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FATTY ACID COMPOSITION OF LAMB MEAT FROM ITALIAN AND GERMAN LOCAL BREEDS

--Manuscript Draft--

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Abstract:	The aim of this study was to evaluate and compare the quality characteristics, chemical composition and lipid profile of lamb meat from Italian (Biellese and Sambucana) and German (Texel-Merino-Blackhead-Charollais [TMBC]) breeds reared in extensive and semi-extensive production systems. Meat samples from 89 animals were analysed. The meat of the lambs from semi-intensively reared Biellese, and extensively reared Sambucana and TMBC breeds produced lean meat, with slightly higher intramuscular fat content in TMBC. The latter also produced meat of darker colour (P<0.05) and higher protein content (P<0.05). The meat of Sambucana lambs presented the lowest total cholesterol content (P<0.05). The fatty acid profile of the meat showed a clear advantage of both extensively reared breeds, which had substantially lower proportion of saturated (SFA) but higher of polyunsaturated fatty acids (PUFA), particularly n-3 (P<0.05). The beneficial effect of the extensive rearing conditions was associated with lower n-6/n3 ratio, and atherogenic (AI) and thrombogenic (TI) indices, thereby suggesting that production system can be used successfully to modify the fatty acid profile to achieve a positive effect for the human health.

20 October 2020

Editor

Small Ruminant Research

Dear Dr. S. Y. Landau

I wish to submit to the reputed Small Ruminant Research journal the manuscript entitled: "FATTY ACID COMPOSITION OF LAMB MEAT FROM ITALIAN AND GERMAN LOCAL BREEDS", whose objective was to evaluate the fatty acid profile and chemical composition of lamb meat originating from 3 Italian and German breeds typically raised in extensive, semi-extensive or intensive regime.

This work has not been submitted to any other journal either in English or in any other language.

Yours sincerely,

Dr. Ursula Gonzales-Barron

Mountain Research Centre (CIMO)

Polytechnic Institute of Bragança

Portugal

13 March 2021

Editor in Chief

Small Ruminant Research

By means of this communication, I wish to provide responses to the comments raised by the reviewers in relation to the manuscript Rumin-D-20-11389. The responses are given in bold font, as follows.

Reviewer #1:

The present study deals with the effects lamb meat quality of three different breeds produced under different production systems. The results presented in the study highlight some relevant differences in some parameters that are relevant for the consumer. However, there are some issues related to the discussion of results which should be completed/amended before being published in SRR.. FAME profile should be expressed in both forms (% of FAMES and mg of FAME/100 g of meat) and discussion modified to avoid confusion between total amount and percentage of each FAME, which at some point are used like synonyms (which indeed they are not).

Thank you very much for your comment. The methodology used in this study to analyse fatty acids aims at the qualitative traits, namely the percentage of the fatty acids in the meat. To present the content of the fatty acids as mg per 100 g meat a completely different method for calculation and calibration of the GC is required which makes it not possible to present the fatty acid profile in this study like it is recommended. Furthermore, the term "amount" is used to present the amount of the total SFA, PUFA, MUFA and this is the relative amount. To avoid the confusion we have replaced "amount/content" with "percentage/ proportion" where relevant in the text marked in red.

All the discussion should be based on the total fatty acid content per serving size (100 g of meat) to avoid confusion between profile of FAME and the total amount of each FAME consumed per serving size (which is what really matters).

Thank you for this comment. As we mentioned above we should apply completely different methods (standard curves, etc. for each fatty acid) to calculate the fatty acid content in meat if we should interpret the fatty acid profile based on the serving size of the meat. The aim of our study here is to present some differences in the intrinsic attributes of lamb meat derived from different breed in Europe (particularly in Italy and Germany) that are reared in their specific systems and based on this information to be able to derive recommendations in the future concerning possible improvement of the meat quality in regard to extrinsic factors.

It is very surprising the different cholesterol amounts observed for the three meat obtained from different breeds. In my opinion, this result should be double checked to be completely sure about the lack of errors. If it is correct, then discussion should be improved and extended. **Thank you very much for the comment. Although a stable characteristic, the cholesterol content might be influenced by the breed of the animals. Such results have been observed in in sheep (Arsenos et al., 2000; Aksoy et al., 2019). However, in our study we could due to the specificities of the experimental design (each breed reared in specific conditions) we might speculate about the factors affecting the cholesterol content and compare with other studies, as we did in the text.**

Reviewer #3: There were many problems with the description of the experiment, making it impossible to determine its validity.

- How were animals grouped (by diet, by weight), did animals stay in one pen or collective, what were intake and performance rates, what were is the live animal performance and carcass data (this is need to help explain group differences in meat quality)? Many details od location, size ant timing of meat sampling were missing. A number od fatty acids were not reported, for example branched chain fatty acids, individual cis 9-18:2 was not separated from cis 9, trans 11-18:2.

Thank you very much for your comment. The respective details about the diet of the animals and the rearing strategy have been added (lines 101-108, 113-114; 117-118). The details about the timing and sampling of meat are presented (lines 132-143). The CLA are not separated in the study. As it is known the main CLA someres that are biologically active and in the greatest proportion in ruminant products are cis 9 trans 11 c18:2and trans 10 cis 12 CLA; however the former is mainly influenced by the feed/ rearing system. In this study, we are focusing on cis 9 trans 11 c18:2 and trans vaccenic acid.

- Surely for such a study the number of lambs for each "genotype" would be provided to demonstrate that you were not just reporting individual lambs effects? This is fundamental.

Thank you very much for your comment. The number of the lambs from each breed is presented in the section materials and methods: 16 Biellese and 20 Sambucana lambs were reared in 2018, and 12 Biellese and 12 Sambucana lambs were reared in 2019. Texel-Merino-Blackhead-Charollais (TMBC) - 29 lambs (lines 113-114).

- Meat characteristics of Italian (Biellese and Sambucana) x German (Texel-Merino-Blackhead-Charollaishas) been well studied, as you amply not in your discussion section. Your introduction section is broad and indicates that these characteristics are not well known, when really the information that is lacking is the comparison of influenced crossbreeds to these other well-studied types. This is particularly relevant for the author's environment and other similar environment. None of this is justified or brought up for consideration in the introduction. This makes it hard to support the main objective statement, therefore a full revision of this section needs, to occur for stronger justification of the study.

Thank you very much for your recommendation. What we aim in the study is to present the meat quality characteristics of some local breeds, according to their specific rearing systems. The aim of the study was modified accordingly (lines 83-86).

- The statistical model provided is unclear and little detailed. Furthermore, doesn't diet or other management groupings play a factor here outside of breed type? Did the authors even investigate this? Based on the manuscript, I would assume that they were not and would be a large issue with interpreting model outcomes.

Thank you for your comment. Indeed we have applied one way ANOVA that provides information about the differences between the studied groups. We are highly aware that two-way ANOVA is appropriate when we want to discuss the effects of the factors breed and rearing system as well as the interaction between them. However, the aim of this study is to present some of the specific Italian and German breeds reared in their specific system and conditions on farm level, with the characteristic differences between them.

On behalf of all authors, I wish to thank the reviewers for they have helped improve the quality of our manuscript.

Best regards,

Dr. Ursula Gonzales-Barron

Principal Investigator

CIMO Mountain Research Centre

HIGHLIGHTS

- Lamb meat quality of three Italian and German breeds was evaluated
- Meat of lambs reared in extensive system had the lowest SFA content
- Meat of lambs reared in extensive system had the highest n-3 PUFA content
- Meat from the Italian Sambucana lambs presented the lowest cholesterol content
- Meat from the three breeds was of high quality with low fat content

FATTY ACID COMPOSITION OF LAMB MEAT FROM ITALIAN AND GERMAN LOCAL BREEDS

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HIGHLIGHTS

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ABSTRACT

The aim of this study was to evaluate and compare the quality characteristics, chemical composition and lipid profile of lamb meat from Italian (Biellese and Sambucana) and German (Texel-Merino-Blackhead-Charollais [TMBC]) breeds reared in extensive and semi-extensive production systems. Meat samples from 89 animals were analysed. The meat of the lambs from semi-intensively reared Biellese, and extensively reared Sambucana and TMBC breeds produced lean meat, with slightly higher intramuscular fat content in TMBC. The latter also produced meat of darker colour ($P<0.05$) and higher protein content ($P<0.05$). The meat of Sambucana lambs presented the lowest total cholesterol content ($P<0.05$). The fatty acid profile of the meat showed a clear advantage of both extensively reared breeds, which had substantially lower proportion of saturated (SFA) but higher of polyunsaturated fatty acids (PUFA), particularly n-3 ($P<0.05$). The beneficial effect of the extensive rearing conditions was associated with lower n-6/n3 ratio, and atherogenic (AI) and thrombogenic (TI) indices, thereby suggesting that production system can be used successfully to modify the fatty acid profile to achieve a positive effect for the human health.

Keywords: Sheep; meat; quality characteristics; fatty acid profile

1. INTRODUCTION

A renewed interest in local sheep breeds has been prompted by the EU's recent promotion of a sustainable development of otherwise marginal areas and policies supporting extensive systems of animal production (Marino et al., 2008). Local sheep production is more likely to be found in low farming areas, where sheep graze semi-natural vegetation and are dominant with other low input extensive grazing livestock (Cruz et al., 2019). Even though sheep farming represents only a small contribution to Europe's gross domestic product (<0.5%; (Eurostat, 2019)), the sector is of great importance to rural development and the environment. The indigenous sheep breeds, in addition to contributing to the diversity of production systems, are important genetic resources that must be preserved because of their local adaptation, disease resistance, high fertility and unique product qualities (Mendelsohn, 2003). Some of these breeds have small body and good adaptation to adverse climatic and orographic environments, which makes them particularly suited to the use and enhancement of natural pastures (Cruz et al., 2019).

One strategy for attaining sustainability of the local sheep farming sector is through high quality standard of the products. Enhancing the quality of meat from autochthonous breeds, making it more attractive to consumers, could contribute to the preservation of the rural world and its diversity, the conservation of endangered breeds, as well as improving the profitability and living standards of the sheep farmers that remain in these rural areas. Nonetheless, meat quality is a very ample concept defined by industry and the final consumer, which is regulated by a series of factors that are intrinsic and extrinsic to the animal (Webb et al., 2005). Fatty acid composition alone has a strong impact on the nutritive value and the organoleptic characteristics of meat (Díaz et al., 2005). The flavour profile, an important attribute of meat, is regulated by variations in the absolute concentration and the relative proportions of different fatty acids (Fisher et al., 2000). Several studies have confirmed that meat fatty acid composition can be influenced by production system, animal breed and sex, slaughter age and live weight, and level of fatness (Atti and Mahouachi, 2009; Boughalmi and Araba, 2016; Domínguez et al., 2015, 2018b; Ekiz et al., 2013).

Therefore, the objective of this study was to evaluate and compare the physicochemical attributes (ultimate pH, colour, proximate composition and cholesterol content) and fatty acid profile of lamb meat originating from three Italian and German breeds typically raised in extensive or semi-extensive production systems. Three sheep breeds exploited for meat production, in their typical production systems, were utilised in the present study: Sambucana lambs raised in extensive system and Biellese lambs raised in semi-extensive system from the Alpine and the Continental bioregions of Italy, respectively, and crossbred Texel-Merino-Blackhead-Charollais (TMBC) lambs reared in extensive system from the Continental bioregion of Germany.

2. METHODOLOGY

2.1 Lamb rearing and feeding

All animals' management and procedures were carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Italy

In the Continental bioregion, located in Turin, Biellese lambs were raised in CISRA, Teaching Animal Farm of the Veterinary Science Department, University of Turin. In the Alpine bioregion, located in Val Maira, Western Alps, at an altitude of 1800-2000 m, Sambucana lambs were bred during the summer season. The lambs from both breeds were raised in collective pens with their mothers. The production system used for Biellese breed was semi-extensive (i.e., the lambs consumed about 500 g of milk per day the first 15 days after birth and then 1000 g per days until weaning (2 months of age)). After weaning, the lambs were fed with ~200 g of concentrate per day and 700 g of hay until the slaughtering (4.5-5 months of age). The production system for Sambucana lambs was based on grazing on natural pasture, with no additional concentrate or hay. The lambs were fed 400 g of milk per day the first 15 days after birth and until 2 month of age the animals received 800 g of milk per day. In the semi-extensive system for Biellese lambs, the flocks would be released to graze outside in autumn-winter season (period of investigation); whereas in the extensive system, Sambucana lambs would leave at dawn and graze until evening; then they would be recovered in a fence in summer season (period of investigation). The lambs were not weaned during the grazing season in the Alpine bioregion. For this investigation, 16 Biellese and 20 Sambucana lambs were reared in 2018, and 12 Biellese and 12 Sambucana lambs were reared in 2019.

Germany

The lambs, all crossbred Texel-Merino-Blackhead-Charollais (TMBC), were raised in a farm at an abandoned military training area on the Swabian Alb near Münsingen; and held in one collective flock. The animals stayed in a pen overnight and during the day they roam freely. The study area belongs to bio-region Kuppenalb, located in the eco-region called the “Western European Broadleaf Forests”. The study pasture of 170 ha is managed extensively, and grazed by a mid-sized flock of around 500 ewes. The insemination is carried out naturally in spring. During winter, traditional transhumance is practiced to the lower Swabian areas of Nördlingen. The animals graze all year long, and the only additionally provided fodder is mineral feed. On this farm 29 lambs were reared.

2.2 Preparation of lamb meat samples

All lambs were four-to-five months old when slaughtered. Lambs were slaughtered in batches ranging from 5 to 12 animals in local abattoirs of Italy and Germany, and the whole experiment was conducted in the fall and spring seasons of 2018 and 2019. A total of 89 lambs were employed in this study, from the following breeds and production systems: Biellese semi-extensive (28), Sambucana extensive (32), TMBC extensive (29).

Lambs were slaughtered after an overnight lairage period, where they had free access to water but not to feed. In the local abattoirs, lambs were electrically stunned, dressed, washed and chilled at 4ºC. Twenty-four hours after slaughter, carcasses were split and the *Longissimus thoracis et lumborum* muscles removed from the 6^{th} to the 13^{th} vertebra under aseptic conditions. The right side was divided into two parts for subsequent laboratorial analysis. They were vacuum packed in transparent gas-tight polyamide and polyethylene vacuum bags (Orved®, Spain, with permeability of 84 ± 4.20 cc/m^2/24h/atm for O_2, 361 ± 18.05 cc/m^2/24h/atm for CO_2, 22 ± 1.10 cc/ m^2/24h/atm for N_2 and 9.0 ± 0.45 cc/m^2/24h/atm for H_2O and density of ± 100µm), and stored at 4±0.5 °C. Within 24 hours, the meat samples were subjected to the following determinations: pH and instrumental colour measurement, proximate composition, cholesterol content and fatty acid profile. All the essays were carried out in the same laboratory.

2.3. Physicochemical analyses of meat

2.3.1. pH and instrumental colour measurements

The pH was measured in triplicate using a digital portable pH-meter (Hanna Instruments, Eibar, Spain) equipped with a penetration glass probe. A portable colorimeter (Konica Minolta CR-600d, Osaka, Japan) was used to measure in triplicate the meat colour in the CIELAB space (lightness L*, redness a* and yellowness b*). The device was set to pulsed xenon arc lamp, 10° viewing angle geometry, standard illuminant D65 and aperture size of 8 mm. Samples were allowed to bloom for 30 min before measuring.

2.3.2. Proximate composition

Moisture, intramuscular fat, protein and ashes contents were determined according to ISO (1997), AOCS (2005), ISO (1978) and ISO (1998), respectively. Determinations were made in triplicate per meat sample.

2.3.3. Cholesterol analysis

Total cholesterol was analysed (saponification, separation and quantification) following the procedure described by Domínguez et al. (2018a). Two grams of homogenised meat sample were placed in a screw Teflon-lined cap tube and 0.25 g of L-ascorbic acid and 5 mL of saponification solution [11% potassium hydroxide in ethanol solution (55%)] were added. The saponification step was carried out in a shaking water bath at 85 ºC for 45 min. After cooling, cholesterol was extracted with 1.5 mL of hexane. Cholesterol was separated and quantified using normal phase-HPLC technique, according the chromatographic conditions reported by Domínguez et al. (2018a). The HPLC systems used was an Alliance 2695 model (Waters, Milford, USA) equipped with a 996 Photodiode Array Detector (Waters Milford, USA). The cholesterol analysis was performed using a normal-phase silica column (SunFireTM Prep Silica, 4.6 mm ID × 250 mm, 5 µm particle size, Waters, Milford, MA, USA). The mobile phase (2% v/v 2-propanol in n-hexane) flow rate was 1 ml/min, the run last for 15 min and the temperature of the column oven was adjusted at 30 ºC. The detection of cholesterol was carried out using Photodiode Array detector at 208 nm. Results were expressed as mg of cholesterol/100 g of meat.

2.4. Fatty acid composition analysis

Intramuscular lipids were extracted following the method described by Bligh & Dyer (1959) with the modifications proposed by Barros et al. (2020). The procedure described by Barros et al. (2020) was used for fatty acids determination. Briefly, 20 mg of extracted fat were trans-esterified with sodium methoxide (0.5 N) and H_2SO_4 solution (10% of H_2SO_4 in methanol). Then, the fatty acid methyl esters (FAMEs) were extracted with hexane and transferred to a gas chromatography vial. Separation and quantification of FAMEs were carried out using a gas chromatograph (GC-Agilent 7890B, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and PAL RTC-120 auto sampler. One microliter of sample was injected in split mode (1:50). For the separation of FAMEs, a DB-23 fused silica capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies) was used. Chromatographic conditions were as follows: initial oven temperature of 50 °C (held for 1 min), first ramp at 25 °C/min to 175 °C, second ramp at 4 °C/min to 230 °C (held for 5 min) and third ramp at 4 °C/min to a final temperature of 240 °C (held for 2.75 min). Helium was used as a carrier gas at a constant flow-rate of 1.2 mL/min. The FID detector was maintained at 280 °C, while the operational flows were set as 40 mL/min of H_2, 450 mL/min of air and 30 mL/min of makeup flow. Individual FAMEs were identified by comparing their retention times with those of authenticated standards and the results were expressed as % of total FAME.

The total contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), PUFA n-6 and PUFA n-3 were calculated. The PUFA n-6/n-3 ratios were also calculated. The atherogenic (AI) and thrombogenic (TI) indices, as proposed by Ulbricht & Southgate (1991), were calculated according to the following equations:

$$AI = (C12{:}0+4\times C14{:}0+C16{:}0)/[MUFA+\Sigma(n\text{-}6)+\Sigma(n\text{-}3)]$$

$$TI = (C14{:}0+C16{:}0+C18{:}0)/[0.5\times MUFA+0.5\times (n\text{-}6)+3\times(n\text{-}3)+(n\text{-}3/n\text{-}6)]$$

2.5. Statistical analysis

Each physicochemical attribute and fatty acid component of meat was subjected to one-way analysis of variance (ANOVA). Differences between the least square means of the quality attributes in the three breeds were compared by the Tukey's Honest Significant Difference test (α=0.05).

3. RESULTS AND DISCUSSION

3.1 Ultimate pH and colour

The pH values measured 24 h post mortem differed significantly between the three examined breeds ($P<0.001$) as presented in Table 1. The lowest pH was observed in the muscle of the German TMBC lambs (5.56), while the two Italian breeds showed higher values of this parameter (5.62 and 5.69, respectively for the Biellese and Sambucana). Apart from the ante-mortem stress, it is suggested that the production system of the animals can affect the ultimate pH of the meat. The extensively reared animals have relatively small but sufficient glycogen reserves to ensure a gradual post mortem decline of the pH in muscle producing meat with a slightly though not significantly higher pH compared to intensively reared animals (Priolo et al., 2001). This partially agrees with the results observed in Sambucana breed reared under extensive system but also in the semi-intensively reared Biellese. On the other hand, the extensively reared TMBC lambs produced meat with considerably lower pH when compared to the other two breeds. In contrast, other authors conclude that the rearing system (semi-extensive system vs. extensive system) did not influence pH values of foal meat (Franco et al., 2011).

Ultimate pH is used to assess the shelf life and quality of meat as well as its suitability for processing. The normal pH decline is from 7.0-7.2 to 5.5-5.7 over about 24 h (Boles and Pegg, 1999). According to Mullen and Troy (2005), the meat with an ultimate pH around 5.5-5.7 possesses the most desirable fresh quality characteristics. Earlier, Devine et al. (1993) reported that young lambs with ultimate pH lower than 5.7 gave the crossbred TMBC most tender meat. The values of ultimate pH measured in this study fall within the recommended range for high quality of the meat.

Meat colour is one of most important characteristics used by the consumers as a visual quality indicator for freshness of meat. Significant difference in the lightness (L*) and redness (a*) of the meat among the three breeds was observed in this study ($P<0.0001$). Regardless of the rearing system, both breeds from Italy showed similar L* values (45.95 and 45.75, for the Biellese and Sambucana respectively); however, the TMBC lambs had significantly darker meat (L*=40.63), corresponding with the highest a* value observed in this breed (12.31 vs. 9.91 and 10.31 for the Biellese and Sambucana). The colour parameters of the meat in the examined breeds are similar to the reported by other authors in lamb meat (Luciano et al., 2012; Teixeira et al., 2005; Tejeda et al., 2008). However, in Sambucana lambs, Battaglini et al. (2004) observed considerably lower L* (36.27) and higher a* (14.47).

3.2 Proximate composition and cholesterol content

With exception of the IMF content, all the chemical components of the meat differed significantly between the two indigenous Italian breeds and the TMBC lambs, as presented in Table 2 ($P<0.0001$). The meat of Biellese and Sambucana lambs had low fat content (1.40%-1.60%), which did not differ one from another despite the different production systems.

Similar low IMF contents of m. *L. thoracis et lumborum* in these breeds were previously reported by Battaglini et al. (2004) and Brugiapaglia et al. (2019). The IMF in the muscles of TMBC lambs tended to be higher (P=0.087 at α=0.10) than both indigenous breeds. Italian breeds showed similar content of protein (19.60%-19.64%), ashes (1.06%-1.08%) and moisture (77.15%-77.30%). When compared to them, the meat from TMBC lambs presented significantly higher protein content (21.38%) corresponding to the higher ashes (1.22%), but lower moisture (75.43%). The advantage of the meat from TMBC lambs regarding the chemical composition and in particular protein content over the Italian autochthonous breeds might be due to their crossbreed origin, and especially the presence of Texel and Charollais breeds. When comparing indigenous Segurena breed with its Texel crossbreed, Blasco et al. (2019) observed higher protein content in the meat of the crossbreed lambs.

The cholesterol content of meat was significantly different among the breeds (P<0.0001) and varied between 28.53 mg/100g and 53.38 mg/100g. The lowest value was measured in the muscles of Sambucana lambs. Data from several studies suggested that breed is the most important factor affecting the cholesterol content in lamb carcass. However, Salvatori et al. (2004) demonstrated clear differences in the cholesterol levels among three muscles in extensively reared crossbred lambs, with values determined in m. LD within the range of 56.5-63.0 mg/100g. Furthermore, in indigenous breeds and crosses, Costa et al. (2009) observed 65.88-67.88 mg/100g, with no effect of the energy levels of the feed. These values are slightly higher from the ones in this study, and particularly in comparison with the cholesterol levels in the meat of Sambucana lambs. The latter was similar to the one reported by Serra et al. (2014), in Massese sucking lambs slaughtered at 11-17 kg.

3.3 Fatty acid composition

The fatty acid profile presented in Table 3 revealed substantial differences among the breeds. In comparison to the two breeds reared under extensive systems, the meat of semi-intensive Biellese lambs is characterised by higher saturation of the fatty acid profile. This was associated with increased percentage of C16:0 (25.21%) and C18:0 (18.57%), which are the major saturated fatty acids in the muscle tissue. The proportion of these two fatty acids also differed significantly between the two breeds reared in extensive system. The meat of Sambucana lambs had a lower percentage of C16:0 than TMBC (21.38% vs. 22.79%), however the opposite was observed for the content of C18:0 (17.12 % vs. 15.37%). The other saturated fatty acid present in considerable proportions in the muscle tissue (C14:0) tended to differ among the breeds ($P=0.086$), and showed lower amounts in the meat from Sambucana lambs, when compared with the other two breeds (3.99% vs. 4.49% and 4.64%, respectively for the Biellese and TMBC). Lauric acid (C12:0) also differed among breeds ($P=0.039$), with the lowest percent observed in Sambucana lamb meat, and the highest values for the Biellese lamb meat ($P<0.05$). The TMBC lamb meat remained in intermediate position with regards to the content of C12:0. Our results contradict the observations of Pompa-Roborzyński and Kędzior (2006) who showed increased proportion of saturated fatty acids, and in particular C18:0 and C14:0 in lambs reared under extensive system. However, Lorenzo et al. (2010) reported similar results to those observed in the present study. Lambs reared in extensive system had lower SFA content ($P>0.05$) in comparison with those reared in semi-extensive system, mainly due to significantly lower C16:0 values in the animals reared under extensive production system. Significant differences among breeds were also observed in regard to other fatty acids that are present in relatively low proportions, but with no consistent trends (Table 3). The percentage of C15:0 was higher in Sambucana lamb meat (0.66%) in comparison to the other two breeds (0.58%), while the content of C17:0 was lower in TMBC (1.04% vs 1.20-1.26%).

Substantial differences ($P<0.0001$) among breeds existed with regards to monounsaturated fatty acids (MUFA). The lowest proportion was observed in the meat of Sambucana lambs (34.10%) in comparison to Biellese (36.97%) and TMBC (37.89%), which could be explained with the significantly lower percentage of C18:1n-9 in this breed (26.91% vs. 34.24% and 30.86%, for the Biellese and TMBC, respectively). Meat of Sambucana animals also presented the lowest percent of C16:1n-7 compared to the other breeds. On the other hand, despite the lower proportions of C18:1n-9 and C16:1n-7, the muscles of Sambucana presented a significantly higher proportion of trans-vaccenic acid (11t-C18:1) than Biellese and TMBC ($P<0.05$), corresponding to the high content of conjugated linoleic acid (9c,11t-C18:2; CLA) observed in this breed. Trans-vaccenic acid as well as CLA are intermediate compounds in the biohydrogenation process of C18:2n-6 and its conversion to C18:0 (Song and Kennelly, 2003). CLA are found in increased amounts in the ruminants reared on pasture, which is confirmed also in this study with significantly higher proportion of CLA in both extensively reared breeds (1.48%-1.67%), in comparison to Biellese.

It should be noted, however, that the proportion of trans-vaccenic acid is only significantly higher in Sambucana and not in TMBC when compared to the semi-intensive Belliese. Furthermore, the TMBC lamb meat showed significantly lower percentage of trans-vaccenic acid than Sambucana. It could be suggested that the conversion of CLA to trans-vaccenic acid is more intensive in Sambucana lambs, and the high relative amounts of CLA and trans-vaccenic could be attributed to the milk in the diet of these lambs. The high percentage of CLA and trans-vaccenic acid in the pastured breeds is a clear advantage with respect to a healthy human diet. Numerous studies have demonstrated the beneficial effect of

CLA on cardiovascular health, cancer and obesity (Benjamin et al., 2015). Emerging evidence also revealed that *trans* fats derived from milk and ruminant body fats, such as trans-vaccenic acid, have pronounced cytotoxic effect in some types of cancer or might suppress tumour growth (Blewett et al., 2009; Lim et al., 2014).

Significant differences in the PUFA amounts were also observed among breeds ($P<0.0001$). With regards to the proportion of total PUFA, the breeds could be ranked as Sambucana>TMBC>Biellese. In addition to the CLAs, the essential C18:2n-6 and C18:3n-3, significantly contributed to the highest PUFA amount in the meat of Sambucana lambs. While the differences in the proportion of C18:2n-6 were significant only between Sambucana and Biellese, the percentage of C18:3n-3 differed substantially among all three breeds, with highest values in Sambucana (3.00%) followed by TMBC (2.50%). The lowest proportion of C18:3n-3 was observed in Biellese (1.09%). These discrepancies are not surprising and are due to the production systems. Generally, the ruminants reared under extensive conditions display higher levels of C18:3n-3, which is more abundant in grass than in grain feed (Aurousseau et al., 2007; Domínguez et al., 2015; Popova, 2014). On the other hand, the content of C18:3n-3 in the meat of Biellese lambs had also relatively high proportion, when compared to lambs reared in intensive conditions. This is also attributed to the pastures, despite the additional concentrate in the diet (Popova, 2014, 2007). Similarly, in a previous study that compares the effect of livestock production system in lamb meat quality, the authors observed higher C18:3n-3 content in animals reared in extensive than in semi-extensive system (Lorenzo et al., 2010). Results of other studies, however, do not confirm differences in the content of the essential fatty acids due to the rearing systems (Kaczor et al., 2010) or observe decreased amount of C18:3n-3 in semi-intensive systems vs. intensive systems (Borys et al., 2012).

The elevated amounts of C18:3n-3 quantified in meat from the extensively reared breeds beneficially affected the proportion of the long chain n-3 PUFA, in particular C20:5n-3 and C22:5n-3 ($P<0.0001$). For these fatty acids, meat from Sambucana lambs showed the highest contents, while the lowest values were observed in the meat of Biellese lambs. The percentage of C22:6n-3 remained the lowest in Biellese (0.34%) and the difference was significant when compared to Sambucana (0.49%). The level of C22:6n-3 in TMBC was 0.44%. The higher long chain n-3 PUFA content in meat from the extensively reared breeds in comparison to the Biellese ($P<0.0001$) shows that a significant amount of C18:3n-3 escapes biohydrogenation and is further desaturated and elongated to long chain derivatives. Significant differences among breeds was observed as well with regards to n-6 PUFA ($P=0.0041$), being more pronounced between Sambucana (11.85%) and Biellese (8.50%). The differences were due to the elevated content of C18:2n-6 and C20:4n-6 in the meat of Sambucana lambs. Despite the high percentage of total n-6 PUFA, the ratio n-6/n-3 was favourably decreased in both Sambucana (1.68) and TMBC (1.74) when compared to Biellese (3.39) ($P<0.05$). In all three breeds n-6/n-3 ratio is below the recommended value of 4.0, which is considered beneficial for human health (Simopoulos, 2009). The discrepancies in the fatty acid profile were also reflected in the P/S ratio, which was above the recommended minimum of 0.4 in the meat of Sambucana lambs, and in the lower AI and TI in the two breeds reared extensively. Atherogenic and thrombogenic indices take into account the different effects that single fatty acids might have on human health and, in particular, on the probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation (Pilarczyk and Wójcik, 2015). In agreement with our results, beneficially decreased values of AI and TI were observed by Liotta et al. (2020) in m. *Logissimus dorsi* of lambs reared under extensive systems compared to semi-extensive. Other studies with lambs (Fiori et al., 2013; Margetin et al., 2014; Margetín et al., 2018) also observed lower atherogenic or thrombogenic potential in meat of lambs reared on pasture.

4. CONCLUSION

The comparative analysis of the meat from the Biellese, Sambucana and TMBC lambs reared under semi-intensive and extensive production systems showed that, regardless of the rearing conditions, the three breeds render high quality meat with low fat content. Moreover, the fatty acids composition, and the atherogenic and thrombogenic indices of the meat showed significant differences and a clear advantage of the meat from the animals reared under extensive production system. Such variability indicates that rearing strategy can be used successfully to influence the lipid profile in a way that it can positively affect human health.

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FATTY ACID COMPOSITION OF LAMB MEAT FROM ITALIAN AND GERMAN LOCAL BREEDS

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HIGHLIGHTS

- Lamb meat quality of three Italian and German breeds was evaluated
- Meat of lambs reared in extensive system had the lowest SFA content
- Meat of lambs reared in extensive system had the highest n-3 PUFA content
- Meat from the Italian Sambucana lambs presented the lowest cholesterol content
- Meat from the three breeds was of high quality with low fat content

ABSTRACT

The aim of this study was to evaluate and compare the quality characteristics, chemical composition and lipid profile of lamb meat from Italian (Biellese and Sambucana) and German (Texel-Merino-Blackhead-Charollais [TMBC]) breeds reared in extensive and semi-extensive production systems. Meat samples from 89 animals were analysed. The meat of the lambs from semi-intensively reared Biellese, and extensively reared Sambucana and TMBC breeds produced lean meat, with slightly higher intramuscular fat content in TMBC. The latter also produced meat of darker colour ($P<0.05$) and higher protein content ($P<0.05$). The meat of Sambucana lambs presented the lowest total cholesterol content ($P<0.05$). The fatty acid profile of the meat showed a clear advantage of both extensively reared breeds, which had substantially lower proportion of saturated (SFA) but higher of polyunsaturated fatty acids (PUFA), particularly n-3 ($P<0.05$). The beneficial effect of the extensive rearing conditions was associated with lower n-6/n3 ratio, and atherogenic (AI) and thrombogenic (TI) indices, thereby suggesting that production system can be used successfully to modify the fatty acid profile to achieve a positive effect for the human health.

Keywords: Sheep; meat; quality characteristics; fatty acid profile

1. INTRODUCTION

A renewed interest in local sheep breeds has been prompted by the EU's recent promotion of a sustainable development of otherwise marginal areas and policies supporting extensive systems of animal production (Marino et al., 2008). Local sheep production is more likely to be found in low farming areas, where sheep graze semi-natural vegetation and are dominant with other low input extensive grazing livestock (Cruz et al., 2019). Even though sheep farming represents only a small contribution to Europe's gross domestic product (<0.5%; (Eurostat, 2019)), the sector is of great importance to rural development and the environment. The indigenous sheep breeds, in addition to contributing to the diversity of production systems, are important genetic resources that must be preserved because of their local adaptation, disease resistance, high fertility and unique product qualities (Mendelsohn, 2003). Some of these breeds have small body and good adaptation to adverse climatic and orographic environments, which makes them particularly suited to the use and enhancement of natural pastures (Cruz et al., 2019).

One strategy for attaining sustainability of the local sheep farming sector is through high quality standard of the products. Enhancing the quality of meat from autochthonous breeds, making it more attractive to consumers, could contribute to the preservation of the rural world and its diversity, the conservation of endangered breeds, as well as improving the profitability and living standards of the sheep farmers that remain in these rural areas. Nonetheless, meat quality is a very ample concept defined by industry and the final consumer, which is regulated by a series of factors that are intrinsic and extrinsic to the animal (Webb et al., 2005). Fatty acid composition alone has a strong impact on the nutritive value and the organoleptic characteristics of meat (Díaz et al., 2005). The flavour profile, an important attribute of meat, is regulated by variations in the absolute concentration and the relative proportions of different fatty acids (Fisher et al., 2000). Several studies have confirmed that meat fatty acid composition can be influenced by production system, animal breed and sex, slaughter age and live weight, and level of fatness (Atti and Mahouachi, 2009; Boughalmi and Araba, 2016; Domínguez et al., 2015, 2018b; Ekiz et al., 2013).

Therefore, the objective of this study was to evaluate and compare the physicochemical attributes (ultimate pH, colour, proximate composition and cholesterol content) and fatty acid profile of lamb meat originating from three Italian and German breeds typically raised in extensive or semi-extensive production systems. Three sheep breeds exploited for meat production, in their typical production systems, were utilised in the present study: Sambucana lambs raised in extensive system and Biellese lambs raised in semi-extensive system from the Alpine and the Continental bioregions of Italy, respectively, and crossbred Texel-Merino-Blackhead-Charollais (TMBC) lambs reared in extensive system from the Continental bioregion of Germany.

2. METHODOLOGY

2.1 Lamb rearing and feeding

All animals' management and procedures were carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Italy

In the Continental bioregion, located in Turin, Biellese lambs were raised in CISRA, Teaching Animal Farm of the Veterinary Science Department, University of Turin. In the Alpine bioregion, located in Val Maira, Western Alps, at an altitude of 1800-2000 m, Sambucana lambs were bred during the summer season. The lambs from both breeds were raised in collective pens with their mothers. The production system used for Biellese breed was semi-extensive (i.e., the lambs consumed about 500 g of milk per day the first 15 days after birth and then 1000 g per days until weaning (2 months of age)). After weaning, the lambs were fed with ~200 g of concentrate per day and 700 g of hay until the slaughtering (4.5-5 months of age). The production system for Sambucana lambs was based on grazing on natural pasture, with no additional concentrate or hay. The lambs were fed 400 g of milk per day the first 15 days after birth and until 2 month of age the animals received 800 g of milk per day. In the semi-extensive system for Biellese lambs, the flocks would be released to graze outside in autumn-winter season (period of investigation); whereas in the extensive system, Sambucana lambs would leave at dawn and graze until evening; then they would be recovered in a fence in summer season (period of investigation). The lambs were not weaned during the grazing season in the Alpine bioregion. For this investigation, 16 Biellese and 20 Sambucana lambs were reared in 2018, and 12 Biellese and 12 Sambucana lambs were reared in 2019.

Germany

The lambs, all crossbred Texel-Merino-Blackhead-Charollais (TMBC), were raised in a farm at an abandoned military training area on the Swabian Alb near Münsingen; and held in one collective flock. The animals stayed in a pen overnight and during the day they roam freely. The study area belongs to bio-region Kuppenalb, located in the eco-region called the "Western European Broadleaf Forests". The study pasture of 170 ha is managed extensively, and grazed by a mid-sized flock of around 500 ewes. The insemination is carried out naturally in spring. During winter, traditional transhumance is practiced to the lower Swabian areas of Nördlingen. The animals graze all year long, and the only additionally provided fodder is mineral feed. On this farm 29 lambs were reared.

2.2 Preparation of lamb meat samples

All lambs were four-to-five months old when slaughtered. Lambs were slaughtered in batches ranging from 5 to 12 animals in local abattoirs of Italy and Germany, and the whole experiment was conducted in the fall and spring seasons of 2018 and 2019. A total of 89 lambs were employed in this study, from the following breeds and production systems: Biellese semi-extensive (28), Sambucana extensive (32), TMBC extensive (29).

Lambs were slaughtered after an overnight lairage period, where they had free access to water but not to feed. In the local abattoirs, lambs were electrically stunned, dressed, washed and chilled at 4ºC. Twenty-four hours after slaughter, carcasses were split and the *Longissimus thoracis et lumborum* muscles removed from the 6^{th} to the 13^{th} vertebra under aseptic conditions. The right side was divided into two parts for subsequent laboratorial analysis. They were vacuum packed in transparent gas-tight polyamide and polyethylene vacuum bags (Orved®, Spain, with permeability of 84 ± 4.20 $cc/m^2/24h/atm$ for O_2, 361 ± 18.05 $cc/m^2/24h/atm$ for CO_2, 22 ± 1.10 $cc/ m^2/24h/atm$ for N_2 and 9.0 ± 0.45 $cc/m^2/24h/atm$ for H_2O and density of ± 100µm), and stored at 4±0.5 °C. Within 24 hours, the meat samples were subjected to the following determinations: pH and instrumental colour measurement, proximate composition, cholesterol content and fatty acid profile. All the essays were carried out in the same laboratory.

2.3. Physicochemical analyses of meat

2.3.1. pH and instrumental colour measurements

The pH was measured in triplicate using a digital portable pH-meter (Hanna Instruments, Eibar, Spain) equipped with a penetration glass probe. A portable colorimeter (Konica Minolta CR-600d, Osaka, Japan) was used to measure in triplicate the meat colour in the CIELAB space (lightness L*, redness a* and yellowness b*). The device was set to pulsed xenon arc lamp, 10° viewing angle geometry, standard illuminant D65 and aperture size of 8 mm. Samples were allowed to bloom for 30 min before measuring.

2.3.2. Proximate composition

Moisture, intramuscular fat, protein and ashes contents were determined according to ISO (1997), AOCS (2005), ISO (1978) and ISO (1998), respectively. Determinations were made in triplicate per meat sample.

2.3.3. Cholesterol analysis

Total cholesterol was analysed (saponification, separation and quantification) following the procedure described by Domínguez et al. (2018a). Two grams of homogenised meat sample were placed in a screw Teflon-lined cap tube and 0.25 g of L-ascorbic acid and 5 mL of saponification solution [11% potassium hydroxide in ethanol solution (55%)] were added. The saponification step was carried out in a shaking water bath at 85 ºC for 45 min. After cooling, cholesterol was extracted with 1.5 mL of hexane. Cholesterol was separated and quantified using normal phase-HPLC technique, according the chromatographic conditions reported by Domínguez et al. (2018a). The HPLC systems used was an Alliance 2695 model (Waters, Milford, USA) equipped with a 996 Photodiode Array Detector (Waters Milford, USA). The cholesterol analysis was performed using a normal-phase silica column (SunFireTM Prep Silica, 4.6 mm ID × 250 mm, 5 µm particle size, Waters, Milford, MA, USA). The mobile phase (2% v/v 2-propanol in n-hexane) flow rate was 1 ml/min, the run last for 15 min and the temperature of the column oven was adjusted at 30 ºC. The detection of cholesterol was carried out using Photodiode Array detector at 208 nm. Results were expressed as mg of cholesterol/100 g of meat.

2.4. Fatty acid composition analysis

Intramuscular lipids were extracted following the method described by Bligh & Dyer (1959) with the modifications proposed by Barros et al. (2020). The procedure described by Barros et al. (2020) was used for fatty acids determination. Briefly, 20 mg of extracted fat were trans-esterified with sodium methoxide (0.5 N) and H_2SO_4 solution (10% of H_2SO_4 in methanol). Then, the fatty acid methyl esters (FAMEs) were extracted with hexane and transferred to a gas chromatography vial. Separation and quantification of FAMEs were carried out using a gas chromatograph (GC-Agilent 7890B, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and PAL RTC-120 auto sampler. One microliter of sample was injected in split mode (1:50). For the separation of FAMEs, a DB-23 fused silica capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies) was used. Chromatographic conditions were as follows: initial oven temperature of 50 °C (held for 1 min), first ramp at 25 °C/min to 175 °C, second ramp at 4 °C/min to 230 °C (held for 5 min) and third ramp at 4 °C/min to a final temperature of 240 °C (held for 2.75 min). Helium was used as a carrier gas at a constant flow-rate of 1.2 mL/min. The FID detector was maintained at 280 °C, while the operational flows were set as 40 mL/min of H_2, 450 mL/min of air and 30 mL/min of makeup flow. Individual FAMEs were identified by comparing their retention times with those of authenticated standards and the results were expressed as % of total FAME.

The total contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), PUFA n-6 and PUFA n-3 were calculated. The PUFA n-6/n-3 ratios were also calculated. The atherogenic (AI) and thrombogenic (TI) indices, as proposed by Ulbricht & Southgate (1991), were calculated according to the following equations:

$$AI = (C12{:}0+4\times C14{:}0+C16{:}0)/[MUFA+\Sigma(n\text{-}6)+\Sigma(n\text{-}3)]$$

$$TI = (C14{:}0+C16{:}0+C18{:}0)/[0.5\times MUFA+0.5\times (n\text{-}6)+3\times(n\text{-}3)+(n\text{-}3/n\text{-}6)]$$

2.5. Statistical analysis

Each physicochemical attribute and fatty acid component of meat was subjected to one-way analysis of variance (ANOVA). Differences between the least square means of the quality attributes in the three breeds were compared by the Tukey's Honest Significant Difference test (α=0.05).

3. RESULTS AND DISCUSSION

3.1 Ultimate pH and colour

The pH values measured 24 h post mortem differed significantly between the three examined breeds ($P<0.001$) as presented in Table 1. The lowest pH was observed in the muscle of the German TMBC lambs (5.56), while the two Italian breeds showed higher values of this parameter (5.62 and 5.69, respectively for the Biellese and Sambucana). Apart from the ante-mortem stress, it is suggested that the production system of the animals can affect the ultimate pH of the meat. The extensively reared animals have relatively small but sufficient glycogen reserves to ensure a gradual post mortem decline of the pH in muscle producing meat with a slightly though not significantly higher pH compared to intensively reared animals (Priolo et al., 2001). This partially agrees with the results observed in Sambucana breed reared under extensive system but also in the semi-intensively reared Biellese. On the other hand, the extensively reared TMBC lambs produced meat with considerably lower pH when compared to the other two breeds. In contrast, other authors conclude that the rearing system (semi-extensive system vs. extensive system) did not influence pH values of foal meat (Franco et al., 2011).

Ultimate pH is used to assess the shelf life and quality of meat as well as its suitability for processing. The normal pH decline is from 7.0-7.2 to 5.5-5.7 over about 24 h (Boles and Pegg, 1999). According to Mullen and Troy (2005), the meat with an ultimate pH around 5.5-5.7 possesses the most desirable fresh quality characteristics. Earlier, Devine et al. (1993) reported that young lambs with ultimate pH lower than 5.7 gave the crossbred TMBC most tender meat. The values of ultimate pH measured in this study fall within the recommended range for high quality of the meat.

Meat colour is one of most important characteristics used by the consumers as a visual quality indicator for freshness of meat. Significant difference in the lightness (L*) and redness (a*) of the meat among the three breeds was observed in this study (P<0.0001). Regardless of the rearing system, both breeds from Italy showed similar L* values (45.95 and 45.75, for the Biellese and Sambucana respectively); however, the TMBC lambs had significantly darker meat (L*=40.63), corresponding with the highest a* value observed in this breed (12.31 vs. 9.91 and 10.31 for the Biellese and Sambucana). The colour parameters of the meat in the examined breeds are similar to the reported by other authors in lamb meat (Luciano et al., 2012; Teixeira et al., 2005; Tejeda et al., 2008). However, in Sambucana lambs, Battaglini et al. (2004) observed considerably lower L* (36.27) and higher a* (14.47).

3.2 Proximate composition and cholesterol content

With exception of the IMF content, all the chemical components of the meat differed significantly between the two indigenous Italian breeds and the TMBC lambs, as presented in Table 2 (P<0.0001). The meat of Biellese and Sambucana lambs had low fat content (1.40%-1.60%), which did not differ one from another despite the different production systems. Similar low IMF contents of m. *L. thoracis et lumborum* in these breeds were previously reported by Battaglini et al. (2004) and Brugiapaglia et al. (2019). The IMF in the muscles of

TMBC lambs tended to be higher (P=0.087 at α=0.10) than both indigenous breeds. Italian breeds showed similar content of protein (19.60%-19.64%), ashes (1.06%-1.08%) and moisture (77.15%-77.30%). When compared to them, the meat from TMBC lambs presented significantly higher protein content (21.38%) corresponding to the higher ashes (1.22%), but lower moisture (75.43%). The advantage of the meat from TMBC lambs regarding the chemical composition and in particular protein content over the Italian autochthonous breeds might be due to their crossbreed origin, and especially the presence of Texel and Charollais breeds. When comparing indigenous Segurena breed with its Texel crossbreed, Blasco et al. (2019) observed higher protein content in the meat of the crossbreed lambs.

The cholesterol content of meat was significantly different among the breeds (P<0.0001) and varied between 28.53 mg/100g and 53.38 mg/100g. The lowest value was measured in the muscles of Sambucana lambs. Data from several studies suggested that breed is the most important factor affecting the cholesterol content in lamb carcass. However, Salvatori et al. (2004) demonstrated clear differences in the cholesterol levels among three muscles in extensively reared crossbred lambs, with values determined in m. LD within the range of 56.5-63.0 mg/100g. Furthermore, in indigenous breeds and crosses, Costa et al. (2009) observed 65.88-67.88 mg/100g, with no effect of the energy levels of the feed. These values are slightly higher from the ones in this study, and particularly in comparison with the cholesterol levels in the meat of Sambucana lambs. The latter was similar to the one reported by Serra et al. (2014), in Massese sucking lambs slaughtered at 11-17 kg.

3.3 Fatty acid composition

The fatty acid profile presented in Table 3 revealed substantial differences among the breeds. In comparison to the two breeds reared under extensive systems, the meat of semi-intensive Biellese lambs is characterised by higher saturation of the fatty acid profile. This was associated with increased percentage of C16:0 (25.21%) and C18:0 (18.57%), which are the major saturated fatty acids in the muscle tissue. The proportion of these two fatty acids also differed significantly between the two breeds reared in extensive system. The meat of Sambucana lambs had a lower percentage of C16:0 than TMBC (21.38% vs. 22.79%), however the opposite was observed for the content of C18:0 (17.12 % vs. 15.37%). The other saturated fatty acid present in considerable proportions in the muscle tissue (C14:0) tended to differ among the breeds (P=0.086), and showed lower amounts in the meat from Sambucana lambs, when compared with the other two breeds (3.99% vs. 4.49% and 4.64%, respectively for the Biellese and TMBC). Lauric acid (C12:0) also differed among breeds (P=0.039), with the lowest percent observed in Sambucana lamb meat, and the highest values for the Biellese lamb meat (P<0.05). The TMBC lamb meat remained in intermediate position with regards to the content of C12:0. Our results contradict the observations of Pompa-Roborzyński and Kędzior (2006) who showed increased proportion of saturated fatty acids, and in particular C18:0 and C14:0 in lambs reared under extensive system. However, Lorenzo et al. (2010) reported similar results to those observed in the present study. Lambs reared in extensive system had lower SFA content (P>0.05) in comparison with those reared in semi-extensive system, mainly due to significantly lower C16:0 values in the animals reared under extensive production system. Significant differences among breeds were also observed in regard to other fatty acids that are present in relatively low proportions, but with no consistent trends (Table 3). The percentage of C15:0 was higher in Sambucana lamb meat (0.66%) in comparison to the other two breeds (0.58%), while the content of C17:0 was lower in TMBC (1.04% vs 1.20-1.26%).

Substantial differences (P<0.0001) among breeds existed with regards to monounsaturated fatty acids (MUFA). The lowest proportion was observed in the meat of Sambucana lambs (34.10%) in comparison to Biellese (36.97%) and TMBC (37.89%), which could be explained with the significantly lower percentage of C18:1n-9 in this breed (26.91% vs. 34.24% and 30.86%, for the Biellese and TMBC, respectively). Meat of Sambucana animals also presented the lowest percent of C16:1n-7 compared to the other breeds. On the other hand, despite the lower proportions of C18:1n-9 and C16:1n-7, the muscles of Sambucana presented a significantly higher proportion of trans-vaccenic acid (11t-C18:1) than Biellese and TMBC ($P<0.05$), corresponding to the high content of conjugated linoleic acid (9c,11t-C18:2; CLA) observed in this breed. Trans-vaccenic acid as well as CLA are intermediate compounds in the biohydrogenation process of C18:2n-6 and its conversion to C18:0 (Song and Kennelly, 2003). CLA are found in increased amounts in the ruminants reared on pasture, which is confirmed also in this study with significantly higher proportion of CLA in both extensively reared breeds (1.48%-1.67%), in comparison to Biellese.

It should be noted, however, that the proportion of trans-vaccenic acid is only significantly higher in Sambucana and not in TMBC when compared to the semi-intensive Belliese. Furthermore, the TMBC lamb meat showed significantly lower percentage of trans-vaccenic acid than Sambucana. It could be suggested that the conversion of CLA to trans-vaccenic acid is more intensive in Sambucana lambs, and the high relative amounts of CLA and trans-vaccenic could be attributed to the milk in the diet of these lambs. The high percentage of CLA and trans-vaccenic acid in the pastured breeds is a clear advantage with respect to a healthy human diet. Numerous studies have demonstrated the beneficial effect of CLA on cardiovascular health, cancer and obesity (Benjamin et al., 2015). Emerging evidence also revealed that *trans* fats derived from milk and ruminant body fats, such as trans-vaccenic acid, have pronounced cytotoxic effect in some types of cancer or might suppress tumour growth (Blewett et al., 2009; Lim et al., 2014).

Significant differences in the PUFA amounts were also observed among breeds (P<0.0001). With regards to the proportion of total PUFA, the breeds could be ranked as Sambucana>TMBC>Biellese. In addition to the CLAs, the essential C18:2n-6 and C18:3n-3, significantly contributed to the highest PUFA amount in the meat of Sambucana lambs. While the differences in the proportion of C18:2n-6 were significant only between Sambucana and Biellese, the percentage of C18:3n-3 differed substantially among all three breeds, with highest values in Sambucana (3.00%) followed by TMBC (2.50%). The lowest proportion of C18:3n-3 was observed in Biellese (1.09%). These discrepancies are not surprising and are due to the production systems. Generally, the ruminants reared under extensive conditions display higher levels of C18:3n-3, which is more abundant in grass than in grain feed (Aurousseau et al., 2007; Domínguez et al., 2015; Popova, 2014). On the other hand, the content of C18:3n-3 in the meat of Biellese lambs had also relatively high proportion, when compared to lambs reared in intensive conditions. This is also attributed to the pastures, despite the additional concentrate in the diet (Popova, 2014, 2007). Similarly, in a previous study that compares the effect of livestock production system in lamb meat quality, the authors observed higher C18:3n-3 content in animals reared in extensive than in semi-extensive system (Lorenzo et al., 2010). Results of other studies, however, do not confirm differences in the content of the essential fatty acids due to the rearing systems (Kaczor et al., 2010) or observe decreased amount of C18:3n-3 in semi-intensive systems vs. intensive systems (Borys et al., 2012).

The elevated amounts of C18:3n-3 quantified in meat from the extensively reared breeds beneficially affected the proportion of the long chain n-3 PUFA, in particular C20:5n-3 and C22:5n-3 (P<0.0001). For these fatty acids, meat from Sambucana lambs showed the highest contents, while the lowest values were observed in the meat of Biellese lambs. The percentage of C22:6n-3 remained the lowest in Biellese (0.34%) and the difference was significant when compared to Sambucana (0.49%). The level of C22:6n-3 in TMBC was 0.44%. The higher long chain n-3 PUFA content in meat from the extensively reared breeds in comparison to the Biellese (P<0.0001) shows that a significant amount of C18:3n-3 escapes biohydrogenation and is further desaturated and elongated to long chain derivatives. Significant differences among breeds was observed as well with regards to n-6 PUFA (P=0.0041), being more pronounced between Sambucana (11.85%) and Biellese (8.50%). The differences were due to the elevated content of C18:2n-6 and C20:4n-6 in the meat of Sambucana lambs. Despite the high percentage of total n-6 PUFA, the ratio n-6/n-3 was favourably decreased in both Sambucana (1.68) and TMBC (1.74) when compared to Biellese (3.39) (P<0.05). In all three breeds n-6/n-3 ratio is below the recommended value of 4.0, which is considered beneficial for human health (Simopoulos, 2009). The discrepancies in the fatty acid profile were also reflected in the P/S ratio, which was above the recommended minimum of 0.4 in the meat of Sambucana lambs, and in the lower AI and TI in the two breeds reared extensively. Atherogenic and thrombogenic indices take into account the different effects that single fatty acids might have on human health and, in particular, on the probability of increasing the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation (Pilarczyk and Wójcik, 2015). In agreement with our results, beneficially decreased values of AI and TI were observed by Liotta et al. (2020) in m. *Logissimus dorsi* of lambs reared under extensive systems compared to semi-extensive. Other studies with lambs (Fiori et al., 2013; Margetin et al., 2014; Margetín et al., 2018) also observed lower atherogenic or thrombogenic potential in meat of lambs reared on pasture.

4. CONCLUSION

The comparative analysis of the meat from the Biellese, Sambucana and TMBC lambs reared under semi-intensive and extensive production systems showed that, regardless of the rearing conditions, the three breeds render high quality meat with low fat content. Moreover, the fatty acids composition, and the atherogenic and thrombogenic indices of the meat showed significant differences and a clear advantage of the meat from the animals reared under extensive production system. Such variability indicates that rearing strategy can be used successfully to influence the lipid profile in a way that it can positively affect human health.

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Table 1. Ultimate pH and instrumental colour measurements of m. *Longissimus thoracis et lumborum* in lambs from Biellese, Sambucana and Texel-Merino-Blackhead-Charollais (TMBC) breeds

Attribute[1]	Biellese	Sambucana	TMBC	SEM[2]	P-value
pH_{24}	5.62[a]	5.69[b]	5.56[c]	0.06	<0.0001
L*	45.95[a]	45.47[a]	40.63[b]	2.83	<0.0001
a*	9.91[a]	10.32[a]	12.31[b]	1.72	<0.0001
b*	12.56	12.57	12.49	1.38	0.972

[1]Values with different superscript letters differ significantly, P<0.05
[2]Standard error of the means

Table 2. Proximate composition (%) and cholesterol content (mg/100 g meat) of m. *Longissimus thoracis et lumborum* in lambs from Biellese, Sambucana and Texel-Merino-Blackhead-Charollais (TMBC) breeds

Attribute[1]	Biellese	Sambucana	TMBC	SEM[2]	P-value
IMF	1.60	1.40	1.84	0.76	0.087
Protein	19.64[a]	19.60[a]	21.38[b]	0.57	<0.0001
Moisture	77.30[a]	77.15[a]	75.43[b]	0.95	<0.0001
Ash	1.06[a]	1.08[a]	1.22[b]	0.06	<0.0001
Cholesterol	51.73[a]	28.53[b]	53.38[a]	22.54	<0.0001

[1]Values with different superscript letters differ significantly, P<0.05

[2]Standard error of the means

Table 3. Fatty acid composition (% FAME) of m. *Longissimus thoracis et lumborum* in lambs from Biellese, Sambucana and Texel-Merino-Blackhead-Charollais (TMBC) breeds. Only the fatty acids that represented more than 0.1% are presented.

Fatty acid[1]	Biellese	Sambucana	TMBC	SEM[2]	P-value
C10:0	0.23[a]	0.16[b]	0.20[ab]	0.07	0.0046
C12:0	0.55[a]	0.41[b]	0.46[ab]	0.19	0.0390
C14:0	4.49	3.99	4.64	1.20	0.086
C14:1n-5	0.14	0.12	0.14	0.07	0.2812
C15:0	0.58[a]	0.66[b]	0.58[a]	0.12	0.0070
C15:1n-5	<0.001[a]	0.63[b]	0.87[b]	0.78	0.0002
C16:0	25.21[a]	21.38[b]	22.79[c]	2.16	0.0013
C16:1n-7	1.43[a]	1.18[b]	1.26[a]	0.25	<0.0001
C17:0	1.26[a]	1.20[a]	1.04[b]	0.13	<0.0001
C17:1n-7	0.37[a]	0.19[b]	0.49[a]	0.23	<0.0001
C18:0	18.57[a]	17.12[b]	15.37[c]	2.19	<0.0001
9t-C18:1	0.45[a]	0.55[a]	0.76[b]	0.23	<0.0001
11t-C18:1	2.31[a]	3.41[b]	2.48[a]	0.85	<0.0001
C18:1n-9	31.24[a]	26.91[b]	30.86[a]	2.68	<0.0001
C18:1n-7	0.86	0.91	0.89	0.16	0.5403
9t, 11t-C18:2	0.23[a]	0.51[b]	0.24[a]	0.11	<0.0001
C18:2n-6	5.88[a]	7.61[b]	6.53[ab]	1.99	0.0043
C20:0	0.15[a]	0.20[b]	0.12[c]	0.03	<0.0001
C20:1n-9	0.10[a]	0.15[b]	0.09[b]	0.04	<0.0001
C18:3n-3	1.09[a]	3.00[b]	2.50[c]	0.59	<0.0001
9c, 11t-C18:2 (CLA)	0.69[a]	1.48[b]	1.67[c]	0.28	<0.0001
C20:2n-6	0.05[a]	0.15[b]	0.15[b]	0.11	0.0007
C22:0	0.25[ab]	0.32[a]	0.18[b]	0.17	0.0075
C20:3n-6	0.17[a]	0.29[b]	0.23[ab]	0.10	0.0001
C20:3n-3	0.02[a]	0.06[b]	0.20[c]	0.04	<0.0001
C20:4n-6	2.01[a]	3.09[b]	2.25[a]	1.28	0.0037
C24:0	0.01[a]	0.05[b]	0.01[a]	0.03	<0.0001
C20:5n-3	0.41[a]	1.73[b]	1.12[c]	0.64	<0.0001
C22:5n-6	0.10	0.14	0.11	0.11	0.7269
C22:5n-3	0.65[a]	1.78[b]	1.22[c]	0.58	<0.0001
C22:6n-3	0.34[a]	0.49[b]	0.44[ab]	0.18	0.0058

[1]Values with different superscript letters differ significantly, P<0.05
[2]Standard error of the means

Table 4. Total proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), PUFA n-3, PUFA n-6, PUFA n-6/n-3 ratios, and atherogenic (AI) and thrombogenic (TI) indices in lamb meat from Biellese, Sambucana and Texel-Merino-Blackhead-Charollais (TMBC) breeds

Attribute[1]	Biellese	Sambucana	TMBC	SEM[2]	P-value
SFA (%)	51.33^{a}	45.54^{b}	45.43^{b}	3.30	<0.0001
MUFA (%)	36.97^{a}	34.07^{b}	37.85^{a}	3.07	<0.0001
PUFA (%)	11.70^{a}	20.39^{b}	16.72^{c}	4.81	<0.0001
n-3 (%)	2.51^{a}	7.06^{b}	5.48^{c}	1.88	<0.0001
n-6 (%)	8.50^{a}	11.85^{b}	9.57^{ab}	2.19	0.0041
n-6/n-3	3.39^{a}	1.68^{b}	1.74^{b}	1.51	<0.0001
P/S	0.23^{a}	0.45^{b}	0.37^{c}	0.14	<0.0001
AI	0.91^{a}	0.71^{b}	0.79^{b}	0.16	<0.0001
TI	1.58^{a}	0.95^{b}	1.05^{b}	0.20	<0.0001

[1]Values with different superscript letters differ significantly, $P<0.05$
[2]Standard error of the means

CRediT author statement

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Ursula Gonzales Barron, On behalf of all authors