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Use of mirrors into free-range areas: effects on rabbit meat quality and storage stability

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ABSTRACT

Nowadays, animal welfare is driving consumers' purchase choice, hence the challenge to recover rabbit sector from many years of downturn is to find a valuable compromise between animal welfare, farmers' needs, and meat quality. Among the efforts to improve rabbit welfare, mirrors have been proposed as cages or pens enrichment; however, it is unclear if they affect rabbit meat quality. Hence, the present study aimed to evaluate the effect of the use of mirrors into free-range areas on color, weight loss, pH, water holding capacity (WHC), fatty acid profile (FA), conjugated dienes (CD) and thiobarbituric acid reactive substances (TBARS) of both fresh and stored rabbit meatballs. Specifically, rabbits were divided in three farming groups: in the open group (OG) the rabbits of three replicates could see and smell each other; in the closed group (CG), plastic sheets isolated each replicate; the replicates of the mirror group (MG) were confined as the CG but animals could mirror themselves thanks to two mirrors (120 × 40 cm each) placed inside the area. After 49 days, nine rabbits from each group were slaughtered, their carcass meat was minced to form 40 meatballs for each group destined to be analyzed (n = 10) immediately (T0), and after 20 (T20), 40 (T40), and 80 (T80) days of frozen storage (-10°C). The meatballs physical traits were similar among the farming groups, while the pH value was lower in MG (6.02) than OG (6.07), and CG (6.07) meat ($P < 0.05$). The farming slightly affected the FA content, being the PUFAn-3 amount between 60 and 121 mg/100 g ($P > 0.05$). Concerning the oxidative status, both CG and MG groups had higher ($P < 0.01$) TBARS than the OG, while the CDs were unaffected. The duration of storage modified all the physical traits, for instance the weight loss increased ($P < 0.001$) and the WHC diminished (-5.86%) during the early 20 days. Only the OG group showed a specific ability to maintain its redness value till the T40. Even though the overall PUFAn-3 fraction did not vary, both C20:5n-3 and C22:5n-3 were halved ($P < 0.01$) and CD ($P < 0.001$) doubled during the early 20 days. In conclusion, the OG farming was the best method for improving meat quality; however, mirrors can minimize the negative effects of a confined housing on the quality items, hence representing a valuable environmental enrichment.

Introduction

The size of rabbit meat production in the European Union (EU) amounts to 180 million rabbits annually, predominantly concentrated in few Member States, namely Spain, France and Italy (European Commission, 2017). Supporting the economy and providing jobs in rural areas are among the main goals of this livestock sector, frequently highlighted by different institutions, such as the European Commission and European Food Safety Authority Panel on Animal Health and Welfare (EFSA AHAW Panel, 2020). Despite the European undertaking, the rabbit meat consumption in Europe need to recover from many years of downturn, which means that the market demand, needs to be carefully analyzed. From one side, this crisis coincides with consumers' increasing inclination to look at rabbit as a pet (Cullere and Dalle Zotte, 2018). From the other side, meat purchases are more often driven by ethical statements, being animal welfare one of these ones. In this regard, 85% of total EU production is still carried out into traditional cages, 9% in enriched cages, while only the remaining 6% in enriched pen housing systems (European Commission, 2017). It is well established that conventional cages limit two points of the "five freedoms" of the animals (freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behavior; freedom from fear and distress), so that they cannot be considered "animal friendly". A recent EFSA Opinion (EFSA AHAW Panel, 2020) concluded that both traditional and enriched cages prominently restrict movements, gnawing and resting behaviors compared

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to floor or outdoor pens. Furthermore, the social contact, which is a fundamental rabbit attitude, is inhibited by the conventional farming systems enough to cause stress, frustration and stereotypic behaviors (Hansen and Berthelsen, 2000). However, despite the EU Parliament suggestion to abandon cages in favor of collective pens (European Commission, 2017), the stocking densities and the difficulties in hygienic condition maintenance can compromise growth performance (Loponte et al., 2018) and carcass quality traits (Combes et al., 2010) of floor and outdoor farmed animals.

Among the efforts to improve rabbit welfare, either in cages or in pens, mirrors have been successfully proposed as environmental enrichment for fattening rabbits (Dalle Zotte et al., 2009) by increasing their behavioral complexity (Jones and Phillips, 2005). The hypothesis is that the use of mirrors might deceive the animals of a greater presence of conspecific without increasing the actual available space (Dalle Zotte et al., 2009). This practice theoretically results in a good compromise between rabbit welfare and farmers' needs. To prove it, Musco et al. (2019) compared the growth performance and haematological parameters of rabbits grown in freerange area with the possibility to see each other (open group), isolated from the other replicates (blind group), and isolated from the other replicates but with the possibility to see themselves reflected in a mirror (mirror group). The authors showed that the rabbits from the mirror group had the best Feed Conversion Ratio (FCR) and net dressing out values due to their moderated locomotion activity, finally proposing the use of mirrors as a valid model for "small group" free-range farming system, able to maximize the animals' health and productive performance. Nevertheless, rabbit meat quality and its shelf life might be influenced by the farming system in various extents (Matics et al., 2014; Paci et al., 2014; Secci et al., 2019). Hence, we continued the trial of Musco et al. (2019) through the evaluation of the main physical and chemical quality parameters performed on both fresh and stored meat.

Materials and Methods

The experimental procedures were approved by the Ethical Animal Care and Use Committee of the University of Napoli Federico II, Italy (prot. N. 2019/0058989) according to the principles stated by the EC Directive 2010/63/UE, regarding the protection of animals used for experimental and other scientific purposes.

This work supplements the information provided by Musco et al. (2019) where the growing trial, conducted in a private farm located in Avellino (Italy), has been fully described. Briefly, 81 male rabbits (New Zealand White × California) were weaned at 32 days of age, then moved from their bi-cellular cages into free-range areas (2.0 × 1.5 m) at 45 days of age (mean weight 1,422 ± 79 g) and they remained there until 94 days of age. Overall, the experimental farming trial lasted 49 days. The animals were randomly distributed in three experimental groups (27 rabbits each) constitutes by nine replicates of three rabbits each one (available space 1 m^2/head). In the first group, the rabbits of each replicate could see and smell the rabbits of the other replicates (open group, OG); in the second group, plastic sheets were placed along the entire perimeter of the housing system in order to isolate one replicate from another (closed group, CG); the replicates of the last group were confined as the CG but two mirrors (120 × 40 cm each) were placed in a corner of the perimeter, near the feeding area, to form a 90 degree angle, hence the animals could mirror themselves (mirror group, MG).

Supplementary information about farming condition as free-area confinement, feeding points, floor material, temperature and humidity conditions can be retrieved from Musco et al. (2019). All the groups were fed *ad libitum* the same commercial diet (showed in Musco et al., 2019) administered since the first period of the bi-cellular cage farming. The calculated Feed Conversion Ratio (FCR) values were 5.36 (ab), 5.88 (a), and 4.97 (b) for OG, CG, and MG, respectively ($P < 0.05$).

At 94 days of age, nine rabbits per group (one rabbit for replicate) were moved to a specialized slaughterhouse where head, liver, whole heart, lungs, esophagus, trachea, thymus gland and kidney free of the perirenal fat of the individuals were removed. For our purposes, we decided to consider the remaining meat of the carcass after the separation of hind legs and *Longissimus thoracis et lumborum* muscles. Overall, 1,490 g, 1,632 g, and 1,565 g of meat were obtained from OG, CG, and MG, respectively. These amounts were then minced by a manual grinder (0.5-hole diameter; Westmark, Elspe, Germany) before being processed as meatballs (around 35 g) with a Meatball Maker (Snips, LO, Italy) equipped with 16 shapes. Globally, 40 meatballs for each group were formed and weighed prior to be separated into 4 groups (n = 10) that were analyzed immediately (T0), and after 20 (T20), 40 (T40), and 80 (T80) days of frozen storage (-10°C). The stored ones were individually inserted into common aluminum bowls (Cuki, Volpiano, Italy) and covered with an aluminum foil. We decided to adopt this scheme to verify a possible interaction between the farming system and the oxidative stability during the storage. Hence, the meat was minced to facilitate lipid oxidation, we set the storage temperature at -10°C to observe the oxidative changes in a feasible time and to slow down the microbiological growth, and we use only the meat of carcass residual after the hind legs and *Longissimus thoracis et lumborum* muscles removal because it is frequently sold as processed meat and not as whole one. The patties underwent the measurements of the physical and chemical parameters detailed below.

2.1. Physical and chemical characterization

Weight, color and pH were recorded at each sampling time, after 12 h of thawing at 4°C in the case of the T20, T40 and T80 meatballs. Briefly, the meatballs were gently removed from the bowls, manually wetted with common kitchen paper, and then weighed. Drip loss (%) was calculated as the difference between the initial weight of the ball and its weight after the storage, divided for the initial weight. Color measurements were carried out through a Konica Minolta colorimeter (Chiyoda, Japan) used on three different superficial points of the patties expressed as lightness (L*), red (a*) and yellowness (b*) indexes, according to the CIELab system (Commission Internationale de l'Éclairage, 2004). Similarly, pH was measured in three different points of each meatball, using a pH meter Mettler-Toledo (Schwerzenbach, Switzerland). Water holding capacity (WHC) was determined after centrifugation (210 × *g*, 5 min) of 2 g of minced meat, as described in Santos et al. (2019). WHC (%) was calculated as

(water content – water loss after centrifugation) × 100/water content.

Water content was determined gravimetrically by weighing 2 g of sample before and after evaporation at 105°C overnight (AOAC, 2012). The analysis was conducted in triplicate for each sample.

Total lipids were extracted from each sample according to Folch et al. (1957) method, and gravimetrically quantified. Around 400 mg of each lipid extract were esterified to fatty acid methyl esters (FAME) using the method proposed by Christie (1982) to determine the fatty acid (FA) profile. The FA composition was determined by gas chromatography (GC) using a Varian GC 430 gas chromatograph (Varian Inc., Palo Alto, CA, USA), equipped with a flame ionization detector (FID) and a Supelco Omegawax™ 320 capillary column (30 m × 0.32 mm i.d., 0.25-μm film and polyethylene glycol-bonded phase; Supelco, Bellefonte, PA, USA). One-μL was injected with a 1:20 split ratio at 220°C. Oven temperature was programmed to rise from 130°C to 230°C in 40 minutes. The detector was set at 300°C, helium was the carrier gas flowed at 1.5 mL/min. Chromatograms were recorded with the Galaxie Chromatography Data System 1.9.302.952 computing integrator software (Varian Inc., Palo Alto, CA, USA). FAs were identified by comparing the FAMEs retention time with the Supelco 37 component FAME mix (Supelco, Bellefonte, PA, USA), then quantified by means of calibration curves using tricosanoic acid (C23:0, 0.4 mg/mL) (Supelco, Bellefonte, PA, USA) as internal standard.

Oxidative stability was analyzed following the primary and secondary oxidation products by means conjugated dienes (primary, CD) and 2-thiobarbithuric acid reactive substances (TBARS), according to the spectrophotometric methods previously proposed by Srinivasan et al. (1996) and Vyncke (1970), respectively. The analyses were performed in duplicate and the results were expressed as mmol hydroperoxides (mmol Hp)/100 g meat and malondialdehyde equivalents (MDA-eq.)/100 g meat, respectively.

2.2. Statistical analysis

The data were processed by a two-way ANOVA, using the PROC GLM of SAS (2011). Farming system (F, three levels: OG, CG, and MG) and storage length (S, four levels: T0, T20, T40, and T80) were utilized as fixed factors. The interaction F × S was also considered but not reported in tables when not significant. The differences between the treatment means were separated using Tukey's test at $P < 0.05$ (SAS, 2011). The overall carcass meat (per group) was considered as the experimental unit.

Results

The color traits of the meatballs belonging to the three experimental groups were similar, as illustrated in Table 1, excepted for lightness that significantly ($P < 0.01$) decreased in the CG and MG groups in comparison with the OG one. Concerning pH, the MG meatballs showed a slight but significantly reduced value ($P < 0.05$) with respect to the other two groups. As expected, the storage significantly modified ($P < 0.001$) all the physical traits as early as the 20th day. The lightness dropped between T0 and T20, then remained unaltered, while the b* index was reduced during the first 20 days of storage then increased at T40 returning at its initial value. The lowest pH value was found at the beginning of the storage, then gradually increased ($P < 0.001$) after 20 days and reached its highest value at the end of the trial. A significant interaction emerged for the a* index, depicted in Fig. 1. All the three groups had the same a* value at the beginning of the trial; however, a different ability in color maintenance emerged during the storage. Indeed, the OG redness index unchanged till the T40, then dropped between T40 and T80. Conversely, both the CG and MG redness index significantly decreased ($P < 0.05$) at the T20. However, the MG meatballs had an a* value ($P < 0.05$) lower than that of the CG ones. Both groups showed unmodified redness values for the following 20 days. Then, the MG group seemed to slight recover its a* initial value so that it assumed an intermediate value between the OG and CG groups at T40, resulting significantly different ($P < 0.05$). Despite these sundry patterns, all the groups showed the same redness index at T80.

Table 1 even shows the results obtained for the weight loss and water holding capacity analyses. Contrary to the other physical analyses, these items were significantly modified ($P < 0.05$) only by the increase in storage time. In particular, the early 20 days increased the weight loss ($P < 0.001$) and diminished the WHC (-5.86%). Looking at the results, it is possible to observe that the highest weight loss was just recorded at the T20, while the variations between T20 and T40, and T40-T80 were 0.31% and 0.64%, respectively. Finally, the WHC did not undergo any further modification for the remaining days of the storage.

Table 2 illustrates the total lipids and the fatty acids contained in 100 g of meatballs. Minor effects of the investigated treatments were observed. In details, lauric acid (C12:0) was more than halved in the CG compared to the OG ($P < 0.05$), while the MG showed an intermediate content. In addition, the lipid content significantly increased after 20 days of storage ($P < 0.01$) even if the final value returned at the initial level. Independently from the treatments, the rabbit meatballs were mostly characterized by saturated (SFA) and polyunsaturated fatty acids of the n-6 series (PUFAn-6), followed by monounsaturated fatty acids (MUFA). Contrariwise, PUFAn-3 fraction varied between 60 and 121 mg/100 g, without any statistical difference among the groups that differed for the n-6/n-3 ratio, the OG group showing the lowest value (11.32). Nevertheless, while observing the fatty acid profile (expressed as percentage of the total FAME) (Table 3), numerous effects of the farming system, storage, and their interaction emerged. Indeed, 20 of 32-fatty acids were significantly modified by the farming system, whereas 16 fatty acids underwent the effect of the storage. Overall, the SFA fraction prevailed in the rabbit meatballs regardless of the farming group, while the MUFA, PUFAn-6, and PUFAn-3 were modified as follows. The open and mirror groups had the same values of MUFA and PUFAn-6, which however were significantly higher and lower, respectively, than the CG. Instead, the OG meatballs were significantly richer in the PUFAn-3 fraction ($P < 0.001$) than the other two groups of meatballs. The SFA percentage was significantly augmented after 40 days of storage ($P < 0.05$). A significant increase of MUFA while a decrease of PUFAn-6 ($P < 0.01$) was evident at T20. Even though the overall PUFAn-3 fraction did not vary during the storage time, it is possible to observe that both C20:5n-3 and C22:5n-3 were halved ($P < 0.01$) during the early 20 days.

Finally, the possible effect of both farming system and storage time on lipid oxidation process was assessed. The results, depicted in Table 4, highlighted that the secondary oxidation products increased ($P < 0.01$) in both CG and MG meatballs, while the farming system did not influence the primary ones. On the contrary, the storage length promoted the increase in conjugated dienes (immediately after 20 days, followed by a further increase between T40 and T80) and TBARS levels (at T40).

Discussion

The effect of the farming system on rabbit meat quality has been recently investigated, especially concerning the comparison between traditional (i.e. cage) and alternative systems (as free-range, open-air cages, organic farming) (D'Agata et al., 2009; Paci et al., 2014; Loponte et al., 2018). However, the effect of the rearing system has

Table 1
Color values, pH, weight loss, and water holding capacity (WHC) of meatballs from rabbits reared with three farming systems and stored at -10°C for different times.

	Farming system, F[1]			Storage, S[2]				*P*-value			RMSE
	OG	CG	MG	T0	T20	T40	T80	F	S	F × S	
L*	48.338 a	45.691 b	46.276 b	50.034 A	45.493 B	45.208 B	46.338 B	**	***	ns	2.097
a*	14.883	14.803	14.037	16.666 A	14.609 B	15.174 B	11.850 C	ns	***	*	1.347
b*	5.809	5.461	5.049	5.822 A	4.405 B	5.544 A	5.988 A	ns	***	ns	0.915
pH	6.07 a	6.08 a	6.02 b	5.98 C	6.06 B	6.07 B	6.13 A	*	***	ns	0.075
Weight loss, %	2.15	2.207	1.81	-	1.63 B	1.94 B	2.58 A	ns	***	ns	0.494
WHC, %	91.01	89.26	90.14	94.09 A	88.23 B	89.78 B	88.44 B	ns	*	ns	3.819

* $P < 0.05$;
** $P < 0.01$;
*** $P < 0.001$; ns: not significant ($P > 0.05$). a, b: means with different lowercase letters are statistically different among the three farming systems. A, B, C: means with different capital letters are statistically different among the four sampling times during the storage. RMSE: Root Mean Square Error. 1 Farming system: open group (OG), closed group (CG), and mirror group (MG). 2 Storage: 0 day (T0), 20 days (T20), 40 days (T40), and 80 days (T80).

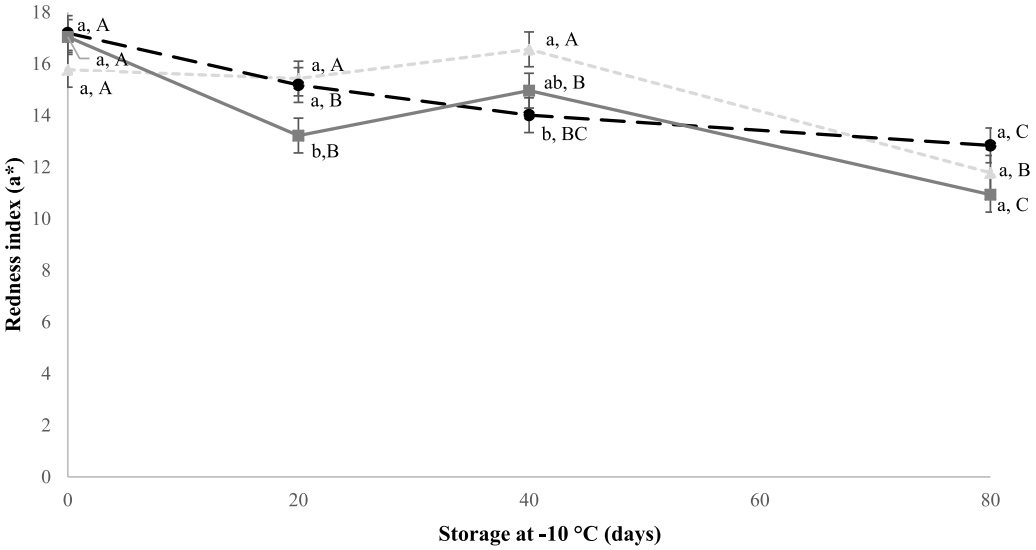

Fig. 1. Interaction between farming systems (open group, light grey line; closed group, dark line; mirror group, grey line) and storage time (F × S) on the meatballs redness index (a*).
a, b: means with different lowercase letters are statistically different among the three farming systems ($P < 0.05$). A, B, C: means with different capital letters are statistically different among the four sampling times during the storage ($P < 0.05$). Vertical bars stand for the standard error of the mean.

Table 2
Total lipids and fatty acid content (g/100 g meat) of meatballs from rabbits reared with three farming systems and stored at -10°C for different times.

	Farming system, F[1]			Storage, S[2]				*P*-value			RMSE
	OG	CG	MG	T0	T20	T40	T80	F	S	F × S	
Total lipids	4.036	2.791	3.350	2.428 B	4.383 A	3.956 AB	2.802 B	ns	**	ns	1.875
C12:0	0.007 a	0.002 b	0.004 ab	0.003	0.006	0.004	0.004	*	ns	ns	0.004
C13:0	0.001	0.0004	0.001	0.0004	0.001	0.0005	0.005	ns	ns	ns	0.001
isoC14:0	0.001	0.001	0.001	0.0008	0.002	0.001	0.001	ns	ns	ns	0.001
C14:0	0.077	0.043	0.057	0.043	0.075	0.064	0.053	ns	ns	ns	0.043
C14:1n5	0.001	0.001	0.001	0.0008	0.015	0.011	0.0009	ns	ns	ns	0.001
C15:0iso	0.002	0.001	0.002	0.001	0.002	0.002	0.002	ns	ns	ns	0.001
C15:0anteiso	0.003	0.002	0.003	0.002	0.004	0.003	0.003	ns	ns	ns	0.002
C15:0	0.021	0.012	0.016	0.013	0.021	0.017	0.015	ns	ns	ns	0.011
C16:0iso	0.005	0.003	0.005	0.003	0.006	0.004	0.004	ns	ns	ns	0.003
C16:0	0.966	0.572	0.747	0.597	0.947	0.837	0.667	ns	ns	ns	0.500
C16:1n-9	0.015	0.009	0.013	0.010	0.017	0.013	0.010	ns	ns	ns	0.008
C16:1n-7	0.035	0.021	0.032	0.023	0.037	0.032	0.025	ns	ns	ns	0.018
C17:0	0.033	0.019	0.027	0.021	0.034	0.028	0.023	ns	ns	ns	0.018
C18:0	0.283	0.179	0.263	0.187	0.319	0.255	0.205	ns	ns	ns	0.168
C18:1n-9	0.985	0.565	0.786	0.588	1.022	0.856	0.649	ns	ns	ns	0.548
C18:1n-7	0.040	0.026	0.035	0.027	0.043	0.036	0.029	ns	ns	ns	0.020
C18:2n-6	1.174	0.684	0.957	0.736	1.224	1.019	0.775	ns	ns	ns	0.644
C18:3n-6	0.004	0.002	0.003	0.002	0.004	0.003	0.003	ns	ns	ns	0.001
C18:3n-4	0.001	0.001	0.001	0.001	0.001	0.001	0.001	ns	ns	ns	0.000
C18:3n-3	0.104 a	0.050 b	0.064 ab	0.056	0.097	0.078	0.061	*	ns	ns	0.056
C20:0	0.005	0.003	0.005	0.003	0.006	0.005	0.004	ns	ns	ns	0.003
C20:2n-6	0.009	0.005	0.007	0.006	0.008	0.007	0.006	ns	ns	ns	0.004
C20:1n-9	0.011	0.005	0.009	0.007	0.011	0.009	0.007	ns	ns	ns	0.006
C20:3n-6	0.010	0.008	0.009	0.010	0.010	0.009	0.009	ns	ns	ns	0.002
C20:3n-3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	ns	ns	ns	0.001
C20:4n-6	0.089	0.085	0.087	0.097	0.086	0.086	0.080	ns	ns	ns	0.018
C20:5n-3	0.002	0.002	0.002	0.002	0.002	0.002	0.001	ns	ns	*	0.001
C22:0	0.002	0.002	0.002	0.002	0.003	0.002	0.002	ns	ns	ns	0.001
C22:1n-9	0.002	0.001	0.002	0.001	0.002	0.002	0.002	ns	ns	ns	0.001
C22:4n-6	0.015	0.012	0.014	0.014	0.015	0.014	0.012	ns	ns	ns	0.004
C22:5n-6	0.005	0.005	0.005	0.005	0.005	0.005	0.004	ns	ns	ns	0.001
C22:5n-3	0.009	0.007	0.008	0.009	0.008	0.008	0.007	ns	ns	ns	0.002
SFA	1.472	0.838	1.133	0.876	1.512	1.220	0.982	ns	ns	ns	0.707
MUFA	1.141	0.630	0.878	0.657	1.203	0.949	0.723	ns	ns	ns	0.566
PUFAn-6	1.363	0.800	1.083	0.870	1.427	1.142	0.889	ns	ns	ns	0.624
PUFAn-3	0.121	0.060	0.072	0.067	0.115	0.085	0.070	ns	ns	ns	0.054
n-6/n-3 ratio	11.32 b	14.43 a	14.24 a	13.93	12.75	13.02	13.62	***	ns	ns	1.017

* $P < 0.05$;
** $P < 0.01$; ns: not significant ($P > 0.05$). a, b: means with different lowercase letters are statistically different among the three farming systems. A, B: means with different capital letters are statistically different among the four sampling times during the storage. RMSE: Root Mean Square Error. 1 Farming system: open group (OG), closed group (CG), and mirror group (MG). 2 Storage: 0 day (T0), 20 days (T20), 40 days (T40), and 80 days (T80).

been frequently summed or confused with other factors, such as the presence of a different stocking density and group size. Contrariwise, the present study evaluated an environmental enrichment which did not require adjusting the number of the individuals or the available space per head. Despite this, Mastellone et al. (2019), who published the ethological results of the same trial, observed that the rabbits from the open group spent around 37.9 min/d in locomotion activity, while the other groups only 25.1 min/d (closed) or 28.5 min/d (mirror) ($P <$

Table 3
Fatty acid profile (g/100 g of total FAME) of meatballs from rabbits reared with three farming systems and stored at -10°C for different times.

	Farming system, F[1]			Storage, S[2]				*P*-value			RMSE
	OG	CG	MG	T0	T20	T40	T80	F	S	F × S	
C12:0	0.16 a	0.09 c	0.12 b	0.12	0.13	0.11	0.12	***	ns	ns	0.03
C13:0	0.02	0.02	0.02	0.01	0.02	0.02	0.11	ns	ns	*	0.01
C14:0iso	0.03 b	0.03 b	0.04 a	0.03 B	0.03 B	0.03 B	0.04 A	*	**	***	0.01
C14:0	1.92 a	1.64 b	1.76 b	1.59	1.84	1.82	1.85	**	ns	*	0.20
C141n5	0.03	0.03	0.04	0.03	0.03	0.03	0.03	ns	ns	ns	0.01
C15:0iso	0.05 b	0.05 b	0.06 a	0.05 B	0.05 B	0.05 B	0.06 A	***	***	*	0.01
C15:0anteiso	0.08 c	0.09 b	0.10 a	0.09 AB	0.09 B	0.09 B	0.10 A	***	**	ns	0.01
C15:0	0.52	0.48	0.48	0.49	0.50	0.46	0.53	ns	ns	ns	0.08
C16:0iso	0.12 c	0.13 b	0.15 a	0.12 B	0.13 A	0.13 AB	0.14 A	***	*	***	0.01
C16:0	24.66	23.91	23.82	23.58	23.90	24.56	24.49	ns	ns	ns	0.94
C16:1n-9	0.39 a	0.37 b	0.40 a	0.37 B	0.40 A	0.38 B	0.39 AB	***	*	**	0.02
C16:1n-7	0.93	0.82	1.09	0.90	0.98	0.97	0.95	ns	ns	ns	0.22
C17:0	0.83	0.81	0.83	0.81	0.83	0.81	0.84	ns	ns	*	0.04
C18:0	7.20 b	8.08 a	8.07 a	7.91	7.79	7.60	7.85	**	ns	ns	0.64
C18:1n-9	25.03 a	22.89 b	24.41 a	22.67 B	25.17 A	24.81 A	23.79 B	***	***	*	1.19
C18:1n-7	1.05 b	1.17 a	1.12 a	1.16	1.08	1.10	1.12	***	ns	ns	0.06
C18:2n-6	29.89	29.21	29.83	29.40	30.15	29.83	29.21	ns	ns	ns	0.86
C18:3n-6	0.09	0.10	0.10	0.09	0.08	0.09	0.12	ns	ns	ns	0.03
C18:3n-4	0.03	0.04	0.04	0.04	0.04	0.04	0.04	ns	ns	ns	0.01
C18:3n-3	2.63 a	1.95 b	1.91 b	2.07	2.37	2.09	2.12	***	ns	ns	0.37
C20:1n-9	0.24	0.19	0.21	0.25 A	0.10 B	0.24 A	0.26 A	ns	***	**	0.06
C20:0	0.12 c	0.15 b	0.17 a	0.14	0.15	0.14	0.16	***	ns	ns	0.02
C20:2n-6	0.22	0.23	0.21	0.24 A	0.22 AB	0.21 B	0.23 A	ns	**	ns	0.02
C20:3n-6	0.28 b	0.47 a	0.34 b	0.50 A	0.26 B	0.30 AB	0.38 A	**	**	*	0.12
C20:3n-3	0.04 a	0.03 a	0.02 b	0.03 A	0.03 AB	0.02 B	0.03 A	***	*	ns	0.01
C20:4n-6	2.49 b	5.33 a	3.40 b	5.78 A	2.53 C	3.01 BC	3.84 B	***	**	**	1.50
C20:5n-3	0.05 b	0.09 a	0.06 b	0.09 A	0.05 B	0.06 B	0.07 AB	**	*	*	0.03
C22:0	0.05 b	0.08 a	0.78 a	0.07 AB	0.06 B	0.07 AB	0.08 A	***	*	ns	0.01
C22:1n-9	0.05	0.07	0.06	0.06 B	0.05 B	0.06 B	0.08 A	ns	**	ns	0.02
C22:4n-6	0.41 b	0.68 a	0.52 b	0.72 A	0.41 B	0.46 B	0.56 AB	**	**	*	0.18
C22:5n-6	0.14 c	0.29 a	0.20 b	0.30 A	0.16 B	0.17 B	0.21 B	***	**	*	0.08
C22:5n-3	0.25 b	0.46 a	0.30 b	0.49 A	0.23 C	0.28 BC	0.35 B	***	**	*	0.13
SFA	35.77	35.54	35.69	35.01 C	35.51 BC	35.89 AB	36.26 A	ns	*	**	0.81
MUFA	27.72 a	25.60 b	27.38 a	25.45 C	27.98 A	27.58 AB	26.61 BC	***	**	*	1.22
PUFAn-6	33.52 b	36.29 a	34.60 b	36.82 A	33.79 B	34.06 B	34.54 B	***	**	*	1.65
PUFAn-3	2.97 a	2.52 b	2.29 b	2.68	2.68	2.44	2.56	***	ns	ns	0.38

* $P < 0.05$;
** $P < 0.01$;
*** $P < 0.001$; ns: not significant ($P > 0.05$). a, b: means with different lowercase letters are statistically different among the three farming systems. A, B: means with different capital letters are statistically different among the four sampling times during the storage. RMSE: Root Mean Square Error. 1 Farming system: open group (OG), closed group (CG), and mirror group (MG). 2 Storage: 0 day (T0), 20 days (T20), 40 days (T40), and 80 days (T80).

0.05). This difference seems to be fundamental to further explain the effect of the farming system on the conventional quality parameters, starting from the color values registered in this trial.

For instance, an enhanced locomotory activity might result in an increased meat lightness (Dal Bosco et al., 2002), as verified in the present study for the OG meatballs, cause of the augmented respiration activity of the muscle fiber which can induce the reduction of oxy-myoglobin in myoglobin (Xiccato et al., 1999). Even though this metabolic adaptation is frequently associated to an increase in the redness index immediately after slaughter (Combes et al., 2010), we found an interesting interaction for the a* value between the farming system and the storage. Specifically, it seemed that the increased locomotory activity of the OG induced a greater storage stability of this meat parameter, which was unvaried until the 40th day of the storage. Despite the hypothetical muscular metabolic adjustment induced by the rearing system, no modification to the myofibrillar structure could be assumed, since both weight loss and water holding capacity were unaffected by the farming method, thus agreeing with the results obtained by D'Agata et al., 2009 and Combes et al. (2010). All the quality changes occurred during the storage, such as meat discoloration, pH and weight loss increase, and WHC reduction are consistent with previous results (Mancini et al., 2015; Wang et al., 2020). The deterioration of meat characteristics during the storage is primarily led by microbial growth, protein and lipid oxidation, whose influence is however a function of the storage temperature (Wang et al., 2020). For instance, bacterial growth was found to be strongly inhibited at -12°C, a

Table 4
Conjugated dienes (mmol Hp/100 g meat) and TBARS (mg MDA-eq./100 g meat) values of meatballs from rabbits reared with three farming systems and stored at -10°C for different times.

	Farming system[1]			Storage[2]				*P*-value		RMSE
	OG	CG	MG	T0	T20	T40	T80	A	C	
Conjugated dienes	0.087	0.087	0.085	0.046 C	0.088 B	0.073 BC	0.137 A	ns	***	0.034
TBARS	0.07 b	0.10 a	0.10 a	0.07 B	0.04 B	0.12 A	0.12 A	**	***	0.34

** $P < 0.01$;
*** $P < 0.001$; ns: not significant ($P > 0.05$). a, b: means with different lowercase letters are statistically different among the three farming systems. A, B, C: means with different capital letters are statistically different among the four sampling times during the storage. RMSE: Root Mean Square Error. 1 Farming system: open group (OG), closed group (CG), and mirror group (MG). 2 Storage: 0 day (T0), 20 days (T20), 40 days (T40), and 80 days (T80).

temperature similar to the one utilized in the present trial, compared to +4°C and -4°C, while the protein oxidation was slowed down but still present throughout 45 days of storage at -12°C (Wang et al., 2020). In a recent study, protein carbonylation of both sarcoplasmic and myofibrillar proteins was observed and correlated to the loss of protein solubility and WHC drop since the early stage (30 days) of a long term frozen storage (175 days at -10°C) (Santos et al., 2019). Moreover, the freezing process damaged the structure of rabbit myofibrils, more severely as the storage time increases and the temperature decreases (Lan et al., 2016). The cell size, the number of gaps inside the cells as well as the detachment of the myofibrils generated during the formation of intra- and extra-cellular ice crystals increased in the frozen rabbit muscle, thus limiting the muscle ability to retain water (Lan et al., 2016). Interestingly, the significant reduction in WHC and, consequently, the major weight loss here observed after 20 days of storage at sub-zero temperature might be ascribable to the protein modifications probably exacerbated by the mincing process.

The nutritional quality of rabbit meat is considered a strength of this livestock production as its chemical composition generally fits consumers' attitude towards healthy food (Cullere and Dalle Zotte, 2018). In this regard, a moderate total lipid content of the meatballs was found irrespective of the farming systems under study, in line with previous studies (Dal Bosco et al., 2002; Dalle Zotte et al., 2015; Loponte et al., 2018). However, the nutritional value also depends on the fatty acid composition of the meat. In comparison to the other livestock production, rabbit meat is commonly considered an important source of unsaturated fatty acids, both MUFA and PUFA (Dalle Zotte, 2014), even if the lipid composition varies among the cuts of the carcass and is affected by other productive factors, especially the diet. Based on the fatty acid content of 100 g of rabbit meatball, it was possible to observe that a portion could provide on average 1.15 g, 0.88 g, and 1.16 g of SFA, MUFA and PUFA, respectively, including important fatty acids such as oleic, linoleic, α-linolenic, and arachidonic acids. The results obtained in the present study showed that the lipid fraction was scarcely affected by the farming system and that it was extremely stable during the storage, thus underlining its resilience and the promising role of rabbit meat in human nutrition.

Nonetheless, it is not easy to retrieve the fatty acid quantification of rabbit meat from the available literature since the data are mainly expressed as relative abundance (i.e. in % of the total fatty acid methyl esters). Hence, also the FA profile of rabbit meatballs of this trial was considered to allow the comparison with the studies in the literature. In this respect, the calculated sums of the fatty acid classes in meat are consistent with the ranges reported for various cuts of the rabbit carcass (Dalle Zotte, 2014), regardless of the rearing method. However, a profound effect of the farming condition was evident, supporting what previously observed in animal housed in conventional bicellular cages, straw bedded pen, and wire netted pen (Dal Bosco et al., 2002), in collective wire net cages and straw bedded pens (Chodová et al., 2014), in pens and cages (Dalle Zotte et al., 2015), and in open-air cages and free-range (Loponte et al., 2018). To date, the lipid fraction modification due to the housing system has been ascribed to the difference in locomotory activity. Indeed, the augmented available space or the reduced stocking density may increase animal movements, thus resulting in a lean meat which is generally rich in phospholipids and consequently in PUFA, especially of the n-3 series (Loponte et al., 2018). The highest PUFAn-3 percentage was found in the OG meat in accordance with the previous assertion and this group coherently also showed better n-6/n-3 ratio. It should be noted that the meat of rabbit grown in free-range areas enriched with mirrors got the overall fatty acid profile closed to the OG one.

As function of storage time, C20 and C22 fatty acids seemed to be the preferential substrate for the early stage of lipid oxidative degradation, by undergoing a strong reduction during the first 20 days of storage. This reduction occurred in parallel with the doubling of CDs and the unchanged TBARS contents, consistently with the well-established lipid oxidation pathway (Min and Ahn, 2005). The CDs can go through various reactions until the formation of the secondary oxidation products, such as TBARS, and this transformation was evident between T20 and T40, when CDs remained almost unaltered while a four-fold increase in TBARS was registered. The lipid degradation timing completely agreed with the one observed by Wang et al. (2020), who reported an increase in TBARS value in rabbit meat after 35 days of storage at -12°C. In addition to the previous evaluations on meat susceptibility to oxidative processes, the present experiment confirmed that the farming system affects the lipid oxidation pattern. Specifically, the highest TBARS levels found in the CG and MG meatballs might have derived from a significantly reduced antioxidant capacity found in meat of rabbit housed into open-air cages in comparison with that of rabbits from free-range system (Loponte et al., 2018). Another possible explanation might be related to the inability of the rabbits to express their social habits inside a completely confined environment (CG), hence causing fearful or anxious behaviors. In this regard, the use of mirrors did not seem to be able to mitigate this stress, which could lead the increase in the reactive oxygen species (ROS), pro-oxidant factors commonly increased by troublesome events (Dal Bosco et al., 2002). Not surprisingly, previous papers underlined that rabbits gradually decreased their interest in mirror because they did not receive confirmatory cues that the mirror was a conspecific (Dalle Zotte et al., 2009; Jones and Phillips, 2005).

Conclusions

Nowadays, animal welfare is strongly driving consumers' purchase choice, especially for meat; hence the farming system can be deemed a quality parameter. The use of mirror into free-range areas housed "small group" rabbits for meat purposes resulted fully analogous to the one of rabbits farmed into confined free-range areas, both immediately after death and during the frozen storage at -10°C. Giving to the rabbits the opportunity to see and smell their conspecific, as in the tested open group, improved the meat quality and its storage stability. Nonetheless, to answer the question if mirrors could represent an environmental enrichment well balancing farmers' needs and animal welfare, the present results should be considered together with the *in vivo* parameters published elsewhere. Hence, since the use of mirrors can enhance growth performance and carcass traits minimizing the effects on quality items, it could represent a valuable environmental enrichment, which however deserves major attention regarding animal welfare issues.

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Giulia Secci: Conceptualization, Investigation, Formal analysis, Writing - original draft. **Fulvia Bovera:** Writing - review & editing. **Nadia Musco:** Investigation. **Yara Husein:** Investigation, Formal analysis. **Giuliana Parisi:** Conceptualization, Data curation, Supervision.

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Supplementary materials

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