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Influence of feeding system on *Longissimus thoracis et lumborum* volatile compounds of an Iberian local lamb breed

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ABSTRACT

The chemical composition and volatile profile of the *Longissimus thoracis et lumborum* muscle of lambs reared in two distinct production systems (intensive and extensive) was evaluated. For this, sixty-six lambs for meat production of the autochthonous Gallega Iberian breed were raised with concentrate and grass in intensive (30 animals) and extensive (36 animals) system, respectively, until 4–4.5 months of age when they were slaughtered. Subsequently, *Longissimus thoracis et lumborum* muscles were excised for the determination of chemical composition (moisture, intramuscular fat, protein, and ash percentages) and volatile substances. The aftermaths obtained evidenced that moisture (75.90% and 75.68%), intramuscular fat (1.68% and 1.76%), and protein (20.62% and 20.97%) contents were not significantly ($P > 0.05$) affected by the production system. However, the extensively-fed lambs displayed a higher ash content (1.35% vs. 1.24%). Additionally, the total volatile content was also not significantly ($P > 0.05$) influenced by feeding system. Despite this, the total content of hydrocarbons, acids, aldehydes, ketones, esters, ethers, furans, sulfur compounds, and others was significantly ($P < 0.05$) affected by diet, being the alcohol family the only group not influenced ($P > 0.05$) by the production system (1321.3 vs. 1211.3 AU $\times 10^4$/g fresh muscle). Specifically, intensively-fed lambs showed significantly higher amounts for all volatile families apart from ketones (2215 vs. 2826 AU $\times 10^4$/g fresh muscle) and sulfur compounds (22.7 vs. 123.7 AU $\times 10^4$/g fresh muscle). In addition, benzyl alcohol and carbon disulfide were proposed as appropriate biomarkers for grass diets, while 1-butanone, 2-heptanone, and furan, 2-penthyl were indicated as suitable tracers for concentrate-based diets.

1. Introduction

At present, there is a growing interest in the "green image" of products of animal origin. In fact, there are progressively more consumers who value the use of techniques that respect the environment and that promote animal welfare, and demand clearer information about the animal production system (Mouzo et al., 2020). In response to this demand, the meat industry in developed countries has changed its focus from quantity to quality (Sosnicki and Newman, 2010). Concretely in lamb production, the use of ancient autochthonous breeds such as Gallega and the employment of extensive techniques with the use of natural pastures, replacing intensive farming with commercial feeds, are highly appreciated by consumers (De-Arriba and Sánchez-Andrés, 2014). For this reason, the possibility of being able to trace the feed supplied to lambs acquires a special interest to protect the consumer against fraud (Vasta et al., 2012).

On this matter, the use of volatile compounds as feeding biomarkers to distinguish between commercial and grass-fed animals are of particular relevance because some of these substances have previously been related to specific diets (Almela et al., 2010; Echegaray et al., 2020; Gravador et al., 2015; Lorenzo et al., 2013, 2014; Sivadier et al., 2010). This occurrence is due to the fact that several volatile compounds can be transferred to animal tissues directly from the diet (Vasta et al., 2010) or they can appear as a consequence of their generation from certain characteristic substances of the feed. In this way, concentrate-based diets have been linked with higher amounts of compounds such as 2, 3-butanedione, lactones, and branched chain fatty acids. On contrary, phenols, terpenes, and substances as 2,3-octanedione (Vasta et al., 2012)

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have been previously proposed as biomarkers of grass-based diets.

On the other hand, volatile compounds are also important in lamb meat because these substances are responsible for its flavor and aroma (Zhang et al., 2020). In this way, since the feed supplied to the lambs can affect the concentration and variety of volatile compounds detected, the acceptability of lamb meat by the consumer could also be influenced by the feeding system (Almela et al., 2010; Watkins et al., 2013). Thus, the aim of current study was to determine the effect of the feeding system (intensive and extensive regimens) on the chemical composition and the volatile compounds in *Longissimus thoracis et lumborum* muscle of Gallega lambs and identify the presence of diet biomarkers and the possible differences in the volatile profile.

2. Material and methods

2.1. Lamb rearing and feeding

The autochthonous breed of Gallega sheep was employed for meat production in the present work. A total of 66 lambs of this native breed were raised under two different production systems (namely intensive and extensive) in two distinct regions of the Iberian Peninsula. Thus, in the fall of 2018 and the spring of 2019, a total of thirty lambs (19 males and 11 females) were reared under intensive conditions at the School of Agriculture holding of the Polytechnic Institute of Bragança, in the Mediterranean region of Bragança (Portugal). For this, the conditions of a commercial farm were simulated. Specifically, the lambs were weaned at 4–6 weeks old and they housed in a feedlot with straw bedding and free access to commercial concentrate, cereal straw, and fresh water. On the other hand, during the winter of 2018, 36 lambs (11 males and 25 females) were raised under an extensive production system in the experimental farm of SERIDA, located in the Atlantic region of Asturias (Spain). Concretely, these lambs were grown suckling their mothers on pasture. The animals would graze with the flock from morning to evening, and at dusk they remained in the barn where they had free access to meadow hay and fresh water.

2.2. Preparation of lamb meat samples

Once the lambs were 4–4.5 months of age, they were transported in batches containing 5–12 lambs to commercial slaughterhouses from Portugal and Spain, as appropriate. Prior to slaughter, the animals were housed with only full access to fresh water for 12 h. After this term, the lambs were electrically stunned and slaughtered by exsanguination of the jugular vein. Afterwards the lambs were skinned, eviscerated, washed and chilled at 4 ± 1 °C. Twenty-four hours then slaughter, carcasses were cut up and the left and right *Longissimus thoracis et lumborum* muscles were extracted from the sixth to the thirteenth vertebra (a total of 132 pieces, 2 muscles for each carcass) under aseptic conditions. Both left and right muscles were individually packed under vacuum conditions in transparent gas-tight polyamide and polyethylene vacuum bags (Orved®, Spain, with a density of ±100 µm, and a permeability of 84 ± 4.20; 361 ± 18.05; 22 ± 1.10; and 9.0 ± 0.45 cc/m^2/24 h/atm for O_2; CO_2; N_2; and H_2O, respectively) and kept at 4 ± 1 °C. The left muscle was utilized for the analysis of the chemical composition 72 h after slaughter, meanwhile the right muscle was employed for the determination of volatile compounds then storage during 15 days.

2.3. Analysis of chemical composition

Moisture, protein (Kjeldahl N × 6.25) and ashes amounts were determined and displayed as percentage of fresh *Longissimus thoracis et lumborum* muscle in accordance with ISO 1442 (1997); ISO 937 (1978); and ISO 936 (1998) standards, respectively. Meanwhile, intramuscular fat was analyzed and showed as percentage of fresh *Longissimus thoracis et lumborum* muscle according to the American Oil Chemistry Society (AOCS) official procedure (AOCS, 2005).

2.4. Volatile compounds analysis

For the quantification of volatile compounds, a previous extraction was performing at 37 °C during 30 min (after equilibration of the samples for 15 min at identical temperature) using solid-phase microextraction device (SPME) (DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) with an autosampler Pal RTC-120. For this, 1 g of chopped meat was weighed in a 20-mL glass vial, and consequently screw-capped with a laminated Teflon-rubber disk. The extracted compounds were then separated, identified, and quantified in a gas chromatograph 7890B GC-System (Agilent Technologies, Santa Clara, CA, USA) equipped with a mass selective detector 5977B MSD (Agilent Technologies). The whole parameters, chromatographic and mass spectrometer conditions employed in this determination were firstly described by Domínguez et al. (2019a). After integration, peak detection and identification of each substance, the extracted ion chromatogram (EIC) from the Quantifier Ion was attained from each peak. The final results were displayed as area units of the EIC × 10^4 per gram of fresh *Longissimus thoracis et lumborum* muscle (AU × 10^4/g of fresh muscle).

2.5. Statistical analysis

A total of 132 samples (66 for the chemical determination, and 66 for the volatile compounds study) were analyzed in triplicate for each parameter. Normal distribution and variance homogeneity had been initially tested (Shapiro–Wilk). The effect of the rearing system on the chemical composition and the volatile profile of the Gallega lamb meat was examined employing a one-way analysis of variance (ANOVA). The outcomes were displayed in terms of mean values, the standard error of the mean (SEM) was show for both productions systems, and significant differences in the attributes were indicated at $P < 0.05$, $P < 0.01$ and $P < 0.001$. All statistical analyses were conducted utilizing SPSS package version 23.0 (IBM SPSS, Chicago, IL, USA).

3. Results and discussion

3.1. Chemical composition

Table 1 display the effect of the rearing system on the chemical composition (expressed as percentage) of the *Longissimus thoracis et lumborum* of Gallega lambs. Statistical analysis shown that the amounts of moisture (75.90% and 75.68%), intramuscular fat (1.68% and 1.76%), and protein (20.62% and 20.97%) were not significantly ($P > 0.05$) affected by the production system, obtaining very similar values in both intensive and extensive diets, respectively. These similarities contrast with what is expected, since it has been previously observed that some chemical parameter as intramuscular fat are frequently affected by the diet supplied (Cividini et al., 2014). However, the absence of significant effects found in this work may be related to the short rearing period of the lambs and to the minor influence of diet has on intramuscular fatty acids of the ruminant animals (Joy et al., 2008). Furthermore, several authors also found that rearing system not significant affect moisture (Wilches et al., 2011), fat (Gkarane et al., 2019), and protein (Wilches et al., 2011) contents. On the other hand, ash

Table 1
Influence of the production system on the chemical composition (expressed as percentages) of *Longissimus thoracis et lumborum* muscle from Gallega lambs.

	Intensive	Extensive	SEM	Sig.
Moisture (%)	75.90	75.65	1.107	ns
Intramuscular fat (%)	1.68	1.76	0.792	ns
Protein (%)	20.62	20.97	0.817	ns
Ash (%)	1.24	1.35	0.106	***

SEM: standard error of the mean. Sig.: significance. *** $P < 0.001$; ns: no significant difference.

amount was the only composition parameter significantly ($P < 0.001$) affected by the feeding system. Concretely, the extensively-fed lambs displayed a higher ash content (1.35% vs. 1.24%). These aftermaths contrast with those obtained by Gkarane et al. (2019) and Wilches et al. (2011), who observed that the production system did not affect the ash percentage. Nevertheless, the outcomes reported in the present research could be a consequence of the higher ash content of the grass compared to the commercial feed (Wilches et al., 2011).

3.2. Volatile profile

Despite the SPME technique is not usually utilized for absolute quantifications, this method allows the determination of the relative amounts between samples when precisely the same extraction methodology is employed in all samples (Domínguez et al., 2014). In this way, the influence of the feeding system on the volatile profile in the head space of *Longissimus thoracis et lumborum* muscle of Gallega lambs (expressed as AU × 10^4/g of fresh muscle) is presented in Tables 2–5. Concretely, a total of 148 volatile substances were isolated and identified using SPME/GC-MS technique, and classified in 10 chemical families: hydrocarbons (lineal, branched, cyclic, and benzene-derived; $n = 55$), acids ($n = 7$), alcohols ($n = 24$), aldehydes ($n = 10$), ketones ($n = 20$), esters ($n = 13$), ethers ($n = 4$), furans ($n = 3$), sulfur compounds ($n = 2$), and a mix named others ($n = 10$).

3.3. Hydrocarbons: lineal, branched, cyclic and benzene-derived

Table 2 shows the influence of the feeding system on hydrocarbons of the *Longissimus thoracis et lumborum* muscle of Gallega lambs. Concretely, a total of 55 substances pertaining to this volatile family were identified in both diets (except for 4-octene, (E)- which was only found in intensively-fed lambs) and were distributed as follows: 8/7 lineal hydrocarbons (for intensive/extensive system, respectively), 28 branched hydrocarbons, 16 cyclic hydrocarbons, and 3 benzene-derived hydrocarbons. As can be seen, the production system significantly ($P < 0.001$) affects the total hydrocarbon content, being higher in intensively-fed lambs (668.89 vs. 327.81 AU × 10^4/g fresh muscle). Similarly, the animals reared in intensive also showed significantly ($P < 0.001$) higher concentrations of the total lineal (284.25 vs. 22.58 AU × 10^4/g fresh muscle), branched (175.88 vs. 79.73 AU × 10^4/g fresh muscle), and benzene-derived (5.29 vs. 2.83 AU × 10^4/g fresh muscle) hydrocarbons, whereas the total cyclic hydrocarbon content was significantly ($P < 0.05$) higher in the extensively-fed lambs (222.67 vs. 203.47 AU × 10^4/g fresh muscle).

Regarding the individual hydrocarbons, these volatile compounds were significantly ($P < 0.05$) influenced by diet in all cases, apart from pentane, 2-methyl; heptane, 2,3-dimethyl; and the nonane, 3,7 dimethyl-. More specifically, in our study intensively-fed lambs showed higher amounts of these substances, since 44 of the 55 detected hydrocarbons were found in significantly ($P < 0.05$) higher concentrations in the meat of intensively-reared lambs. This distinction could be due to the fact that some alkanes greater than 10 carbon atoms can be deposited directly in the animal tissue through the diet supplied. Additionally, some hydrocarbons have previously been directly related to diet. For example, Sivadier et al. (2010) determined that compounds such as tetradecane were really identified as biomarkers of grass-based diets. However, in our study this occurrence is not confirmed, since the lambs fed with concentrate obtained significantly ($P < 0.001$) higher amounts of tetradecane than the grass-fed lambs (2.04 vs. 0.63 AU × 10^4/g fresh muscle). In a similar way, benzene-derived compounds, which were previously linked as tracers of grass-based diets (Sivadier et al., 2010), were found in significantly ($P < 0.001$) higher quantities in intensively-fed lambs. These facts differ with the relationship that the benzene-derived hydrocarbons have with green plants, since these substances are frequently retained in these vegetables because they are environmental pollutants, and therefore they could be related with

Table 2
Influence of the production system on lineal, branched, cyclic and benzene-derived hydrocarbons (expressed as AU × 10^4/g fresh muscle) of *Longissimus thoracis et lumborum* muscle from Gallega lambs.

	LRI	*m/z*	Intensive	Extensive	SEM	Sig.
Lineal hydrocarbons						
Pentane	487	43	32.24	10.45	1.590	***
Heptane	655	71	11.84	2.31	0.776	***
4-Octene, (E)-	827	55	216.57	0.00	13.778	***
Decane	1041	57	7.01	2.90	0.329	***
Undecane	1131	57	5.96	3.13	0.289	***
Dodecane	1213	57	4.89	2.13	0.266	***
Tridecane	1288	71	3.70	1.03	0.195	***
Tetradecane	1358	57	2.04	0.63	0.105	***
Total lineal hydrocarbons			284.25	22.58	16.554	***
Branched hydrocarbons						
Pentane, 2-methyl-	515	71	0.33	0.34	0.016	ns
Pentane, 3-methyl-	524	56	0.60	0.48	0.027	*
Pentane, 2,3-dimethyl-	655	56	6.10	7.67	0.260	**
Pentane, 2,3,4-trimethyl-	743	71	2.56	9.57	0.471	***
Pentane, 2,3,3-trimethyl-	751	71	39.91	17.70	1.841	***
Hexane, 2,3-dimethyl-	758	70	0.36	0.95	0.046	***
Hexane, 2,2,5-trimethyl-	791	57	14.95	11.23	0.620	**
Heptane, 2,3-dimethyl-	837	85	1.33	1.15	0.054	ns
Heptane, 2,6-dimethyl-	854	71	1.51	1.26	0.054	*
Heptane, 3-ethyl-	906	57	2.43	1.33	0.085	***
Nonane, 3,7-dimethyl-	915	57	1.16	1.06	0.046	ns
Heptane, 2,2,4-trimethyl-	923	57	3.42	1.53	0.173	***
Octane, 3,3-dimethyl-	938	71	3.81	1.29	0.186	***
Pentane, 2,2-dimethyl-	939	57	3.09	1.17	0.128	***
3-Ethyl-2-methyl-1-heptene	989	84	1.55	0.45	0.076	***
2,3-Dimethyl-1-hexene	1032	55	2.28	0.62	0.112	***
Pentane, 3,3-dimethyl-	1041	71	3.08	1.36	0.124	***
1-Hexene, 3-methyl-	1058	70	61.48	8.74	3.405	***
(Z)-4-Methyl-2-hexene	1069	98	1.06	0.29	0.055	***
Nonane, 5-butyl-	1094	127	0.83	0.25	0.041	***
Nonane, 5-(2-methylpropyl)-	1094	71	1.46	0.92	0.061	***
Dodecane, 2,6,10-trimethyl-	1095	57	2.14	0.73	0.100	***
Heptane, 2,2-dimethyl-	1098	57	3.40	1.06	0.171	***
2-Undecene, 9-methyl-, (Z)-	1151	98	2.15	0.55	0.110	***
5-Ethyl-1-nonene	1227	83	4.65	1.05	0.241	***
1-Decene, 2,4-dimethyl-	1227	70	9.49	1.76	0.531	***
Heptadecane, 8-methyl-	1230	71	0.43	2.53	0.141	***
Dodecane, 2-methyl-	1261	57	0.30	2.69	0.152	***
Total branched hydrocarbons			175.88	79.73	6.370	***
Cyclic hydrocarbons						
Cyclopentane, 1,2-dimethyl-, trans-	646	56	1.83	0.70	0.093	***
Cyclopentane, 1,2-dimethyl-, cis-	647	70	1.46	0.62	0.077	***
1,4-Cyclohexadiene	688	79	1.22	0.37	0.062	***

(*continued on next page*)

Table 2 (*continued*)

	LRI	*m/z*	Intensive	Extensive	SEM	Sig.
Bicyclo[3.2.0] hepta-2,6-diene	796	91	21.59	13.44	0.688	***
Cyclopentane, 1,2,3-trimethyl-	806	56	120.63	190.33	6.319	***
Cyclohexane, 1,3-dimethyl-, cis-	826	97	11.94	2.19	0.649	***
Cyclohexane, 1,3-dimethyl-	828	55	5.64	0.84	0.310	***
Cyclobutane, 1,1,2,3,3-pentamethyl-	929	70	2.52	1.06	0.102	***
Cyclopropane, 1-methyl-2-pentyl-	933	55	0.64	0.28	0.032	***
Bicyclo[2.2.1] heptane, 2,2-dimethyl-3-methylene-, (1R)-	985	79	0.24	0.93	0.053	***
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	985	93	1.31	5.30	0.295	***
Cyclopentane, 1,2,3,4,5-pentamethyl-	989	69	1.34	0.44	0.064	***
Cyclodecene, (Z)-	1037	67	2.16	0.26	0.127	***
Cyclopropane	1059	41	26.74	5.09	1.437	***
Cyclohexane, 1,2-diethyl-1-methyl-	1071	125	0.60	0.20	0.028	***
Cyclopentane, pentyl-	1081	68	3.61	0.61	0.192	***
Total cyclic hydrocarbons			203.47	222.67	4.828	*
Benzene-derived hydrocarbons						
Ethylbenzene	918	91	1.64	0.80	0.063	***
Benzene, 1,3-dimethyl-	928	91	2.88	1.73	0.151	***
Benzene, n-butyl-	1116	91	0.76	0.31	0.033	***
Total benzene-derived hydrocarbons		5.29	2.83	0.215	***	
Total hydrocarbons			668.89	327.81	22.073	***

LRI: Lineal Retention Index calculated for DB-624 capillary column (30 m × 0.25 mm id, 1.4 μm film thickness; J&W Scientific, Folsom, CA, USA) installed on a gas chromatograph equipped with a mass selective detector. *m/z*: quantification ion. SEM: standard error of the mean. Sig.: significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: no significant difference.

extensively-fed lambs (Engel and Ratel, 2007).

Despite the above discrepancies, our investigation identified two cyclic hydrocarbons that could be linked to the grass-based diet. Concretely, the aforementioned substances are the terpenes bicyclo [2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1R)-; and bicyclo[3.1.1] hept-2-ene, 3,6,6-trimethyl-, which were found in significantly ($P < 0.001$) higher quantities in extensively-fed lambs (0.93 *vs.* 0.24 AU × 10^4/g fresh muscle; and 5.30 vs. 1.31 AU × 10^4/g fresh muscle, respectively). This greater presence may be because terpenes originate mainly from green plants. In fact, previous studies had frequently detected terpenes in grass-fed lambs and proposed them as generic pasture diet biomarkers (Vasta et al., 2011). However, it should be noted that in our study a third terpene was identified, namely bicyclo [3.2.0]hepta-2,6-diene, which was found in a significantly ($P < 0.001$) higher concentration in intensive lambs (21.59 vs. 13.44 AU × 10^4/g fresh muscle, respectively), suggesting that not all terpenes could be used as properly biomarkers of the grass-based diets.

With regards to the contribution of hydrocarbons over the total volatile compounds, this group represented the third most abundant family in both feeding systems as it obtained a total percentage of 13.54% in intensively-fed lambs and 6.75% in extensively-fed lambs (Fig. 1). However, their contribution to the general aroma of lamb meat could be reduced to the presence of benzene-derived hydrocarbons, as previous authors have indicated that lineal, branched and cyclic hydrocarbons have no relevance in the volatile pattern of meat (Domínguez et al., 2019a,b; Flores, 2018). Thus, despite the low presence of benzene-derived hydrocarbons (0.11% and 0.06% of total volatile compounds in lambs fed intensively and extensively, respectively), they could be the most relevant hydrocarbons in terms of aromatic profile owing to their low odor threshold (Karabagias, 2018).

3.4. Acids

In the present research, 7 acids were identified in the *Longissimus thoracis et lumborum* muscle of both intensive and extensive Gallega lambs. As display in Table 3, the feeding system influenced the total acid content, which was significantly ($P < 0.01$) higher in intensively-fed lambs (307.74 vs. 261.54 AU × 10^4/g fresh muscle). Identically, 5 of the 7 detected acids were found in a significantly ($P < 0.001$) higher concentration in intensive lambs, while the remaining 2 acids (namely propanoic acid, 2-methyl-; and acetic anhydride) were not significantly ($P > 0.05$) influenced by diet supplied. These facts are consistent with what is expected, since previous studies have revealed that concentrated diets favor the deposition of some acids such as the branched chain fatty acids due to the low roughage content of this foodstuff (Vlaeminck et al., 2004). In this way, branched chain fatty acids such as butanoic acid, 3-methyl-; and pentanoic acid, 2-methyl-, anhydride identified in this study could be employed as biomarkers of concentrate-based diets, since both obtained significantly higher concentrations in intensively-reared lambs (18.13 vs. 3.66 AU × 10^4/g fresh muscle; and 1.94 vs. 0.14 AU × 10^4/g fresh muscle, respectively). However, another

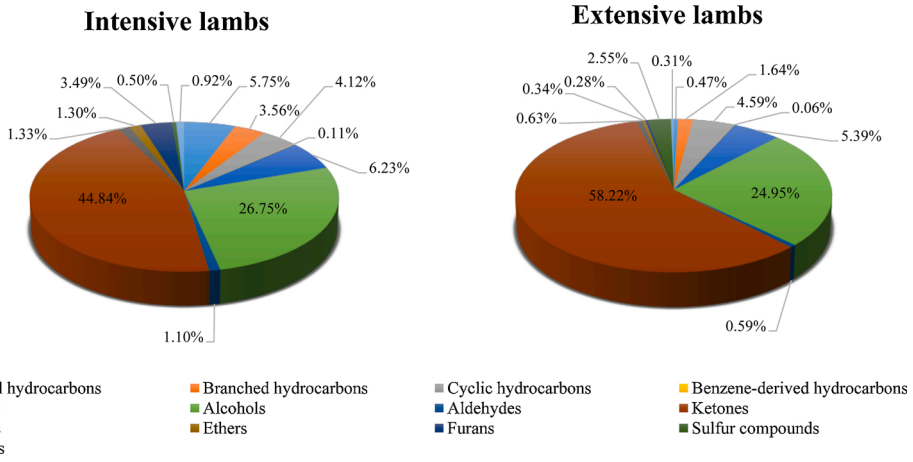

Fig. 1. Volatile families of *Longissimus thoracis et lumborum* muscle from Gallega lambs (displayed as percentages) influenced by the production system.

Table 3
Influence of the production system on acids and alcohols (expressed as AU × 10^4/g fresh muscle) of *Longissimus thoracis et lumborum* muscle from Gallega lambs.

	LRI	*m/z*	Intensive	Extensive	SEM	Sig.
Acids						
Acetic anhydride	563	43	227.94	254.30	8.495	ns
Acetic acid	684	60	2.16	0.40	0.138	***
Propanoic acid, 2-methyl-	888	73	0.47	0.47	0.020	ns
Butanoic acid	920	60	2.24	1.10	0.105	***
Butanoic acid, 3-methyl-	976	60	18.13	3.66	0.931	***
Hexanoic acid	1099	60	54.87	1.47	3.436	***
Pentanoic acid, 2-methyl-, anhydride	1160	99	1.94	0.14	0.124	***
Total acids			307.74	261.54	8.805	**
Alcohols						
1-Propanol	546	59	1.09	0.53	0.064	***
1-Propanol, 2-methyl-	626	74	0.95	1.30	0.063	**
1-Butanol	691	56	9.93	2.15	0.558	***
3-Buten-1-ol, 3-methyl-	795	56	0.72	0.85	0.034	ns
1-Butanol, 3-methyl-	801	55	180.75	1070.82	58.555	***
1-Pentanol	846	55	182.85	22.07	11.542	***
Cyclobutanol, 2-ethyl-	863	56	20.39	2.04	1.189	***
2-Octen-1-ol, (Z)-	863	67	15.44	1.38	0.937	***
2,3-Butanediol, [R-(R*,R*)]-	921	45	4.76	4.16	0.145	*
1-Hexanol	960	56	165.35	27.64	9.969	***
2-Heptanol	987	45	0.63	0.21	0.028	***
1-Heptanol	1059	56	40.57	8.22	2.557	***
1-Octen-3-ol	1066	57	602.65	54.81	36.945	***
2,3,4-Trimethyl-1-pentanol	1096	71	1.54	1.30	0.055	*
1-Hexanol, 2-ethyl-	1111	57	4.72	2.99	0.171	***
4-Ethylcyclohexanol	1123	81	4.52	0.66	0.256	***
5-Methyl-1-heptanol	1143	83	1.71	0.29	0.097	***
Benzyl alcohol	1144	108	0.39	1.33	0.087	***
1-Octanol	1147	56	26.59	3.82	1.624	***
3-Octen-1-ol, (Z)-	1148	81	5.29	1.04	0.292	***
2-Octen-1-ol, (E)-	1149	57	15.08	0.74	0.926	***
1-Heptanol, 4-methyl-	1150	84	13.61	1.49	0.797	***
4-Methyl-5-decanol	1185	83	18.43	0.98	1.150	***
1-Nonanol	1227	56	3.32	0.48	0.187	***
Total alcohols			1321.30	1211.30	29.984	ns

LRI: Lineal Retention Index calculated for DB-624 capillary column (30 m × 0.25 mm id, 1.4 μm film thickness; J&W Scientific, Folsom, CA, USA) installed on a gas chromatograph equipped with a mass selective detector. *m/z*: quantification ion. SEM: standard error of the mean. Sig.: significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: no significant difference.

branched-chain fatty acid (namely propanoic acid, 2-methyl-) was not significantly ($P > 0.05$) influenced by diet in this research, which may indicate that not all branched chain fatty acids would be good indicators of diets based on commercial concentrates.

On the other hand, the group of acids constitutes the fourth most abundant family in both diets, representing 6.23% and 5.39% of the total content of volatile compounds in intensive and extensive lambs, respectively (Fig. 1). This relatively high presence could be important in terms of the aromatic profile of lamb meat, because previous authors found that some branched-chain acids such as 4-methyloctanoic, 4-ethyloctanoic and 4-methylnonanoic acids contribute to the characteristic mutton-like aroma of lamb (Erasmus et al., 2017). Nevertheless, in agreement with Karabagias (2018), not one of these substances were found in the lambs of our investigation, independent of the diet supplied. This occurrence could be owing to the young age of the lambs employed in our analysis (4–4.5 months), since branched-chain fatty acids have previously been related to animals older than 2 years (Watkins et al., 2014). Furthermore, it has also been found that these compounds are principally present in adipose tissue, which could explain their lower presence in muscle tissue. In addition, this absence can be positively related to consumer acceptance, since 4-methyloctanoic, 4-ethyloctanoic and 4-methylnonanoic acids have been associated with low levels of lamb meat acceptance by consumers (Erasmus et al., 2017).

3.5. Alcohols

A total of 24 alcohols were detected in the *Longissimus thoracis et lumborum* muscle of Gallega lambs. As shown in Table 3, the production system did not significantly ($P < 0.05$) affect the total alcohol content since both feedings showed similar values (1321.30 and 1211.30 AU × 10^4/g fresh muscle for intensively- and extensively-fed lambs, respectively). In contrast, all individual alcohols were significantly ($P < 0.05$) affected by diet, except for 3-buten-1-ol, 3-methyl-. Specifically, the lambs fed in intensive obtained higher amounts for almost all the alcohols detected, because 20 of the 24 compounds detected were found in significantly ($P < 0.05$) higher quantities in the meat of these animals.

Regarding the most abundant alcohol in the intensively-fed lambs, this was 1-octen-3-ol. Furthermore, this alcohol was found in significantly ($P < 0.001$) higher amounts in these lambs (602.65 vs. 54.81 AU × 10^4/g fresh muscle). Despite this, prior studies have not determined this volatile compound as a biomarker of concentrated diets, but Sivadier et al. (2009) observed that its presence varied according to the duration of the grazing period. Contrary to these findings, Almela et al. (2010) proposed that there was a correlation between the presence of 1-octen-3-ol and grass-based diets. Moreover, it has previously been observed that this volatile compound can be formed from various precursors through lipid oxidation, being frequently related to different meats and derivatives (Gravador et al., 2015; Karabagias, 2018). Therefore, this alcohol might not be suitable for tracking concentrate-diets used in lamb rearing. However, alcohols such as 1-butanol and 1-pentanol could be expected to be appropriate biomarkers of the concentrate-based diet in this study, since both alcohols were detected in significantly higher concentrations in intensively-fed lambs (9.93 vs. 2.15 AU × 10^4/g fresh muscle for 1-butanol; and 182.85 vs. 22.07 AU × 10^4/g fresh muscle for 1-pentanol). This fact could be justified because 1-butanol and 1-pentanol can be generated from linoleic acid (Domínguez et al., 2019a), which is typically found in commercial concentrates employed in animal rearing. Along the same lines, Almela et al. (2010) observed that 1-butanol was only found in animals fed with concentrate, even proposing this volatile compound as a biomarker that permits to distinguish the grass- from concentrate-based feedings. Nevertheless, these same authors did not identify differences with respect to 1-pentanol in lambs fed with grass or with concentrate, contrasting with the facts found in our study. Vasta et al. (2012) even observed a trend for 1-pentanol inverse to that found in the present work, since they saw that grass-fed lambs displayed significantly higher amounts of this substance. Similarly, the volatile compounds 1-hexanol and 1-octanol did not seem to be clear biomarkers of the concentrate-based diet, since although in our study these substances showed significantly ($P < 0.001$) higher amounts in intensively-reared lambs (165.35 vs. 27.64 AU × 10^4/g fresh muscle for 1-hexanol, and 26.59 vs. 3.82 AU × 10^4/g fresh muscle for 1-octanol), different trends were observed in the literature. Thus, Sivadier et al. (2008) and Vasta et al. (2012) found that 1-hexanol prevailed in grass-fed lambs while other studies showed that differences in diet did not affect this volatile compound (Vasta et al., 2011; Wilches et al., 2011). Even other authors observed that 1-hexanol display higher values in intensive lambs (Vasta et al., 2007), in accordance with our research. Additionally, Del Bianco et al. (2020) and Wilches et al. (2011) found

that 1-octanol was not significantly affected by diet, while Sivadier et al. (2008) related this substance to grass lambs.

On the other hand, the most abundant alcohol found in the extensively-fed lambs was 1-butanol, 3-methyl-. Besides its abundance, this alcohol stands out for being the only one found in a significantly ($P < 0.001$) higher quantity compared to intensively-fed lambs, together with 1-propanol, 2-methyl- and benzyl alcohol. Thus, the extensive lambs exhibited values of 1070.82 AU × 10^4/g fresh muscle for 1-butanol, 3-methyl-, while the lambs fed intensively obtained a concentration of 180.75 AU × 10^4/g fresh muscle. Similarly, Wilches et al. (2011) found that in Castellana lambs this compound was also found in extensive and not in intensive lambs. However, these same authors did not find this difference for lambs of the Churra breed. Therefore, 1-butanol, 3-methyl- could be a biomarker in grass-fed lambs, but only in certain lamb breeds. For its part, benzyl alcohol could be a good biomarker of grass-based diets, because this compound was found in significantly ($P < 0.001$) higher amounts in lambs reared extensively (1.33 vs. 0.39 AU × 10^4/g fresh muscle). This occurrence is in agreement with Wilches et al. (2011), since they also found that the Castellana and Churra lambs extensively-reared possessed said volatile compound, while this alcohol does not appear in intensively-reared lambs. This may be due to the fact that phenolic compounds are secondary metabolites of plants (Lorenzo et al., 2018), which can appear in meat owing to the consumption of green plants by animals. In fact, other investigations have positively correlated the presence of different phenolic compounds with grazing activity in ruminants (Raes et al., 2003).

Regarding the contribution of alcohols on the volatile content, this family was the second most abundant in both feeding systems, since it represented 26.75% and 24.95% of the volatile profile for lambs fed in intensive and extensive, respectively (Fig. 1). Nevertheless, this high content contrasts with the low impact that alcohols possess on the aroma of meat, due to their usually high detection thresholds (Selli and Cayhan, 2009). In this way, alcohols such as benzyl alcohol does not have a special interest in the lamb aroma even having a sweet and flora-like aroma (Zhang et al., 2020). In spite of this, certain alcohols have been shown to contribute to the aroma of meat. Thus, 1-pentanol and 1-hexanol could influence the lamb flavor due to their pleasant, sweet or fruit and herbal, fatty odor, respectively, and their low odor threshold (Calkins and Hodgen, 2007). Similarly, 1-octanol could affect the aromatic profile of the meat analyzed, since in previous studies this compound has been strongly correlated with the rancid aroma of lamb meat (Ortuño et al., 2016). In this sense, the aroma of intensively-raised lambs may be more intense owing to the significantly ($P < 0.001$) higher presence of these alcohols (Table 3), and could even be negative due to the greater presence of 1-octanol.

3.6. Aldehydes

Substances belonging to the aldehydes group were displayed in Table 4. As can be seen, the breading system significantly ($P < 0.005$) affected the total aldehyde content, this amount being higher in lambs fed in intensive (54.25 vs. 28.67 AU × 10^4/g fresh muscle). In a similar way, 8 out of 10 determined aldehydes were affected by the diet. Specifically, 6 of them were found in significantly ($P < 0.01$) higher amounts in intensively-reared lambs, while only 2 (namely, butanal, 3-methyl-; and benzeneacetaldehyde) were obtained in significantly ($P < 0.001$) higher concentration in extensive lambs.

Hexanal was the most plentiful aldehyde in the lambs fed in intensive, which showed significantly ($P < 0.001$) higher amounts than those found in extensive lambs (15.98 vs. 2.11 AU × 10^4/g fresh muscle). This

Table 4
Influence of the production system on aldehydes and ketones (expressed as AU × 10^4/g fresh muscle) of *Longissimus thoracis et lumborum* muscle from Gallega lambs.

	LRI	*m/z*	Intensive	Extensive	SEM	Sig.
Aldehydes						
Propanal, 2-methyl-	530	72	1.39	0.74	0.077	***
Butanal, 3-methyl-	638	58	3.36	4.87	0.196	***
2-Butenal	827	70	1.24	1.02	0.040	**
Hexanal	864	56	15.98	2.11	0.919	***
Heptanal	981	70	10.91	11.15	0.396	ns
Hexanal, 3,3-dimethyl-	999	69	1.04	0.36	0.047	***
2-Heptenal, (E)-	1053	83	2.64	1.30	0.110	***
Octanal	1081	84	3.44	3.58	0.115	ns
Benzeneacetaldehyde	1138	91	0.60	2.81	0.190	***
Nonanal	1173	98	13.66	0.73	0.890	***
Total aldehydes			54.25	28.67	1.757	***
Ketones						
2,3-Butanedione	565	86	76.96	131.60	7.224	***
2-Butanone	569	72	1.78	2.18	0.096	*
2-Pentanone	705	86	1.43	0.91	0.081	***
3-Pentanone	718	57	47.21	18.60	2.069	***
Acetoin	778	45	1869.10	2620.15	110.642	***
2,5-Furandione, dihydro-3-methyl-	843	70	110.34	26.88	5.602	***
2-Hexanone	856	58	1.94	0.93	0.084	***
Cyclobutanone, 2,3,3,4-tetramethyl-	885	70	0.58	0.26	0.022	***
Acetyl valeryl	910	57	0.86	1.16	0.061	*
3-Heptanone	966	57	2.19	2.96	0.098	***
2-Heptanone	973	71	25.81	2.64	1.711	***
Butyrolactone	1057	86	4.30	4.30	0.155	ns
3-Heptanone, 5-methyl-	1065	99	52.42	5.55	3.299	***
5-Hepten-2-one, 6-methyl-	1069	108	1.66	0.76	0.073	***
2-Octanone	1073	58	12.84	5.28	0.660	***
Cyclohexanone, 4-ethyl-	1096	69	1.03	0.48	0.049	***
3-Nonanone	1154	113	0.99	0.17	0.060	***
2-Nonanone	1161	58	1.03	0.93	0.052	ns
2(3H)-Furanone, 5-ethyldihydro-	1181	85	1.95	0.43	0.105	***
2-Undecanone	1317	58	0.59	0.17	0.028	***
Total ketones			2215.01	2826.36	110.917	**

LRI: Lineal Retention Index calculated for DB-624 capillary column (30 m × 0.25 mm id, 1.4 μm film thickness; J&W Scientific, Folsom, CA, USA) installed on a gas chromatograph equipped with a mass selective detector. *m/z*: quantification ion. SEM: standard error of the mean. Sig.: significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: no significant difference.

difference between feedings is in accordance with that reported by various authors (Fruet et al., 2018; Gkarane et al., 2019), who related the high presence of hexanal in intensively-reared lambs with the high linoleic acid content of concentrate feeds employed in rearing system (Sivadier et al., 2008), since hexanal is a product of the oxidation of said acid (Domínguez et al., 2019a,b; Schaich, 2013). Nevertheless, Almela et al. (2010) observed opposite trends, since they found that grass-fed lambs had higher amounts of hexanal. Additionally, another compound found in significantly ($P < 0.001$) higher amounts in intensively-fed lambs was nonanal. Concretely, this volatile compound displayed amounts of 13.73 AU × 10^4/g fresh muscle in intensive lambs and values of 0.73 AU × 10^4/g fresh muscle in extensively-fed lambs. These facts contrast with those obtained by other works, which did not find significant differences between both feedings for said aldehyde (Fruet et al., 2018; Gkarane et al., 2019; Vasta et al., 2012, 2011).

For its part, heptanal was the most abundant aldehyde found in the meat of lambs fed extensively, since values of 11.15 AU × 10^4/g fresh muscle were detected in these animals. Despite this high amount compared to the rest of aldehydes, the presence of heptanal in extensive lambs was similar to that shown by the lambs fed intensively (10.91 AU × 10^4/g fresh muscle). These facts agree with those shown by Gkarane et al. (2019) and Vasta et al. (2012), who did not associate this aldehyde with any type of diet. In an identical way, the lambs analyzed in this study also did not show significant ($P > 0.05$) differences regarding the octanal content. Again this fact agrees with previous works, where the octanal was not influenced by diet (Fruet et al., 2018; Gkarane et al., 2019). On the contrary, butanal, 3-methyl; and benzeneacetaldehyde did show significantly ($P < 0.001$) higher amounts in grass-fed lambs (4.87 vs. 3.36 AU × 10^4/g fresh muscle; and 2.81 vs. 0.60 AU × 10^4/g fresh muscle, respectively). These facts contrast with various authors, who did not observe significant differences in these aldehydes due to the feeding of the lambs (Gkarane et al., 2019; Vasta et al., 2012, 2011).

Despite finding some coincidences in the bibliography regarding the relationship of certain aldehydes with the diet supplied to ruminants, in general there are quite a few discrepancies in relation to their correct use as diet tracers. This occurrence may be because aldehydes originate from lipid oxidation, so that their presence may be conditioned by external factors such as the temperature used for the extraction of volatile compounds (Sivadier et al., 2010). Thus, it is not easy to relate a compound of this chemical family to a specific feeding system (Vasta and Priolo, 2006).

With respect to the contribution of aldehydes on the volatile profile, this was relatively low since this group was the second and third least abundant in the lambs fed intensively and extensively, representing percentages of 1.10 and 0.59%, respectively (Fig. 1). This occurrence contrasts with that reported in previous investigations, where aldehydes formed one of the principal families found in ruminant meat (Vasta et al., 2012). Despite this, aldehyde group is probably the most interesting because the concentration of this compounds can be critical for the meat aromatic profile due to their low odor threshold (Du, 2012). Indeed, various saturated aliphatic aldehydes such as hexanal, octanal and nonanal have been suggested as the main indicators of rancid aroma of meat (Ortuño et al., 2016). On this matter, intensively-reared lambs could possess a rancid aroma intensity greater than that of extensively-fed lambs, since they revealed a significantly ($P < 0.001$) higher content of hexanal and nonanal (Table 4).

3.7. Ketones

A total of 20 different ketones were identified in the *Longissimus thoracis et lumborum* muscle of Gallega lambs (Table 4). As it can be observed, the total content of this family was significantly ($P < 0.01$) higher in extensively-fed lambs (2826.36 vs. 2215.01 AU × 10^4/g fresh muscle). However, the general trend of individual ketones was contrary to the total content, since only 5 ketones were found in significantly ($P < 0.05$) higher amounts in extensive lambs, while 13 of these substances obtained significantly ($P < 0.001$) higher values in lambs intensively-fed. Additionally, only 2 ketones were not affected ($P > 0.05$) by the production system (namely, butyrolactone and 2-nonanone), which could suggest that both ketones are related to the specific metabolism of ruminants (Coppa et al., 2011).

The most abundant ketone in meat of concentrate-fed lambs was acetoin (also known as 2-butanone, 3-hydroxy). However, despite this large number, grass-fed lambs showed significantly ($P < 0.001$) higher amounts of this compound (2620.15 vs. 1869.10 AU × 10^4/g fresh muscle), also being the most abundant ketone in these animals. In disagreement with the above, Gravador et al. (2015) observed that acetoin was not influenced by the diet supplied, while Almela et al. (2010) related this compound to diets based on concentrates. Similarly, although 2,3-butanedione (diacetyl) and 2-butanone were previously associated with concentrate diets (Sivadier et al., 2009), in our investigation both ketones were found in significantly higher amounts in grass-fed lambs (131.61 vs. 76.96 AU × 10^4/g fresh muscle for 2,3-butanedione; and 2.18 vs. 1.78 AU × 10^4/g fresh muscle for 2-butanone). Considering these occurrences, neither acetoin, nor 2,3-butanedione, nor 2-butanone, would be judged as suitable diet tracers. For its part, 2-heptanone was found in significantly ($P < 0.001$) higher amounts in concentrated-fed lambs (25.81 vs. 2.64 AU × 10^4/g fresh muscle). This event may be related to the high content of linoleic acid in concentrate feeds (Morand-Fehr and Tran, 2001), since 2-heptanone is a product of the oxidation of this fatty acid (Elmore et al., 2005). Additionally, several authors also identified the use of concentrate-based diets with the highest presence of this ketone (Resconi et al., 2010). Thus, 2-heptanone appears to be an appropriate biomarker for concentrate-based diets.

On the other hand, in the present investigation 3 lactones (2,5-furandione, dihydro-3-methyl-; butyrolactone; and 2(3 H)-furanone, 5-ethyldihydro-) were identified. It is known that this type of ketones is formed in ruminants from the corresponding hydroxy-fatty-acid (Gargouri et al., 2002), which originate in the rumen due to the oxidation of oleic and linoleic acid supplied in the diet. In this way, lactone formation has previously been related to concentrate-based diets (Sebastiàn and Viallon, 2003). According to this knowledge, in our study 2,5-furandione, dihydro-3-methyl-; and 2 (3H) -furanone, 5-ethyldihydro- were determined in significantly ($P < 0.001$) higher amounts in intensively-fed lambs (110.34 vs. 26.88 AU × 10^4/g fresh muscle; and 1.95 vs. 0.43 AU × 10^4/g fresh muscle, respectively). Nevertheless, butyrolactone did not appear to be affected ($P > 0.05$) by diet, since in both feedings the same concentration was obtained for this lactone (4.30 AU × 10^4/g fresh muscle).

Regarding the ketones usually reported as tracers of grass-based diets, 2,3-octanedione has been proposed as an excellent biomarker (Engel and Ratel, 2007). However, in the present research this ketone was not identified in any of the lambs analyzed, thus questioning its suitability as a pasture-fed biomarker. However, acetyl valeryl and 3-heptanone were presented in significantly ($P < 0.05$) higher amounts in grass-fed lambs (1.16 vs. 0.86 AU × 10^4/g fresh muscle; and 2.96 vs. 219 AU × 10^4/g fresh muscle, respectively). Despite this significance differences, these ketones have not previously been linked to grass-based diets. Therefore, in this research no ketones are suggested as a biomarker for extensive lambs.

Additionally, the fraction corresponding to the family of ketones constituted the largest group of volatile substances detected in both intensive and extensive systems (44.48% and 58.22%) (Fig. 1). This high presence, accompanied by the characteristic low threshold odor possessed by these volatile compounds, means that this family has a notable contribution to the meat aroma (Van et al., 2012). Several ketones such as 2-heptanone (Resconi et al., 2010) and 2-nonanone (Gkarane et al., 2018) have even been linked to the characteristic lamb aroma. In this sense, given that the lambs fed on concentrate showed significantly ($P < 0.001$) higher amounts of 2-heptanone

(Table 4), their meat could have a more intense specific lamb aroma.

3.8. Esters, ethers, furans, sulfur compounds, and others

Table 5 shows the influence of the feeding system on esters of the *Longissimus thoracis et lumborum* muscle of Gallega lambs. Concretely, a total of 13 distinct esters were identified, their total content being significantly ($P < 0.001$) higher in intensively-fed lambs (65.74 vs. 30.69 AU $\times 10^4$/g fresh muscle). In a similar way, 10 of the 13 esters found were significantly ($P < 0.01$) higher in intensive lambs, while only 1 ester (pentyl glycolate) was obtained in significantly ($P < 0.001$) higher amounts in extensive lambs. These facts are in accordance with those obtained by Engel and Ratel (2007) and Vasta et al. (2012). However the use of esters as biomarkers of diet could be limited by their scarce presence in lamb, since various authors detected a lower number of esters than those found in the present study (Del Bianco et al., 2020; Krvavica et al., 2015; Ortuño et al., 2016), or even did not reach identify any compound of this chemical family (Vasta et al., 2010; Zhang et al., 2020). Additionally, despite esters have low odor thresholds, it has been previously indicated that their contribution to the aroma of lamb meat may be limited (Gravador et al., 2015; Resconi et al., 2010). Therefore, given that this family only represented 1.33% and 0.63% of the total volatile compounds in intensive and extensive lambs, respectively, (Fig. 1), the significant ($P < 0.001$) difference found in the total concentration of both production systems (Table 5) could not affect the final aroma of the lamb meat.

With respect to ether family, only 4 distinct substances were detected in the *Longissimus thoracis et lumborum* muscle of Gallega lambs (Table 5). As can be seen, both the total content and each individual ether was affected by the diet, being in all cases significantly ($P < 0.001$) higher in the lambs fed intensively. Even though this marked trend, ethers have not been reported as common compounds in lamb meat, and they have not been detected on many occasions (Del Bianco et al., 2020; Gkarane et al., 2019; Vasta et al., 2012; Zhang et al., 2020). Besides, its presence in lamb has previously been related to the use of these compounds as soil fumigants, acaricides and insecticides (Karabagias, 2018). Thus, their use as biomarkers may be inappropriate. In addition, regarding the ether contribution to the total volatile profile, these compounds only represented 1.30 and 0.34% of the total content in intensive and extensive lambs, respectively, (Fig. 1), being proposed as irrelevant in the aromatic profile of lamb meat (Bueno et al., 2011).

On the other hand, 3 furans were identified in this research (Table 5),

Table 5
Influence of the production system on esters, ethers, furans, sulfur compounds, and others (expressed as AU $\times 10^4$/g fresh muscle) of *Longissimus thoracis et lumborum* muscle from Gallega lambs.

	LRI	*m/z*	Intensive	Extensive	SEM	Sig.
Esters						
Acetic acid, (dodecahydro-7-hydroxy-1,4b,8,8-tetramethyl-10-oxo-2(1H)-phenanthrenylidene)-,2-(dimethylamino) ethyl ester	705	71	0.67	0.36	0.028	***
Pentyl glycolate	758	71	0.25	0.80	0.040	***
Butanoic acid, ethyl ester	851	88	1.25	0.77	0.056	***
Butanoic acid, 2-methyl-, ethyl ester	910	102	0.56	0.42	0.024	**
Butanoic acid, 3-methyl-, ethyl ester	915	85	1.09	1.01	0.050	ns
1-Butanol, 3-methyl-, acetate	943	70	28.13	17.76	0.942	***
1-Butanol, 2-methyl-, acetate	947	70	1.45	0.87	0.060	***
Sulfurous acid, 2-ethylhexyl nonyl ester	1082	57	14.87	4.95	0.694	***
1-Butanol, 2-methyl-, trifluoroacetate	1151	70	2.19	0.60	0.125	***
2-Butenoic acid, 2-methyl-, 2-methylpropyl ester	1188	55	12.22	1.05	0.789	***
2-Propenoic acid, 2-methyl-, (tetrahydro-2-furanyl)methyl ester	1303	71	0.45	0.32	0.013	***
Sulfurous acid, hexyl nonyl ester	1303	85	1.36	1.38	0.055	ns
Sulfurous acid, 2-ethylhexyl isohexyl ester	1423	57	1.24	0.41	0.060	***
Total esters			65.74	30.69	2.331	***
Ethers						
Oxirane, (methoxymethyl)-	905	45	0.74	0.42	0.033	***
Propane, 1-(1,1-dimethylethoxy)-2-methyl-	1080	57	15.30	5.99	0.678	***
Ether, 2-ethylhexyl tert-butyl	1087	57	24.75	6.55	1.222	***
Decyl heptyl ether	1169	57	23.58	3.38	1.349	***
Total ethers			64.37	16.33	3.161	***
Furans						
Furan, 2-ethyl-	687	81	20.53	2.85	1.328	***
2-n-Butyl furan	948	81	2.77	0.44	0.165	***
Furan, 2-pentyl-	1050	81	149.22	10.19	10.208	***
Total furans			172.52	13.47	11.396	***
Sulfur compounds						
Dimethyl sulfide	505	62	0.78	0.93	0.062	ns
Carbon disulfide	505	76	23.89	122.76	7.095	***
Total sulfur compounds			24.68	123.68	7.102	***
Others						
Fumaronitrile	625	78	6.75	1.11	0.413	***
Butanimidamide	640	71	10.74	2.91	0.544	***
2-Pyrrolidinone	890	84	0.57	0.30	0.020	***
tert-Butyl hydroperoxide	895	59	1.24	0.47	0.057	***
N-Methyl-2-isopropoxycarbonylazetidine	992	70	3.38	1.01	0.167	***
Oxetane, 2-ethyl-3-methyl-	1019	56	1.11	0.33	0.054	***
Pyrolo[3,2-d]pyrimidin-2,4(1H,3H)-dione	1053	151	9.82	4.74	0.427	***
Benzene, (1,1-dimethylethoxy)-	1136	94	2.64	1.47	0.133	***
Hexane, 1-(hexyloxy)-3-methyl-	1146	57	7.51	2.27	0.381	***
4-Methoxycarbonyl-4-butanolide	1412	85	1.64	0.55	0.075	***
Total others			45.42	15.16	1.945	***

LRI: Lineal Retention Index calculated for DB-624 capillary column (30 m × 0.25 mm id, 1.4 μm film thickness; J&W Scientific, Folsom, CA, USA) installed on a gas chromatograph equipped with a mass selective detector. *m/z*: quantification ion. SEM: standard error of the mean. Sig.: significance. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: no significant difference.

which were affected by diet. Specifically, both the total furan content and the individual compounds were found in significantly ($P < 0.001$) higher amounts in lambs fed intensively. These occurrences contrast with those previously informed, since several authors identified most furans as possible tracers of grass-based diets (Sivadier et al., 2010). However, the higher presence of furan, 2-penthyl in intensively-fed animals (149.22 vs. 10.19 AU × 10^4/g fresh muscle) does agree with what was previously reported by Fruet et al. (2018). This may be due to the higher α-tocopherol content of the grass diets, since different studies have shown a negative correlation between this antioxidant and the formation of furan, 2-penthyl (Vasta et al., 2011). Moreover this volatile compound is a product of the linoleic acid oxidation (Domínguez et al., 2019a,b), thus favoring its appearance in concentrate-fed lambs. Due to this evidence, furan, 2-penthyl could be an ideal indicator of intensive production lambs with concentrate as food. Additionally, the contribution with respect to the volatile profile was different in both diets, since the lambs fed intensively showed a percentage of 3.49%, being the fifth family in abundance, while in the lambs fed extensively, they only represented 0.28%, the family being less abundant (Fig. 1). These differences can have important effects on the final aroma of lamb meat as furans have been reported as potential contributors to the rancid aroma of the meat (Ortuño et al., 2016).

Regarding the sulfur compounds, only 2 substances were identified in the *Longissimus thoracis et lumborum* muscle of Gallega lambs (Table 5). As can be seen, the total content of these substances was influenced by the diet supplied, being significantly ($P < 0.001$) higher in the extensively-fed lambs (123.68 vs. 24.68 AU × 10^4/g fresh muscle). This difference is consistent with that previously reported, since various authors observed that grass-fed animals showed a higher content of sulfur compounds than those fed grain-based diets (Sivadier et al., 2008). Accordingly, dimethyl sulfide and carbon disulfide were found in greater amounts in grass-fed lambs, although this difference was only significant ($P < 0.001$) in the case of carbon disulfide. This difference may be due to the fact that green herbage diets can generate an increase in free amino acids in the rumen (Vasta and Priolo, 2006). Thus, the formation of sulfur compounds would be favored, since these substances derive from the enzymatic proteolysis of the sulfur-containing amino acids (Saraiva et al., 2015). Therefore, since carbon disulfide was significantly ($P < 0.001$) affected by feeding, it could be suggested as a suitable biomarker for grass diet in lambs. In addition, sulfur compounds obtained very different percentages on the volatile profile of intensive (0.50%) and extensive (2.55%) lambs (Fig. 1). These distinction in the percentage may suggest marked differences in the aroma of the lambs, since the sulfur compounds contribute strongly to the meat flavor due to their low sensory detection thresholds (Gijs et al., 2000). In fact, carbon disulfide was frequently reported as a common and important compound in lamb meat (Karabagias, 2018; Saraiva et al., 2015).

Finally, in the present investigation 10 substances were identified and enclosed in the group "others" (Table 5). Both the 10 individual compounds and their total content were affected by the diet supplied, since for all of them significantly ($P < 0.001$) higher amounts were found in intensively-fed lambs. These differences may be due to the presence of different precursors of the Maillard reaction in the lamb meat, such as sugars and amino acids, since pyrroles and carbonyls are typical products of this reaction (Raes et al., 2003). Furthermore, despite their low contribution to the volatile profile (0.92% and 0.31% in intensive and extensive lambs, respectively) (Fig. 1), these group could be important in the general aroma of lamb meat because, for example, compounds containing nitrogen have low sensory perception thresholds (Maggiolino et al., 2020). In this way, lambs fed intensively could have a more intense aroma due to the significantly ($P < 0.001$) higher content of these compounds (Table 5).

Another factor to take into account about the general aroma profile is that the total content of volatile compounds was not significantly ($P > 0.05$) affected by the diet, since very similar values were detected for both production systems (4939.92 AU × 10^4/g fresh muscle for intensively-fed lambs; and 4855.01 AU × 10^4/g fresh muscle for extensively-fed lambs). These facts are in disagreement with reported by several authors, who reported that extensive productions originated higher amounts of volatile compounds (Maiorano et al., 2010). Despite this difference, our results could be justified because similar intramuscular fat content was found in both intensive and extensive animals (Table 1). Nevertheless, the correlations between fat and total volatile compounds were very low in both feedings and were only significant in the case of intensively-fed lambs ($r = 0.369$; $P < 0.05$, for intensively-fed lambs; and $r = -0.235$; $P > 0.05$, for extensively-fed lambs). In addition, although the total content is very similar in the two feeding systems, the lamb meat obtained could possess a distinct aromatic profile, since 9 of the 10 families identified were found in significantly ($P < 0.01$) different concentrations.

4. Conclusions

The present study showed that the production system (intensive and extensive) did not affect the chemical composition of the *Longissimus thoracis et lumborum* muscle of Gallega lambs, except for the ash content. Similarly, the total content of volatile compounds was not influenced by the rearing system. However, all the chemical families of volatile substances detected, with the exception of alcohol group, and most of the individual compounds were significantly affected by the diet. These differences could be mainly due to the different lipid profile of the feed supplied, since various fatty acids (such as linoleic acid) act as precursors of different volatile compounds. In addition, feeding can modify the metabolism of the rumen and therefore modify the volatile profile of the lamb meat. In this sense, in the present study benzyl alcohol and carbon disulfide for grass-based diets and 1-butanone, 2-heptanone, and furan, 2-penthyl for concentrate-based diets were proposed as appropriate biomarkers for the discrimination of intensively and extensively reared lambs, respectively. Additionally, the current work highlights the possible difference in the aromatic profile of Gallega lamb meat from both breeding systems.

Conflict of interest

The authors declare no conflict of interest.

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