

## Article

# Improving Sustainability in Buffalo Finishing: Olive Cake Supplementation and Its Effects on Performance and Meat Quality

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## Abstract

This study aimed to evaluate the effects of olive cake (OC) supplementation on buffalo performance and meat quality. Sixty Italian Mediterranean Buffalo males (thirty/group) were enrolled for 90 days before slaughter and allocated into two homogeneous groups according to body weight and age. The Innova group received concentrate with a 7% inclusion of OC, whereas the Ctrl group received no supplementation. Animal performances were recorded at the beginning and at the end of the trial to assess average daily gain (ADG), final live weight, and carcass weight. The *Longissimus thoracis* muscle samples were harvested and analyzed for chemical composition, fatty acid profile, and total polyphenols content. Dietary inclusion of OC improved animal performances, with greater ADG, final live weight, and carcass weight than the Ctrl group. Furthermore, the Innova meat exhibited a greater polyphenols content and a better acidic profile, represented by greater monounsaturated fatty acids and lower saturated fatty acids. Innova meat had a greater n-3/n-6 ratio, lower atherogenic (AI) and thrombogenic index (TI), and greater hypocholesterolemic/hypercholesterolemic ratio (H/H) compared with Ctrl meat. These results suggest that inclusion of OC in buffalo diet improved the meat's fatty acid profile and nutritional value, contributing to healthier, higher-quality products while supporting circular economy principles.

**Keywords:** olive cake; buffalo meat; polyphenols; growth performances; fatty acids



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## 1. Introduction

The increasing demand for sustainable livestock production has led to a growing interest in the use of agro-industrial by-products as alternative feed ingredients. Among these, olive pomace—a residual material from olive oil extraction—has emerged as a promising candidate due to its abundance in Mediterranean regions and its rich content of bioactive compounds, including polyphenols and unsaturated fatty acids [1–3]. In cattle, dietary supplementation with olive pomace and related by-products has shown positive effects on meat quality, including improved fatty acid profiles, enhanced oxidative stability, and better sensory attributes. For example, Chiofalo et al. [4] reported that the

inclusion of partially destoned olive cake in beef cattle diets increased intramuscular fat and the proportion of unsaturated fatty acids, particularly oleic acid. Similarly, Luciano et al. [5] found that olive cake supplementation improved the oxidative stability of meat and increased the concentration of beneficial fatty acids. The antioxidant properties of olive polyphenols have also been shown to reduce lipid oxidation and improve shelf life [3,6]. These effects are largely attributed to the modulation of ruminal biohydrogenation processes, which allow for greater retention of beneficial fatty acids such as conjugated linoleic acid (CLA) and vaccenic acid [6,7]. Research on dairy ruminants supports these findings. For instance, Nudda et al. [8] demonstrated that olive by-products can improve the nutritional quality of milk fat without negatively affecting production traits. Similar results were observed by Chiofalo et al. [9] in sheep, where olive cake improved milk fatty acid composition and antioxidant status. Despite the growing body of research on cattle and small ruminants, limited data are available regarding the effects of olive pomace supplementation in dairy buffalo diets [10]. Lopreiato et al. [11] showed that olive cake supplementation in beef cattle diets led to maintained growth performance and potentially lowered methane emissions via rumen modulation. However, the authors have suggested that inclusion should not exceed 10%, and further holistic research is needed. Given the physiological and metabolic similarities between buffaloes and cattle, it is plausible to hypothesize that similar benefits may be observed. Buffalo meat is valued for its leanness and nutritional profile, and enhancing its quality through dietary strategies could further improve its market appeal. Furthermore, the use of olive pomace aligns with circular economy principles, promoting waste valorization and reducing the environmental footprint of livestock production. Olive oil production generates substantial quantities of by-products, and their incorporation into animal feed represents a viable strategy for resource efficiency and sustainability [7,12–14]. Italy hosts the largest buffalo population in Europe, with over 120,000 animals registered in official breeding programs, mainly concentrated in southern regions. The sector plays a significant economic role, particularly through the production of Mozzarella di Bufala Campana PDO, and is increasingly involved in sustainable innovation, including the use of agro-industrial by-products to reduce feeding costs and environmental impact. Although dairy production is predominant, buffalo meat is gaining attention for its nutritional value, characterized by high protein content, low cholesterol levels, and favorable fatty acid profiles. Improving meat quality is therefore a growing research focus. This study aims to investigate the impact of dietary inclusion of olive cake on growth performance and meat quality traits in buffaloes. Specifically, it evaluates changes in carcass characteristics and the lipid composition of the meat. The findings are expected to contribute to the development of sustainable feeding practices and to the valorization of olive oil by-products within buffalo production systems.

## 2. Materials and Methods

### 2.1. Ethical Statement

The experimental protocol was approved by the Ethical Committee of the Department of Veterinary Science, University of Messina, Italy (code 07/2024). The research complied with the guidelines of Good Clinical Practices [15] and the Italian and European regulations on animal welfare (Directive 2010/63/EU) [16].

### 2.2. Animals and Diets

The study was conducted on 60 male Italian Mediterranean buffaloes. Twelve group pens were used, each housing five buffaloes with a space allowance of 4 m<sup>2</sup> per head. Animals were blocked by body weight and age and randomly allocated into the two experimental groups: Innova (470.60 ± 61.5 Kg) and Control (Ctrl; 470.01 ± 61.5 Kg).

Hence, six pens constituted the Ctrl group, who received a conventional finishing diet, while the remaining six pens formed the Innova group, whose concentrate included 7% olive cake (crude protein: 11.23%; fat: 16.44%; starch: 15.13%; crude fiber: 28.65%; NDF: 59.40%; ADF: 46.84%; ADL: 21.34%; ash: 4.33%; total polyphenols: 12.01 mg/g) as a partial replacement of standard feed ingredients.

All pens were equipped with permanent straw bedding to ensure optimal comfort and hygiene throughout the trial. Fresh straw was added weekly (35 kg straw/animal), and soiled material was removed at the end of the finishing period.

Both diets were administered as total mixed rations (TMR), formulated to meet the nutritional requirements during the finishing phase, and were isoproteic and isoenergetic. The forage contained 7.41% crude protein, 1.07% fat, 57.01% NDF, 34.93% ADF, 4.06 ADL, and 4.44% ash. The TMR was formulated to reach a forage/concentrate ratio of approximately 30:70 on a dry matter basis. Feed was distributed twice daily, in equal portions, at 0800 and 1500 h, in linear feed troughs. All the animals were allowed *ad libitum* access to water (two drinkers/pen). The trial lasted 90 days, and the detailed composition of the concentrates is presented in Table 1.

**Table 1.** Ingredients and chemical composition (% of DM), fatty acid profile of nutritional interest [g/100 g fatty acid methyl esters (FAME)], and fatty acid classes of the concentrates.

Ingredients, % of DM	Treatment <sup>1</sup>	
	Ctrl	Innova
Corn	58	40
Flaked corn	-	10
Barley grain	-	15
Wheat bran	10	4
Alfalfa flour	8	-
Soybean meal	8	11.5
Olive cake	-	7
Sugar beet pulp	5	2
Sunflower meal	4	3
Minerals supplementation	2.2	-
Carob meal	-	2
Molasses-based additive	1.5	-
Calcium salts	-	1.4
Calcium carbonate	-	0.9
Calcium	0.85	-
Sodium bicarbonate	0.5	0.5
Lithothamnium calcareum	0.4	0.5
Magnesium oxide	0.4	0.25
Urea	0.35	0.35
Sodium chloride	0.3	0.55
Mineral–vitamin premix	0.3	0.70
Dicalcium phosphate	0.2	0.35
<b>Chemical composition, % of DM</b>		
Dry matter	88.65	89.12
Crude protein	15.91	15.27
Ether extract	4.24	5.26
Crude fiber	5.49	6.84
Neutral Detergent Fiber	13.89	19.08
Acid Detergent Fiber	7.59	10.14
Acid Detergent Lignin	2.44	2.53
Starch	49.50	44.19
Ash	6.51	7.83
Total polyphenols (mg GAE/g)	1.09	1.25
NE, MJ/kg SS <sup>2</sup>	9.81	9.43

Table 1. Cont.

Ingredients, % of DM	Treatment <sup>1</sup>	
	Ctrl	Innova
<b>Fatty acid profile, % of total FAME</b>		
C14:0	0.11	0.15
C16:0	15.50	16.50
C18:0	2.71	2.70
C18:1n-9	31.63	39.95
C18:2n-6	47.54	37.80
C18:3n-6	1.50	1.96
Fatty acid classes		
SFAs	18.96	19.97
MUFAs	32.01	40.27
PUFAs	49.04	39.76
UFAs/SFAs	4.27	4.01

<sup>1</sup> Ctrl, no inclusion of olive cake; Innova, inclusion of 7% of olive cake; <sup>2</sup> NE = Net energy (Net energy for meat production in ruminants according to the INRA 2018 system); C14:0 = Myristic acid; C16:0 = Palmitic acid; C18:0 = Stearic acid; C18:1n-9 = Oleic acid; C18:2n-6 = Linoleic acid; C18:3n-3 = alpha-Linolenic acid; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; UFAs/SFAs = unsaturated fatty acids/saturated fatty acids ratio.

### 2.3. Animal Performance and Carcass Traits

Throughout the experimental period, productive performance was monitored by recording individual body weight (Brecknell PS-2000 Veterinary Floor Scale, Brecknell, Fairmont, MN, USA; capacity: 1000 kg, readability: 0.5 kg) at the beginning and end of the trial, as well as the calculation of average daily gain (ADG). At 90 d animals were transported to a commercial EU-licensed slaughterhouse (5.1 km; approximately 6 min from the farm) and were humanely slaughtered according with EU Council regulation No 1099/2009 [17]. At slaughter, when animals were approximately  $23.24 \pm 4.74$  months of age, carcass traits were evaluated according to the SEUROP classification system, including conformation and fatness scores.

### 2.4. Meat Samples and Analysis

All carcasses were subjected to conventional chilling for 24 h at a temperature ranging from 0 to 4 °C. Following the cooling period, for each animal, the left half of each carcass was sectioned, and individual samples of the *Longissimus thoracis* muscle were carefully collected between the 12th and 13th ribs for chemical and nutritional analysis ( $n = 60$ ). All determinations were performed in triplicate for each sample. Moisture content was determined by oven-drying (Memmert UF 110, Memmert GmbH + Co. KG, Schwabach, Germany) samples at 105 °C until constant weight was achieved. Crude protein content was assessed using the Kjeldahl method [18] and expressed as % nitrogen  $\times 6.25$ . Ash content was measured by incineration of the samples in a muffle furnace at 550 °C for 6 h. Lipid extraction was performed using the method described by [19], based on a chloroform-methanol solvent system. Total lipid content was expressed as a percentage of fresh tissue.

Fatty acid composition was determined by gas chromatography (GC) following methylation of fatty acids according to the method of Christie [20]. Fatty acid methyl esters (FAMES) were separated and quantified using a GC (Trace GC Ultra-FID, Thermo Fisher Scientific, Rodano, Italy) equipped with a flame ionization detector (FID) and a capillary column suitable for FAME analysis. Results were expressed as a percentage of total identified fatty acids.

The analysis focused on key nutritional indicators, including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and the n-6/n-3 ratio. Additionally, the atherogenic index (AI) and thrombogenic index (TI) were calculated according to Ulbricht et al. [21]; the hypocholesterolemic/hypercholesterolemic ratio (HH), according to Santos-Silva et al. [22]; and the peroxidation index (PI) as proposed by Luciano et al. [5], in order to assess the potential health implications of meat consumption.

The nutritional indicators were calculated using the following formulas:

$$AI = (C12:0 + (4 \times C14:0) + C16:0) / (\Sigma MUFA + \Sigma n - 6PUFA + \Sigma n - 3PUFA)$$

$$TI = (C14:0 + C16:0 + C18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma n - 6PUFA) + (3 \times \Sigma n - 3PUFA) + (\Sigma n - 3PUFA / \Sigma n - 6PUFA)]$$

$$H/H = (C18:1n - 9 + C18:2n - 6 + C20:4n - 6 + C18:3n - 3 + C20:5n - 3 + C22:5n - 3 + C22:6n - 3) / (C14:0 + C16:0)$$

$$PI = (0.025 \times \% \text{Monoenoic}) + (1 \times \% \text{Dienoic}) + (2 \times \% \text{Trienoic}) + (4 \times \% \text{Tetraenoic}) + (6 \times \% \text{Pentaenoic}) + (8 \times \% \text{Hexaenoic})$$

Polyphenol content in meat samples was determined using the Folin–Ciocalteu colorimetric method, as described by Amato et al. [12]. In detail, 6 g of the homogenized sample was added to 10 mL of methanol/water (80/20, *v/v*) and shaken vigorously; the two phases were separated by centrifugation. A 0.2 mL aliquot of each extract was mixed with 1.8 mL H<sub>2</sub>O and 10 mL Folin–Ciocalteu phenol reagent (2 N); 8 mL saturated Na<sub>2</sub>CO<sub>3</sub> solution (75 g/L) was then added and the mixture was allowed to stand in the dark for 90 min. Absorbances were read on a UV-visible spectrophotometer (UV-2401 PC, Shimadzu, Milan, Italy) at 765 nm using water–methanol as an analytical blank. Appropriate dilutions of a gallic acid standard solution were used to construct a calibration curve. Results were expressed as milligrams of gallic acid equivalents (GAEs) per 100 g of meat.

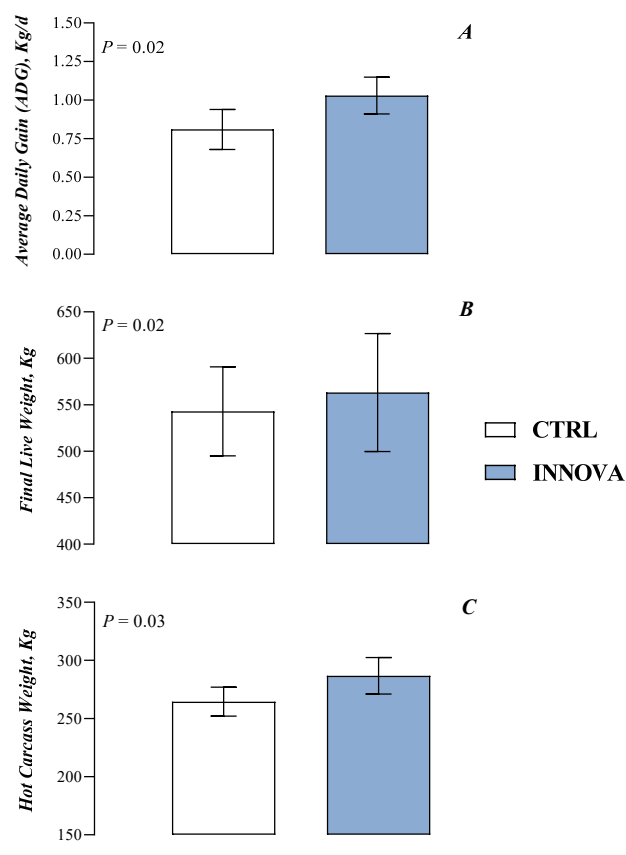
### 2.5. Statistical Analysis

Data were analyzed using SAS software (version 9.4; SAS Institute). The normality of residuals was assessed using the UNIVARIATE procedure of SAS. All data were subjected to ANOVA using the GLIMMIX procedure. The statistical model included the fixed effect of treatment (INNOVA vs. CTRL), whereas buffaloes were included as a random effect. The model initially included pen as a random effect, as animals were housed in groups; however, this factor was not significant ( $p > 0.05$ ) for any variable and was therefore removed from the final statistical model. Differences were considered statistically significant at  $p \leq 0.05$ , and trends were discussed when  $0.05 < p \leq 0.10$ .

## 3. Results

### 3.1. Animal Performance and Carcass Characteristics

As shown in Figure 1, animals in the Innova group exhibited greater performance compared with the Ctrl group. In particular, the average daily gain (ADG) was higher in the Innova group than Ctrl group (1.03 vs.  $0.81 \pm 0.13$  kg/d, respectively;  $p < 0.05$ ; Figure 1A). This improvement resulted in a significant increase in both final live weight (Innova: 563.27 vs. Ctrl:  $542.91 \pm 63.52$  kg;  $p < 0.05$ ; Figure 1B) and carcass weight (Innova: 286.71 vs. Ctrl:  $264.57 \pm 15.71$  kg;  $p < 0.05$ ; Figure 1C). At slaughter, carcass classification according to the SEUROP system yielded consistent results, with both groups receiving an average score of O3, indicating a standard conformation and fatness levels typical of animals finished under conventional conditions.



**Figure 1.** Average daily gain (A), final live weight (B), and hot carcass weight (C) of male Italian Mediterranean buffaloes during the finishing phase fed either a standard diet (Ctrl) or a diet supplemented with olive cake (Innova). Bars indicate the standard errors.

### 3.2. Meat Characteristics

The results of meat characteristics are reported in Table 2.

**Table 2.** Effect of dietary olive cake supplementation on meat composition, fatty acid methyl esters (FAMES), and nutritional indices in the meat of Italian Mediterranean buffaloes at the finishing phase (mean  $\pm$  SEM).

Item	Ctrl	Innova	SEM	<i>p</i> -Value
Chemical composition				
Lipids, %	1.54	1.59	0.09	0.66
Proteins, %	20.58	20.30	0.43	0.65
Total polyphenols, mg/kg	94.39	140.78	3.73	<0.01
Cholesterol, mg/100 g	37.62	37.20	0.79	0.70
FAMES (% of total FAs)				
C14:0	1.53	1.55	0.1	0.91
C16:0	22.83	20.94	0.31	<0.01
C17:0	0.75	1.01	0.06	<0.01
C18:0	27.60	21.38	0.47	<0.01
C20:0	0.1	0.17	0.01	<0.01
C14:1	1.60	3.11	0.21	<0.01
C16:1 n-9	0.86	1.08	0.04	<0.01
C17:1	0.65	0.65	0.03	0.98
C18:1 cis	31.46	36.63	0.70	<0.01
C18:1 cis vaccenic	2.39	2.24	0.15	0.47
C18:1 trans	0.66	0.63	0.02	0.41
C18:2 cis	3.71	4.56	0.3	0.06

Table 2. Cont.

Item	Ctrl	Innova	SEM	<i>p</i> -Value
C18:3 n-6	0.43	0.24	0.02	<0.01
C18:3 n-3	0.39	0.32	0.01	<0.01
C20:3 n-6	0.52	0.33	0.02	<0.01
C20:4 n-6	1.01	1.18	0.07	0.10
C22:5 n-3	0.08	0.08	0.004	0.31
SFAs	52.81	45.05	0.64	<0.01
MUFAs	37.62	44.35	0.81	0.01
PUFAs	6.15	6.71	0.29	0.17
Nutritional indices				
n-3	4.19	4.96	0.3	0.08
n-6	1.96	1.75	0.09	0.09
n-3/n-6	2.15	2.96	0.23	0.02
TI	1.51	1.09	0.04	<0.01
AI	0.38	0.31	0.01	<0.01
H/H	1.55	1.93	0.05	<0.01
PI	11.89	12.64	0.36	0.15

SFAs = Saturated fatty acids; MUFAs = Monounsaturated fatty acids; PUFAs = Polyunsaturated fatty acids; TI = Thrombogenic index; AI = Atherogenic index; H/H = Hypocholesterolemic/Hypercholesterolemic ratio; PI = Peroxidation index.

Although no significant differences ( $p > 0.05$ ) between groups were observed for lipid, protein, and cholesterol level in meat, the meat of the Innova group showed a significantly higher polyphenol content than that of the Ctrl group ( $p < 0.01$ ). Dietary supplementation with OC resulted in significant differences in FAMES composition compared with the Ctrl group. In particular, among the long-chain saturated FAs, palmitic acid (C16:0) was lower in the Innova group than in the Ctrl group ( $p < 0.01$ ), and a similar reduction was observed for stearic acid (C18:0). A different trend was observed for heptadecanoic acid (C17:0) and arachidic acid (C20:0), which, on the contrary, were greater in Innova compared with Ctrl meat ( $p < 0.01$ ). However, no significant differences between groups were observed ( $p > 0.05$ ) for myristic acid (C14:0). Concerning the long-chain monounsaturated fatty acids, Innova meat exhibited greater content ( $p < 0.01$ ) of myristoleic acid (C14:1), palmitoleic acid (C16:1 n-9), and oleic acid (C18:1 cis-9). A tendency was observed for linoleic acid (C18:2 cis), which was higher in Innova compared to Ctrl buffaloes ( $p = 0.06$ ). Conversely, the Innova group showed lower content of  $\gamma$ -Linolenic acid (C18:3 n-6),  $\alpha$ -Linolenic acid (C18:3 n-3), and dihomo- $\gamma$ -linolenic acid (C20:3 n-6) compared with Ctrl ( $p < 0.05$ ). However, no significant differences between groups ( $p > 0.05$ ) were observed for the meat content of heptadecenoic acid (C17:1), vaccenic acid (C18:1 cis vaccenic), elaidic acid (C18:1 trans), arachidonic acid (C20:4 n-6), or docosapentaenoic acid (C22:5 n-3). Moreover, significant differences ( $p < 0.05$ ) between groups were observed for total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). Indeed, the Innova group showed lower SFAs content ( $p < 0.05$ ) and higher MUFAs ( $p < 0.05$ ) than the Ctrl group. However, no significant differences ( $p > 0.05$ ) between groups were observed for polyunsaturated fatty acids (PUFAs). Among the nutritional indices, a tendency was observed for n-3 ( $p = 0.08$ ) which was higher in the Innova group, and for n-6 ( $p = 0.09$ ), which, on the contrary, was lower compared with the Ctrl group. As a consequence, the n-3/n-6 ratio was greater in Innova buffaloes ( $p < 0.05$ ). Overall, the supplementation of OC had an effect on atherogenic (AI) and thrombogenic (TI) indices, both being lower in the Innova group compared with the Ctrl group ( $p < 0.01$ ). Moreover, the hypocholesterolemic/hypercholesterolemic ratio was significantly greater ( $p < 0.01$ ) in the Innova meat compared with the Ctrl, while no differences ( $p > 0.05$ ) were observed for the peroxidation index (PI).

## 4. Discussion

Buffalo farming has a key economic, cultural, and historical role worldwide. Although it has traditionally been focused on milk production for mozzarella cheese, in recent years, buffalo meat has gained increasing importance in recent years. In Italy, annual production has remained relatively stable, with 106,752 buffaloes slaughtered in 2023 and 99,830 in 2024 [23]. Its importance is mainly related to the nutritional benefits of buffalo meat, including its low cholesterol and fat levels as well as its richness in iron [24–26]. In this context, the possibility of enhancing buffalo meat quality through dietary supplementation with a by-product not only may improve the product itself but also contributes to sustainability, thus providing an added value to buffalo meat.

This study demonstrated that the supplementation of a widely produced by-product, olive cake (OC), had positive effects on animal performances, carcass characteristics, and meat quality. Among these aspects, the Innova group exhibited greater growth performances, as shown by greater average daily gain, final live weight, and hot carcass weight (Figure 1). This result agrees with a previous study on Limousin bulls, which reported that dietary supplementation with OC at 7.5% improved body weight, ADG, and carcass characteristics, suggesting positive effects on growth performances and meat quality [4]. As reported in this latter study, this could be attributed to a more efficient use of the energy supplied by OC lipids and better protein utilization, since polyphenols limit their ruminal degradation [27]. Conversely, a recent trial on young Podolian bulls supplemented with 30% OC reported no effects on performance [28], and a study conducted on lambs demonstrated no effects on performances with supplementation of 15% of OC, but at higher inclusion levels (30%), a decrease in ADG and carcass weight was observed [29]. These findings suggest that excessive inclusion levels of OC in the diet may have undesirable effects on animal performance, highlighting the importance of identifying an optimal supplementation rate in order to balance energy supply.

Despite the lack of significant differences in the chemical composition of meat among groups (content of lipids, proteins, and cholesterol), the Innova group showed a significantly higher polyphenol content compared with the Ctrl group. This finding is consistent with previous studies, showing that, after OC supplementation, total polyphenols can be transferred into the milk of dairy cows [12] and into the meat of bulls [4]. The presence of polyphenols in meat is relevant as they can have an important role in the inhibition of lipid oxidation, the main factor influencing meat quality and shelf life [30]. Moreover, polyphenols are also beneficial to human health, as they protect the body against oxidative stress and exhibit notable anti-inflammatory properties [31]. This finding provides an opportunity to valorize the product, in line with consumers' growing demand for healthier and more sustainable foods.

Polyphenols play also a key role in modulating rumen metabolism and the microbial community, by limiting the complete biohydrogenation process in the rumen [32]. Due to this interference, the PUFAs ingested with the diet are not fully isomerized and/or saturated into C18:0, resulting in higher concentrations of MUFAs and PUFAs in the rumen [32]. This finding is consistent with our results, especially for the higher MUFAs content and, conversely, lower SFAs levels, which were observed in the meat of buffaloes supplemented with OC. In detail, among SFAs, a significant decrease in C16:0 and C18:0 was observed in the Innova group, which is consistent with previous studies which observed decreased levels in beef meat [4], probably due to the interference of biohydrogenation by polyphenols. The increase in SFAs such as C17:0 and C20:0 was observed in Innova meat, probably attributed to their greater amount in the concentrate supplemented with olive cake [12]. Specifically, C20:0 is considered neutral from a nutritional standpoint being classified as a very long-chain fatty acid [33], thus its increasing levels should not be considered as indicative of a health risk. Regarding

unsaturated fatty acids (UFAs), the higher MUFAs content in the Innova meat is mainly related to the high concentration of C18:1 cis-9 in the OC, and consequently to its greater deposition in tissue. These findings agree with previous studies on the supplementation of OC in dairy cows [1,12], lamb [7], sheep [34], bulls [4], and dairy buffalo [35], which also reported an increase in C18:1 cis-9. Although to a lesser extent, C14:1 and C16:1 cis-9 likely contributed to the increase in total MUFAs. Their higher proportions in the treated group may be associated with an enhanced  $\Delta 9$ -desaturase activity, which can be stimulated in muscle tissue by the inclusion of polyphenols in the diet [36]. Therefore, it might also be possible that OC supplementation (which in turn leads to an increase in polyphenols content in the diet) resulted in the modification of rumen metabolic pathways via modulation of the absorbed fatty acids, indirectly altering the regulatory mechanism responsible for desaturase expression. Indeed, in the latter study, the authors reported an increase in desaturase activity with the increase in the supplementation of tannins. In addition, a significant decrease in SFAs and an increase in MUFAs and PUFAs content seemed to play a positive role in increasing desaturase activity, which ultimately resulted in increased MUFAs in muscle. Among PUFAs, the Innova meat showed lower content of C18:3 n-3, C18:3 n-6, and C20:3 n-6. Even though the presence of these fatty acids in meat is relevant, it is noteworthy that their reduction did not compromise the overall nutritional quality of the Innova group meat, as reflected by the improved SFAs and MUFAs profiles. It is well known that SFAs increase blood LDL cholesterol levels, thereby raising the risk of coronary heart disease in humans, whereas dietary sources of UFAs have the opposite effect and can reduce this risk [37]. For this reason, the World Health Organization guideline (WHO) recommend that dietary intake of SFAs should be restricted, whereas a greater inclusion in the diet of omega-3 fatty acids can support cardiovascular health [7]. In the present study, the better acidic profile of meat is reflected by the better nutritional indices represented by the higher n-3/n-6 and H/H ratio, and the lower TI and AI in the Innova group. Atherogenic and thrombogenic index are important indicators of the level of atherogenicity and thrombogenicity, since they express the relationship between specific SFAs, which promote atherogenic and thrombogenic processes, and UFAs, which on the contrary exert protective functions [21]. The general improvement in the quality of Innova meat reveals a more favorable fatty acid profile that adds nutritional value to the meat, supporting higher product quality and promoting consumer health. Due to the high content of SFAs in ruminant meat, the opportunity to produce meat with a higher content of bioactive compounds, together with a more favorable fatty acid profile, could make buffalo meat not only a high-quality food which supports human health, but also support a circular economy and reduce the environmental footprint of livestock production. Moreover, the supplementation of OC in the diet of buffaloes could support the extension of the production specifications of the collective brand "*Qualità Sicura Garantita della Regione Siciliana*" (Sicilian Region Guaranteed Safe Quality), approved by the Department of Agriculture of the Sicilian Region in 2018, to include the buffalo beef sector among the supply chains already certified.

## 5. Conclusions

The outcomes of this study highlight the potential of olive cake as a sustainable feed ingredient for the buffalo beef supply chain. Its inclusion in the finishing diet did not compromise carcass yield or meat composition, while contributing to an enhanced lipid profile and the enrichment of bioactive compounds. These results emphasize that the valorization of agro-industrial by-products such as olive cake can represent an effective strategy to enhance meat quality and promote sustainability. Furthermore, the use of olive cake aligns with the goals of the regional quality program, supporting the development of a more sustainable and circular buffalo supply chain in Sicily.

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