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## Impact of innovative rearing strategies for the Italian heavy pigs: Technological traits and chemical composition of dry–cured hams

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#### ABSTRACT

To explore the influence of 4 feeding strategies on dry-cured ham quality, 336 barrows and gilts (3 batches, 112 pigs/batch) of 90 kg body weight (BW), were divided into 4 groups and housed in 8 pens with automated feeders. In the control group (C), the pigs were fed restrictively medium-protein feeds and slaughtered at 170 kg BW (SW) and 265 d of slaughter age (SA). With the older age (OA) treatment, the pigs were restrictively fed low protein feeds and slaughtered at 170 kg BW (SW) and 265 d of slaughtered at 170 kg SW and 278 d SA. The other two groups were fed ad libitum high protein feeds, the younger age (YA) group was slaughtered at 170 kg SW and 237 d SA, the greater weight (GW) at 265 d of SA and 194 kg SW. The hams were dry-cured and seasoned for 607 d, weighed before and after seasoning and deboning. Sixty hams were sampled and sliced. The lean and the fat tissues were separated and analyzed for proximate composition and fatty acid profile. The model of analysis considered sex and treatment as fixed factors. With respect to C: i) OA lowered the ham weight, the lean protein content, increased marbling and decreased the PUFA proportion in intramuscular and subcutaneous fat; ii) YA hams had thicker fat cover with lower PUFA in intramuscular and subcutaneous fat, without alteration of the lean moisture content. Sex had a negligible impact.

#### 1. Introduction

The industry for the production of dry–cured ham originated in the Mediterranean area, however, it is now common in many regions of the world (Toldrà, 2010). Some product specifications necessitates that pigs attain heavier weights for the dry–cured ham production (Toldrà, 2010). In Italy, the consortia for the dry–cured ham protection indicate that pigs should be slaughtered at the minimum age of 9 months and at 160  $\pm$  16 kg body weight (BW) (Bosi & Russo, 2004; European Commission EC, 1992; Mordenti et al., 2003). To comply with these specifications a restricted feeding strategy is practiced in order to achieve pigs of high quality hams with the best aptitude for the dry–curring (Dalla Bona, Schiavon, Carraro, & Gallo, 2016; Gallo et al., 2014, 2015, 2016). If the standard required by the Protected Denomination Origin (PDO)

specification produces a high quality ham, following those guidelines has a significant impact on production cost (+20% compared to the EU average), mainly due to the prices of the feed ingredients and the duration of the fattening cycle (AHDB, 2022). In recent years, given the diffusion of modern pig genotypes in the production chain, the proportion of pigs too lean at slaughter increased with a contextual increase in the proportion of hams not labeled as PDO (INEQ, 2015). To address this issue, innovations in rearing strategies that ensure the traditional qualities of the dry–cured hams is needed.

Previous studies evidenced that pig's breed, age, body weight, diet composition and rearing system influence the carcass quality, the thigh characteristics and their seasoning aptitude (Carrapiso, Bonilla, & García, 2003; Latorre, Medel, Fuentetaja, Lázaro, & Mateos, 2003; Peloso, Lopes, Gomide, Guimarães, & Carneiro, 2010), and the resulting

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physical–chemical and sensory attributes of the dry–cured ham (Čandek-Potokar & Škrlep, 2012; Toldrà, 2010). The traditional feed restriction and reduction in dietary crude protein levels in feeding heavy pigs to reduce feed cost, environmental impact and increased marbling have been extensively studied (Bava, Zucali, Sandrucci, & Tamburini, 2017; Schiavon et al., 2015; Wood et al., 2013). However, optimizing conventional feeding practices will not alleviate the problem of excessive leanness of the pigs at slaughter and the high cost of production. In our recent study, we evinced innovative strategies that could offer the possibility to improve the pig performances, the feed efficiency and the quality of the thighs for heavy pigs destined for dry–cured ham production compared to the traditional one (Malgwi et al., 2021). In Malgwi et al. (2021, 2022) the impact of these innovative strategies on growth performance, carcass quality and green ham traits have been reported.

Data from the same pig population of Malgwi et al. (2021) experiment were used in the current manuscript to evaluate the impact of these innovative rearing strategies on the technological traits and the main chemical characteristics of seasoned ham, in comparison with the traditional rearing practice.

#### 2. Materials and methods

All experimental procedures performed in this study were approved by the animal ethics committee of the University of Padova ("Organismo preposto per il Benessere Animale", OPBA – approval document #36/ 2018) and they were conducted following the European Union directive for animal experiments (European Union EU, 2010/63/EU). The data used in this study were obtained from hams processed and dry–cured in our in vivo experiment that involved 336 (112 pigs  $\times$  3 batches) purebred Goland C21 barrows and gilts as detailed in Malgwi et al. (2021).

#### 2.1. Pig rearing and management

The in vivo experiment (Malgwi et al., 2021), was arranged as a splitplot design with treatment and sex within a pen, included 4 treatments, control and 3 groups, representing 3 alternative rearing strategies. On their arrival at the experimental station, the 112 pigs of each batch were divided into 8 pens for a total of 14 pigs/pens, with 2 pens per treatment and with an equal presence of barrows and gilts in each pen. An acrossbatch rotation scheme was used to assign treatment groups to pens of different batches so that each treatment was assigned to each pen. Within batch, means and standard deviations of initial BW were similar across the pens.

Pigs of the conventional rearing practice (C) were restrictively fed medium protein diets up to 170 kg BW and approximately 9 months of age (265 d). The OA pigs were fed restrictively low protein diets to achieve the target 170 kg slaughter weight (SW) at an older age (278 d) than C. It was hypothesized that a protein restriction could improve carcass and ham fat tissue deposition, partitioning a greater proportion of the dietary energy toward fat deposition (Bosi & Russo, 2004; Malgwi et al., 2021). Conversely, YA pigs were fed ad libitum high protein diets up to 170 kg SW and slaughtered at a lower slaughter age (SA, 237 d) than C. The increased dietary energy availability would promote a better carcass and ham adiposity. Similarly, GW pigs were fed ad libitum the same high protein diets of the YA group, but were slaughtered at 265 d of SA at a greater SW (194 kg) than C. The rationale of this treatment was that a greater slaughter weight would promote a greater ham weight, better fat covering and marbling, leading to a better overall quality of the ham (Malgwi et al., 2021, 2022).

The low protein diet was formulated to provide SID lysine below the estimated requirement for maintenance and growth, the medium protein diet provided SID lysine close to the requirements and the high protein diet provided SID lysine well exceeding the requirements (Schiavon et al., 2022). Criteria for feed formulation, the ingredient and the chemical composition of the diets are given in Malgwi et al. (2021), while the fatty acid composition of the feeds is given in Table 1. Feeds Table 1

Major nutrient contents	(% as-fed,	unless	otherwise	indicated)	of the	early	and
late finishing feeds <sup>1</sup> .							

Item <sup>1</sup>	Early finishing feeds (90 to 120 kg body weight)			Late finishing feeds (over 120 kg body weight)			
	OA <sup>3</sup> C <sup>4</sup>		YA and GW <sup>5</sup>	OA <sup>3</sup>	C <sup>4</sup>	YA and GW <sup>5</sup>	
Analyzed composition							
Dry matter	90.4	90.4	90.6	90.4	90.2	90.6	
Net Energy (MJ/kg)	10.1	10.0	10.0	9.9	10.0	10.1	
Crude protein (N $\times$	11.3	12.8	16.2	10.4	11.9	13.8	
6.25)							
Ether Extract	4.4	4.6	4.3	4.8	5.0	4.8	
Fatty acid profile <sup>2</sup>							
16:0	8.0	8.0	7.3	7.8	7.7	7.34	
18:0	2.9	3.0	2.8	2.8	2.8	2.8	
c18:1	13.0	13.0	12.2	12.6	12.6	12.3	
c18:2n-6	13.1	12.7	11.4	13.0	12.7	11.7	
c18:3n-3	0.8	0.8	0.7	0.8	0.8	0.7	

<sup>1</sup> The complete nutrient composition of the diets is given in Malgwi et al. (2021).

 $^2$  Computed based on the NRC (2012) tabular values of each feed ingredient.

<sup>3</sup> OA = low protein diet assigned to older age pigs.

 $^4$  C = medium protein diet assigned to control pigs.

 $^5\,$  YA and GW = high protein diet assigned to younger age and greater weight pigs.

were formulated maintaining the dietary fat content and its fatty acid profile constant across diets.

During the experiment, 11 animals were moved for health issues to the infirmary and were not considered in the statistical analysis. The pigs were slaughtered according to commercial practices (Malgwi et al., 2021), and the thighs were cooled at 4 °C, trimmed the day after slaughtering, and subsequently sent to the ham factory ("Attilio Fontana prosciutti", Montagnana, Padova, Italy) to be dry–cured according to the product specification of the "Prosciutto Veneto" (European Commission EC, 1992). Information about the growth performance, the carcass and fresh ham quality of the pigs of this study, are detailed presented in works of Malgwi et al. (2021, 2022) and Schiavon et al. (2022).

#### 2.2. Ham processing

A total of 325 experimental left thighs were processed into dry–cured ham. No selection for defects was practiced. At their arrival at the ham factory the thighs were weighted, trimmed again according to the factory practice, weighed again, completely covered with sea salt and stored at 2-3 °C for 6 d at a humidity of about 75 to 95% ("Salagione" or "Salting"; Fig. 1 – 1). Then the hams were brushed from the salt and massaged to favor a homogeneous salt absorption and complete bleeding using mechanical equipment. The hams were salted again ("Ripasso" or "Repetition"; Fig. 1–2) and kept at 2-3 °C for additional 5 to 8 d under salt. The total duration of the salting + repetition treatment was 11-14 d, according to the thumb rule of 1 d of salt exposure for each kg of ham weight.

The hams were desalted with compressed air (Fig. 1–3), to avoid microbial contamination, and pressed again to complete the ham bleeding and the salt absorption. The distal part of the legs ("Gambetto") was perforated, according to product specifications of the Veneto ham consortia, and the hams were hanged in a room at 1–3 °C for two weeks ("Pre–riposo" or "Pre–resting"; Fig. 1–4). This phase was followed by a grooming, particularly in the proximity of veins, and to a reduction of the iliac bone to make homogeneous the ham surface and drying. After that, the hams were rested ("Riposo" or "Resting"; Fig. 1–5) in refrigerated rooms at 2/4 °C for 90 days at a humidity in a range between 70 and 80%. The resting treatment was aimed to assure the shelf–life of the hams. In the past the resting treatment was performed using the cold conditions of the four winter months which are typical in the Veneto



Fig. 1. Timeline of dry-cured ham processing: from thighs arrival to the end of seasoning.

areal of ham production.

After these first 4 months, the hams were washed with water at 40 °C ("Lavaggio" or "Washing"; Fig. 1–6). This procedure is done for several purposes: i) to soften hams; ii) to prevent the formation of crust; iii) to clean the surface from salt; iiii) to trigger the proteolysis process. The hams were kept overnight at 20 °C at very high humidity (90%, "Asciugatura" or "Drying"; Fig. 1–7), and for about 40 days at 12–16 °C ("Pre–Stagionatura" or "Pre–Seasoning"; Fig. 1–8), to favor the progressive ham drying.

After these 40 days the parts of hams not covered by the skin were grouted with a dough composed by rice flour and lard ("Stuccatura" or "Grouting"; Fig. 1–9). At the end of this phase, the hams were ready for curing (Fig. 1–10). The following seasoning period of about 13 months is aimed to complete the ham drying and to favor the development of the flavor and the sensorial characteristics appreciated by the consumer. After about 4/5 months of curing a repetition of the grouting was performed to avoid the crust formation and to assure the homogenous hardness of the ham at slicing. The curing period was traditionally completed in 8 to 12 months in rooms miming the traditional cellars of the past, with the presence of wood, and opening or closing the windows according to the climatic conditions. However, according to the most recent procedures, the entire duration of the process in the current study was greater (20 months) because the salt exposure was shorter compared to what practiced in the past (European Commission EC, 1992). The lower salt exposition, aimed to produce hams with less salt content and sweeter, is desired for consumer health concerns (Di Vita et al., 2022).

#### 2.3. Ham measurements and evaluations

At the end of seasoning, the hams were weighted, deboned, and weighed again. The weight loss was computed as the weight of the ham trimmed at the ham factory less the weight at the end of seasoning. This loss, which is almost exclusively due to dehydration, was expressed as a proportion of the initial trimmed ham weight. The seasoned hams were scored by an expert for fat cover depth (1 to 5, 1 =thin; 5 = thick).

#### 2.4. Chemical analysis

Chemical analysis was performed on a sample of 60 hams, with 15 hams per treatment (7 or 8 hams per pen) and with gilts and barrows equally represented, randomly chosen among those of the second batch. The hams of each different treatment were collected in different times, according to the different durations of the pig raising periods but maintaining constant the duration of the dry-curing process. The hams were vacuum sealed in plastic bags and moved to the laboratory. A few days later the bags were opened, and the hams were sliced, 14 mm depth, in proximity of the head of the femoris.

One slice was used for chemical analysis after separation of the lean from the fat tissues, the other slice was used for physical analysis not presented in the current paper. The lean tissue, composed by the *biceps femoris, semimembranosus, quadriceps femoris* and *semitendinosus* muscles, was analyzed for dry matter (# 950.46), total protein (N  $\times$  6.25; #

981.10), lipid (#991.36), ash (# 920.153) according to AOAC (2012). The soluble N was assessed using a 10% trichloroacetic acid solution and then used to compute soluble protein as soluble N  $\times$  6.25. The proteolysis index was computed as the percentage ratio between the soluble and total protein.

Sodium was determined using inductive coupled plasma–optical emissions spectrometer (ICP–OES; Ciros Vision EOP, Spectro Analytical Instruments GmbH, Kleve, Germany) on 1 g of minced slice mixed with 7 mL of 67% nitric acid and 2 mL of 30% hydrogen peroxide and mineralized at 200 °C for 15–18 min in a microwave digestion system (Milestone Start, Sorisole, Bergamo, Italy). The samples were cooled to 35 °C and made up to volume with distilled water. Salt was calculated as Na  $\times$  2.5043 (European Union EU, 2011).

Fat was extracted from both the subcutaneous depot, and the lean part of the slice and analyzed for fatty acid (FA) profile as detailed in Carcò et al. (2019). After separate collection, grinding, homogenization, freezing, storing and thawing, fat was extracted from four g of samples of each tissue following an accelerated solvent extraction procedure (ASE, Thermo fisher Scientific Inc., Waltham, MA, USA) using petroleum ether as the solvent. Aliquots of 40 mg of extracted fat were methylated according to Christie (1993) using 2 mL of 2% sulphuric acid in methanol, resting overnight at 50 °C. The day after 2 mL of n-heptane and 4 mL of water with 2% potassium bicarbonate were added to the mixtures. These solutions were centrifuged (2834 g for 10 min), the supernatant was collected with a micropipette and transferred into vials for gas chromatographic analysis. The analysis was performed using an Agilent 7820 GC system (Agilent, Palo Alto, CA, USA) equipped with a flame--ionization detector and an Omegawax 250 capillary column (Omegawax 250, Supelco, Bellefonte, PA, USA; 30 m, 0.25 mm i.d.; film thickness 0.25 µm).

A split/splitless injector with a split ratio of 1:80 was used to inject an aliquot of the sample into the GC system under the following conditions: initial oven temperature 60 °C held for 1 min, then increased to 173 °C at a rate of 2 °C/min and held for 30 min, then increased to 185 °C at 1 °C/min and held for 5 min, and finally increased to 220 °C at a rate of 3 °C/min and held for 19 min. The injector temperature was set at 270 °C and the detector temperature at 300 °C. The identification of individual FA methyl esters in the chromatogram was obtained by comparison with a standard mixture (18918–1AMP 595 N, Supelco, Bellefonte, PA, USA) and expressed as grams per 100 g of total FAs. The FA methyl esters were quantified using methyl 12-tridecenoate as internal standard, and the area of each peak was corrected using flame ionization detector (FID) relative response factors determined after calibration from five serial dilutions for each standard fatty acid (all  $\mathbb{R}^2 > 0.997$ ).

The fat extracted from the lean part of the slice was also analyzed for the thiobarbituric acid (TBA) content, which reflects the presence of secondary lipid oxidation products, according to the procedure described in Carcò et al. (2019).

#### 2.5. Statistical analysis

The data of the 325 hams were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) using the following linear mixed model  $y_{ijklm} = \mu + treat_i + sex_j + (treat \times sex)_{ij} + batch_k + pen(treat \times batch)_{l:ik} + e_{ijklm}$ (A)

where  $y_{ijklm}$  was the observed trait;  $\mu$  was the overall intercept of the model, treat<sub>i</sub> was the fixed effect of the i<sup>th</sup> treatment (i = 1, ..., 4), sex<sub>j</sub> was the fixed effect of the j<sup>th</sup> sex (j: 1 = gilts, 2 = barrows), (treat × sex)<sub>ij</sub> was the interaction effect between treatment and sex, batch<sub>k</sub> was the random effect of the k<sup>th</sup> batch (k = 1, ..., 3), pen<sub>l</sub> was the random effect of the k<sup>th</sup> batch (k = 1, ..., 3), pen<sub>l</sub> was the random effect of the l<sup>th</sup> pen (l = 1, ...,8) within the (treat × batch)<sub>ik</sub> interaction, and e<sub>ijklm</sub> was the random residual. The pen, batch and residual effects were assumed to be independently and normally distributed with a mean of zero and variance  $\sigma_1^2$ ,  $\sigma_k^2$  and  $\sigma_e^2$ , respectively.

The chemical data (60 hams sample) were analyzed with a simplified model (Model B) that considered the treatment, the sex and their interactions as fixed effects, and the residual error as a random effect. The variance of the pen was always negligible, and thus this effect was excluded from the model.

With Model A, the effect of the rearing strategy was tested on the pen (treat  $\times$  batch) variance, whereas the effect of sex and of the rearing strategy  $\times$  sex interaction were tested on the residual variance. With model B the effects of treatment, sex and treatment  $\times$  sex interaction were tested on the residual variance. The 3 degrees of freedom due to the treatment were used in orthogonal contrasts to test mean differences of the 3 alternatives treatments compared to the conventional one.

#### 3. Results

#### 3.1. Ham weight, seasoning losses and fat cover depth score

In comparison with C, no differences were induced by the OA and YA treatment on the whole seasoned ham weight, whereas the deboned ham weight was decreased (P = 0.033) by OA, and the cover fat depth score was increased (P = 0.014) by YA (Table 2). The GW treatment increased the whole (P < 0.001) and deboned (P < 0.001) dry–cured ham weight, decreased the weight loss (P < 0.001) and markedly increased the cover fat depth score (P < 0.001).

The barrows had lower (P = 0.028) weight loss and greater (P = 0.033) cover fat depth than gilts (Table S1), whereas the sex × treatment interaction had negligible influence on the investigated traits.

#### 3.2. Chemical composition of the lean parts of the 60 sliced seasoned ham

The chemical composition of the lean tissue of the dry–cured hams is presented in Table 3.

Pigs subjected to the OA treatment, which were fed restrictively low protein diets, produced hams with lower contents of protein (P = 0.022), soluble protein (P = 0.005) and greater contents of lipids (P = 0.013) than C. The OA hams had also a greater TBARS index (P = 0.010) compared to C.

No differences were observed between YA and C. Despite the observed difference in weight of dry–cured hams, the lean tissue of the GW seasoned hams evinced minor modification compared to C. For instance, the soluble protein, the ash content and the proteolysis index of GW treatment were lower (P = 0.022, P = 0.030 and P = 0.031, respectively) than the C.

No significant differences in the chemical composition of the lean tissue were observed by effects due to sex and to sex  $\times$  treatment interaction (Table S2).

# 3.3. Fatty acid profile of intramuscular and subcutaneous fat of dry-cured hams

The mean values for the FA composition of intramuscular and subcutaneous fat of the 60 sliced hams are presented in Table 4.

The OA increased and decreased the intramuscular fat percentage of monounsaturated FA (MUFA; P < 0.001) and of polyunsaturated FA (PUFA; P = 0.008), respectively, compared to the C. These changes were also reflected in the main FA of the respective categories, with an increase in oleic acid (P = 0.001) and a decrease in linoleic and linolenic acid (P = 0.007 and P = 0.010, respectively). The YA treatment increased the proportions of saturated FA (SFA; P = 0.030) and in particular of palmitic acid (P = 0.020). A decrease in PUFA (P = 0.007) and linoleic acid (P < 0.001) was also observed. Compared to C, the GW treatment increased the MUFA fraction (P = 0.016), oleic acid (P = 0.024) contents and decreased the PUFA (P < 0.001), linoleic (P < 0.001) and linolenic acid (P = 0.032) contents. Although the increase in SFA of GW compared to C was not statistically significant, a remarkable increase in palmitic acid (P = 0.032) was detected in hams from the GW pig group.

The lipid content of subcutaneous fat was similar across rearing strategies, averaging 90 g/100 g, being the rest mainly represented by water. No differences in the FA profile were observed between OA and C. The YA treatment increased the SFA content (SFA; P < 0.001) and decreased the polyunsaturated one (P < 0.001) compared to C, and such variations were mainly due to an increase of the palmitic and stearic acid (P < 0.001) and linolenic acid (P = 0.021) content. The influence of the GW treatment was similar to that observed for the YA treatment. The SFA contents were increased (P < 0.001) and the PUFA were decreased (P < 0.001) compared to C as a consequence of higher palmitic (P < 0.001) and stearic acid (P = 0.039) levels and reduction in the levels of linoleic and linolenic acid (P < 0.001) contents.

The sex had minor influence on these traits. However, gilts had lower intramuscular fat MUFA content (P = 0.011) in and greater subcutaneous fat PUFA (P = 0.006), linoleic and linolenic acid content (P = 0.005 and P = 0.05, respectively) than barrows (Table S3). The sex × treatment interaction did not significantly affect the acid profile of intramuscular and subcutaneous fat of dry–cured hams.

Table	2
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Least-sq	uare means and	P-values of t	he effects of	different rearing	ng strateg	ies on weig	ht, weig	ht loss and	cover fat de	pth score of d	v-cured hams (	(n = 325).
					() ()		- / - / /					· · · · · · · · · · · · · · · · · · ·

Item	Rearing strategy				SEM <sup>5</sup>	<i>P</i> -value			
	$C^1$	OA <sup>2</sup>	YA <sup>3</sup>	GW <sup>4</sup>		C vs OA	C vs YA	C vs GW	
Age at slaughter, d	265	278	237	265	-	-	-	-	
Whole seasoned ham, kg	9.21	8.97	9.40	10.52	0.11	0.12	0.22	< 0.001	
Deboned seasoned ham weight, kg	6.90	6.65	7.07	8.00	0.091	0.033	0.17	< 0.001	
Weight loss, % of green ham weight	29.3	28.8	28.3	27.2	0.35	0.39	0.063	< 0.001	
Cover fat depth (1 thin,, 5 thick)	2.59	2.62	3.15	3.49	0.27	0.89	0.014	< 0.001	

 $^{1}$  C = control group.

<sup>2</sup> OA = older age.

<sup>3</sup> YA = younger age.

<sup>4</sup> GW = greater weight.

<sup>5</sup> SEM = pooled standard error of the means.

Table 3

Least-square means and P-values of the effects of different rearing strategies on the chemical composition of the lean tissue of seasoned dry-cured hams (n = 60).

Item	Rearing stra	tegy			SEM <sup>5</sup>	P-value		
	$C^1$	OA <sup>2</sup>	YA <sup>3</sup>	GW <sup>4</sup>		C vs OA	C vs YA	C vs GW
Dry matter, %	49.2	50.2	49.8	49.3	0.45	0.11	0.35	0.89
Protein (N $\times$ 6.25), %	29.7	28.6	30.1	29.2	0.34	0.022	0.44	0.33
Soluble protein, %	8.92	8.22	8.75	8.35	0.17	0.005	0.47	0.022
Proteolysis index, %	30.1	28.8	29.2	28.5	0.50	0.08	0.21	0.030
Lipid, %	10.4	12.6	11.5	11.4	0.59	0.013	0.20	0.24
TBARS <sup>6</sup> , %	2.31	4.18	2.89	2.77	0.50	0.010	0.41	0.52
Ash, %	6.96	6.79	6.69	6.60	0.11	0.29	0.10	0.031
Na, %	2.27	2.23	2.20	2.22	0.07	0.64	0.43	0.54
NaCl (Na $\times$ 2.50), %	5.68	5.58	5.50	5.54	0.16	0.64	0.43	0.54

 $^{1}$  C = control group.

 $^2$  OA = older age.

<sup>3</sup> YA = younger age.

<sup>4</sup> GW = greater weight.

<sup>5</sup> SEM = pooled standard error of the means.

<sup>6</sup> TBARS = thiobarbituric acid reactive substances.

#### Table 4

Least-square means and P-values of the effects of different rearing strategies on the fatty acid (FA) profile (%) of the intramuscular and subcutaneous fat of the 60 sliced seasoned ham.

Item <sup>1</sup>	Rearing strategy				SEM <sup>6</sup>	<i>P</i> -value			
	$C^2$	OA <sup>3</sup>	YA <sup>4</sup>	GW <sup>5</sup>		C vs OA	C vs YA	C vs GW	
Intramuscular fat									
ΣSFA	35.05	34.81	36.42	36.14	0.437	0.70	0.030	0.08	
16:0	22.56	22.36	23.44	23.37	0.26	0.61	0.020	0.032	
18:0	10.30	10.22	10.77	10.58	0.22	0.80	0.13	0.37	
ΣMUFA	49.77	51.64	50.04	50.96	0.337	< 0.001	0.56	0.016	
c18:1	41.22	41.72	41.61	42.23	0.30	0.001	0.36	0.024	
ΣPUFA	15.19	13.55	13.54	12.90	0.420	0.008	0.007	< 0.001	
c18:2n-6	12.50	11.12	11.11	10.59	0.35	0.007	< 0.001	< 0.001	
c18:3n-3	0.91	0.77	0.83	0.76	0.04	0.010	0.15	0.009	
Subcutaneous fat									
Lipid, %	89.84	90.63	91.01	90.61	0.51	0.27	0.11	0.29	
ΣSFA	33.48	32.99	35.31	35.07	0.310	0.27	< 0.001	< 0.001	
16:0	21.95	21.60	23.13	22.97	0.20	0.21	< 0.001	< 0.001	
18:0	8.80	8.67	9.33	9.29	0.16	0.58	0.024	0.039	
ΣMUFA	49.81	50.41	49.80	50.44	0.310	0.16	0.99	0.14	
c18:1	42.20	42.82	42.12	42.73	0.29	0.14	0.86	0.21	
ΣPUFA	16.71	16.60	14.88	14.49	0.220	0.79	< 0.001	< 0.001	
c18:2n-6	13.48	13.40	11.97	11.68	0.26	0.80	< 0.001	< 0.001	
c18:3n-3	1.18	1.13	1.08	1.01	0.03	0.17	0.021	< 0.001	

<sup>1</sup>  $\Sigma$ SFA = sum of saturated fatty acids;  $\Sigma$ MUFA = sum of monounsaturated fatty acids;  $\Sigma$ PUFA = sum of polyunsaturated fatty acids.

<sup>2</sup> C = control group.

<sup>3</sup> OA = older age.

<sup>4</sup> YA = younger age.

<sup>5</sup> GW = greater weight.

<sup>6</sup> SEM = pooled standard error of the mean.

#### 4. Discussion

#### 4.1. General considerations

Dry–cured hams are a traditional Mediterranean food, although they are also produced and consumed globally. Each country produces a distinctive type of dry–cured ham based on its unique traditions and market preferences. The primary variations across nations are the pig breeds used, the method of pig training and feeding, the dietary and feed ingredient specifications, and the curing techniques, which may include the inclusion of substances other than salt and the use of smoke (Toldrà, 2010).

Most dry–cured hams in Italy are produced in accordance with the PDO standards of the Parma and San Daniele consortia and other smaller traditional dry–cured ham PDO specifications, such as the Prosciutto Veneto. Regardless of the PDO specifications, the most significant criteria are the admitted pig genetic lines, the slaughter weight, and the slaughter age. To meet weight and age criteria, feed intake is restricted, however this lowers the growth rate, feed efficiency, and ham fat cover depth of modern pig lines (Malgwi et al., 2021). The dry-curing procedure for Italian PDO dry cured hams is based only on NaCl addition and ambient temperature and humidity regulation. Because this process is insufficient to correct for problems in fresh hams, the quality of the raw material is critical (Bosi & Russo, 2004). The effects of slaughter age, ham weight, and adiposity on ham quality were often statistically conflated. Generally, hams of older and heavier pigs offer better seasoning aptitude due to increased adiposity (Candek-Potokar & Škrlep, 2012). Adiposity is also positively correlated with fat saturation, which is desired to avoid rancidity and oiliness. Thus, these authors showed that, among the three possible determinants of ham quality, adiposity would play the major role. The level of subcutaneous and intra- and inter-muscular fat plays an essential role in the dehydration, salt penetration, and related physical effects such as pastiness and softness dynamics (Gou, Guerrero, & Arnau, 1995).

This study's experiment was designed to control, at least in part, the effects of slaughter age, slaughter weight (ham weight), and ham adiposity (fat cover depth and marbling) to ensure a better assessment of the determinants of ham quality. Because of the length of the dry–curing process, most existing literature focused on characteristics of the green hams, while data on the characteristics of seasoned hams remained lacking.

#### 4.2. Weight and major chemical components of the conventional hams

Seasoned ham weights in the current research ranged from 8 to 11 kg consistent with specifications of the major dry–cured ham consortiums (Table 2). The PDO specification of the Parma dry-cured ham indicates that the weight of the dry-cured ham must be in the range 8.2 to 12.5 kg, which is quite large. In agreement with others, the seasoning weight losses were approximately 29% of the ham weight at the start of processing (Sabbioni et al., 2004; Schivazappa et al., 2002). Deboning resulted in a 25% loss in dry–cured ham weight, which was significantly more than the 9–15% loss observed by Sabbioni et al. (2004), but similar to those reported by Carcò et al. (2019) and by the ham manufacturing operators at the time of weighing.

The chemical composition of the C ham lean portion given in Table 3 were similar to those reported in Carco et al. (2019) who used hams seasoned for 18 months according to the San Daniele PDO product specification. The chemical composition of the hams reported by Corino, Magni, Pastorelli, Rossi, and Mourot (2003), evidences a greater dry matter content (54%) however, the chemical analysis was performed on the whole slice that included the separable fat. Virgili and Schivazappa (2002) found that the moisture content of the lean fraction of different types of European dry-cured hams ranged between 45.2 and 60.8%. The moisture content of the lean fraction of the ham is subjected to a gradual reduction during seasoning and it is influenced by the amount of salt used and the thickness of the fat cover (Benedini, Parolari, Toscani, & Virgili, 2012). In recent years, the amount of salt used or the duration of salt exposure have been reduced to ensure consumer health (Martuscelli, Lupieri, Chaves-Lopez, Mastrocola, & Pittia, 2015). Current product specifications indicate that the NaCl content of the seasoned ham at biceps femoris muscle must be in the range 4.2-6.0%. There are no product specifications about the amount of salt to be used, but the thumb rule to reach these contents is one day of salt exposure per kg of green ham weight. However, the reduction of salt exposure inhibits proteolysis, decreases water extraction from the ham muscles, and alters the sensory qualities of the ham (Pinna, Saccani, Schivazappa, Simoncini, & Virgili, 2020). As a result, the seasoning period must be extended to compensate for the lower exposure to the salt (Benedini et al., 2012). The hams in the current study were seasoned for about 20 months, longer than the 14-month minimum required by the various PDO consortia (European Commission EC, 1992).

The FA composition (given in Table 4) is essential for sensory and nutritional ham quality. Increasing PUFA levels is responsible for the undesirable degree of oiliness and softness of the fat (Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000). A greater degree of adiposity is often related to a greater proportion of SFA, which is desirable since it reduces the incidence of oxidation, rancidity, and oiliness caused by a prolonged maturation process (Virgili & Schivazappa, 2002). A lower adiposity is related to a greater proportion of PUFA, mainly phospholipids, which can be easily oxidized (Pastorelli et al., 2003). To prevent such problems, several consortia's production specifications stipulate that the maximum level of linoleic acid in the subcutaneous fat of green hams must not exceed 15% of total FA, and that the maximum level of linoleic acid in the ration must not exceed 2% of FA (European Commission EC, 1992). Notwithstanding, the ham factory operators frequently complain about an increase in the proportion of hams with insufficient fat cover depth and an excess of linoleic acid in recent years, which is most likely due to the gradual increase in lean pig genotypes together with the conventional feed restriction strategy.

#### 4.3. Older age ham traits

To improve the quality and sensorial characteristics of the ham and to mitigate the environmental impact of pig production some authors proposed a protein restriction in addition to an energy restriction (Lebret, Juin, Noblet, & Bonneau, 2001; Malgwi et al., 2021; Wood et al., 2013). In the current study, protein restriction significantly reduced the rate of growth and feed efficiency, with pigs reaching their targeted BW approximately one month later C. There were few changes on the proportions of lean and fat carcass constituents (Malgwi et al., 2021). No research was undertaken to assess the effect of OA treatment on the characteristics of the hams at the end of the dry-curing process. Our results suggest that the energy-protein restriction had minor effect on the overall weight of the seasoned ham, but had greater effect on the weight of the deboned ham, which reduced by 3.6% compared to C. This suggests that, in addition to decreasing protein growth, protein restriction had an effect on the bone formation and the protein:bone ratio of the pigs, which is consistent with previous research (Rouy et al., 2014). This finding is also in agreement with the close allometric relationship that relates body ash to body protein mass, that is characterized by a coefficient of allometry close to the unit (NRC, 2012). Thus, the statement that ash deposition proceeds at a constant ratio to protein deposition (Ferguson, Gous, & Emmans, 1994) that is valid at animal level, would be also valid for some anatomical parts of the body such as the legs.

The protein restriction had little or no effect on the dry-curing weight losses and cover fat depth score of the dry-cured ham compared to C. Most noteworthy were the effects of dietary protein restriction on the chemical composition of lean tissue hams, with crude protein and soluble protein levels decreasing by 3.7 and 7.8%, respectively, and lipid and TBARS content increasing by 21% and 81%, respectively, when compared to C. It was previously evidenced that with low protein diets, lipogenic enzymes are expressed more readily in muscle than in subcutaneous fat, suggesting that intramuscular fat may increase more than fat in other depots when this feeding strategy is used (Schiavon et al., 2015; Wood et al., 2013). This increase in fat deposition in lean tissue is of interest since it would improve the technological and sensory properties of the ham (Cernadas, Fernández-Delgado, Fulladosa, & Muñoz, 2022). This data confirms that insufficient dietary protein supply shifts energy partitioning toward greater synthesis of intramuscular fat (Wood et al., 2013).

On one hand, the OA treatment had a negligible impact on the FA profile of the subcutaneous fat. On the other hand, this strategy changed the intramuscular fat content and its FA profile. In fact, when compared to C, the percentage of MUFAs increased while the percentage of PUFAs decreased. These changes could also be observed in the relative proportions of the two FA categories' individual FA (e.g., oleic +1.2% and linoleic -11%). However, despite these variations in the fatty acid profile, the OA treatment evidenced a strong increase in the TBARS variable, which is a measure of fat oxidation. The compounds produced during lipid oxidation are associated with the development of rancid taste and they are one of the main factors limiting the acceptability of meat products (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Toldrà, 2010).

#### 4.4. Younger age ham traits

Meat from young and lean pigs is inadequate for ham processing due to high moisture content, which raises the activity of hydrolytic and proteolytic enzymes, promotes dry–curing water losses, and compromises the end–sensory product's characteristics (Čandek-Potokar & Škrlep, 2012). This strategy was designed to answer the question raised by Bosi and Russo (2004): could an younger subject with an optimal fat covering be suitable for the typical production? Previous research with heavy pigs for dry–cured ham production found that greater age at slaughter was often associated with increased body weight, green ham weight, and ham adiposity, therefore the impacts of these parameters were statistically ambiguous (Čandek-Potokar & Škrlep, 2012; Malgwi et al., 2021). In this study, it was observed that the early slaughter age of the YA treatment (8 months of age) had a limited impact on the whole and deboned ham weight when compared to the C treatment. Despite some improvements in the green ham's cover fat depth score (Malgwi et al., 2021), the YA treatment had minimal effect on ham weight loss and the final water content of the dry–cured ham's lean tissue. It could be possible that the prolonged duration of the seasoning period may have reduced the difference between the C and the YA treatment.

Concerning the FA composition of the YA compared to the C treatment, an increase in the relative amount of SFA at the expense of PUFA might important as a factor to improve the ham shelf life. In fact, the SFA are less prone to oxidation and rancidity than PUFA. As a result, the fat becomes "whiter" and less "oily." Regardless, an increase in visible fat in the slice may be an unappealing trait for customers (Pastorelli et al., 2003).

From a practical viewpoint, this YA treatment is of interest to the dry–cured ham pig producers as it offers the possibility of enhancing feed efficiency and environmental sustainability with negligible impact on the green and dry–cured ham quality compared to the conventional feed restriction (Malgwi et al., 2021). However, there is need for further investigation to ascertain the effect of this treatment on the physical and sensory characteristics of the dry–cured ham in comparison with the conventional rearing practices.

#### 4.5. Greater weight seasoned ham traits

The GW pigs were slaughtered at the same age, but at a greater slaughter weight (193 kg) compared to C (170 kg). This was due to the effect of ad libitum feeding compared traditional restricted feeding, and this was associated with an increase in the weight and adiposity of the dry-cured ham (Malgwi et al., 2021). Generally, the fat content of the ham is an important factor in determining the technological and sensory quality of the ham. The subcutaneous fat, as well as the intermuscular and intramuscular fat, is a barrier that limits salt penetration and water diffusion (Bosi & Russo, 2004). However, an insufficient fat covering reduces water seasoning losses because rapid dissection promotes the formation of a crust, which limits dehydration during seasoning (Bosi & Russo, 2004). Once this occurs, the inner part of the ham becomes soft, impairing the slicing quality (Carcò et al., 2019). The GW had 7.2% lower ham weight loss, but the dry matter, protein, lipid, and salt content of the lean parts of the hams did not differ from those of the C. This might imply that the water content of the green ham was lower than C at the start of the processing, most likely due to a greater proportion of adipose tissue in the ham, which is lower in moisture than muscle tissue. When compared to C hams, the soluble protein concentration and the proteolysis index were significantly lower in the GW. Proteolysis, or the release of peptides and free amino acids, is advantageous to the sensory quality of dry-cured ham since it is associated with distinctive aroma and taste (Harkouss et al., 2015). However, an excessive proteolytic process results in unpleasant aroma and off-taste and it reduces the slicing aptitude of the ham (Pérez-Santaescolástica et al., 2018). Nevertheless, a proteolysis value between 24 and 31% is considered the optimal range by some product specifications (European Commission EC, 1992).

In general, higher dietary energy allocation resulted in an increase in the proportion of MUFA at the cost of the PUFA fraction, with a 2% reduction in the proportion of linoleic acid. This might be used as an effective method for enhancing the quality of hams. From a practical standpoint, the findings of this study, along with those obtained in vivo by Malgwi et al. (2021), indicate that the GW strategy would result in beneficial effects on the weight, fat cover depth, and proteolysis index, as well as the FA profile of the hams, with little influence on feed efficiency when compared to C pig feeding practiced.

#### 5. Conclusions

This study evaluated the impact of 3 innovative feeding and management strategies on the technological and chemical characteristics of dry-cured hams. All the treatments caused an increased ham adiposity, except the OA, which was instead associated to a production of lighter hams with greater marbling fat, but with a strong worsening of the feed efficiency. This would limit the implementation of the OA strategy in practice. The YA treatment produced hams with greater fat cover depth score, but with little difference in the chemical lean composition compared to C. The YA treatment, based on ad libitum feeding diets with 138 g/kg of crude protein from 120 to 170 kg BW, would permit to achieve a product similar to the traditional one, but with a greater fat cover and a strong improvement of feed efficiency. The GW, on the other hand, resulted in heavier hams, thicker fat cover and marbling, less seasoning losses, and similar feed efficiency than C. Further research is needed to investigate the effects of such modifications of rearing strategies on quality and sensorial attributes of dry-cured hams.

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#### **Declarations of Competing Interest**

None.

#### CRediT authorship contribution statement

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#### **Declaration of Competing Interest**

None.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

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