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Meat quality and sensory traits in rabbits fed with two different percentages of bovine colostrum

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ABSTRACT

The nutritional, antimicrobial, and antioxidant properties of bovine colostrum (BC) have encouraged its use in animal nutrition as a functional food in recent years. Nonetheless, the potential implications of BC supplementation on meat quality remain to be thoroughly assessed. To address this, thirty-nine New Zealand White rabbits (n = 13/group) were fed different dietary regimens until slaughter.: commercial standard diet for the control group (C) and C with 2.5% and 5% w/w of BC for BC-2.5 and BC-5 groups, respectively. Rabbits were slaughtered at 91 days of age and meat quality, and sensory characteristics were evaluated at days 2 (48 h after slaughter), 5, and 10 of refrigerated storage at 4 °C.

The addition of colostrum in the diet resulted in a reduction of the total viable count, albeit only at the highest concentration and at the final detection, whereas for *Lactobacillus* spp. and *Pseudomonas* spp., there was little or no effect. The colour coordinates showed no differences between the groups, but they varied over time according to diet. Some differences between groups emerged in the definition of sensory attributes but did not affect the overall liking and overall scores of individual descriptors. These results indicate that the use of colostrum in rabbit feeding does not significantly impart meat quality and sensory attributes, but the potential of this valuable by-product for the food industry needs further investigation.

1. Introduction

The need for the food industry to constantly raise the quality of the food offered to the consumer has led to research efforts focused on exploring effective, cost-efficient, and environmentally-friendly solutions. Existing studies in the literature primarily revolve around two main strategies: one intervenes in the preparation and packaging phase of the product (Arcan & Yemenicioğlu, 2011; Arroyo et al., 2019; Jafarzadeh et al., 2020; Messinese et al., 2023), and the other acts on the primary production level through the supplementation of phytochemicals or bioactive compounds in animal feed (Agradi et al., 2023; Serra et al., 2023). Intervening on the feed, not only holds the potential to elevate production quality but also offers advantages in terms of performance, health, and animal welfare (Agradi et al., 2022; Cremonesi et al., 2022). The proven antimicrobial and antioxidant activity of molecules derived from the plant and animal world has encouraged research in recent years to experiment with their use in animal feed, to optimize these otherwise by-products resources. From a circular economy perspective, finding these substances from by-products would help reduce costs, giving added value to the operation. In this regard, bovine colostrum (BC) is an extremely interesting by-product containing multiple biologically active substances (Langer, 2009). In particular, BC is known to be a nutrient-rich liquid secreted by female mammals after giving birth that contains macro- and micronutrients,

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immunomodulators, growth factors, and other bioactive molecules (Stelwagen, Carpenter, Haigh, Hodgkinson, & Wheeler, 2009). Its derivatives have been studied for their potential use in functional foods, pharmaceuticals (Antonio, Sanders, & Van Gammeren, 2001; Ashraf, Mahalanabis, Mitra, Tzipori, & Fuchs, 2001; Buckley, Abbott, Brinkworth, & Whyte, 2002; Filipescu et al., 2018; Menchetti et al., 2016; Otto, Najnigier, Stelmasiak, & Robins-Browne, 2011; Playford et al., 1999; Rocha, 2016), and animal feed. They play a significant role in the development of innovative products aimed at promoting animal health and welfare (Boudry et al., 2007; Bridger & Brown, 1981; Huguet, le Dividich, & le Huërou-Luron, 2012; Johnson et al., 2022; Solomons, 2002; Sugiharto, Poulsen, Canibe, & Lauridsen, 2015; Yang, Zou, Wu, Li, & Cao, 2015). The existing body of literature states that current breeding methods allow large quantities of BC production for clinical and veterinary use (Kim, Jeon, & Kim, 2005; Kindlein et al., 2018; Menchetti et al., 2020; Nagaraja, Dar, Pandey, & Mondal, 2011; Zhu et al., 2022). The components derived from BC and utilized in livestock feed production play a crucial role in providing the necessary proteins for muscle development and overall bodily growth (Bridger & Brown, 1981; Huguet et al., 2012; Nagaraja et al., 2011; Solomons, 2002; Sugiharto et al., 2015), without causing mortality (Bridger & Brown, 1981; Sugiharto et al., 2015). In recent decades, dietary supplementation with BC has been evaluated in the zootechnical field to improve production performance (Castrica et al., 2022). However, the effects of BC supplementation on meat quality in terms of microbiological, physicochemical, and sensory profile are, to date, still little investigated. In recent research, the addition of 5% BC to the rabbit diet modified the fatty acid profile, with an increase in Saturated Fatty Acids (SFAs) at the expense of Monounsaturated Fatty Acids (MUFAs) but gave the meat greater oxidative stability during storage (Castrica et al., 2022).

From a consumer perspective, rabbit meat is highly palatable, high in protein, and low in calories, with high levels of unsaturated fatty acids and low levels of sodium and cholesterol (Hernàndez & Gondret, 2006; Petraci & Cavani, 2013). Furthermore, rabbit meat can be classified as a "functional food" due to its delivery of bioactive substances that offer health benefits to consumers. These include conjugated linoleic acid (CLA), vitamins, antioxidants, and a balanced ratio of n-6 to n-3 polyunsaturated fatty acids (PUFA) (Domeneghini, Di Giancamillo, & Corino, 2006; Hernández, 2008). Consequently, it is imperative to comprehend the implications of dietary supplementation on meat characteristics and how these evolve during storage. For these reasons, the study aimed to evaluate the effect of dietary supplementation with two different percentages of bovine colostrum, 2.5% and 5% *w*/w, respectively on microbiological quality, physical, and sensory traits of rabbit meat. In addition, histochemical changes of muscle fibers were evaluated. In our opinion, it is necessary to expand knowledge on the use of this valuable by-product, to understand whether its use in livestock can improve meat quality and become a resource for the food industry as well.

2. Material and methods

2.1. Rabbits farming and slaughtering

Post-weaned, thirty-nine New Zealand White (NZW) rabbits were farmed in an experimental farm at the Department of Agricultural, Food, and Environmental Science of the University of Perugia (Italy). The experimental study was conducted in compliance with the Legislative Decree No. 146, implemented by the Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes and was approved by the Body for the Protection of Animals (OPBA) of the University of Milan (OPBA_42_2021, 7 May 2021). As described by Castrica et al. (2022), the experimental design involved the allocation of the rabbits into three dietary groups (13 animals/group): standard commercial formulation for the control group (C group) and standard commercial formulation with 2.5% and 5% bovine colostrum (Skim Immulox Powder, APS BioGroup Main Offices and Manufacturing Facilities: 2235 South Central Ave Phoenix, AZ 85004 US), added for the BC-2.5 and BC-5 experimental groups, respectively. Skim Immulox Powder, a colostrum powder rich in Proline-rich Polypeptide (PRP), is a water-soluble, pasteurized, and reduced-fat product derived exclusively from the first milking colostrum. This agglomerated, instantized powder undergoes processing at low pressures and temperatures, followed by spray drying using indirect steam to preserve its maximum bioactivity, resulting in a free-flowing powder.

Each rabbit was held in individual cages (600 × 250 × 330 mm) and water was provided ad libitum (Dal Bosco et al., 2015). Ingredients (percentage wet weight) of each diet were as described in Serra et al. (2023) and were reported in Table 1. After 91 days of rearing, all rabbits were slaughtered.

2.2. Samples preparation

Rabbits were slaughtered at an official slaughterhouse after stunning by electronarcosis and the carcasses were immediately chilled (4 °C) until the analysis time. Forty-eight h after slaughter, the animals were sectioned and $n = 26$ *Longissimus thoracis et lumborum* (LTL) muscles and $n = 26$ hind leg muscles belonging to each group were removed and individually packed in sterile plastic bags at room at room atmosphere. After that, $n = 156$ samples were transported in refrigerated boxes to the Food Inspection laboratory at the Department of Veterinary Medicine and Animal Sciences, University of Milan (Italy), where they were stored in dark conditions, at the temperature of 4 ± 1 °C. An observation period of 10 days, from slaughter, was planned, during which three analysis sessions were performed: the first 48 h from slaughter (day 2), the second after 120 h (day 5), and the third at the end of shelf life (240 h, day 10). Microbiological and physical investigations were conducted on LTL muscles (13 right and 13 left loins, respectively). Meanwhile sensory assessments and histological examinations (only at D0) were performed on the hind leg muscles (*Semimembranosus muscle)*.

Table 1
Ingredients (percentage wet weight) of control (C) and experimental diets (BC-2.5 and BC-5) of post-weaned rabbits.

Ingredients	C	BC-2.5	BC-5
Dehydrated alfalfa meal	32.40	32.40	32.40
Barley	22.40	19.90	17.40
Wheat bran	20.50	20.50	20.50
Sunflower meal	8.00	8.00	8.00
Soybean meal	4.00	4.00	4.00
Cane molasses	3.00	3.00	3.00
Carob pods	2.70	2.70	2.70
Wheat meal	2.50	2.50	2.50
Calcium Carbonate	2.00	2.00	2.00
Vitamin–mineral premix [1]	1.60	1.60	1.60
Soybean oil	0.50	0.50	0.50
Sodium chloride	0.40	0.40	0.40
Bovine colostrum	–	2.50	5.00
Chemical composition [2]			
Dry matter	92.34	91.71	91.69
Crude protein	14.82	14.76	15.23
Ether extract	2.79	2.95	3.02
Ash	7.04	7.23	7.62
NDF	40.00	36.81	35.79
ADF	27.04	24.92	24.31
ADL	12.02	10.03	9.11

C: group without supplemented diet; BC-2.5: C supplemented with 2.5% of bovine colostrum; BC-5: C supplemented with 5.0% of bovine colostrum; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin. [1] Contains, per kg of feed: retinyl acetate, 4800 IU; vitamin D3, 1800 IU; calcium-D pantothenate, 6.6 mg; choline chloride, 300 mg; ferrous sulfate monohydrate, 37.5 mg; manganous sulphate monohydrate, 54.0 mg; copper sulphate pentahydrate, 6.0 mg; zinc oxide, 36.0 mg; potassium iodide, 0.66 mg; sodium selenite, 0.12 mg; butylhydroxytoluene, 30.0 mg; butylhydroxyanisole, 30.0 mg. [2] Analyzed.

2.3. Microbial evaluation

At each shelf life day selected, the right rabbit loins were processed according to the protocol described by (Castrica et al., 2020; Castrica et al., 2022) and the following parameters were enumerated: (i) total viable count (TVC) using Petrifilm (3 M, St. Paul, MN, USA) following the AFNOR certification no. 3 M 01/01–09/89, (ii) *Pseudomonas* spp. utilizing Pseudomonas Agar Base (Biolife Italiana s.r.l., Milan, Italy) with CFC Pseudomonas Supplement (Biolife), then incubating the plates at 25 °C for 48 h., and (iii) *Lactobacillus* spp. that have been enumerated on de Man, Rogosa and Sharpe agar (Biolife) anaerobically incubated at 37 °C for 48 h. Results were expressed as Log CFU/g meat.

2.4. pH and colour analysis

On a portion of each left rabbit loin, pH and colour were measured. The pH was tested in triplicate using the portable pH meter HI 99163 with a specific probe suitable for meat (Hanna Instruments, Italy), properly calibrated using buffer solutions of pH 4.01 (HI70004, Hanna Instruments, Italy) and 7.01 (HI70007, Hanna Instruments, Italy). Regarding the colour evaluation, three measurements were taken on transverse cuts of the loin's caudal portion after the samples were allowed to oxygenate for 5 min at room temperature. The coordinates of lightness (L^*), redness (a^*), and yellowness (b^*) according to the CIEL^* a^* b^* system (CIE, 1976), were evaluated using a colorimeter (Minolta CR400 Chromameter, Osaka, Japan—light source of D65 calibrated against a standard white tile).

Total colour difference (ΔE) was calculated between groups and between storage times, as previously shown (Castrica et al., 2020; Castrica, Menchetti, et al., 2020; Sharma, 2017):

$$\Delta E_{0-1} = \sqrt{(L_0 - L_1)^2 + (a_0 - a_1)^2 + (b_0 - b_1)^2}$$

A score of 2.3 was used as a threshold for human noticeable differences.

2.5. Sensory analysis

Fresh leg muscles of rabbits were used for the sensory test at the Department of Veterinary Medicine and Animal Sciences, University of Milan (Italy). At each time points during shelf-life (day 2, day 5 and day 10) the evaluations were carried out by a group of 9 trained panellists, always the same during the 3 sessions, to highlight any differences resulting from animal nutrition that might be perceptible to the consumer at the time of purchase. Before the sensory sessions, the panel group identified the descriptors to be evaluated, which were: texture consistency, odour/smell, and colour, and each of them chose 8 specific definitions (Table 2). The model proposed by Iqbal et al. (2013) and Brenda and Lyon (2001), with minor adaptations, was used to elaborate the sensory sheet. In the assessment sessions, assessors were presented with samples from each group simultaneously. Their responsibility was to assign the most suitable definition to the descriptors while concurrently providing an acceptability score on a scale ranging from 1 (not acceptable) to 10 (acceptable). The sessions concluded with the judges answering the following questions, *"Which one did you appreciate the most?"* and *"Which one did you like the least?"* and scoring them to overall liking & disliking.

Table 2
Definition to be attributed to texture, odour/smell, and colour.

Texture	Odour/smell	Colour
a: extremely low	a: pungent	a: white
b: very low	b: bad	b: light yellow
c: moderately low	c: moderately unpleasant	c: pale
d: slightly low	d: smell-less	d: creamy
e: slightly high	e: slightly pleasant	e: brown
f: very high	f: pleasant	f: pink
g: moderately high	g: very pleasant	g: orange
h: extremely high	h: sweet	h: red

2.6. Histochemical analysis

Rabbits' leg muscles were sampled for *Semimembranosus muscle* (SM): 1cm^3 samples were covered in Killik O.C.T. (Bio-Optica, Milan, Italy) embedding medium and gradually frozen in – 80 °C isopentane, previously cooled in liquid nitrogen. Samples were finally stored at −20 °C. Succinate Dehydrogenase histochemical staining was performed on 5um fresh transverse sections (n = 4/treatment) cut at the cryostat and let dry. They were then rinsed in distilled water and incubated for 1 h at 37 °C in the dark in a saline solution enriched with Sodium Succinate (Sigma-Aldrich, Milan, Italy) and Nitro-Blue-Tetrazolium (Sigma-Aldrich, Milan, Italy). After the incubation, samples were rinsed in distilled water and mounted in glycerol (Sigma-Aldrich, Milan, Italy). Images were acquired by epifluorescent microscopy at 100× magnification (Optika S.r.l., Ponteranica, Italy). Histometrical analyses were performed to evaluate the percentage (%) of SDH-positive and SDH-negative fibers using *Optika Proview x64* imaging software. For each sample, we considered 10 microscopic fields at a magnification of 40×. The percentage of negative and positive fibers was evaluated in each field and the obtained results were averaged for each animal.

2.7. Statistical analysis

The distribution of microorganisms in the meat was characterized by two key parameters: as prevalence (percentage of units that contain the target organism above a predetermined microbiological limit) and microbial count (expressed as Log CFU/g) (Food and Agriculture Organization of the United Nations, 2016).

Prevalences were analyzed by generalized estimating equations (GEE) using binomial as probability distributions and logit as a link function. These analyses assessed the effects of the group (with three levels: C, BC-2.5, and BC-5), the sampling time (with three levels: 2, 5, and 10 days), and their interaction. Regarding microbial count, pH, and colour related parameters were analyzed using full factorial models by GEE procedures where normal and identity were, respectively, set as a probability distribution and link function. Only the samples with positive results (>1 Log CFU/g) for each microorganism were considered for this analysis. Sidak correction was used for pairwise comparisons.

Regarding the histochemical results, an ordinary one-way analysis of variance (ANOVA followed by Tukey's multiple comparisons test was performed using the statistical software GraphPad Prism 8.0.1. The considered fixed effect was the treatment, i.e., the % of bovine colostrum in the diet.

A non-parametric approach was used for the analysis of sensory parameters. The scores obtained for the Overall liking and acceptability were presented as median and range while Kruskal-Walli's test was used to compare these scores among groups. Panellists' consistency assessment of acceptability scores was evaluated using Cronbach's alpha (Pinto, Fogliatto, & Qannari, 2014). Values of $\alpha > 0.55$ were considered acceptable (Pinto et al., 2014). Fisher's exact test was used to assess whether there was a difference between the three groups in the texture quality, odour, and colour attributed to the meat by the panellists. The proportions of each group were compared by pairwise z-tests. Finally, distributions of answers for the question *"Which did you appreciate the most?"* and *"Which one did you like the least?"* were analyzed using Chi-Square Goodness of Fit Tests. The null hypothesis assumed that there is no significant difference between the observed and the expected value (all groups equal). Statistical analyses were performed with SPSS Statistics version 25 (IBM, SPSS Inc., Chicago, IL, USA). The level for statistical significance was set at $P < 0.05$.

3. Results and discussion

The pH of meat is an important factor that can have implications for other quality traits such as water-holding capacity, colour, tenderness, and shelf life (Debrecéni, Lípová, Bucko, Cebulska, & Kapelánski, 2018; Ewa Czarniecka-Skubina, Przybylski, Jaworska, Kajak-Siemaszko, & Wachowicz, 2010). It is thus essential that after the animal's demise, the pH progressively decreases to allow the muscle to attain an appropriate level of acidification. In this study, the initial mean pH (day 2) was 5.48, and it gradually increased to 5.70 on day 10 ($P < 0.001$), but the effect of the diet was not significant ($P = 0.665$; Fig. 1). The moderate increase in pH observed during the storage period suggests relatively low enzyme activity on the nitrogen component, with values aligning well with this type of meat. Castrica, Menchetti, et al. (2020) and Menchetti et al. (2020) reported average pH values for rabbit meat at 5.90 and 5.66, respectively, with no or minimal effects linked to dietary treatments. There are several studies in the literature in which pH is not affected by dietary antioxidant supplementation (Cullere et al., 2018; Dal Bosco et al., 2015). In contrast, Nutautaitė, Racevičiūtė-Stupelienė, Pockevičius, and Vilienė (2023) found a decrease in pH 48 h after slaughter in loins of rabbits fed with a diet supplemented with 8% biomass of *C. glomerata*.

As with pH, the colour was not significantly affected by diet, but a^*, b^*, and L^* coordinates changed over time (Fig. 2). In particular, the marginal mean of a^* increased after 5 days of storage ($P = 0.027$) although the values were quite variable, and the group effect was not significant ($P = 0.316$), thus the pairwise comparisons did not reveal any significant differences (Fig. 2b). The progressive increase over time was more evident for b^* (from 6.5 at day 2 to 7.5 at day 10; $P < 0.001$; Fig. 2c) and L^* (from 52.7 at day 2 to 55.5 at day 10; $P < 0.001$; Fig. 2a), even if the group effect remained not significant ($P = 0.866$ and $P = 0.203$ for b^* and L^*, respectively). It can also be noted that for b^* coordinate, the pairwise comparisons showed significant overtime increase only in the control group (Fig. 2c), while for L^*, no increase was recorded in the BC-5 group (Fig. 2a). Colour changes in fresh meat during storage depend on lipid, protein, and myoglobin oxidation phenomena and the interaction between them (Ramanathan, Suman, & Faustman, 2020; Wang, He, Gan, & Li, 2018), which tend to lead to a loss of lightness and redness and an increase in yellowness (Wang, Tu, Zhou, Lu, & Xu, 2021). However, colour does not always evolve in that direction, as the findings of this work, and animal diet may be to a greater or lesser extent implicated in this development. In a recent study by Nutautaitė et al. (2023), a diet supplemented with 8% *C. glomerata* reduced the lightness of rabbit meat compared with the standard diet 24 h after slaughter, but after 48 h, the L^* coordinate was similar to that of the control, which had meanwhile become more yellow. In other rabbit studies, Castrica, Menchetti, et al. (2020) observed increases in L^*, a^*, and b^* during storage and slightly lower L^* and b^* values due to the animals' diets supplemented with goji berries, but only 48 h after slaughter; similarly, Cullere et al. (2018) reported increases in L^*, a^* and b^* over time, but with no effect related to 3% flax included in the diet and the type of packaging. Furthermore, Cullere, Singh, Gerencsér, Matics, and Cappellozza (2021) found that replacing sunflower oil with silkworm oil in the diet of growing rabbits increased the lightness and yellowness of the loins at day 0 of the shelf life. However, after 3 and 7 days, no significant differences were observed between the dietary groups.

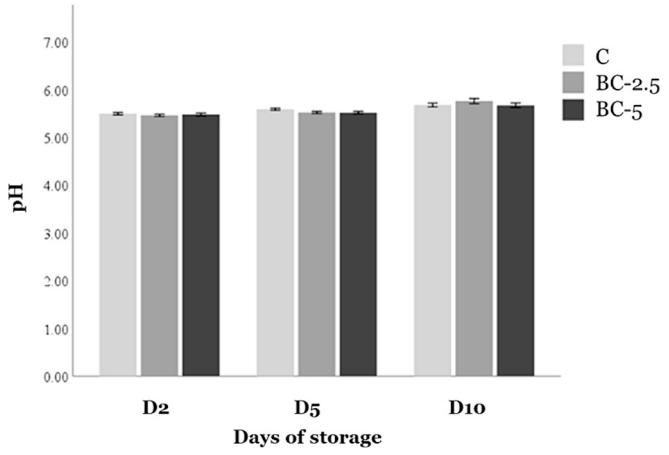

Fig. 1. Effect of colostrum inclusion in the diet of rabbits on pH of the meat at 48 h *post-mortem* (day 2), day 5, and day 10 of shelf-life. C = control diet; BC-2.5 = diet supplemented with 2.5% of bovine colostrum; BC-5 = diet supplemented with 5% of bovine colostrum. The graph show mean ± standard error.

White meat, such as rabbit meat, contains more polyunsaturated fats, which are prone to oxidation, compared to red meat (Alasnier & Gandemer, 1998; Wood et al., 2004). On the other hand, white meat, in particular rabbit L. *thoracis et lumborum* muscles, has a low content of lipids and heme-proteins (Cullere & Dalle Zotte, 2018), this results in a reduced presence of free iron which can act as a pro-oxidant (Domínguez et al., 2019; Ouhayoun, 1992). These conditions likely contribute to the meat's resilience to colour changes during storage, as observed in a study by Cullere et al. (2018). In this regard, it is interesting to note that in the colostrum groups, yellowness, which in rabbit meat is closely linked to lipid oxidation (Lan, Shang, Song, & Dong, 2016; Wang et al., 2018) remained unaltered over time (Fig. 2c).

This trend is consistent with a previous study according to which the inclusion of colostrum in the diet of rabbits decreased the share of polyunsaturated fatty acids (PUFAs) in favour of saturated fatty acids (SFAs) and, at the same time, lipid oxidation during meat storage (Castrica et al., 2022).

The changes in L^*, a^*, and b^* coordinates recorded over time within each group ultimately resulted in total colour differences that could be perceptible to the human eye upon visual assessment. In fact, from the calculation of ΔE (Table 3), it appears that the colour of the meat remained similar to the initial colour until the third day of shelf-life ($\Delta E_{2-5} < 2.3$). However, as storage continued, the colour coordinates continued to change, so that at the last detection the ΔE_{2-10} of the control and BC-2.5 groups exceeded the threshold of 2.3 points. In the BC-5 group, on the other hand, the evolution of L^*, a^*, and b^* coordinates reversed between the first and second parts of the conservation, so that the ΔE exceeded the threshold of 2.3 points between the second and third detections ($\Delta E_{5-10} > 2.3$). In contrast, when comparing between groups, ΔE always remained below 2.3 at all times of analysis. These results on the whole show that the addition of colostrum to the diet does not make any changes to the colour characteristics of the meat and that the substances possibly transferred through the diet are unable to arrest the colour changes that occur during storage.

In the histochemical analysis, red fibers tested positive to the histochemical staining, appearing as blue-stained. Conversely, those fibers that did not exhibit this reaction were considered negative and identified as white fibers (Fig. 3).

The statistical analysis revealed that the percentage of positive red fibers is significantly higher in the BC-5 group than in the C group (Fig. 4). This means that the treatment at 5% led to a significant difference ($P < 0.05$) in the number of slow oxidative fibers. Though apparently, the percentage of red fibers in the BC-2.5 group seemed higher than in the control group, no significant differences were observed, neither between the control group and BC-2.5 nor between BC-2.5 and BC-5.

For what concerns white fibers, a significant difference ($P < 0.05$) was found between the C group and the BC-5 group, with a lower percentage of white fibers in the latter; no significant differences in the percentage of white fibers were found neither between the C group and the BC-2.5 group, nor between the two treated groups.

The nutritional properties of feed can influence animal muscle fibers in several aspects. For example, in recent research of Nutautaitė et al.

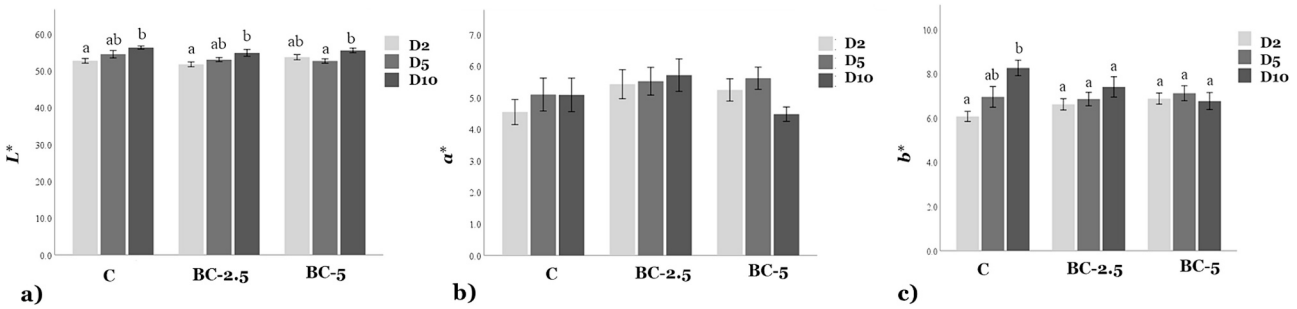

Fig. 2. Effect of colostrum inclusion in the diet of rabbits on lightness (L^*, a), redness (a^*, b), and yellowness (b^*, c) at 48 h *post-mortem* (day 2), day 5, and day 10 of shelf-life. C = control diet; BC-2.5 = diet supplemented with 2.5% of bovine colostrum; BC-5 = diet supplemented with 5% of bovine colostrum. The graphs show mean ± standard error. Bars not sharing any superscript within each group are significantly different at $P < 0.05$.

Table 3
Total colour difference (ΔE) between groups and between storage time. C = control diet; BC-2.5 = diet supplemented with 2.5% of bovine colostrum; BC-5 = diet supplemented with 5% of bovine colostrum.

	Storage days		
	Day 2	Day 5	Day 10
C vs BC-2.5	1.41	1.53	1.79
C vs BC-5	1.46	1.91	1.80
BC-2.5 vs BC-5	1.98	0.46	1.54
	Day 2 vs Day 5	Day 2 vs Day 10	Day 5 vs Day 10
C	2.07	4.26[1]	1.31
BC-2.5	1.30	3.22[1]	1.93
BC-5	1.13	1.98	3.10[1]

[1] Value over the threshold (2.3 points) with a noticeable difference in colour between the samples.

(2023), 8% of *C. glomerata* inclusion in the rabbit's diet stimulated muscle growth by increasing the fiber length of the LTL. Colostrum is rich in n-3 PUFAs (Wilms et al., 2022) and some studies have proved that PUFAs can act on muscle metabolism (Tachtsis, Camera, & Lacham-Kaplan, 2018), promoting muscle fiber type switching (Castillero, Martín, López-Menduiña, Villanúa, & López-Calderón, 2009; Wang et al., 2022). Peoples and McLennan (2010) observed a higher oxidative capacity of hindlimb muscles in rats whose diet had been enriched with fish oil, which is rich in n-3 PUFAs. Similarly, Mizunoya et al. (2013) demonstrated that a fish oil rich diet increased the level of oxidative fibers in rats glycolytic *Digitorum longus* muscle. They observed an upregulation of those genes linked to metabolic and contractile properties of skeletal muscles, thus proving that PUFAs in fish oil can positively affect the oxidative properties of white muscles (Mizunoya et al., 2013). To our knowledge, there have been no previous studies conducted on the effect of colostrum administration on muscle metabolism. However, the histological characterization we performed on the glycolytic muscle *Semimembranosus* suggest that BC supplementation can influence the fiber type composition, promoting the shift toward red oxidative fibers. It is reasonable to assess that BC PUFAs, similarly to those in fish oil, can act on muscle fiber profile by upregulating metabolism related genes. Nevertheless, further studies are still needed to confirm the role of BC supplementation on muscle fibers types.

From a microbiological point of view, all plates were positive for TVC at all time points. Regardless of group, TVC count increased from 2.40 ± 0.16 Log CFU/g at day 2 to 4.68 Log CFU/g at day 10 ($P < 0.001$). However, in the latter part of the storage period, microbial growth in the treated groups slowed down (BC-2.5 and BC-5 groups: TVC day 5 vs TVC day 10, $P > 0.05$), resulting in a significant effect of the group ($P < 0.001$) and the interaction between group and time ($P = 0.028$) significant. Consequently, at day 10, the Control group had higher values of TVC than the BC-5 group ($P = 0.001$) and, although with weak evidence ($P = 0.051$), also compared to the BC-2.5 group (Fig. 5a). As regards the *Pseudomonas* spp. count (Fig. 5b), all samples were negative at day 2.

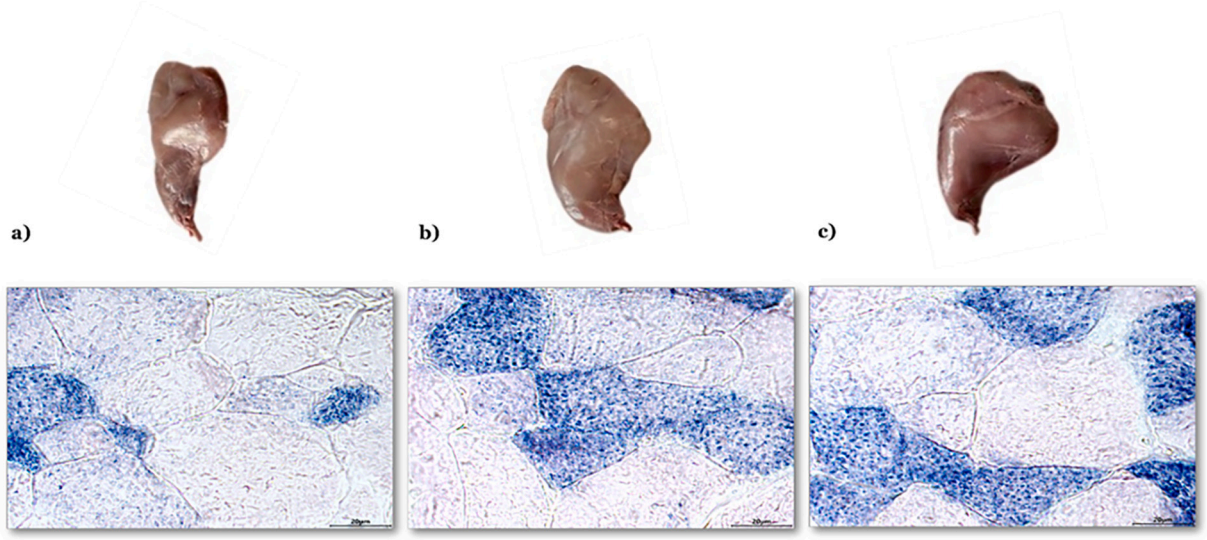

Fig. 3. Effect of bovine colostrum inclusion in the diet of rabbits on fibers of *Semimembranosus muscle*. C = control diet (a); BC-2.5 = diet supplemented with 2.5% of bovine colostrum (b); BC-5 = diet supplemented with 5% of bovine colostrum (c). Blue staining red fibers, and no staining white fibers. Scale bar 20 μm. 100× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

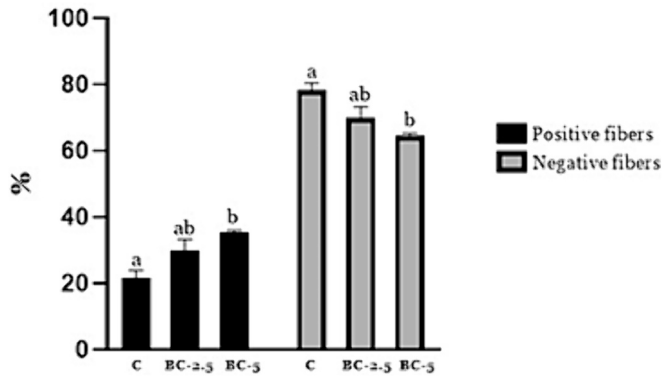

Fig. 4. Effect of bovine colostrum inclusion in the diet of rabbits on % fibers of the hind leg muscles. C = control diet (a); BC-2.5 = diet supplemented with 2.5% of bovine colostrum (b); BC-5 = diet supplemented with 5% of bovine colostrum (c). Positive fibers: red fibers. Negative fibers: white fibers. The graph show mean ± standard error. Values followed by the same letter between groups do not differ significantly ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Subsequently, their prevalence ($P < 0.001$) and count progressively increased ($P = 0.004$) but without the effect of the group ($P = 0.884$ and $P = 0.718$ for prevalence and count, respectively) or its interaction with the time ($P = 0.305$). Likewise, at day 2, all samples were negative for *Lactobacillus* spp. (Fig. 5c) but subsequently their count progressively increased ($P < 0.001$) thereafter the group did not exhibit significant differences ($P = 0.193$) although a trend toward significance was found for its interaction with time ($P = 0.054$). At day 10, *Lactobacillus* spp. counts of the BC-5 group tended to be lower than the control ($P = 0.082$).

Overall, these results highlight a slight slowdown in microbial development exerted by dietary colostrum supplementation on rabbit meat during storage, particularly on the total viable count. However, the limited impact on the other evaluated parameters raises questions about the effectiveness of colostrum as an antimicrobial agent in this context. The unconditional growth of *Pseudomonas* spp., which are considered to be among the main spoilage bacteria of refrigerated meat (Corry, 2007), raises doubts about the real antimicrobial effect of colostrum and even more about any extension of the shelf life of the meat. These results are consistent with a previous study by Castrica et al. (2022), in which rabbit diets supplemented with colostrum resulted in some variation in microbial counts over time, but with no differences between groups, so the authors concluded that no molecules with antimicrobial activity are transferred from feed to meat. As regard the sensory profile, up to the third day of storage, the inclusion of colostrum in the diet did not generate changes in texture and odour, with the judgments of the evaluators being quite varied within each group in the first two sessions (Tables S1 and S2). In general, at day 2, at least 55% of the judges described meat texture as more or less consistent, and odour as more or less pleasant, with no differences between groups ($P = 0.498$ and $P = 0.389$ respectively). However, as the shelf life progressed, these attributes evolved differently within the groups; in particular, the texture of the BC-5 group became less consistent, such that, at the end of the observation period, most assessors continued to define the texture of the control and BC-2.5 groups as "*moderately and slightly high*", while no assessors defined the meat of the BC-5 group in this way, actually perceiving it as more tender ($P < 0.009$). Similarly, odour, at the day 10 of shelf life was also perceived with differences: 89% of the assessors called the odour of the control group "*bad*" and 66.7% "*moderately unpleasant*" that of the treated groups.

Conversely, regarding colour (Table S3), between-group differences in assessments were evident right from the first observation. At day 2, a greater proportion of meat of the control group was defined as pale than the meat of the BC-2.5 and BC-5 groups ($P = 0.003$), which was defined as pink. As storage continued, the colour gradually intensified, so that in subsequent observations, most judges also defined the meat of the control group as pink and the meat of group BC-5 as red ($P < 0.01$). These results are consistent with the instrumental assessment of colour, which had shown an increase in a^* coordinate over time, but no difference with diet. In addition, the varying descriptions of meat colour by the judges across the three groups are in line with the results of histological examination, which showed an increase in red fibers at the expense of white fibers directly proportional to the addition of colostrum in the diet. Consumers pay a lot of attention to meat colour, which can be decisive at the time of purchase. In our study, the differences noticed based on the diet did not adversely affect the acceptability of colour or other assessed attributes, as illustrated in Table 4. It was more the time that influenced their acceptability, as shown by the decrease in scores incurred in all groups in the second part of conservation (Table 4).

Assessors' preferences for overall liking were highly heterogeneous within each group (Table S4), therefore no significant differences in scores attributable to diet were found at each time point (Table 4). Particularly, at the beginning of the sensory session, the medians were around the score of 5. However, going ahead with shelf-life days, the medians fluctuated first upwards and then downwards, so much so that the last recorded measurement values were significantly lower than previously recorded ones ($P < 0.001$; Table S5). It is worth noting that

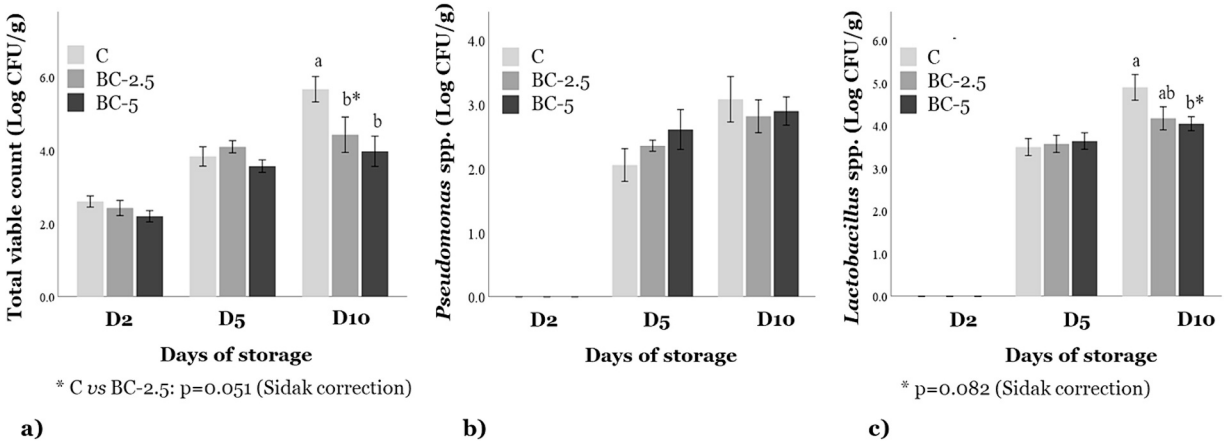

Fig. 5. Effects of colostrum supplementation in rabbit diet on TVC (a), *Pseudomonas* spp. (b), and *Lactobacillus* spp. (c) of meat at 48 h *post-mortem* (day 2), day 5, and day 10 of shelf-life. C = control diet; BC-2.5 = diet supplemented with 2.5% of bovine colostrum; BC-5 = diet supplemented with 5% of bovine colostrum. The graphs show mean ± standard error. Bars not sharing any superscript within each time are significantly different at $P < 0.05$.

Table 4
Effect of colostrum inclusion in the diet of rabbits on acceptability score at 48 h *post-mortem* (day 2), day 5, and 10 days of shelf-life. C = control diet; BC-2.5 = diet supplemented with 2.5% of bovine colostrum; BC-5 = diet supplemented with 5% of bovine colostrum.

Acceptability score	Storage days	C			BC-2.5			BC-5			*P* value
		Md	Min	Max	Md	Min	Max	Md	Min	Max	
Overall liking	2	5	3	7	5	3	7	5	3	8	0.803
	5	6	4	7	6	3	6	7	5	8	0.113
	10	2	1	7	4	2	7	4	3	7	0.069
Texture	2	8	7	9	8	7	10	8	7	9	0.821
	5	8	7	9	8	7	9	8	7	10	0.464
	10	6	5	8	6	5	7	6	4	7	0.867
Odour/smell	2	9	7	10	9	7	10	9	7	10	0.948
	5	8	7	10	8	7	10	8	7	9	0.754
	10	6	5	8	7	5	8	6	5	8	0.578
Colour	2	9	7	10	8	7	10	9	8	10	0.748
	5	8	7	9	8	7	10	9	7	10	0.732
	10	6	4	7	6	4	7	7	4	8	0.604

Abbreviations: Md = median value; min = minimum; value; max = maximum value.

over half of the panellists consistently named the meat of the BC-5 group as their favourite and that of the control group as the least liked meat (Table 5). However, Chi-Square Goodness of Fit Tests were not significant at any time point.

4. Conclusion

The addition of bovine colostrum to the rabbit diet influenced some meat quality traits during storage, although it did not lead to significant improvements. When the diet contained the highest concentration of bovine colostrum, some antimicrobial activity was transferred to the meat, effectively slowing bacterial growth. However, this effect was only significant for TVC at the end of the observation period, making the antimicrobial action too slight and it did not have a substantial impact on other quality traits, but these findings need further verification. Concerning colour, dietary regimen affected the evolution of coordinates during the shelf life, but the differences that emerged over time were only within groups. It is plausible that the inclusion of bovine colostrum did not confer antioxidant properties to the meat, or in any case not in quantity sufficiently to contrast the changes in colour, which remained influenced mainly by the time variable. On a sensory level, the diet generated some differences between the groups in terms of how descriptors were defined, but this did not significantly affect the overall liking or the overall scores of individual descriptors, which were affected by storage time. Given these results, the inclusion of up to 5% colostrum in growing rabbit nutrition does not present any contraindications from a sensory point of view, although it does not seem to be an all-sufficient strategy for improving meat quality or preserving it during storage.

Nevertheless, it is essential to assess additional quality aspects beyond the scope of this study, including alternative dietary formulations and diverse methods of feeding animals. Expanding the studies to include other species of livestock is also imperative. This broader examination will yield data, currently insufficient, necessary for a comprehensive cost-benefit analysis to determine whether bovine colostrum can serve as a valuable resource for the food industry.

Finally, to understand the potential of its bioactive compounds, the authors of the present study are currently investigating the effects of bovine colostrum in formulations applied directly on meat during preparation and packaging.

Table 5
Effect of colostrum inclusion in the diet of rabbits on appreciation percentage, at 48 h *post-mortem* (day 2), day 5, and day 10 of shelf-life. C = control diet; BC-2.5 = diet supplemented with 2.5% of bovine colostrum; BC-5 = diet supplemented with 5% of bovine colostrum.

		Storage days		
		Day 2 (% respondents)	Day 5 (% respondents)	Day 10 (% respondents)
"Which did you appreciate the most?"	C	33.3	22.2	0.0
	BC-2.5	11.1	22.2	33.3
	BC-5	55.6	55.6	66.7
"Which one did you like the least?"	C	55.6	55.6	66.7
	BC-2.5	33.3	44.4	22.2
	BC-5	11.1	0.0	11.1

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Ethics statement

Ethic Committee Name: Università degli Studi di Milano, approval Code: OPBA_42_2021, 7 May 2021.

CRediT authorship contribution statement

Marta Castrica: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Laura Menchetti:** Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. **Stella Agradi:** Validation, Visualization, Writing – review & editing. **Giulio Curone:** Conceptualization, Validation, Visualization, Writing – review & editing. **Daniele Vigo:** Validation, Visualization, Writing – review & editing. **Grazia Pastorelli:** Validation, Visualization, Writing – review & editing. **Margherita Pallaoro:** Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alessia Di Giancamillo:** Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Silvia Clotilde Modina:** Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Federica Riva:** Validation, Visualization, Writing – review & editing. **Valentina Serra:** Validation, Visualization, Writing – review & editing. **Egon Andoni:** Validation, Writing – original draft, Writing – review & editing. **Gabriele Brecchia:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Validation, Writing –

review & editing. **Claudia Maria Balzaretti:** Validation, Visualization, Writing – review & editing. **Dino Miraglia:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest in this article.

Data availability

The data sets that support the findings of this study are available from the corresponding author upon reasonable request.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2024.109512.

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