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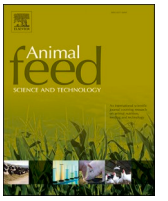

Silages of agro-industrial by-products in lamb diets – Effect on growth performance, carcass, meat quality and in vitro methane emissions

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ABSTRACT

The use of agro-industrial by-products in animal feed is an opportunity to reduce imports, food waste and promote a clean and circular economy, turning worthless raw materials into high-quality and low-cost animal feeds, which does not compete with human food. This study aims to produce silages with by-products of carrot, sweet potato, potato, and tomato pomace and integrate them in lamb diets, replacing 50% of dry matter (DM) of a concentrate-based diet. Effects on growth performance, meat quality and methane production were evaluated. Three silages were produced using 350 g/kg tomato pomace, 200 g/kg wheat bran, 150 g/kg alfalfa hay and 300 g/kg potato (Psil) or 300 g/kg sweet potato (SPsil) or 300 g/kg carrot (Csil). Thirty-two lambs were housed individually and divided into four groups (8 animals/group) on the following diets: Control - 850 g/kg concentrate and 150 g/kg hay; P - 500 g/kg concentrate and 500 g/kg Psil in DM; SP – 500 g/kg concentrate and 500 g/kg SPsil in DM; C – 500 g/kg concentrate and 500 g/kg Csil in DM. The trial lasted 6 weeks after 1 week of adaptation. Methane production was assessed in vitro by the Ankom system, using as inoculum rumen content samples collected individually from 6 lambs on each diet at slaughter. Diet had no effect on DM intake, average

Abbreviations: DM, dry matter; CP, crude protein; CF, crude fiber; NDF, neutral detergent fiber without sodium sulphite, without alpha amylase and expressed with residual ash; ADF, acid detergent fiber; ADL, acid detergent lignin; EE, ether extract; OMD,, organic matter digestibility; Psil, silage containing 350 g/kg tomato pomace, 200 g/kg wheat bran, 150 g/kg of alfalfa hay and 300 g/kg potato; SPsil, silage containing 350 g/kg tomato pomace, 200 g/kg wheat bran, 150 g/kg of alfalfa hay, 300 g/kg sweet potato; Csil, containing 350 g/kg tomato pomace, 200 g/kg wheat bran, 150 g/kg of alfalfa hay, 300 g/kg carrot; P, diet containing 500 g/kg concentrate and 500 g/kg Psil in DM; SP, diet containing 500 g/kg concentrate and 500 g/kg SPsil in DM; C, diet containing 500 g/kg concentrate and 500 g/kg Csil in DM; KKCF, kidney knob channel fat; IMF, intramuscular fat; LL, *Longissimus lumborum*; TBARS, thiobarbituric acid reactive substances; L^*, lightness; a^*, redness; b^*, yellowness; C^*, chroma; H^*, hue angle; ADG, daily weight gains; LW, live weight; ScF, subcutaneous fat; ΔE_3 and $\Delta E_{7,}$, color stability index of meat evaluated after 3 and 7days of storage respectively; MDA, malondialdehyde.

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daily gain and DM conversion ratio, averaging 1055 ± 248 g, 320 ± 61.1 g and 3.35 ± 0.600, respectively. Also, carcass characteristics were not affected by the diet except for the lightness of subcutaneous fat which was increased by the silage diets (L* value, $P = 0.016$). The meat parameters, pH, intramuscular fat, shear force, cooking losses and sensory attributes, were not affected by the diet. In meat color, the parameters a* and Chroma were highest in animals fed diet C, have an intermediate value with Control diet and are lowest with diets P and SP ($P = 0.027$ and $P = 0.012$, respectively). Diets had no impact on total methane emissions. The costs of the silage diets per kg live weight gain were lower than those of Control diet ($P = 0.018$). In summary, by-product silage can be a good option to replace concentrated feed in lamb diets without altering the growth performance and meat quality or methane production and thus contributing to the sustainability of livestock farms and the environment.

1. Introduction

In Mediterranean region, the lambs in the fattening phase are frequently fed with diets based on commercial concentrates, composed of mainly of imported raw materials (cereals and oilseed cake). The high dependence of this sector on external sources and the high price of raw materials in international markets, have brought more difficulties to the sector, compromising the viability of livestock farms (Braz, 2021). According FAO (2011), on a global level 1.3 billion tons of food are lost or wasted each year. The use of agro-industrial by-products as animal feed, particularly for ruminants, is an option that should be considered by the livestock sector and is encouraged by the European Union. It is a way to reduce imports, food waste and promote a clean and circular economy by transforming worthless raw materials into high quality, low cost animal feed that does not compete with human food (Vastolo et al., 2022). However, many of the available agro-industrial by-products have characteristics that hinder their use in animal feed. Among these characteristics, we highlight the seasonal production, the lack of homogeneity, the high moisture content and, often, the low acceptance by the animal, either due to low palatability or the presence of undesirable odors (Salami et al., 2019; Vastolo et al., 2022). In products with high moisture content, it is essential to apply preservation methods to stabilize the product and mitigate seasonal availability. Preservation techniques such as dehydration requires high energy inputs, resulting in high economic and environmental costs (Bremer et al., 2011; Salami et al., 2019). Ensiling by-products in mixtures with other feeds, is a strategy to overcome many of these limitations, obtaining stable and nutritionally balanced silages that can be produced without effluent losses and in which animal self-selection is avoided. Furthermore, through the fermentations that occur during ensiling, palatability can be improved and odors that may exist can be mitigated (Cao et al., 2009). Potato, sweet potato and carrot wastes are the products rejected during harvesting, packaging and processing as they do not meet the required marketing standards with regard to quality, size, shape, color, among other aspects. Tomato pomace is the by-product of the tomato concentrate industry consisting of seeds, skins and pulp waste. All these products are available in Portugal from August to October. They have a high moisture content and therefore deteriorate rapidly, requiring a method of preservation if they are to be used in animal feed outside the season of production. The objective of this experiment was to produce silages based on carrot, sweet potato and potato wastes and tomato pomace, and integrate them in diets for lambs during fattening, replacing partially commercial concentrate. The effects of the diets on the productive performance of the

Table 1
Chemical composition of raw materials used in silages.

	Potato	Sweet potato	Carrot	Tomato pomace	wheat bran	alfalfa hay
Nutrient composition, g/kg of DM, unless otherwise stated						
Dry matter (g/kg)	188	150	92.5	291	876	896
Crude protein	98.4	79	137	191	169	192
NDF	134	150	150	559	410	474
ADF	68.6	84.3	158	507	126	378
ADL	20.1	11.6	11.2	296	32.5	79.5
sugar	9.92	193	318	54.8	103	62.9
starch	497	381	362	17.3	312	17.4
Ether extract	2.80	7.2	18.6	116	33.3	10.7
Ash	86.4	64.0	114	32.0	49.0	101
Ca	0.68	4.18	5.96	1.4	1.00	12.1
P	2.48	1.90	4.76	4.5	8.91	2.97
Total phenol (mg GAE/g DM)[1]	2.24	12.7	52.4	10.1	-	-
α-tocopherol (μg/g DM)	14.5	19.3	135	127	-	-
β-carotene (mg/g DM)	nd[4]	9.40	60.6	8.97	-	-
FRAP[2]	7.04	42.0	128	28.0	-	-
TEAC[3]	6.64	21.6	90.5	14.5	-	-
Organic matter digestibility (g/kg)	763	769	857	579	726	551

[1] GAE, Gallic acid equivalents;
[2] FRAP, ferric reducing antioxidant power in μmol of Fe^{2+} equivalents/g of the diet;
[3] TEAC, trolox equivalent antioxidant capacity in μmol of trolox equivalents/g of the diet;
[4] nd, not detectable.

animals, carcass, meat quality and methane emissions were evaluated.

2. Methodology

2.1. Silages

The silages were carried out in the Estação Zootécnica Nacional in Santarém (INIAV-Fonte Boa), during the September 2020. The carrot, sweet potato and potato were the fresh produce waste intended for the domestic market and export, acquired from local companies. The tomato pomace is the by-product of the tomato concentrate industry, collected on the day of its production. Wheat bran and lucerne hay were purchased from the domestic market and were used as absorbents in all mixtures to increase the DM content. Carrot, sweet potato and potato were previously crushed into pieces of about 1 cm diameter in a semi-automatic industrial crusher (Talleres Cato S.A.) to facilitate compaction in the silo. Samples of all raw materials were collected for chemical and nutritional characterization and the results are presented in Table 1. Three mixtures were prepared for ensiling, to contain 400 g/kg DM and 160 g/kg crude protein (CP) in DM: Psil - 300 g/kg potato + 350 g/kg tomato pomace + 200 g/kg wheat bran + 150 g/kg alfalfa hay; SPsil - 300 g/kg sweet potato + 350 g/kg tomato pomace + 200 g/kg wheat bran + 150 g/kg alfalfa hay; Csil - 300 g/kg carrots + 350 g/kg tomato pomace + 200 g/kg wheat bran + 150 g/kg alfalfa hay. The mixtures were ensiled in black plastic bags of about 40 kg/bag using an ensiling machine. The bags started to be opened at the beginning of the animal trial on 3 March 2021. Each time a silage bag was opened, a silage sample was taken for chemical characterization. At the end of the trial, two composite samples were formed from each silage for chemical and fermentative characterization and the results are presented in Table 2.

2.2. Animals, diets and sample collection

This experiment was approved by the "Organisation Responsible for the Welfare of Experimental Animals" (ORBEA) which is the Ethics Committee of the National Institute for Agricultural and Veterinary Research (INIAV) in Portugal. The animal husbandry followed the Directive, /63 (2010)/EU on the protection of animals used in scientific experiments. Thirty-two Romane lambs weaned at 70 days of age were used. During the suckling period the animals were vaccinated against pasteurellosis and clostridioe (Heptavac® P Plus., MSD Saúde Animal, Portugal) and dewormed against coccidiosis (Toltrazutil, 20 mg/kg body weight). After weaning the lambs were transported to the facilities of INIAV-Fonte Boa, Vale de Santarem, where the experiment was conducted. At 78 days of age, they were weighed, randomly divided into four groups (8 animals/group) and individually housed in pens with the floor covered with wood chips and with permanent access to clean water. One of the following diets was distributed to each group: 1) Control – 850 g/kg

Table 2
Raw materials, chemical and fermentative composition of silages.

Raw materials (g/kg)	Silage		
	Psil	SPsil	Csil
Potato	300	-	-
Sweet potato	-	300	-
Carrots	-	-	300
Tomato pomace	350	350	350
Wheat bran	200	200	200
Alfalfa hay	150	150	150
Nutrient composition, g/kg of DM, unless otherwise stated			
Dry matter (g/kg)	444	453	431
Crude protein	163	166	171
NDF	415	436	435
ADF	270	276	305
sugar	13.4	20.1	14.5
starch	18.6	11.9	9.06
Ether extract	42.1	42.7	42.3
Ash	79.4	64.6	67.8
Ca	2.90	2.86	4.60
P	6.24	6.76	6.66
Total phenol (mg GAE/g DM)[1]	13.0	14.5	17.2
α-tocopherol (μg/g DM)	19.3	27.0	30.3
β-carotene (mg/g DM)	2.56	6.57	6.13
FRAP[2]	19.5	22.5	26.3
TEAC[3]	17.7	18.8	24.0
pH	4.16	3.97	4.10
Soluble-N (g/kg total N)	530	470	541
NH_3-N (g/kg total N)	4.72	3.29	4.52
Organic matter digestibility (%)	62.4	63.5	64.0

[1] GAE, gallic acid equivalents;
[2] FRAP, ferric reducing antioxidant power, μmol of Fe^2 +equivalents/g of the diet;
[3] TEAC, Trolox equivalent antioxidant capacity, μmol of trolox equivalents/g of the diet;

commercial concentrate and 150 g/kg grass hay; 2) P – 500 g/kg concentrate and 500 g/kg Psil in DM; 3) SP - 500 g/kg concentrate and 500 g/kg SPsil in DM; 4) C - 500 g/kg concentrate and 500 g/kg silage Csil in DM. The concentrate feed with 880 g/kg DM, 160 g/kg CP, 151 g/kg NDF and 614 g/kg starch in DM consisting of corn (283 kg/ton), wheat (250 kg/ton), barley (260 kg/ton), 44% soybean meal (179 kg/ton), calcium carbonate (14 kg/ton), sodium bicarbonate (8 kg/ton), salt (4.5 kg/ton) and a premix (2 kg/ton) contained Vit A (4000000 UI), Vit D3 (1100000 UI), Vit E (7.5 g/kg), Vit B1 and B2 – 250 mg/kg; Zn – 35 g/kg; Fe – 12.5 g/kg; Mn – 17.5 g/kg; I – 200 mg/kg; Co – 250 mg(kg; Se – 100 mg/kg. Grass hay distributed with the concentrate contained 910 g/kg DM, 79 g/kg CP and 683 g/kg NDF in DM. The costs of the diets were calculated based on the market prices of by-products, raw material and hay, practiced in Portugal in April 2022. Tomato pomace had no market value and so was considered as 0 cost. Daily silage was mixed in an electric feed mixer with concentrate. The diets were offered ad libitum, once a day at 9:00 am. The feed offered daily was calculated based on the previous day intake, weighing the amounts offered and refused in each pen, considering 10% of leftover. The trial was carried out for 7 weeks, comprising a 1 week of adaptation period to the experimental conditions, followed by a 6-week period of data collection. Samples of diets were collected daily, frozen at −20 °C and, at the end of the trial, a composite sample was analyzed for chemical characterization. The chemical composition of the diets is presented in Table 3. The animals were weighed weekly, before the distribution of diets.

During the adaptation period one animal from group P and another from group SP were withdrawn from the trial because got sick with urolithiasis.

2.3. Slaughter, carcass evaluation and sample collection

At the end of the trial, the lambs were weighed and transported to the experimental slaughterhouse at INIAV-Fonte Boa, where they were slaughtered after electrical stunning. After skinning, the carcasses were immediately weighed to obtain the hot carcass weight and placed at 10 °C for 24 h. After this period, they were weighed again to obtain the cold carcass weight and were classified according to EU carcass classification system for lamb carcasses (EC regulations Nº1234/2007 and Nº1249/2008 (E.C., 2011a, 2011b)) and then placed at 2 °C. On the third day after slaughter, the kidney knob channel fat (KKCF) and kidneys were removed and the carcasses were divided in two halves along the vertebral column and separated into 8 joints as described by Santos-Silva et al. (2002). The weight of the higher price joints was evaluated as the sum of the leg + chump + loin +ribs. The shoulder was vacuum packed and frozen at -20 °C for further dissection into muscle, bone, subcutaneous and intermuscular fat. The subcutaneous fat color was evaluated. The left and right loin joints containing the *Longissimus lumborum* (LL) were individually vacuum-packed and refrigerated at 2 °C during 7 days and after were frozen at − 20 °C during approximately 60 days until shear force, cooking losses and sensorial analysis. Samples of *Longissimus thoracis* muscle (LT) isolated from left rib joints were used for color and lipid stability, pH and chemical composition. For

Table 3
Chemical composition of the experimental diets.

Raw materials (g/kg DM	Diets			
	Control	P	SP	C
Concentrate	850	500	500	500
Grass hay	150	-	-	-
Psil	-	500	-	-
SPsil	-	-	500	-
Csil	-	-	-	500
Nutrient composition, g/kg of DM, unless otherwise stated				
Dry matter (g/kg)	887	588	575	553
Organic matter	944	936	961	940
Crude protein	160	171	162	164
Ether extract	18.6	28.3	30.7	32.3
Crude Fiber	99.5	140	158	170
NDF	228	279	308	298
ADF	121	173	184	188
ADL	13.7	56.7	56.7	61.4
Sugar	63.6	32.3	37.1	27.0
Starch	517	396	329	328
Ash	56.4	64.0	38.6	60.7
Ca	5.02	4.38	3.90	5.14
P	3.83	5.34	5.13	5.41
Total phenol (mg GAE/g DM)[1]	2.11	6.97	8.22	9.35
α-tocopherol (μg/g DM)	13.9	10.5	15.4	14.1
β-carotene (mg/g DM)	nd[4]	1.00	2.50	2.97
FRAP[2]	2.54	9.08	10.8	12.2
TEAC[3]	2.54	9.83	10.8	13.6
Organic matter digestibility (%)	82.4	76.9	74.4	74.1

[1] GAE, Gallic acid equivalents;
[2] FRAP, Ferric reducing antioxidant power, μmol of Fe^2 +equivalents/g of the diet;
[3] TEAC,Trolox equivalent antioxidant capacity, μmol of trolox equivalents/g of the diet;
[4] nd, not detectable.

lipid and color stability, three sub-samples of meat of around 1,5 cm in thickness were collected and color a lipid oxidation evaluated after 0, 3 and 7 days of storage. At day 0 of storage, meat color was evaluated after 1 h of blooming and then samples were vacuum packed and stored at − 80 °C until analysis. Other samples were individually placed on polystyrene trays, wrapped with oxygen permeable polyvinyl chloride film, and stored at 2 °C in an illuminated cooler for 3 and 7 days. These samples were vacuum packed and stored at − 80 °C until lipid oxidation analysis, after color evaluation.

For chemical analysis muscle samples were minced with a food processor and stored at – 20 °C until pH, DM and intramuscular fat (IMF) analysis.

2.4. Measurement of chemical, physical and sensory meat quality

The colors of subcutaneous fat and meat were evaluated using a CR-400 chromometer (Konica Minolta, Japan) with a 10 mm diameter aperture, a D65 illuminant and 2∘ standard observer. The CIELAB system was used, where L* is lightness, a^* redness and b* yellowness. Color saturation (chroma, C*) was calculated as $(a^{*2} + b^{*2})^{1/2}$ and hue angle (H*) was calculated as $\tan^{-1}(b^*/a^*)$ x $(180/\pi)$ (AMSA, 2012). The color stability index (ΔE) was determined between each day of storage ($n = 3$ or 7) and the day 0 of measurements, was calculated as $\Delta E_{(n\text{-}0)} = ((\Delta L^*_{(n\text{-}0)})^2 + ((\Delta a^*_{(n\text{-}0)})^2 + ((\Delta b^*_{(n\text{-}0)})^2)^{1/2}$. Lipid oxidation over storage time was assessed through the quantification of thiobarbituric acid reactive substances (TBARS), according to Grau et al. (2000) and the results were expressed as mg of malondialdehyde (MDA)/kg of muscle. For pH and chemical analysis, frozen samples of LT were thawed for 24 h at 4 °C and the pH measured in a suspension of 5 g of minced meat in 50 ml of potassium chloride 0.1 M, using a Metrohm 744 pH meter (Metrohm AG, Switzerland) equipped with a combined glass electrode and according to ISO 2917 (1999). Dry matter was determined according to ISO 1442 (1973). For assessment of cooking losses, the left loin joints were thawed for 24 h at 4 °C, the LL were isolated and cleaned of all external fat and connective tissue and weighed. Then, were cooked in an electric oven at 280 °C and the meat internal temperature was monitored using an internal type T thermocouple (Thermometer Omega RDXL4SD, Manchester, USA), until reaching 70 °C. After that, samples were cooled in a refrigerator at 4 °C during 20 h and were weighted (AMSA, 2015). The cooking losses were determined as the differences of samples weights before and after cooking and were expressed as percentage of the initial weight.

To shear force, samples of cooked meat with about 1 × 1 × 3 cm in dimension, cut parallel to the direction of the muscle fibers were sheared by using a texturometer TA-XT2 (Stable Micro System, Surrey, England) equipped with a load cell of 25 kgf and a Warner-Bratzler shearing device, running at a crosshead speed of 2 mm/s, along 25 mm. Results were expressed in N/cm2, and the mean of 25–30 measurements per sample was used for statistical analyses (Almeida et al., 2018).

Sensory evaluation was carried-out by a trained panel of eight members, three men and five women, with age ranging from 22 to 63 years. There were 6 trial sessions. Each judge received four or five different samples representing the various treatment combinations. *Longissimus lumborum* samples from the right loin joints were thawed and prepared as described above for shear force and cooking losses determinations. After cooked and after 10 min of stabilization at 40 °C LL samples were cut into 1 × 1 × 1 cm subsamples and two cubes were placed in a pre-heated disposable Petri dish, covered and held at 40 °C until evaluation (no longer than 30 min) (Almeida et al., 2018). Different meat properties were scored in a scale of 1–8 according to AMSA (2015): odor intensity (1 = extremely mild; 8 = extremely intense), juiciness (1 = extremely dry; 8 = extremely juicy), tenderness (1 = extremely tough; 8 = extremely tender), flavor intensity (1 = extremely mild; 8 = extremely intense), flavor acceptability, and global acceptability (1 = extremely inacceptable; 8 = extremely acceptable).

2.5. Chemical analysis of feed

Raw materials, silage and diets were analyzed for DM (ISO 6496, 1999), ash (ISO 5984, 2002), Crude protein (CP) (ISO 5983, 1997), ether extract (EE) (ISO 6492, 1999) sugar and starch (Clegg, 1956). Calcium was determined by atomic absorption spectrometry (ISO 6869, 2000) and P by UV/Vis spectrometry (ISO 6491, 1995). The NDF, ADF and ADL were analyzed according to Van Soest et al. (1991) with NDF analysis performed without sodium sulfite, without alpha amylase and expressed with residual ash. *In vitro* organic matter digestibility (OMD) was determined by the Tilley and Terry method modified by Alexander and McGowan (1961). In silages were measured the pH, N-NH_3 by the method of Conway (1957) and soluble-N by solubilization in artificial saliva (Dulphy and Demarquilly, 1981).

In the by-products, silages and experimental diets were determined the total phenols, α-tocopherol and ß-carotene contents following the methodologies described in Santos-Silva et al. (2022). In extracts prepared to total phenols analysis were evaluated the antioxidant activity using the ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) assays, which were performed according to Luciano et al. (2011).

2.6. Methane production

Total gas and methane production were evaluated in vitro using the Ankom system that measures the kinetics of the microbial fermentation in an automated fashion by monitoring the gas pressure produced in glass bottles that contain an inoculum (buffered rumen fluid) and the substrate (diet samples). The gas pressure is recorded in a computer spreadsheet. The system is also equipped with a temperature sensor, which is also registered. The inoculums used were rumen juices individually collected after slaughter of 6 rams from each diet. Rumen fluid taken from the animals consuming the same diet were mixed two by two (3 mixtures per diet). In the glass bottles were placed 30 ml of each rumen mixture, after filtered through four layers of gauze and 60 ml of a buffer solution as described by Menke and Steingass (1988), kept under anaerobic conditions by continuously flushing with CO_2. Diet samples (1 g of DM) were

incubated in duplicate in the buffered rumen solutions in the glass bottles placed in a shaking water bath at 39 ^{0}C. The diet samples incubated in each bottle were the same as those given to the rumen donors during the experiment. Two bottles without sample diets were used as blanks. Gas production was recorded every 20 min for 48 h and corrected with blanks. After the 48 h of incubation a sample of gas was collected from each bottle with a syringe into a Vacutainer® tube and analyzed for CH_4 concentration by GC-FID (HP6890A, Agilent, Avondale, PA, USA), equipped with a capillary column (TG-Bond Q 30 m x 0.53 mm × 20 μm, Thermo Scientific). The temperature of the injector was 200 °C and of the detector was 220 °C, in an isothermal run at 150 °C. In each run were injected 100 μL of gas injection, using a syringe Pressure lock series A-2 (1 ml), considering 5 replicates per sample.

2.7. Statistical analysis

Data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The experimental unit was the lamb and the level of statistical significance was set at $P < 0.05$. The variance homogeneity was tested for a level of $P = 0.01$, and when significant were accommodated in the model using the group option within the repeated statement of the Proc Mixed. The Individual daily weight gains (ADG) were determined using a random intercept regression model for the analysis of the individual weights recorded during the experiment. Individual ADGs were analyzed using a model that included initial weight as a covariate, diet as a fixed effect and pen as a random effect. Dry matter and nutrients intake were analysed using a model that included the diet as fixed effect. Intake data corresponded to repeated measurements on time in each pen. Therefore, the model used for intake also included the day of trial, considering a first order autoregressive (AR(1)) covariance structure, selected based on the Akaike information criteria (AICC). Diet was considered as the single fixed effect for slaughter weight, feed conversion ratio, carcass and meat quality traits data. Slaughter weight (BW) and hot and cold carcass weight were adjusted to the lambs' initial weight. The dressing percentage, KKCF (%), shoulder composition data were adjusted to the hot carcass weight. Shear force and cooking losses were adjusted for meat pH. For meat sensorial attributes the model included the diet and the panel judge were included as fixed effects and the session day as random block. For analysis of data from meat color and lipid oxidation, the model included diet and storage time (0, 3 and 7 days) as fixed effects and the diet x storage time interaction and considering the autoregressive (AR(1)) covariance structure. When the effect of the diet was significant, least square means were compared ($P < 0.05$).

3. Results

3.1. Nutrient intake and growth performance

Table 4 shows the amounts of DM and nutrients ingested by the animals fed with the experimental diets, their productive performance, the feed conversion ratio and the price of the diets reported to kg of live weight gain. Intake of DM and CP was similar among diets, with an average 1055 ± 248 g, 171 ± 40 g, respectively. The daily intake of NDF and total phenols was different between diets, being higher with SP and C diets, intermediate with P diet and lower with the Control diet. Intake of β-carotene was higher in C diet, followed by SP and by P. In Control diet β-carotene was not detected (Table 3). The α-tocopherol intake was higher with C diet,

Table 4
Nutrient intake, average daily gain and feed conversion and growth performance of lambs fed with the experimental diets.

	Diets				*P*-value
	Control[1]	P[2]	SP[3]	C[4]	
Intake (g/day)					
Dry matter (DM)	1024 ± 41.8	972 ± 46.4	1079 ± 48.7	1099 ± 63.4	0.328
Crude protein	157 ± 6.0	169 ± 10.3	176 ± 6.4	180 ± 10.4	0.137
NDF	183 ± 12.8^a	272 ± 13.7^b	333 ± 13.7^c	328 ± 18.9^c	< .0001
Starch	531 ± 17.9^b	388 ± 21.7^a	417 ± 21.7^a	456 ± 27.2^a	< .0001
Sugar	65.4.4 ± 4.63^c	31.5 ± 4.46^a	40.0 ± 2.79^b	29.7 ± 4.65^a	< .0001
Total phenol	2.17 ± 0.073^a	6.80 ± 0.517^b	8.85 ± 0.517^c	10.3 ± 0.484^c	< .0001
β-carotene	-	1.01 ± 0.061^a	2.70 ± 0.125^b	3.26 ± 0.188^c	< .0001
α-tocopherol (mg/day)	14.2 ± 0.57^b	10.2 ± 0.63^a	16.7 ± 0.61^c	26.5 ± 1.53^d	< .0001
Initial LW[5] (kg)	22.1 ± 0.86	19.9 ± 0.92	20.7 ± 0.92	21.2 ± 0.86	0.532
Slaughter weight, (kg)	36.0 ± 1.29	33.0 ± 1.38	34.8 ± 1.38	34.7 ± 1.29	0.118
ADG[6] (g/day)	327 ± 23.1	310 ± 24.6	303 ± 24.0	334 ± 22.4	0.781
Dry matter conversion ratio[7]	3.14 ± 0.217	3.19 ± 0.232	3.50 ± 0.232	3.56 ± 0.217	0.445
Diet cost /kg of weight gain (€)	1.40 ± 0.07^b	1.03 ± 0.080^a	1.14 ± 0.080^a	1.16 ± 0.075^a	0.018

[1] Control, 850 g/kg DM of concentrate and 150 g/kg DM of grass hay;
[2] P, 500 g/kg DM of concentrate and 500 g/kg DM of Psil;
[3] SP, 500 g/kg DM of concentrate and 500 g/kg DM of SPsil;
[4] C, 500 g/kg DM of concentrate and 500 g/kg DM of Csil;
[5] LW, live weight;
[6] ADG, average daily gain;
[7] Dry matter intake (kg) / weight gain (kg)
[a,b, c] Values within a row with different superscripts differ significantly at $P < 0.05$.

followed by SP and by Control and was lower with P diet.

The initial e slaughter weights were not affected by the diet, averaging 21 ± 2.5 kg and 35 ± 3.6 kg, respectively. The average daily weight gain and DM conversion ratio were similar between diets, averaging 320 ± 61.1 g and 3.35 ± 0.600, respectively. Diet costs per kg live weight gain (LW) were calculated individually, by multiplying the value of feed conversion ratio by the respective unit cost of the diet. The silage diets had lower prices than control diet.

3.2. Carcass and meat quality traits

Hot and cold carcass weights and dressing percentage were not affected by the experimental diets, averaging 16.9 ± 1.78 kg, 16.4 ± 1.76 kg and 48.9 ± 1.43%, respectively (Table 5). Also, no significant differences were found in the amount of higher price joints (540 ± 15.2 g/kg average), in KKCF (average 17 ± 05.2 g/kg) and in shoulder tissue composition, averaging 601 ± 37.0 g/kg for muscle, 212 ± 37.6 g/kg for bone, 99 ± 24.1 g/kg for intermuscular fat and 88 ± 21.7 g/kg for subcutaneous fat. In Fig. 1 are presented the conformation and fat cover scores of the lambs. For conformation, 77% of total lambs were graded as class R (good), 20% as U (very good) and 3% as O (regular). Of the 6 animals of class U, which correspond to 20% of the total, 3 are from the control group, 2 from the SP group and 1 from the C group. In the C diet animal, one carcass was graded as O. For fat cover, 97% of the carcasses were graded in classes 2 or 3 corresponding to slight and average levels of fat cover, respectively and 3% of the carcasses were graded in class 4.

In animals fed the control diet the subcutaneous fat (ScF) color, presented lower value for L^* than animals fed diets with silage diets. For a^* , b^* , C^* and H^* parameters, no significant differences were found averaging 3.9 ± 0.98, 7.0 ± 1.79, 8.1 ± 1.75 and 60.4 ± 6.5, respectively.

Chemical and physical meat characteristics were not affected by diet (Table 6). Meat pH, DM, and IMF averaged respectively, 5.88 ± 0.058, 237 ± 7.8 and 20.1 ± 5.59 g/kg of fresh meat. Shear force and cooking losses were 31.8 ± 12.90 N and 2.25 ± 0.21 g/kg, respectively. Data relative to meat color parameters and lipid stability are presented in Table 7. Interactions between diet and storage time did not show any significant effect on meat color parameters, color stability and TBARS, thus they are not reported. In meat color, the parameters a* and C* are highest in animals fed with C diet, have an intermediate value with control diet, and are lowest with diets P and SP. The L* , b* and H* parameters were not affected by diet, averaging 44.5 ± 2.54, 10 ± 1.94 and 31.9 ± 5.25, respectively. With storage time, these parameters as well as C* , increased until the 3rd day of storage. The a* parameter was not affected by storage time averaging 16.0 ± 0.25. The color stability index of the meat evaluated after 3 (ΔE_3) and 7 (ΔE_7) days of storage was not influenced by either diet or storage. Meat lipid oxidation increased over storage time, but did not differ among diets, averaging 0.84 ± 0.177 mg MDA/kg of meat.

The sensorial meat characteristics of lambs were not affected ($P > 0.05$) by diets. Average values for sensory attributes were 3.1 ± 1.65 for odour, 6.3 ± 1.21 for juiciness, 6.9 ± 1.06 for tenderness, 3.2 ± 1.75 for flavor, 6.01 ± 1.42 for flavor acceptability and 6.2 ± 1.21 for overall acceptability.

Table 5
Carcass traits and shoulder composition of lambs fed with the experimental diets.

	Diets				*P*-value
	Control[1]	P[2]	SP[3]	C[4]	
Carcass traits					
Hot carcass wt, kg	17.3 ± 0.47	16.5 ± 0.50	17.1 ± 0.49	16.8 ± 0.45	0.715
Cold carcass wt, kg	16.8 ± 0.46	16.0 ± 0.49	16.6 ± 0.47	16.3 ± 0.44	0.677
Dressing (%)	49.6 ± 0.53	48.6 ± 0.57	48.5 ± 0.55	48.7 ± 0.51	0.471
Higher price joints, (g/kg)	539 ± 5.8	535 ± 6.2	542 ± 6.0	544 ± 5.6	0.729
KKCF[5] (%)	1.72 ± 0.19	1.67 ± 0.21	1.73 ± 0.20	1.92 ± 0.19	0.792
Shoulder composition (g/kg)					
Muscle	590 ± 13.5	610 ± 14.4	602 ± 14.4	603 ± 13.5	0.762
Bone	232 ± 13.3	207 ± 14.2	208 ± 14.2	202 ± 13.3	0.417
Intermuscular fat	98.7 ± 8.76	106 ± .9.4	101 ± 9.4	91.0 ± 8.76	0.710
Subcutaneous fat	80.0 ± 7.06	77.2 ± 7.55	88.9 ± 7.55	104 ± 7.1	0.062
Subcutaneous fat color					
L*	75.3 ± 0.71[a]	78.6 ± 0.79[b]	78.3 ± 0.76[b]	77.7 ± 0.71[b]	0.016
a*	3.88 ± 0.360	3.87 ± 0.385	3.67 ± 0.385	4.09 ± 0.360	0.887
b*	7.34 ± 1.110	7.02 ± 0.308	6.84 ± 0.445	6.78 ± 0.417	0.944
Chroma (C*)	8.45 ± 0.64	8.03 ± 0.69	7.78 ± 0.69	7.94 ± 0.64	0.881
Hue angle (H*)	59.6 ± 2.39	61.2 ± 2.55	62.0 ± 2.55	59.1 ± 2.39	0.824

[1] Control, 850 g/kg DM of concentrate and 150 g/kg DM of grass hay;
[2] P, 500 g/kg DM of concentrate and 500 g/kg DM of Psil;
[3] SP, 500 g/kg DM of concentrate and 500 g/kg DM of SPsil;
[4] C, 500 g/kg DM of concentrate and 500 g/kg DM of Csil;
[5] KKCF, kidney knob channel fat;
[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$.

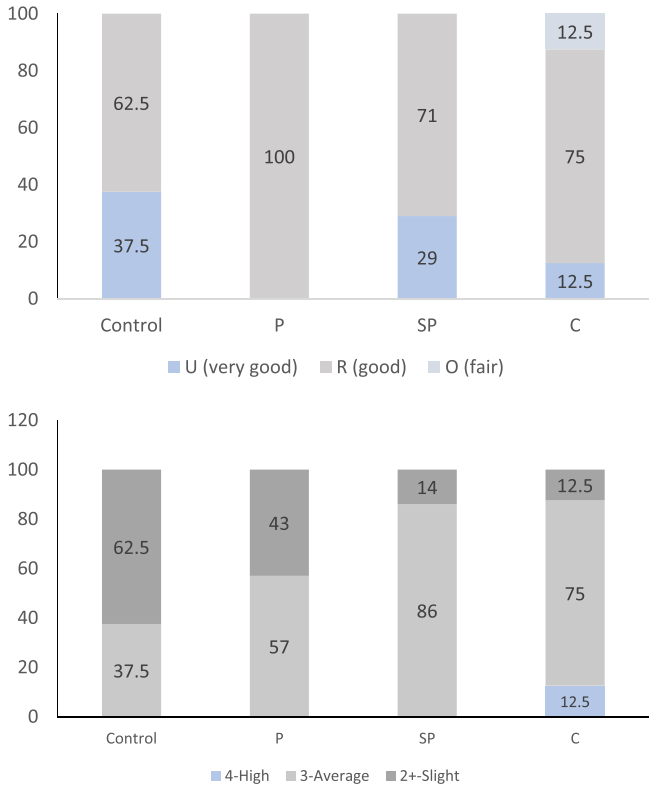

Fig. 1. Conformation and fat cover of lamb carcasses fed with the experimental diets evaluated according EUROP classification system for lamb carcasses weighing > 13 kg. Control, 850 g/kg DM of concentrate and 150 g/kg DM of grass hay; P, 500 g/kg DM of concentrate and 500 g/kg DM of Psil; SP, 500 g/kg DM of concentrate and 500 g/kg DM of SPsil**;** C, 500 g/kg DM of concentrate and 500 g/kg DM of Csil;.

Table 6
Physical, chemical and sensorial meat characteristics of lambs fed with the experimental diets.

	Diets				*P*-value
	Control[1]	P[2]	SP[3]	C[4]	
Meat quality					
pH	5.87 ± 0.021	5.88 ± 0.023	5.87 ± 0.023	5.90 ± 0.021	0.766
Dry matter (g/kg)	239 ± 2.7	232 ± 2.9	238 ± 2.9	238 ± 2.7	0.328
Intramuscular fat (g/kg)	20.4 ± 1.95	21.7 ± 2.09	21.8 ± 2.09	17.0 ± 1.95	0.309
Shear force (N)	41.7 ± 5.56	25.4 ± 2.79	37.9 ± 6.77	29.7 ± 2.60	0.080
Cooking loss (g/kg)	2.27 ± 0.071	2.21 ± 0.076	2.39 ± 0.076	2.16 ± 0.071	0.180
Sensory quality					
Odour intensity	3.18 ± 0.235	2.85 ± 0.253	2.90 ± 0.292	2.85 ± 0.257	0.053
Juiciness	6.39 ± 0.183	6.59 ± 0.197	6.51 ± 0.221	6.74 ± 0.197	0.130
Tenderness	6.89 ± 0.171	7.11 ± 0.184	6.96 ± 0.207	7.24 ± 0.184	0.063
Flavour	2.86 ± 0.171	2.73 ± 0.184	2.73 ± 0.206	2.83 ± 0.183	0.783
Flavour acceptability	6.09 ± 0.101	6.00 ± 0.107	6.00 ± 0.108	6.01 ± 0.101	0.906
Overall acceptability	6.33 ± 0.173	6.44 ± 0.186	6.20 ± 0.211	6.31 ± 0.187	0.410

[1] Control, 850 g/kg DM of concentrate and 150 g/kg DM of grass hay;
[2]P, 500 g/kg DM of concentrate and 500 g/kg DM of Psil;
[3] SP, 500 g/kg DM of concentrate and 500 g/kg DM of SPsil**;**
[4] C, 500 g/kg DM of concentrate and 500 g/kg DM of Csil;

3.3. In vitro methane production

The total gas produced, total methane and proportion of methane in total gas were not affected by diets (Table 8) averaging 212 ± 44.0 ml, 49 ± 18.6 ml and 22 ± 6.3%, respectively.

Table 7
Meat color and lipid stability over storage time.

	Diets				Storage time			*P value*	
	Control[1]	P[2]	SP[3]	C[4]	0	3	7	*Diet*	*Time*
L*	44.3 ± 0.47	44.9 ± 0.50	44.1 ± 0.50	44.6 ± 0.47	42.8 ± 0.42[a]	45.1 ± 0.42[b]	45.6 ± 0.42[c]	0.703	< .0001
a*	16.2 ± 0.27[ab]	15.7 ± 0.29[a]	15.5 ± 0.29[a]	16.6 ± 0.27[b]	16.1 ± 0.25	15.9 ± 0.25	16.1 ± 0.25	0.027	0.708
b*	9.99 ± 0.188	10.1 ± 0.201	9.71 ± 0.200	10.4 ± 0.18	7.63 ± 0.168[a]	11.1 ± 0.168[b]	11.4 ± 0.168[b]	0.069	< .0001
Chroma (C*)	18.4 ± 0.62[ab]	18.1 ± 0.60[a]	17.9 ± 0.52[a]	19.2 ± 0.54[b]	17.3 ± 0.55[a]	18.8 ± 0.55[b]	19.1 ± 0.55[b]	0.012	< .0001
Hue angle (H*)	33.5 ± 0.91	34.4 ± 0.89	33.4 ± 0.77	33.6 ± 0.79	27.1 ± 0.81[a]	36.9 ± 0.81[b]	37.2 ± 0.81[b]	0.531	< .0001
ΔE_3	4.45 ± 0.709	4.49 ± 0.731	4.88 ± 0.818	5.29 ± 0.685	5.68 ± 0.614	4.04 ± 0.614	4.61 ± 0.686	0.812	0.189
ΔE_7	5.44 ± 0.504	4.18 ± 0.521	4.72 ± 0.582	5.77 ± 0.487	5.83 ± 0.437	4.33 ± 0.437	4.91 ± 0.488	0.156	0.077
TBARS[5]	0.97 ± 1.157	0.62 ± 0.833	1.00 ± 1.056	0.78 ± 1.064	0.05 ± 0.028[a]	0.56 ± 0.438[b]	1.93 ± 1.063[c]	0.548	< 0.001

[1] Control, 850 g/kg DM of concentrate and 150 g/kg DM of grass hay;
[2] P, 500 g/kg DM of concentrate and 500 g/kg DM of Psil;
[3] SP, 500 g/kg DM of concentrate and 500 g/kg DM of SPsil;
[4] C, 500 g/kg DM of concentrate and 500 g/kg DM of Csil;
[5] In mg MDA/kg meat;
[a,b,c] Values within a row with different superscripts differ significantly at $P < 0.05$.

Table 8
Effect of diets on the in vitro gas and methane production after 48 h of incubation.

	Diets				*P*-value
	Control[1]	P[2]	SP[3]	C[4]	
Gas production (ml/g DM)	221 ± 21.3	208 ± 15.3	187 ± 17.1	225 ± 23.4	0.542
Methane total (ml/g DM)	45.5 ± 8.06	56.1 ± 8.83	47.7 ± 9.88	48.0 ± 8.83	0.838
Methane (ml/l gas)	189 ± 24.9	261 ± 27.3	242 ± 30.5	209 ± 27.3	0.260

[1] Control, 850 g/kg DM of concentrate and 150 g/kg DM of grass hay;
[2]P, 500 g/kg DM of concentrate and 500 g/kg DM of Psil;
[3] SP, 500 g/kg DM of concentrate and 500 g/kg DM of SPsil;
[4] C, 500 g/kg DM of concentrate and 500 g/kg DM of Csil.

4. Discussion

This study aimed to integrate potatoes, sweet potatoes, carrots wastes and tomato pomace into nutritionally balanced mixtures preserved as silage for use in lamb diets in partial replacement of concentrate based feed. All these by-products have in common a high moisture content which makes it difficult to achieve a high-nutritive value and stable silage (Khorvash et al., 2006). So, to reduce moisture in the mixtures, wheat bran and alfalfa hay were added. The quality of the silage obtained after five months of storage was good with low pH values (between 3.97 and 4.16), less than 10% of total N as NH_3-N and less than 60% of total N as soluble-N, values that define a good quality silage (Demarquilly and Andrieu, 1990). Also, the OMD and CP levels were high (> 60% and 160 g/kg in DM, respectively).

Due to the higher fiber content (NDF and ADF), lower starch and lower OMD of silages than the concentrate, inclusion of silage in the diet replacing 50% of the concentrate based diet provided a more fibrous diet. It is known that when intake is limited by the filling capacity of the rumen, diets with a high fiber content are associated with lower intake by animals (Bernes et al., 2012; da Silva et al., 2015). Also, foreign odors or palatability problems could be associated with the presence of the by-products and could affect intake. However, in our study no differences were observed between diets in the intake of DM, indicating a good feed acceptability by the animals and that the fiber content of silage diets did not limit intake. Also, ADG and DM conversion ratio were not affected by the inclusion of silage in the diets, and values are in accordance to those reported by Alves (2020) (345 g/day and 3.0, respectively for ADG and DM conversion) for Romane lambs during finishing stage and fed with a concentrate based diet.

It is known that feeding costs represent more than 63% of the total production costs in sheep (Raineri et al., 2015; Ellison et al., 2022). The use of non-conventional feedstuffs like agro-industry by-products, with zero or low price, is a potential strategy to reduce the feed costs and simultaneously reduce the competition between humans and animals for food (Yang et al., 2021). In our study, the use of silage diets instead of conventional concentrate based diet in finishing lamb led to a reduction of €0.29 in feed costs per kg live weight, which corresponds to 20% reduction.

Diets had no impact on carcass quality parameters such as hot and cold carcass weight, dressing percentage or higher price joints proportion. By visual evaluation, the carcasses were mostly classified as very good or good with light and medium fat cover (class 2 and 3) (Fig. 1). In group C there were predominantly carcasses with medium fat cover and one of the animals was classified with high fat content. These results are in line with the values of subcutaneous fat proportion determined on the shoulder of animals in group C which shows a tendency ($P = 0.06$) for higher subcutaneous fat in this group.

Animals fed silage-concentrate diets showed lighter subcutaneous fat (higher L^*), characteristic appreciated by consumers (Hopkins, 1996). Yellowness (b^*) was not affected by diet and the values obtained are clearly below the threshold value of 14.2

reported by Dunne et al. (2004), above which carcass may be depreciated in Italian market. Thus, the higher levels of plant pigments present in the silage diets, did not compromise carcass value by its deleterious effects on subcutaneous fat color. In the meat, the parameters a* and L* have the greatest influence on consumer choice (Hopkins, 1996). In this study, the a* and L* values of all lamb meat after seven days of storage, were greater than 14.5 and 44, respectively, values that, according to Khliji et al. (2010), indicate a good acceptance by consumers. Also, ΔE3 and ΔE7, parameters that express the combined changes of L* , a* and b* occurring among 0 and 3 and 7 days of meat under refrigerated storage, were not different between diets and the average values (below 6), indicate that color changes are not detectable by the consumer with the naked eye (Abril et al., 2001). No significant effect of the diet was observed on the lipid oxidation evaluated by the TBARS methodology. The TBARS values increased with storage time. However, the value observed at day 7 is lower than 2.3 mg MDA/kg meat, which is the value reported by Campo et al. (2006) as a threshold for sensory perception of lipid oxidation in meat. It is known that the oxidative stability of meat can be affected by compounds of food origin, such as carotenoids and phenolic compounds that have antioxidant properties (Jerónimo et al., 2012, Reda et al., 2022, Liadakis et al., 2022). Tomato pomace was the major source of tocopherols (α-tocopherol) in silage diets, while lucerne is a source of carotenoids (Prache et al., 2021). Moreover, carrots contained high levels of α-tocopherol, β-carotene and polyphenols. So, in diets with silage, the levels of total phenol, β-carotene, and the antioxidant activity expressed by FRAP and TEAC, were higher than in the Control diet so, one would expect some antioxidant effect of the silage-concentrated diets on meat oxidation. However, no effect was observed suggesting that the high amount of these compounds available in silage diets was not sufficient to produce any effect. Our results agree with those obtained by Valenti et al. (2018) and Forwood et al. (2021) who did not observe an effect of inclusion of tomato pomace and carrots in lamb diets on the lipid oxidative stability of meat. According to Noziere et al. (2006) in ruminants, the transfer of carotenoids from diet to animal product is relatively low due to the different steps including ruminal digestion, intestinal absorption and tissue metabolism. Moreover, the ensiling process may reduce the concentration of carotenoids (Nozière et al., 2006; Łozicki et al., 2015).

The chemical parameters of meat quality traits were not affected by the diet. The pH values are within the acceptable range (5.5–5.8) reported for lamb meat (Hopkins and Fogarty, 1998). No significant differences were detected for IMF. Reference values per IMF from Romane breed are not available to the best of our knowledge but the average, 20 g/kg in fresh meat, agrees with the results obtained in Merino Branco lambs raised under similar conditions and with similar carcass weights (Francisco et al., 2015; Santos-Silva et al., 2019). The value of IMF is low, less than 50 g/kg, the reference value for considering the meat as a low-fat meat (Food Advisory Committee, 1990). Although no significant differences were detected for shear force between diets, with all meat being considered tender with a shear point below 49 N/cm2, the threshold value for considering meat as tender (Hopkins et al., 2006) meat from the P and C groups tended to have a lower shear force than meat from the control and SP groups. The higher tenderness of meat from the P and C groups was later detected by some panel members in the sensory analysis. Juiciness, flavor, and overall acceptability were not affected by the diets. The meat odor from animals fed silage-concentrate diets tended to be less intense than that from animals fed the Control diet, which may be a differentiating and advantageous factor for consumers more sensitive to lamb meat odor.

The livestock sector is responsible for about 14.5% of total anthropogenic greenhouse gases (GHG) emissions and, within livestock productions, ruminants are the main source of GHG, mainly CH_4 (Gerber et al., 2013). These CH_4 emission, besides the greenhouse effect, also represent a substantial loss of energy and contribute to decreased feed efficiency (Waghorn and Hegarty, 2011). Methane production from ruminants is the result of microbial fermentation of feed in the rumen and therefore depends mainly on the composition of diets. Diets with higher fibrous components and lower digestibility are normally associated with higher CH_4 emissions (Gerber et al., 2013). Therefore, due to their nutritional and environmental effect, efforts to mitigate CH_4 emissions from ruminants, mainly through dietary manipulation, are receiving great attention. Thus, the evaluation of new nutritional strategies will have to take into consideration not only the effect on the productive performance of animals and the quality of the final product but also their environmental impact. In this study, the impact of silage-based diets on CH_4 production was evaluated in vitro. It would be expected that with the silage-concentrate diets, with a higher level of fibrous components and lower digestibility, CH_4 emissions could be increased. However, no significant differences between diets were detected probably due the high digestibility of all diets (>70%).

5. Conclusions

Tomato, potato, sweet potato and carrot are agro-industrial by-products with high moisture content and therefore highly perishable. Ensiling these by-products in mixtures is a good method of preservation, producing stable and nutritionally balanced silages. These silages can partially replace concentrate feed in lamb diets, without altering productive performance, carcass trait and meat quality or CH_4 production. The use of the silages byproducts as feed allowed to improve environmental sustainability, reducing feed prices and thus production cost.

CRediT authorship contribution statement

M.T.P.Dentinho: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition; **K. Paulos:** Methodology, Investigation, Data curation. **C. Costa:** Investigation. **J. Costa**: Investigation. **L. Fialho:** Investigation. **L. Cachucho:** Investigation. **A.P. Portugal:** Investigation. **J. Almeida:** Investigation. **I. Rehan**: Investigation. **A.T. Belo**: Writing – review & editing. **E. Jerónimo**: Methodology, Investigation, Writing – review & editing. **J. Santos-Silva**: Conceptualization, Methodology, Formal analysis, Writing – review & editing.

Conflict of interest

The authors have declared that no conflict of interests exist.

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