between October 2011 and January 2012, when the weather conditions were generally good (light or no rain) and without snow cover. Environmental and weather conditions were recorded in three weather stations located in the areas. Wild boars were hunted in wild conditions in compliance with Italian rules for hunting and containment programmes. The dog drive hunting technique, used in this trial, represents the hunting

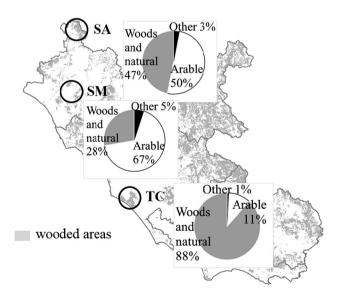


Fig. 1 Hunting areas and main land cover classes (in the frames)

Table 1 Land use (70) of the united number $areas (COS 200)$	Table 1	hunting areas (CUS 2004)
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Hunting area	SM	SA	TC
Land use			
Natural surfaces			
Broadleaf	18	29	48
Other wood cover	2	3	2
Moors and heathland	2	3	12
Chestnut	1	3	0
Thermophilic pinewood	0	2	18
Pasture and shrubs	4	1	8
Sparse vegetation	1	6	_
Arable surfaces			
Arable land	46	47	11
Permanent crops	15	1	-
Other arable lands	3	2	2
Semi-natural crops	6	2	_
Other	3	5	1

SM southern-Maremma hunting area, *SA* sub-Apennine hunting area, *TC* National Reserve of Castelporziano hunting area

technique most widely adopted for wild boar harvesting in Italy (Scillitani et al. 2010).

2.3 Data collection

All the animals were ear tagged after shooting, and a form was filled in with the most relevant hunting data. Before evisceration, the following data were individually recorded: age (estimated from tooth eruption and wear according to Boitani and Mattei 1992), sex and weight (partially blooded). The animals were exsanguinated and eviscerated and, within 2 h of being shot, were transported to a commercial abattoir where they were processed further. Carcasses were weighed and dissected, and a portion of left side *Longissimus thoracis* muscle (LT) of nearly 300 g, between the 2nd and the 4th rib, was collected. Samples were vacuum packed in plastic bags and stored at 2 ± 1 °C by skilled hunters.

All the samples were transported to the laboratory within 3 days of shooting and, following pH and weight determination, were stored at -80 °C under vacuum condition for 3 months before analysis.

2.4 Sample analysis

Individual meat samples were thawed in vacuum packaging for 24 h at 4 °C and submitted to the following physical and chemical measures: thawing loss, colour, cooking loss, shear force on cooked meat and proximate composition (dry matter, ash, fat and protein). The pH was measured on the third day after the arrival of the meat samples at the laboratory, using a portable pH metre (Hanna HI98240) equipped with automatic temperature compensation.

To estimate the colour parameters, the CieLab indexes (lightness L*, redness a* and yellowness b*) were determined by a Minolta CM-2006d reflectance spectrophotometer on raw meat using D65 illuminant after 1 h of air exposure (Cassens et al. 1995).

The thaw loss, expressed as a percentage, was measured for the frozen slice of meat by calculating the difference in weight, before storage and after thawing at 4 $^{\circ}$ C.

The cooking loss was calculated on a portion of a slice of meat using a sealed polyethylene bag. The loss value was obtained by weighing the sample before and after it was cooked in a water bath at 75 °C until the internal temperature reached 70 °C and then cooled for 40 min (Lundström and Malfors 1985). The losses were measured by the differences in weight and were expressed as percentages.

The shear force (WBS) on cooked samples, as above, was determined in four samples with a 1×1 cm cross section and 2 cm in length, using an INSTRON 5543 texturometer equipped with a triangular-shaped blade. A 50 kg compression load cell and a crosshead speed of 100 mm/min were used, and the results were expressed in Newtons (Chrystall et al. 1994).

The proximate composition (dry matter, fat, protein and ash) was determined according to AOAC methods (AOAC 1995). The lipids were extracted from two samples of 5 g of meat according to the procedure of Folch et al. (1957). Lipid extracts were methylated by adding 1 ml of n-hexane and 0.05 ml of 2 N methanolic KOH for 100 mg of lipid, according to IUPAC procedure (IUPAC 1992). The transmethylation was achieved in 5 min at room temperature.

Gas chromatographic analysis was performed on a GC 6890 N (Agilent, Inc., California, USA) instrument. A fused silica capillary column coated with 100 % cyanopropylpolysiloxane (Supelco, 2560; 100 m, 0.25 mm (i.d.), 0.25 μ m film thickness) was used to analyse the methylated fatty acid content. Operating conditions were helium flow rate of 1 ml/min, FID detector at 300 °C, split–splitless injector at 250 °C and injection volume of 1 μ l. The temperature programme of the column was 4 min at 140 °C with a subsequent increase to 220 °C at 4 °C/min and then held at 220 °C for 10 min.

The individual fatty acid peaks were identified by comparison with the retention times of a known mixture of standard fatty acids (FAME mix37, Supelco). The fatty acids were expressed as a percentage of the total methylated fatty acids.

Lipid quality indexes, i.e., atherogenic index (AI) and thrombogenic index (TI), were calculated according to Ulbricht and Sauthgate (1991).

2.5 Statistical analysis

The data were processed by covariance analysis using age as the covariate and geographical areas as factors. The GLM procedure was performed by SAS (2002), and the mean values were compared by Fisher's LSD test, using P < 0.05 as the significant difference level. Stepwise discriminant analysis was also performed on the variables showing significant differences, to identify the principal components for classifying the samples by area. Wilks' lambda was used for the statistical selection of the variables. The robustness of the classifications obtained was tested by cross-validation of the 'leave-one-out' type, which is appropriate for small populations (Celeux and Turlot 1990).

3 Results

3.1 Physical quality and chemical composition

Geographical hunting area did not affect pH value, thawing loss or WBS values (Table 2) on LT muscle of wild boar. The cooking loss percentage, reported in Table 2, was higher in animals hunted in SM area (P < 0.05) than the TC group, while the SA group did not show significant differences compared to the others. In our study, hunting area had a limited effect on colour parameters (Table 2); in fact, only meat samples from animals culled in the SM area showed lower redness index compared to the others.

Dry matter content (Table 3) was lower in meat from the animals of the SA group (P < 0.05) than the others, while protein content was significantly (P < 0.05) different only between the SA and TC groups. No statistical difference was observed for fat and ash contents.

Table 2 Effect of the hunting area on physical quality of wild boar of

 Longissimus thoracis

Hunting area	SM	SA	TC	RMSE
рН	5.61	5.59	5.64	0.083
Thawing loss (%)	8.18	8.03	7.17	2.512
Cooking loss (%)	31.77 ^a	31.16 ^{a, b}	28.48 ^b	2.905
WBS N	41.05	47.08	40.19	7.749
Lightness (L*)	38.64	44.47	40.38	6.642
redness (a*)	5.52 ^b	9.94 ^a	9.83 ^a	3.677
yellowness (b*)	10.48	14.71	13.22	3.377

Different letters within rows mean significant difference (P < 0.05)

SM southern-Maremma hunting area, *SA* sub-Apennine hunting area, *TC* National Reserve of Castelporziano hunting area, *WBS* shear force obtained with Warner–Bratzler apparatus

 Table 3 Effect of the hunting area on chemical composition of Longissimus thoracis muscle

Hunting area (%)	SM	SA	TC	RMSE
Dry matter	25.59 ^a	24.58 ^b	25.66 ^a	0.788
Fat	3.13	2.66	2.54	1.164
Protein	21.20 ^{a, b}	20.67 ^b	21.84 ^a	0.945
Ash	1.26	1.25	1.28	0.131

Different letters within rows mean significant difference (P < 0.05) SM southern-Maremma hunting area, SA sub-Apennine hunting area, TC National Reserve of Castelporziano hunting area

3.2 Fatty acid composition

Fatty acid composition is shown in Table 4. As regards the saturated fatty acid, the level of lauric acid (C12:0; P < 0.05) is higher in meat from SM animals than those in other groups. Furthermore, the geographical hunting area did not affect myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18: 1*cis*-9) and linoleic (C18:2*n*-6) fatty acid contents (P > 0.05). Vaccenic acid (C18:1*trans*-11, P < 0.05) occurred in a higher proportion in wild boar meat from the SA and TC areas than from the SM area.

The percentage of polyunsaturated α -linolenic (C18:3*n*-3; P < 0.001) was higher in meat from wild boar hunted in the SA group than in the SM group, while the TC group showed an intermediate value.

A higher percentage of arachidonic acid (C20:4*n*-6; P < 0.05) was noticed in the SM group when compared to the others

Also, the level of eicosapentaenoic acid (C20:5*n*-3; P < 0.05) was higher in the SM group than the SA group, whereas docosadienoic (C22:2*n*-6; P < 0.05) and docosahexaenoic (C22:6*n*-3; P < 0.05) acid contents were significantly higher in the SM group than the TC group.

Concerning nutritional evaluation of fat fractions, we observed that the n-6 PUFA proportion was quite similar among the groups, whereas the levels of long-chain n-3 PUFA (Table 4) in meat from wild boars gathered in the SA area were significantly higher than those from the SM area, and the meat of the TC group showed intermediate value.

As regards the *n*-6/*n*-3 ratio, it was higher (P < 0.05) in the SM group than in the SA group, while the TC group showed an intermediate value.

Geographical hunting area did not affect the lipid quality index, such as atherogenic and thrombogenic indexes.

Discriminant analysis was used to identify a classification criterion for meat samples, using the geographical area as a grouping variable. The discriminant factorial analysis selected a subset of discriminant variables, namely C20:5*n*-3, C18:1*trans*-11, C12:0 proportion and protein content to classify (after cross-validation) the samples according to geographical hunting area (Fig. 2).

 Table 4 Effect of the hunting area on fatty acid profiles of Longissimus thoracis muscle (expressed as percentage on total methylated fatty acids)

Hunting area	SM	SA	TC	RMSE
C12:0	0.14 ^a	0.10 ^b	0.12 ^b	0.044
C14:0	0.79	0.87	0.85	0.333
C16:0	20.66	20.54	20.81	1.594
C16:1	2.35	2.11	2.13	0.998
C17:0	0.38	0.49	0.34	0.158
C17:1	0.17	0.23	0.16	0.100
C18:0	14.04	14.35	14.15	2.615
C18:1trans-11	0.16 ^b	0.45^{a}	0.40^{a}	0.223
C18:1cis-9	25.83	26.76	26.42	2.434
C18:1 <i>n</i> -7	4.43	3.71	4.30	0.862
C18:2 <i>n</i> -6	20.66	21.17	21.69	5.137
C18:3 <i>n</i> -3	0.61 ^b	1.74 ^a	0.94 ^{a, b}	0.827
C20:0	0.17	0.16	0.16	0.029
C20:2n6	0.11	0.18	0.12	0.082
C20:3n3	0.50	0.51	0.65	0.238
C20:4 <i>n</i> -6	6.21 ^a	4.23 ^b	4.39 ^b	1.583
C20:5 <i>n</i> -3	0.22 ^a	0.11 ^b	0.16^{ab}	0.099
C21:0	0.36	0.38	0.25	0.152
C22:2 <i>n</i> -6	0.61 ^a	$0.49^{a, b}$	0.40^{b}	0.185
C22:4n6	0.38	0.29	0.38	0.123
C22:5n3	1.14	1.05	0.96	0.418
C22:6n-3	0.18 ^a	0.13 ^{a, b}	0.11 ^b	0.052
SFA	36.54	36.88	36.79	3.110
MUFA	32.94	33.25	33.42	8.247
PUFA	30.53	29.86	29.80	6.685
<i>n</i> -6	27.97	26.35	26.98	6.269
<i>n</i> -3	2.56 ^b	3.51 ^a	2.82 ^{a, b}	0.171
<i>n-6/n-3</i>	10.95 ^a	7.50 ^b	9.56 ^{a, b}	2.881
PUFA/SFA	0.84	0.81	0.80	0.170
AI	0.377	0.381	0.386	0.053
TI	0.925	0.873	0.927	0.103

Different letters within rows mean significant differences (P < 0.05) SM southern-Maremma hunting area, SA sub-Apennine hunting area, TC National Reserve of Castelporziano hunting area, SFA (C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0), MUFA (C16:1 + C17:1 + C18:1trans-11 + C18:1cis-9 + C18 :1n-7), PUFA (C18: 2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-3 + C20:4n-6 + C20:5n3 + C22:2n-6 + C22:4n6 + C22:5n3 + C22:6n-3), AI atherogenic index = (C12:0 + 4*C14:0 ++C16:0)/((n-6 + n3) + C18:1 + (MUFA-C18:1)), TI thrombogenic index = (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5n-6 +3n-3 + (n-3/n6))

4 Discussion

As a general consideration, we must underline that diet, one of the major factors affecting meat quality of animals, is not controllable in field studies on wild animals. As is well known, animals living in natural condition in the wild are not fed by humans but search for feeds available in the area

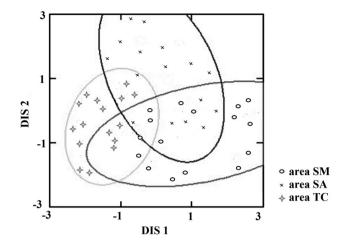


Fig. 2 Discriminant analysis on *Longissimus thoracis* muscle from wild boar culled in different geographical areas

where they live. The major trait of wild boar feeding habits is opportunistic behaviour, inducing the animals to use resources that are attractive and easily available in the area according to the phenological status of plants. Although the feeding habits of wild boar have previously been studied, including compositional (Abaigar et al. 1994; Herrero et al. 2006; Laurent and Roper 2003) and behavioural traits (Primi et al. 2009; Scillitani et al. 2010), in this study, we refer to different feeding habits related to the geographical hunting area considered, as reported by CUS (2004).

4.1 Chemical composition and meat quality

Our results (Table 3) indicate that cooking losses were affected by hunting area. Values of cooking losses were similar to those reported by Tarricone et al. (2010) in wild boar groups reared in outdoor pens.

Muscle colour is an important criterion by which many consumers evaluate meat quality and acceptability. Meat colour depends on myoglobin concentration and the meat's chemical state, surface structure and intramuscular fat (Mancini and Hunt 2005). Myoglobin is mainly responsible for meat colour, and a darker muscle (high a* value) could be attributed to higher myoglobin concentrations (Warriss et al. 2006).

Diet, animal age and exercise could have an effect on meat colour. High-energy diets decrease heminic pigment concentrations and consequently reduce a* values (Lawrie 1998). The lower a* value detected in meat from the SM group compared to the others may be related to an increased availability of feed in this geographical area, due to a higher percentage of arable land compared to wooded areas, as reported in Fig. 1. Although we used frozenthawed samples, the L*, a* and b* values detected in our experimental trial are consistent with those reported by Marsico et al. (2007) in fresh samples. In our study, dry matter and protein content differed between areas. Our results were consistent with Dannenberger et al. (2013), which reported a protein content range of 21.4 to 23.6 %, and those authors support the hypothesis that longlasting different feeding conditions can affect meat protein content in free-living animals.

4.2 Fatty acid composition

The fatty acid composition observed in wild boar LT muscle (Table 4) is within the range of values reported in wild boar hunted in Germany (Dannenberger et al. 2013), pen reared wild boar (Tarricone et al. 2010) and pork (Wood et al. 2008). Some interesting differences are detectable when compared to Razmaite et al. (2012), which found, in wild boar hunted in Lithuania, a higher content of oleic and lower proportion of linoleic acids. The major fatty acids in wild boar LT muscle are oleic, linoleic, palmitic and stearic acids (Dannenberger et al. 2013). In our studies, high levels of oleic and linoleic acids, which accounted for 47 % of the total fatty acid, were found in all groups.

The level of vaccenic acid (C18:1*trans*-11) detected was consistent with the value reported by Quaresma et al. (2011) in meat from wild boars hunted in Portugal. Vaccenic acid is the only known dietary precursor of c9,t11 conjugated linoleic acid (CLA), which has purported anticarcinogenic, anti-atherogenic, anti-inflammatory and positive immune modulatory properties (Benjamin and Spener 2009). Furthermore, recent data (Field et al. 2009) suggest that consumption of this trans fat may impart health benefits beyond those associated with CLA.

The meat concentration of n-3 PUFA was similar to the values observed in wild boars (Tarricone et al. 2010; Dannenberger et al. 2013), domestic pigs reared on pasture under organic production (Oksbjerg et al. 2005) or under the traditional Iberian free-range system (Daza et al. 2007), but higher than the contents found in average retail pork from pigs raised under intensive production systems (Nilzen et al. 2001).

The levels of polyunsaturated α -linolenic, arachidonic, eicosapentaenoic, docosadienoic, docosahexaenoic and vaccenic fatty acids were influenced by hunting area. These results suggest that this is due to feed diversity among the geographical hunting areas and/or individual feeding habits of wild boar.

The consumer, for health reasons, increasingly prefers products with a higher unsaturated fatty acid content, especially the polyunsaturated n-3 fatty acids because of their beneficial effect in preventing cardiovascular diseases (Simopoulos 2008).

Nutritional evaluation of fat fractions of foods is frequently based on nutritional indexes such as the polyunsaturated/saturated (P/S) ratio and the *n*-6/*n*-3 ratio (WHO 2003). According to the recommendations of the Department of Health (1994), the P/S ratio should be above 0.40. The obtained averaged value was 0.80, similar to the value found in feral and reared wild boar by Marsico et al. (2007) and Skewes et al. (2009). On the other hand, the *n*-6/*n*-3 ratio in meat from wild boars harvested in the TC and SA areas was lower than in the SM area, which is 2.5 times higher than the nutritional recommendations (Wood et al. 2004). Our result is similar to the *n*-6/*n*-3 ratio obtained by Marsico et al. (2007) in Italian feral wild boar and by Oksbjerg et al. (2005) in pork raised under organic and free-range production systems.

The atherogenic and thrombogenic indexes, as measures of lipid quality, which could serve as predictors of cardiovascular risks (Sales and Kotrba 2013), were lower than 1.00. The thrombogenic index was lower (0.907) than the value reported by Ulbricht and Sauthgate (1991) in pork (1.66); thus, wild boar meat could be a very useful food in human diets.

As shown in Fig. 2, the stepwise discriminant analysis could separate 75 % of meat samples according to geographical hunting areas, despite the feral conditions of the wild boar, including the variable related to the different feed availability and feeding behaviour.

The considerable differences in land use (Fig. 1) of the three areas considered in this paper justify the organoleptic and nutritional differences of wild boar meat. In particular, the percentage of cultivations adjacent to the hunting areas in which the wild boar occasionally feed represents the main source of variability; in fact, the wooded areas are similar and consist primarily of oaks. Among the three areas considered, the TC area strongly differs both for land use composition and for altitude and shows a limited amount of cultivated land.

As expected, characteristics of wild animal meat showed very high variability due to the environmental factors, including diet, that are not controllable. Nevertheless, colour parameters, cooking loss, dry matter, protein contents and fatty acid profiles of meat showed some differences in relation to hunting area as a probable effect of feed diversity. In terms of human health, meat from animals hunted in SA and TC areas showed desirable fatty acid compositions (high contents of linolenic and vaccenic acid and lower n-6/n-3 ratio).

Acknowledgments The Authors thank the National Reserve of Castelporziano, Roma, Italy.

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