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Diet supplemented with olive cake as a model of circular economy: Metabolic and endocrine responses of beef cattle

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Introduction: Integrating by-products into livestock diet represents a great opportunity for implementing the concept of circular economy while reducing feed costs. Olive cake (OC) is considered an agro-industrial waste, but the high content of valuable metabolites makes it a promising feed integration. Therefore, this study investigated the effect of OC integration in beef cattle diet on different blood parameters.

Methods: Forty-eight young growing fattening Limousines-−24 bulls (body weight 350 ± 15 kg) and 24 heifers (280 ± 10 kg)—, aged 240 ± 20 days, were randomly allocated to 1 of 3 dietary treatments: concentrate at 0% (Control group: CTR), 10% (Low-olive cake group: L-OC), or 15% (High-olive cake group: H-OC) of OC inclusion. Blood samples and body weights were collected before administrating the supplemented diet (0 d), at the end of the stocker growing phase (56 d), and at the end of the fattening (147 d). After being slaughtered, animal carcasses were weighted. A linear regression model was fitted for each blood parameter with the 0 d as covariate and diet, time, sex, diet × time, and diet × sex as fixed effects.

Results: In males, body weight was highest in CTR, but carcass weight was similar in all the groups. All the blood parameters were within physiological ranges, independently from the animal diet. CTR group showed the highest alanine aminotransferase (ALT, $P = 0.0027$) and creatine kinase ($P = 0.0119$), whereas total bilirubin ($P = 0.0023$) was higher in H-OC than in CTR. Moreover, ALT was highest in CTR at 56 d, becoming similar in all the groups at 147 d ($P = 0.0280$). Instead, the increase observed in total cholesterol from 56 to 147 d was lower in H-OC compared with CTR and L-OC ($P = 0.0451$). A significant effect of diet × sex interaction was observed on triglycerides, urea, liver enzymes, and insulin. These data support the OC inclusion of up to 15% of the concentrate with no detrimental effect on beef cattle metabolic status.

Discussion: In conclusion, OC can be considered as a component in beef diet giving an opportunity to improve agriculture sustainability.

KEYWORDS

beef cattle, olive cake, metabolism, circular economy, olive by-products

1. Introduction

In the Mediterranean area, the production of olive oil is significant; indeed, it contributes to 76% of the total production of olive oil, with Spain, Italy, and Greece being the main producers (Berbel and Posadillo, 2018). It is estimated that in European countries about 11.8 million tons of biomass are produced from the olive tree pruning process (Berbel and Posadillo, 2018) and since the production of olive oil is an important source of energy and water consumption, the increase in the olive oil industry has led to an inevitable rising of environmental impact. Besides the considerable emissions, it generates a relevant amount of waste that must be disposed (Salomone and Ioppolo, 2012). The main by-products generated from olive oil process are olive mill wastewater and olive cake (Foti et al., 2022). However, because of its properties, processing waste like olive cake (OC) could be used in different sectors, such as energy generation, food products, pharmaceutical industry, and animal feed (Espeso et al., 2021). In particular, the use of olive cake as animal feed is an interesting and sustainable alternative to its disposal, because it may decrease the costs associated with animal feeding, valorizing a waste biomass and at the same time improving the quality of the products (Keleş, 2015; Castellani et al., 2017; Vastolo et al., 2019). In fact, the spread of its use as integrator in animal feeding is mainly related to the presence of substances with antioxidant and radical scavenging activity, and to its richness in monounsaturated fatty acids (MUFAs) (Ghanbari et al., 2012). There are many studies on the incorporation of this by-product in livestock diet, for example in broilers (Al-Harthi, 2016), pigs (Joven et al., 2014; Liotta et al., 2019), goats (Alkhtib et al., 2021; El Otmani et al., 2021), sheep Chiofalo et al., 2004; Vargas-Bello-Pérez et al., 2013, dairy cattle (Zilio et al., 2015; Chiofalo B. et al., 2020; Neofytou et al., 2020), and beef cattle (Estaún et al., 2014; Branciari et al., 2015; Chiofalo V. et al., 2020).

According to Estaún et al. (2014), the addition of pitted and dehydrated olive cake at rate of 10 or 20% in the concentrate led to no differences in terms of either animal performance for the whole growth period or rumen fermentation parameters in Frisian steers from 100 to 450 kg of body weight. In addition, lack of differences found at rumen fermentation level (pH and volatile fatty acids) suggests that olive cake supplementation (10 or 20% in the concentrate) does not negatively affect the activity of microbial populations, at least in Frisian steers. From these outcomes it is reasonable to suppose that including pitted and dehydrated olive cake in the diet of beef cattle should not affect animal performance and at the same time might be suitable to implement a supply chain system at the economic and sustainable level.

However, to the best of our knowledge, a few studies have been carried out to investigate the effect of olive cake on the metabolic and endocrine traits of beef cattle. Hence, the aim of this study was to determine if the changes in plasma triglycerides (TG), total cholesterol (TCHOL), glucose (GLU), total bilirubin (TBIL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), and urea in heifers and bulls are substantial enough to indicate the need to establish specific reference intervals also for young growing fattening Limousine bulls and heifers. The direction and magnitude of the changes in these parameters could be used as diagnostic tools for the detection and monitoring of metabolic and endocrine traits that could arise during growth in this species fed concentrate with different percentages of olive cake inclusion.

2. Materials and methods

The experimental protocol was approved by the Ethical Committee of the Department of Veterinary Science, University of Messina, Italy (code 041/2020). The research complied with guidelines of Good Clinical Practices (The European Agency for the Evaluation of Medicinal Products, 2000) and the Italian and European regulations on animal welfare (Directive 2010/63/EU) (Council of the European Union, 2010).

2.1. Animal management and experimental design

The experiment was carried out using a total of 48 Limousine young growing fattening animals (24 bulls and 24 heifers), aged 250 ± 20 days at the time of the first sampling. The initial body weight (BW) was on average 350 ± 15 kg for bulls and 280 ± 10 kg for heifers. This breed was chosen because it is the most diffused in Sicily for breed production. All animals were housed in the same pen of a semi-open straw-bedded barn located in Santa Croce Camerina (Ragusa, Italy, 36°49′38″N 14°31′26″E). The farm is located within the Hyblean Mediterranean area with an unequally distributed rainfall throughout the year. Annual mean rainfall is about 700 mm. The annual average temperature is 19.58°C (with an average of maximum and minimum temperature of 21 and 17°C, respectively).

Animals were raised according to an approved UE disciplinary called "QS Sicilia," which allows the inclusion of olive cake in feed for beef cattle not <10% of the diet, as a strategy for the recovery of agro-industrial by-products.

Abbreviations: OC, olive cake; FA, fatty acids; TG, triglycerides; TCHOL, total cholesterol; GLU, glucose; TBIL, total bilirubin; ASP, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; BW, body weight; CTR, control group (0% inclusion); L-OC, low-olive cake group (10% inclusion); H-OC, high-olive cake group (15% inclusion); ADG, average daily gain; CW, carcass weight; LSM, least square mean.

After a 2-week adaptation period, animals were randomly allocated to one of three dietary treatments according to their BW: inclusion of olive cake at 0% (Control group: CTR), 10% (Low-olive cake group: L-OC), or 15% (High-olive cake group: H-OC) in the concentrate. The compositions of the concentrates and of the OC used for the study are reported in Table 1. All the animals were allowed *ad libitum* access to straw and water. After adaptation, animals received their concentrate (equal to 2% of their body weight, on average) twice daily (7.00 a.m. and 3.00 p.m.). During the whole experimental period (147 days), none of the animals had health problems. When animals reached the endpoint, they were transported to a licensed slaughterhouse located 20 min (19.2 km) away from the farm. Animals fasted for 12 h before slaughter, which was performed according to EU Regulations. The interval between the first and the last slaughter was 18 days.

TABLE 1 Feed and nutrient composition of concentrate with 0% (Control group: CTR), 10% (Low-olive cake group: L-OC), or 15% (High-olive cake group: H-OC) of olive cake (OC) inclusion fed to growing fattening Limousine bulls and heifers.

	CTR	L-OC	H-OC
Feed composition (% DM)			
Corn flour	34.00	35.00	35.00
Soybean meal 44	18.00	15.00	15.00
Corn flakes	13.00	13.00	13.50
Destoned olive cake	–	10.00	15.00
Wheat bran	11.00	4.00	4.00
Barley	10.00	9.00	8.00
Sunflower	7.00	5.00	1.40
Vitamin and mineral mix*	4.00	4.00	3.30
Soybean flakes	2.00	4.00	4.00
Carob	1.00	1.00	0.80
Saccharomyces cerevisiae, live yeast	0.40	0.40	0.40
Sodium bicarbonate	0.80	0.80	0.50
Sodium chloride	0.50	0.50	0.50
Sodium propionate	0.30	0.30	0.40
Calcium carbonate	0.30	0.30	0.30
Dicalcium phosphate	0.20	0.20	0.20
Nutrient composition			
Dry matter	89.20	88.50	89.00
Crude protein (% DM)	18.50	18.20	18.30
Crude fat (% DM)	5.00	5.40	6.10
Ash (% DM)	5.00	5.10	4.90
Acid detergent fiber (% DM)	8.50	10.60	11.50
Neutral detergent fiber (% DM)	44.30	46.70	45.30
Starch (% DM)	44.00	43.90	43.40
Net energy (UFV/kg of DM)**	1.09	1.08	1.08

*Vitamin E (1,500 UI/head/d), Selenium (0.30 ppm/head/d), Zinc (1,000 ppm/head/d).
**The UFV/kg of dry matter intake of concentrate is the value of energy density according to the INRA feeding system, corresponding to the Net energy for meat production (in kcal/kg)/1,760.

2.2. Animal measurements in vita and post-mortem

Animals of each pen, before feed distribution, were individually weighed at the beginning of the trial, after 56 days, and at the end of the trial (147 days) using Brecknell PS-2000 Veterinary Floor Scale (capacity: 1,000 kg, readability: 0.5 kg). Individual average daily gain (ADG) was also calculated. At the end of the trial, animals were slaughtered, and after slaughter the hot carcass weight (CW) was recorded, and the dressing percentage was calculated. pH was determined 1 and 24 h after slaughter using a portable pH meter (Hanna Instruments, Woonsocket, RI, USA) and combined glass electrode, inserted ∼5 cm into the *longissimus thoracis* muscle.

2.3. Blood samples collection and analysis

The blood samples were collected at the beginning (day 0, before administrating treated concentrates) and on days 56 and 147 of the trial, in the morning before dispensing the concentrate. Blood was collected from the tail vein by trained veterinarians through venipuncture into 10-mL tubes containing clot activator and separating gel (Terumo Corporation, Tokyo, Japan). Blood samples were centrifuged for 10 min at 2,000 × g; the supernatant serum was collected and stored at −20°C until analyses.

Biochemical lipids (TG, TCHO), liver (TBIL, AST, ALT, LDH), renal (Urea), and muscular (CK), parameters were analyzed by enzymatic colorimetric method, using an automated spectrophotometry (Biotechnical Instrument BP 3500) and reagents of the same commercial house.

Glucose concentration was measured by automated spectrophotometry (Biotecnica Instruments BT 3500) using the colorimetric enzymatic method GOD-POD.

Insulin concentration was analyzed using the Immulite® two-site chemiluminescent immunometric assay (Maglium 800), with a sensitivity of 0.5 μIU/mL and intra- and inter-assay coefficients of variation equal to 1.56 and 4.07% at insulin concentrations of 16.54 and 45.804 μIU/mL.

2.4. Statistical analysis

Statistical analyses were performed with JMP®, Version 16 (SAS Institute Inc., Cary, NC). Appropriate descriptive statistics

TABLE 2 Means and results of the model for all the measured performance traits: body weight (BW), average daily gain (ADG), carcass weight (CW), dressing, and pH.

	Bulls			Heifers			SEM*	Diet	Sex	Diet x Sex
	CTR	L-OC	H-OC	CTR	L-OC	H-OC				
BW (kg)	630.51	549.46	553.23	467.32	481.75	486.30	15.64	0.0794	**<0.0001**	**0.0042**
ADG (kg/d)	1.62	1.42	1.41	1.00	1.25	1.06	0.08	0.3829	**<0.0001**	**0.0276**
CW (kg)	358.12	333.52	344.98	277.07	267.63	286.68	10.76	0.2477	**<0.0001**	0.5657
Dressing (%)	56.86	60.62	62.23	59.28	55.84	58.95	1.01	**0.0308**	**0.0312**	**0.0028**
pH at 1 h	6.66	6.48	6.37	6.40	6.33	6.43	0.08	0.2199	0.0829	0.1629
pH at 24 h	5.66	5.81	5.82	5.78	5.70	5.76	0.05	0.1341	0.5652	**0.0096**

Factors' significant P-values (< 0.05) are reported in bold.
*Greatest standard error of the mean.

was generated for all the variables. The correlation between all the parameters was expressed using Pearson's coefficient (r).

The blood parameters of interest (Y) were modelized as follows:

$$Y_{ijk} = m + D + T + S + DT + DS + T0 + e$$

Where m is the mean, D is the diet (CTR, L-OC, or H-OC), T is the time of sampling (day 56 or day 147 of the trial), S is the sex of the animal (male or female), DT is the interaction between the diet and the time of sampling, DS is the interaction between the diet and the sex, $T0$ is the covariate representing the parameter level at the beginning of the trial (before administering the supplemented concentrated), and e is the random residual. With regard to the performance parameters, only the diet (D), the sex (S), and their interaction (DS) were included as factors. A logarithmic transformation was applied when necessary. When factors or interactions resulted significant, the Tukey-Kramer *post-hoc* test was used to identify the significantly different levels.

Differences were considered to be statistically significant when $P < 0.05$.

3. Results

Results of animal performance are reported in Table 2. As expected, differences between heifers and bulls were observed for all the variables with the exception of pH. Even though the diet was significant for dressing ($P = 0.0308$, with higher values for H-OC than CTR), when we considered heifers only, we observed similar values for all the performance-related variables. Instead, within bulls, we observed that the body weight was higher in the CTR group, but this was not supported by the average daily gain and the carcass weight, which were similar in all the diet groups. Consequently, the dressing resulted higher in H-OC than CTR bulls, with intermediate values for L-OC ones. One factor possibly affecting BW and CW is dry matter intake; several studies on ruminants showed no difference in feed intake between traditional and OC-integrated diets (Yáñez Ruiz et al., 2004; Awawdeh and Obeidat, 2013; Mele et al., 2014; de Evan et al., 2020) but we cannot exclude it, since it was not assessed in the present study, it being carried out in a commercial farm. It is possible that this result might also have been influenced by a difference in the feed intake among the groups; however, it was not possible to assess this data because the trial was performed in a commercial farm.

In this study, a wide range of blood plasma biomarkers associated with the metabolic variation was chosen. The descriptive statistics of the blood metabolic biomarker concentrations by sex, physiological stage, and diet, as well as the P-values and R^2 resulted from the model applied to each blood analyte are reported in Table 3. The parameters significantly affected by the diet, the diet × time interaction (indicating a different effect of the diet according to the time of sampling), and the diet × sex interaction (indicating a different effect of the diet on heifers than on bulls) are plotted in Figures 1–3, respectively.

Data obtained showed that AST, TBIL, urea, TCHO, TG and insulin significantly increased from 56 to 147 d, whereas ALT and GLU decreased ($P < 0.05$).

CTR group showed the highest ALT ($P = 0.0027$) and CK ($P = 0.0119$), whereas TBIL ($P = 0.0023$) was higher in H-OC than in CTR group (Figure 1). Moreover, ALT was higher in CTR at 56 d ($P = 0.0280$), but it was similar in all the groups at 147 d ($P = 0.028$ for diet × time interaction, Figure 2B). Instead, the increase in the level of TCHO observed from 56 to 147 d was lower in H-OC compared with both CTR and L-OC groups ($P = 0.0451$ for diet x time interaction, Figure 2A). Compared with bulls, heifers showed higher TCHO ($P = 0.0131$), TG ($P < 0.0001$), and GLU ($P = 0.0002$) and lower insulin ($P = 0.0075$) concentrations.

The diet × sex interaction was significant for AST ($P = 0.0005$), ALT ($P = 0.0015$), LDH ($P = 0.0102$), and urea

TABLE 3 Mean and standard deviation of all the blood analyte measurements, and *P*-values (in bold when significant) and R^2 of the applied model.

Sex	Heifers						Bulls						P-values						
Time of sampling	Day 56			Day 147			Day 56			Day 147									
Diet	CTR	L-OC	H-OC	CTR	L-OC	H-OC	CTR	L-OC	H-OC	CTR	L-OC	H-OC	Diet	Time	Sex	Diet × Time	Diet × Sex	T0	R^2
AST (UI/l)	82.88 ± 13.77	122.00 ± 46.00	105.63 ± 21.51	96.38 ± 49.53	153.25 ± 93.32	92.75 ± 18.89	79.63 ± 15.80	83.75 ± 14.80	92.63 ± 22.49	122.88 ± 82.07	101.25 ± 26.38	147.75 ± 45.03	0.6284	**0.0071**	0.9583	0.9613	**0.0005**	0.0096	0.27
ALT (UI/l)	34.38 ± 7.82	31.75 ± 4.23	32.13 ± 4.29	26.50 ± 4.72	35.25 ± 8.92	26.75 ± 4.92	28.50 ± 11.51	29.38 ± 8.11	28.75 ± 3.45	22.13 ± 6.98	25.63 ± 6.32	29.88 ± 4.64	**0.0027**	**0.0048**	0.7078	**0.028**	**0.0015**	**<0.0001**	0.53
LDH (UI/l)	2,987.00 ± 362.33	3,102.50 ± 362.05	3,347.50 ± 400.66	2,588.38 ± 788.48	3,430.38 ± 1,031.14	2,823.63 ± 835.99	2,888.25 ± 463.15	3,024.50 ± 389.81	3,010.25 ± 445.75	3,022.63 ± 408.94	2,987.25 ± 378.14	3,589.88 ± 632.96	0.4343	0.9063	0.1878	0.62	**0.0102**	**0.0006**	0.21
CK (UI/l)	328.75 ± 129.95	224.63 ± 75.93	333.00 ± 175.50	279.43 ± 110.34	311.50 ± 197.26	200.25 ± 65.66	328.75 ± 84.85	191.13 ± 53.34	321.13 ± 187.75	276.25 ± 71.92	243.88 ± 174.92	265.25 ± 214.03	**0.0119**	0.1988	0.6779	0.0696	0.4939	0.3498	0.16
Total bilirubin (mg/dl)	0.14 ± 0.03	0.16 ± 0.04	0.19 ± 0.03	0.23 ± 0.05	0.26 ± 0.05	0.24 ± 0.05	0.13 ± 0.01	0.16 ± 0.02	0.17 ± 0.02	0.22 ± 0.05	0.23 ± 0.05	0.25 ± 0.03	**0.0023**	**<0.0001**	0.139	0.1558	0.9434	0.5509	0.59
Urea (mg/dl)	15.00 ± 1.69	18.25 ± 2.05	14.38 ± 1.85	27.75 ± 36.92	18.38 ± 2.33	15.50 ± 2.73	14.88 ± 2.10	13.00 ± 1.93	14.00 ± 2.14	14.63 ± 2.67	15.38 ± 2.26	16.63 ± 2.07	0.0797	**0.033**	0.118	0.1493	**<0.0001**	0.1462	0.36
Total Cholesterol (mg/dl)	109.50 ± 22.51	93.88 ± 11.10	106.00 ± 18.15	158.25 ± 34.43	136.38 ± 36.54	98.13 ± 31.45	95.75 ± 10.94	92.50 ± 22.17	91.50 ± 20.62	116.13 ± 32.43	119.88 ± 29.40	119.25 ± 16.39	0.3035	**<0.0001**	**0.0131**	**0.0451**	0.1754	**<0.0001**	0.51
Triglycerides (mg/dl)	26.63 ± 6.28	27.88 ± 2.90	26.75 ± 4.71	27.38 ± 4.37	33.75 ± 4.83	23.50 ± 5.98	19.75 ± 3.20	19.50 ± 2.93	22.25 ± 4.56	23.38 ± 4.24	25.88 ± 2.95	26.50 ± 7.21	0.7072	**0.003**	**<0.0001**	0.0548	**0.0061**	0.0506	0.41
Glucose (mg/dl)	76.13 ± 8.43	78.75 ± 13.02	85.13 ± 15.99	66.75 ± 11.30	58.88 ± 12.54	76.13 ± 15.68	64.63 ± 9.07	63.25 ± 7.52	68.50 ± 6.65	52.63 ± 25.72	52.88 ± 6.20	61.00 ± 10.11	0.1915	**<0.0001**	**0.0002**	0.4932	0.3517	**<0.0001**	0.5
Insulin (mUI/ml)	1.11 ± 0.72	1.16 ± 0.34	0.91 ± 0.14	1.30 ± 0.73	1.87 ± 0.52	1.20 ± 0.43	0.76 ± 0.07	0.83 ± 0.24	1.09 ± 0.50	2.27 ± 0.28	1.73 ± 0.47	2.29 ± 0.34	0.418	**<0.0001**	**0.0075**	0.8163	**0.0007**	**0.0053**	0.5

CTR, control group, olive cake inclusion; L-OC, low olive cake group, 10% inclusion; H-OC, high-olive cake group, 15% inclusion.

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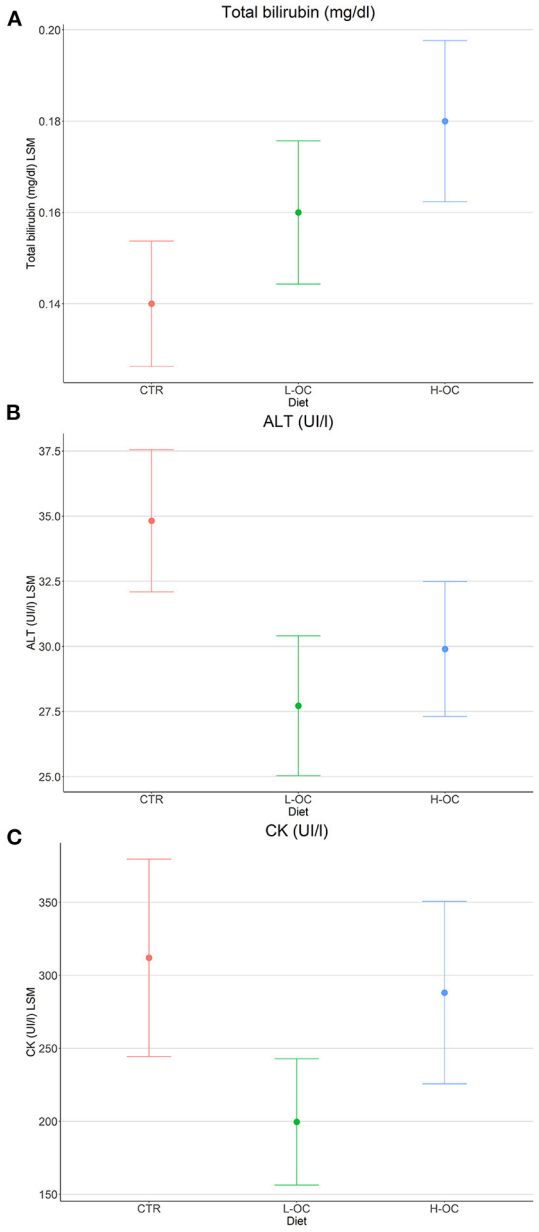

FIGURE 1
Significant effect of the diet on total bilirubin **(A)**, ALT **(B)**, and creatin kinase [CK, **(C)**]. The parameters' least square mean (LSM) and standard error are represented for all the groups with different olive cake inclusions, namely control (CTR, 0%), Low-olive cake (L-OC, 10%), and High-olive cake (H-OC, 15%) groups.

($P < 0.0001$) (Figures 3B–E). Specifically, their concentrations were similar in heifers and bulls of CTR and H-OC groups, but higher in heifers than bulls of L-OC group. Considering bulls only, ALT was higher in CTR than L-OC group (Figure 3C), whereas LDH and TG ($P = 0.0061$) were higher in H-OC than L-OC group (Figures 3A, E); instead, no differences among the three diets were observed in heifers. On the other and, in heifers, urea was higher in L-OC than H-OC (Figure 3B), whereas bulls showed similar values independently from the diet. Insulin ($P = 0.0007$) was higher in bulls than in heifers of CTR and H-OC groups but did not differ between sex in L-OC group; in addition, within heifers, L-OC group showed the highest concentration of insulin, whereas within bulls we observed the highest values in H-OC (Figure 3F).

Pairwise correlations between all the included parameters are reported in Table 4. Hepatic enzymes showed significant positive correlations among them and with CK, urea, and TCHO. CK was also positively correlated with TG. TBIL was positively correlated with TG as well, but negatively correlated to GLU, TCHO, and LDH. Lastly, we observed a negative correlation of insulin with glucose, but a positive correlation with AST, LDH, urea, and TCHO.

4. Discussion

Olive by-products, including olive cake (OC), have raised the attention of the scientific community for their possible use as livestock diet integration, giving the opportunity to reduce feed costs while improving product quality and sustainability. Mediterranean countries are among the major producers of olive oil, and the processing of this product generates a large quantity of co-products with a high environmental impact (1–2). To answer to the current need to increase the sustainability of Mediterranean agricultural sectors by implementing solutions which result in a valuable output, and in this case, in terms of benefits for animal feeding from OC utilization.

Several ruminants (Yáñez-Ruiz and Molina-Alcaide, 2007; Awawdeh, 2011; Abbeddou et al., 2011a,b; Estaún et al., 2014; Castellani et al., 2017; Kotsampasi et al., 2017; Awawdeh et al., 2020; Chiofalo B. et al., 2020; Chiofalo V. et al., 2020; Neofytou et al., 2020; Alkhtib et al., 2021; Symeou et al., 2021; Tzamaloukas et al., 2021) and monogastric meat species (Rupić et al., 1999; Paiva-Martins et al., 2014; Parsaei et al., 2014; Ait-Kaki et al., 2018; Reda et al., 2020) have been investigated, but beef cattle appear to be under-represented. Moreover, most of the studies mainly focus on animal performance and/or the final product or the rumen function (Estaún et al., 2014). For this reason, there is little knowledge about the metabolic effect that OC integration might have on beef cattle metabolism, which represents the main subject of this work.

In all the enrolled Limousine beef cattle the serum concentrations of lipids (TG, TCHO), parameters of liver functions associated with energy and protein metabolism (GLU, TBIL, AST, ALT, LDH), renal function (urea), indicators of muscle protein turnover and energy utilization (CK), and lipogenic hormones (insulin) were in line with physiological

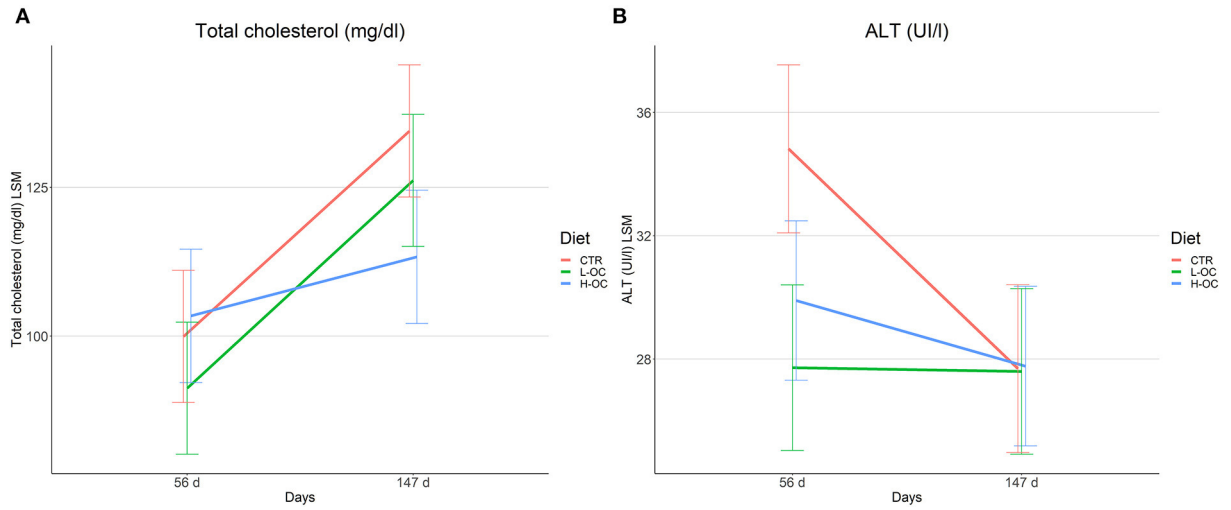

FIGURE 2
Significant effect of the diet x time interaction on total cholesterol **(A)** and ALT **(B)**. The parameters' least square mean (LSM) and standard error are represented for both the sampling times (56 and 147 days) and all the groups with different olive cake inclusions, namely control (CTR, 0%), Low-olive cake (L-OC, 10%), and High-olive cake (H-OC, 15%) groups.

ranges for bovine species (Kaneko et al., 2008), independently from the absence or the 10 or 15% integration of OC in their diet, becoming a relevant result of the current study. Specifically, values of urea and AST were in the range found by Doornenbal et al. (1988) and Gonano et al. (2014) in beef heifers. CK activity was higher than those observed in most studies but still within the range set by Latimer (2011) in cows and in beef heifers.

We also observed that diet or its interaction with sex and/or time of sampling proved to slightly, yet significantly, affect some of these parameters, which are described below.

It is to be considered that, according to our results, the inclusion of the OC did not negatively affect metabolism, as well as the growth of the enrolled animals, consistently with previous studies on beef cattle and lambs (Awawdeh, 2011; Estaún et al., 2014; Kotsampasi et al., 2017; Chiofalo V. et al., 2020; Tzamaloukas et al., 2021). Particularly, no differences were observed in body and carcass weights in heifers. Instead, body weight was higher in CTR bulls, but the carcasses had similar weights independently from the administered diet.

4.1. Lipids (TG, TCHO)

In the present study, growth of animals was characterized by an increase in triglyceridemia and total cholesterolemia. The increase in TCHO concentration can be explained by the marked growth of animals during the finishing phase and the consequent intensification of the anabolic activity of fat and liver metabolism (Van Soest, 1994). Moreover, several studies observed that a high level of TCHO during the early stages of growth is also related to the precocity of puberty attainment (Anderson et al., 2015; Rodríguez-Sánchez et al., 2015) and that TCHO concentrations increase with advancing age in beef cattle, according to the start of puberty and related sexual maturity. Concerning the effect of the diet, we also observed that the increase over time of TCHO was to a lesser extent in the H-OC group only. Accordingly, previous studies investigating olive by-products in sheep diets found no variation or a decrease in TG and TCHO concentrations (El-Tarabany et al., 2018; Awawdeh et al., 2020; Alkhtib et al., 2021). This may be due to the OC's high content in unsaturated fatty acids and phytosterols (Viveros et al., 2009; Cedó et al., 2019).

When considering the effect of sex, heifers showed significant higher TCHO and TG concentrations than bulls. Many aspects of sex differences in physiology arise due to the mechanisms of the sex hormones, including TG and TCHO biology. Estrogen has pleiotropic effects on many tissues and pathways that govern lipid and lipoprotein metabolism and also affects cholesterol efflux capacity (Matthews et al., 2000; Zhu et al., 2018). Heifers may potentially have the capacity to undergo different metabolic adjustments according to their sexual maturity, compared to bulls, as confirmed by the significant effect of age at first service on heifers' plasma concentrations of TG and TCHO observed in cattle and buffalos (Talavera et al., 1985; Wehrman et al., 1991; Ryan et al., 1992; Campanile et al., 2010; Hussein and Abdel-Raheem, 2013). This might explain why we could observe a slight

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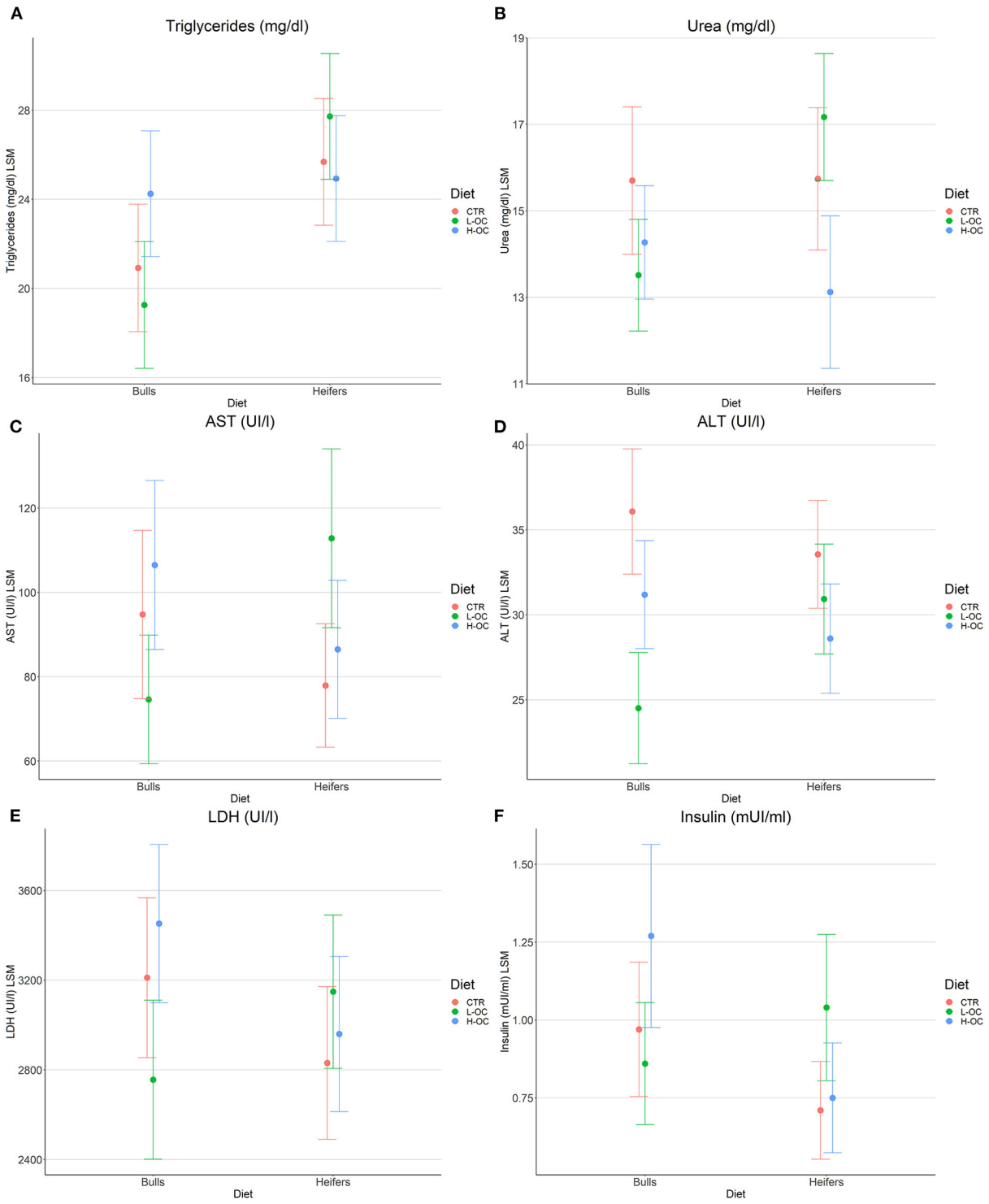

FIGURE 3
Significant effect of the diet x sex interaction on total triglycerides **(A)**, urea **(B)**, AST **(C)**, ALT **(D)**, LDH **(E)**, and insulin **(F)**. The parameters' least square mean (LSM) and standard error are represented for both the sexes and all the groups with different olive cake inclusions, namely control (CTR, 0%), Low-olive cake (L-OC, 10%), and High-olive cake (H-OC, 15%) groups.

TABLE 4 Pairwise correlations for all the included blood parameters.

	AST (UI/l)	ALT (UI/l)	LDH (UI/l)	CK (UI/l)	TBIL (mg/dl)	Urea (mg/dl)	GLU (mg/dl)	TCHO (mg/dl)	TG (mg/dl)
ALT (UI/lh)	**0.48**								
	***P* < 0.0001**								
LDH (UI/l)	**0.58**	**0.53**							
	***P* < 0.0001**	***P* < 0.0001**							
CK (UI/l)	**0.28**	**0.29**	**0.31**						
	***P* = 0.0008**	***P* = 0.0005**	***P* = 0.0002**						
TBIL (mg/dl)	−0.02	−0.08	**−0.33**	−0.06					
	P = 0.7947	*P* = 0.3221	***P* < 0.0001**	*P* = 0.4631					
Urea (mg/dl)	**0.30**	**0.26**	**0.29**	0.04	0.03				
	***P* = 0.0003**	***P* = 0.0017**	***P* = 0.0005**	*P* = 0.6708	*P* = 0.7477				
GLU (mg/dl)	−0.03	0.14	−0.01	0.01	**−0.19**	0.06			
	P = 0.7168	*P* = 0.0944	*P* = 0.9121	*P* = 0.9037	***P* = 0.0206**	*P* = 0.5023			
TCHO (mg/dl)	**0.35**	**0.27**	**0.54**	**0.21**	**−0.2**	**0.20**	−0.07		
	***P* < 0.0001**	***P* = 0.0013**	***P* < 0.0001**	***P* = 0.0124**	***P* = 0.0153**	***P* = 0.0158**	*P* = 0.4353		
TG (mg/dl)	0.07	0.13	−0.06	**0.18**	**0.37**	0.04	0.09	0.04	
	P = 0.3929	*P* = 0.1126	*P* = 0.493	***P* = 0.0325**	***P* < 0.0001**	*P* = 0.6059	*P* = 0.3006	*P* = 0.6341	
Insulin (mUI/ml)	**0.41**	0.13	**0.40**	0.07	−0.15	**0.25**	**−0.22**	**0.49**	−0.01
	***P* < 0.0001**	*P* = 0.1217	***P* < 0.0001**	*P* = 0.4167	*P* = 0.0784	***P* = 0.0028**	***P* = 0.0076**	***P* < 0.0001**	*P* = 0.8585

Significant correlations (P < 0.05) are reported in bold. TBIL, total bilirubin; GLU, glucose; TCHO, total cholesterol; TG, triglycerides.

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difference in TG concentration due to the diet in bulls but not in heifers.

4.2. Total bilirubin

We observed that TBIL concentration increased from 56 to 147 d and was positively related to the OC inclusion. However, it is important to underline that all the measurements remained within the physiological range.

There are few studies reporting the effect of the diet on this parameter, and no study concerns the integration of olive by-products. It is known that, in cattle, a negative energy balance affects fat mobilization and liver function, leading to an increase in TBIL serum concentrations (Mayasari et al., 2019; Marcato et al., 2021; Hisadomi et al., 2022). It has been observed that unsaturated fatty acids, largely present in the OC, reduce bilirubin conjugation, thus increasing its serum concentration (Hargreaves, 1973; Oliveira et al., 2021). In fact, a similar effect was also observed in new-born calves fed with banana extracts (Keivani Rad et al., 2021).

4.3. Urea

The observed significant increase of urea concentrations from 56 to 147 d is consistent with data previously recorded in both beef and dairy cattle (Swali et al., 2008; Brickell et al., 2009; Gonano et al., 2014). A difference in urea concentration was observed in bulls fed with L-OC and H-OC diet, which showed the highest and lowest values, respectively. Instead, heifers showed similar concentrations regardless the OC inclusion. Urea is one of the few blood parameters that was often evaluated in studies regarding the integration of olive by-products in livestock diet, with most of them reporting a decrease in uremia, which supports our results (Yáñez-Ruiz and Molina-Alcaide, 2007; El-Tarabany et al., 2018; Awawdeh et al., 2020; Alkhtib et al., 2021). This effect was associated to a possible reduction in nitrogen intake or protein rumen degradability due to the bind between tannins and dietary proteins (Yáñez-Ruiz and Molina-Alcaide, 2007; Correddu et al., 2020).

4.4. Liver enzymes (AST, ALT, and LDH)

With regard to hepatic enzymes, we observed that ALT was higher in control animals, but the differences among the groups disappeared when we considered the last sampling period. Accordingly, animals of several species, both ruminant (El-Tarabany et al., 2018; Lipińska and Józwik, 2018; Awawdeh et al., 2020; Alkhtib et al., 2021) and monogastric (Paiva-Martins et al., 2014; Parsaei et al., 2014), showed similar or slightly lower ALT activity when fed with olive or chokeberry by-products. It can be hypothesized that this enzyme decreases due to the effect of antioxidants and polyphenols on the liver.

Interestingly, we also observed a similar effect of sex-diet interaction on all the hepatic enzymes. Particularly, lower activity of all of them were measured with the 10% integration (L-OC), in bulls only. Most of the studies on olive by-products administration showed no differences in these parameters (Paiva-Martins et al., 2014; El-Tarabany et al., 2018; Awawdeh et al., 2020; Alkhtib et al., 2021), but the extent of the integration might be important; in fact, in broilers, a small quantity of olive cake has been associated to a decreased AST, whereas a great quantity to increased AST activity (Parsaei et al., 2014). Moreover, a reduction of AST and LDH activities was also recorded in lambs fed with chokeberry by-products (Lipińska and Józwik, 2018). It might be speculated that these alterations depend on a lower diet nitrogen content, which can influence the hepatic metabolism decreasing AST and ALT activities (Puppel and Kuczyńska, 2016).

4.5. Creatin kinase

As well as AST, CK activity was higher in CTR animals than L-OC and H-OC. The CK is an indicator of muscle protein turnover and is also associated with energy utilization (Baird et al., 2012). Its activity can vary according to the time of the day, age, growth rate, and pregnancy (Gonano et al., 2014); nevertheless, in the present study it is possible to exclude the effect of these variables on the changes of CK activity, because animals were homogenous for age, growth rate, time, and physiological conditions.

In addition, elevated CK activity due to the muscle damage, which can be caused by the physical activity-related oxidative stress, can be prevented or reduced by antioxidant supplementation (Ostojic et al., 2008; Wang et al., 2008; Gupta et al., 2009; Marius-Daniel and Stelian Dragomir, 2010; Bentley et al., 2012). For example, red fruit oil, a natural source of antioxidant, showed to decrease significantly the CK activity during exercise when supplemented to mice (Sinaga and Purba, 2018). On this evidence, it is possible to presume that the antioxidants present in the OC contributed to decrease the activity of CK in both L-OC and H-OC. What is more, diets supplemented with olive or chokeberry by-products led to no variation or a decrease of creatin kinase activity, similarly to our study (Lipińska and Józwik, 2018; Awawdeh et al., 2020).

4.6. Glucose and insulin

Insulin is known to accelerate anabolic processes, such as the synthesis of muscular protein contributing to muscle

development, and the synthesis and deposition of fat along developmental processes. Insulin is essential for the regulation of glucose and lipid metabolism and also affects growth through its promotion of the uptake of nutrients into body tissues as recorded by Martin et al. (Martin et al., 1984) and as shown by the existence of significant correlations between insulin and glucose, TCHO, TG, AST, and TBIL. On the other hand, glucose is the favored substrate for lipogenic adipocytes in muscle, whereas acetate is preferred in subcutaneous adipocytes and as precursor of *de novo* fatty acid synthesis (Smith and Crouse, 1984; Gilbert et al., 2003; Ladeira et al., 2016; Bionaz et al., 2020).

In the present study, insulin was higher in bulls and increased from 56 to 147 d, whereas the opposite was true for glucose, in accordance with the literature (Roy et al., 1983; Gray et al., 1986; Beeby et al., 1988; Plouzek and Trenkle, 1991; Shingu et al., 2001). Bulls, in fact, show higher concentrations of insulin-like growth factor 1, growth hormone, and insulin compared with cows, probably due to the effect of sex-dependent steroid, and this might partially explain their increased growth rate (Ronge and Blum, 1989; Plouzek and Trenkle, 1991; Sirotkin et al., 2002). Fat accumulation in different depots is also sexually dimorphic: in humans, men accumulate more visceral fat, whereas women accumulate more subcutaneous fat and have a higher overall percentage of body fat. There is also evidence indicating that insulin sensitivity differs between males and females (Sirotkin et al., 2002; Macotela et al., 2009). In mice, it was observed that the intra-abdominal depot is regulated by physiological levels of sex steroid and that female mice are more insulin-sensitive that males and their adipocyte show higher expression of glucose and lipid metabolism genes (Mittendorfer, 2005). However, how insulin action differs between males and females and how these differences account for a sex-specific regulation of adipose tissue development and function are not completely clear.

In the present study, we observed a similar insulin concentration in heifers and bulls of the L-OC group, even if diet or its interaction had no effect on glycemia. It should be noticed that, as far as we know, this is the first study analyzing the effect of an olive by-product inclusion on insulin in livestock, but on the other hand it has been demonstrated the phenols contained in olive leaves have a hypoglycemic effect in several species (Parsaei et al., 2014; Ait-Kaki et al., 2018; Lipińska and Józwik, 2018; Alkhtib et al., 2021). Moreover, it is known that a diet rich in carbohydrates tends to increase insulin (Mori et al., 2007) and that glucose kinetics is influenced by the intake of nitrogen and nutrients as well as the fasting length (Brickell et al., 2009; Zanton and Heinrichs, 2017); therefore, we cannot exclude that other mechanisms can be involved in the observed insulin alteration.

5. Conclusions

In the present study we demonstrated that olive cake inclusion in shows no detrimental effect on beef cattle performance and metabolism, according to several blood parameters analyzed at different time points. However, small changes in some analytes have been observed in groups fed with different amount of olive cake, sometimes with different effects in heifers and bulls or at different growing stages, opening the possibility of further research on this topic. Moreover, we provided data about the metabolic profile of beef cattle in the context of diet supplemented with olive cake, improving knowledge about the physiological characterization of these animals at different stages of development. According to the economic analysis, profitability level could be considered interesting, and it should be taken into account in Mediterranean regions. In this sense, in the last years there has been new research exploring the possibilities of further use of the olive residues, like the olive cake, to obtain a valuable outcome on the physiological responses of ruminants, also in terms of endocrine and metabolic adaptations.

Therefore, according to our results, we support an olive cake integration up to 15% of the concentrate in beef cattle diet, thus leading to several advantages also in terms of feed costs and environmental sustainability, as a model of circular economy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Ethical Committee of the Department of Veterinary Science, University of Messina, Italy (code 041/2020). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

EF and LL: conceptualization. VC: methodology. PC: software. AB and VL: validation. AB and MO: formal analysis. VL: investigation. AB, PC, AA, and VL: data curation. EF: writing—original draft preparation. EF, AB, and VL: writing—review and editing. AB and LL: visualization. LL: supervision, project administration, and funding acquisition.

All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

Author VC was employed by Consortium of Research for Meat Chain and Agrifood (CoRFilCarni).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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