Effect of olive cake supplementation on faecal microbiota profile of Holstein and Modicana dairy cattle

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ABSTRACT

The present study aimed to investigate the effect of olive cake supplementation on faecal microbiota of Holstein (n = 16) and Modicana (n = 16) dairy cows. Although no difference in richness was detected, within breeds and between the two dietary treatment, the PERMANOVA analysis applied to the beta diversity allowed to discriminate samples according to breeds (p < 0.001) and treatment (p < 0.001). In Holstein cows, the olive cake supplementation led to the increase of *Pseudobutyrivibrio* and *Christensenellaceae_R7-group* genera (p < 0.05) recognized as health-promoting or associated with feed efficiency. Differently, no difference was detected between control and treated groups for Modicana suggesting a high adaptive capacity to diet changes. In addition, the higher prevalence of *Firmicutes* phyla in the Modicana microbiota reflected its better capacity to digest the fibrous sources. Our study supports the suitability of olive cake as a feed supplement for cows and could help validating a sustainable livestock system in the Mediterranean area, characterized by a relevant oil production and by a native breeds reared with extensive systems.

1. Introduction

The gut microbial ecology plays an essential role in both host health and performance.

In cattle, the complex ecosystem inhabiting the gut microbiota interacts symbiotically with the host participating in several metabolic processes, such as the production of volatile fatty acids, the synthesis of vitamin and microbial protein, as well as the immune regulation and prevention of pathogen colonization (Nicholson et al., 2012; Pickard et al., 2017).

Although the rumen is involved in the transformation of the ingested dietary components into energy for the host, the microbiota of the lower gut aids both the health and the physiology of the host (Deepthi et al., 2023). Recently, high-throughput sequencing-related studies, confirmed the comparability among lower gut, cecum, and faecal microbiomes of ruminants therefore, the aforementioned sites can be considered valid alternatives to intrusive sampling techniques (Monteiro et al., 2022).

Moreover, it was established that the faecal microbiota provides a basic overview of the digestion of nutrients in the upper gut tract (Wu et al., 2022). Studies applying a metagenomics approach revealed that intrinsic (e.g. host genetics, age, sex) and extrinsic factors strongly affect the diversity of the gut microbial community (De Filippo et al., 2010; Goodrich et al., 2014; Odamaki et al., 2016; Org et al., 2016; Li et al., 2019; Xue et al., 2020). Among extrinsic factors, diet strongly influences both health and productivity traits (Chalupa et al., 1996; Pitta et al., 2018; Leeming et al., 2019; Li et al., 2020; Nowacka-Woszuk, 2020; Xu et al., 2021; D'Alessandro et al., 2022).

Recently, the suitability of agro-industrial by-products, such as citrus fruit pulp, molasses (Vastolo et al., 2019; Liotta et al., 2019a), and olive cake (Castellani et al., 2017; Foti et al., 2022), was extensively evaluated in order to be included in livestock feeding to reduce costs satisfying, at the same time, nutritional needs (Gómez-García et al., 2021). The Mediterranean area boasts a significant production of both olive oil and table olives (Randazzo et al., 2017; Berbel and Posadillo, 2018;

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Vaccalluzzo et al., 2020;) and olive cake is the main by-product used not only to generate energy and for pharmaceutical purposes but also in animal feeding strategies (Espeso et al., 2021). The Agriculture Department of the Sicilian Region approved in 2018 a collective mark called "Qualità Sicura Garantita della Regione Siciliana" (Sicilian Region Guaranteed Safe Quality), which regulated livestock production; particularly, it was addressed to producers who intended to comply with parameters that exceeded the minimum standards established by the regulations in force on animal welfare, environmental sustainability, and intrinsic product quality. In our specific case, the Disciplinary of production of crude cow milk and derivatives, foresaw in Chapter 5 (Feeding Techniques), the possibility of including citrus and olive processing scraps in the diet at the maximum percentage of 10%. To date the olive cake, as a feed supplement, was revaluated on different animals' species and its suitability was confirmed on pigs, by evaluating both performance and quality production (Liotta et al., 2019b), on beef cattle, by studying performance, carcass characteristics, and meat quality (Chiofalo et al., 2020a), and on dairy cows through the study of the milk composition profile (Chiofalo et al., 2020b) and the gut microbiota composition (Russo et al., 2023). In addition, the effect of olive cake supplementation on metabolic and endocrine responses of beef cattle (Bionda et al., 2022) as well as on the physicochemical and microbiological profile of Provola cheese (Calabrese et al., 2023) was recently investigated.

Modicana is an indigenous breed, capable of adapting to unfavourable environmental conditions using poor food resources. This capability is linked to the different metabolic and digestive capacities compared to Holstein which is the most reared breed for milk production (Brito et al., 2021; Erasmus and van Marle-Köster, 2021; Deepthi et al., 2023). Our hypothesis was that a certain degree of difference in the microbiota of two different genetic types, one cosmopolitan and the other of limited diffusion, could have a different response to the use of agro-industrial waste in the diet, hence, the present study aimed to investigate the potential variation of the taxonomy and structure of the microbial communities with the inclusion of olive cake in the diet of Holstein and Modicana dairy cows.

2. Materials and methods

2.1. Animal management and diet

The study was approved by the Ethical Committee of the Department of Veterinary Science of the University of Messina (code 041/2020) according to the European guidelines for the care and use of animals in research (Directive 2010/63/EU).

The present study was performed in a commercial dairy farm with 150 lactating Holstein and Modicana cows located in the province of Ragusa, Siciliy, Italy (36° 56′ 49′ N 14° 41′ 50′ E) under the traditional semi-intensive farming system.

A total of 32 multiparous dairy cows, 16 Holstein and 16 Modicana, were randomly allocated in 1 of 2 dietary treatment according to breed (Holstein and Modicana), and within each breed according to body condition score (Holstein: 2.5 ± 0.3, and Modicana: 2.6 ± 0.5; using the 5-point scoring system) (Buonaiuto et al., 2022), age (Holstein: 4.2 ± 1.5, and Modicana: $5.2\ 1.8$ years) parity (Holstein: 2.4 ± 0.5, and Modicana: 2.8 ± 0.8), and milk yield (Holstein: 28.8 ± 3.3, and Modicana: 14.7 ± 1.8). Cows in this study were examined daily for health-related problems by visual observation, temperature check, and monitoring milk yield. However, cows involved herein did not suffer from any acute health disorders during the entire experimental period. Additionally, no antibiotic treatments were applied.

The 32 animals chosen were divided into four groups (8 individuals each): two control groups (Holstein: CTRL_H; Modicana: CTRL_M) and two treatment groups (Holstein: TRT_H; Modicana: TRT_M).

In detail, Holstein and Modicana animals allocated to control groups (CTRL_H and CTRL_M, respectively) were fed as total mixed ration (TMR) composed of concentrate and meadow hay (20 kg of Dry Matter (DM)/head per day as Total Mixed Ration) whereas the two treated Holstein (TRT_H) and Modicana (TRT_M) groups received the aforementioned diet supplemented with enriched olive cake as 8% on a Dry Matter (DM).

The feed and nutrient composition of the concentrate of both control (CTRL_H and CTRL_M) and treatment (TRT_H and TRT_M) groups are reported in Table 1. As reported in the trademark flowchart n° 0001428707 (https://www.olioconsoli.com/home-italiano/azienda/in-breve/) the enriched olive cake was obtained by a mechanical two-stage process applied to produce extra virgin olive oil. After the addition of about 5% of vegetation water, the olive cake was pitted by centrifugation and dried in the open air. The detailed chemical composition, fatty acids, and polyphenols content of the olive cake are reported by Calabrese et al. (2023).

All the raw feed ingredients were analysed for aflatoxins content from the feed supplier and, according to the certification, the detected levels were below the maximum content regulated by the Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed (European Commission, 2002; Girolami et al., 2022).

2.2. Faecal samples collection, DNA isolation and sequencing

The faecal samples were collected at the beginning of the trial (0 d, in February) and at the end of the experimental period (60 d, in April) directly from the rectal ampoule from each animal. The samples were placed into sterile tubes and transferred under refrigerated conditions to the Laboratory of Microbiology of the Department of Agricultural Food and Environment (University of Catania, Italy) and then frozen at $-80\ °C$ until further analysis. For total genomic DNA extraction, about 180 mg of stool were weighed and processed using the commercial QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany). To increase the DNA isolation yield, a repeated bead beating (RBB) step was performed as reported by Randazzo et al., (2002, 2015). DNA concentration was evaluated using the fluorimeter Qubit 4.0 (Invitrogen, Carlsbad, CA, United States) and before storing at $-20\ °C$ until use. To in depth study the microbiota composition, the V3-V4 region of the 16 S rRNA gene was

Table 1
Feed and nutrient composition of concentrate of control groups (CTRL_H and CTRL_M) and treatment groups (TRT_H and TRT_M).

	CTRL	TRT
Feed composition (gkg⁻¹ as fed)		
Cornmeal	39.00	38.00
Soybean meal (0.48 CP[1])	19.00	18.00
Wheat middling	15.00	14.00
Barley meal	12.00	10.00
Olive cake	–	8.00
Sunflower meal	6.00	5.00
Carob pulp	3.00	2.00
Beet pulp	3.00	2.00
Vitamin premix[2]	3.00	3.00
Chemical composition, g/kg of Dry Matter (DM)		
Moisture	109	107
Starch	407	407
Crude protein	194	196
Ether extract	45.8	51.1
Non-Fiber Carbohydrates	465	440
Crude Fiber	60.0	72.0
Acid detergent fiber	78.2	105
Ash	64.1	70.2
NEL[3], milk UFL/kg of DM	1.09	1.07

[1]Crude protein. [2]Providing per kg of diet: 32,000 U vitamin A, 3200 U vitamin D3, 120 mg vitamin E, 8 mg vitamin B1, 1.6 mg vitamin B2, 0.016 mg vitamin B12, 400 mg niacin, 4 mg pantothenic acid, 400 mg choline chloride. [3]NEL: net energy lactation. Milk production efficiency was calculated based on the net energy system, where one milk forage unit (UFL) of energy is defined as the net energy content of 1 kg of standard barley for milk production, equivalent to 1700 kcal.

amplified and subjected to MiSeq (Illumina) sequencing at the facilities of GenProbio Srl (www.genprobio.com). The obtained raw data were deposited at NCBI Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under accession code PRJNA928233.

2.3. Bioinformatic analysis

The bioinformatics analysis foresaw the utilization of Quantitative Insights Into Microbial Ecology (QIIME2) tool, version 2022.2 (Bolyen et al., 2019). Particularly, the raw reads were quality filtered (phred score > 20), trimmed, and denoised using Divisive Amplicon Denoising Algorithms 2 (DADA2) which is implemented into QIIME2. The high-quality sequences (phred score > 20) were used to construct the amplicon sequence variant (ASVs) feature table. The SILVA reference database (v138) (Robeson et al., 2021) was used to taxonomic classification and the 75%, 87%, and 95% (Henderson et al., 2019) was used as percentage of identity to classify at phylum, family, and genus levels, respectively. After the classification, the ASVs, which had a relative abundance lower than 0.1%, were grouped as "others".

2.4. Alpha diversity, beta diversity and differential analysis

The differences in the microbiota profile among control and experimental samples of the same breed and within the same diet regime (CTRL_H vs CTRL_M; TRT_H vs TRT_M) were investigated, at the genus level, using the RStudio software (version 4.1.2). Alpha and beta diversities were calculated using the phyloseq packing of R (McMurdie and Holmes, 2013). The alpha diversity, based on the Shannon index, was plotted using the ggplot2 package (Wickham, 2016). The beta diversity was evaluated based on the Bray-Curtis distance and graphically represented by Principal Coordinate Analysis (PCoA), using "Breed" and "Diet" as PCoA grouping variables. Differential analysis was performed using the DESeq2 package in R (Love et al., 2014) and the differences, between breeds and diets, were analysed using separate models (Table 2).

2.5. Statistical analysis

The One-way nonparametric Wilcoxon test was applied to alpha diversity analysis. Pemutational multivariate analysis of variance (PERMANOVA), with 999 permutations, was applied to beta diversity data using the vegan package (Oksanen, 2017). Differential abundance was considered with a false discovery rate (FDR) cut-off < 0.05 and a log2 fold-change (FC) cut-off > ± 0.59. Significance was determined at P ≤ 0.05.

3. Results

3.1. Taxonomy classification

A total of 1.827.013 high-quality sequences (phred score > 20), obtained by QIIME2, have been assigned to 9 phyla, 41 families, and 69 genera and their relative abundance is shown in Fig. 1 (panels A-C) according to breed and treatment group. The sample metadata, denoising statistics, and the relative abundance at each taxonomic level (phylum, families, and genera) are reported in Supplementary data 1.

Overall, as reported in Fig. 1 panel A, *Firmicutes, Bacteroidota,*

Table 2
DESeq2 differential abundance models for each contrast.

	Model design
Effect of diet on Holstein cows	~ *Diet (TRT_H vs CTRL_H)*
Effect of diet on Modicana cows	~ *Diet (TRT_M vs CTRL_M)*
Effect of breed within CTRL group	~ *Breed (CTRL_H vs CTRL_M)*
Effect of breed within TRT group	~ *Breed (TRT_H vs TRT_M)*

Verrucomicrobiota, Spirochaetota, Actinobacteriota, Proteobacteria, Fibrobacterota, Patescibacteria, and *Cyanobacteria* were detected in all samples. *Firmicutes* and *Bacteroidota* were the most abundant phyla although differences in their occurrence were detected among samples. In particular, the *Firmicutes* phylum showed the highest relative abundance in both Modicana control and treated groups as well as in Holstein treated group.

At the family level, the ASVs were classified into 41 families (Fig. 1 panel B). Particularly, all the identified families were revealed in faecal samples of the Modicana control group (CTRL_M) whereas the *Bifidobacteriaceae* family was not detected in the experimental one (TRT_M). Differences were also revealed among Holstein samples, highlighting the absence of *Gastranaerophilales* family in the control group (CTRL_H) and of *Enterobacteriaceae, Fibrobacteraceae, Peptococcaceae, WCHB1–41*, and *Succinivibrionaceae* families in the treated group (TRT_H).

A total of 69 genera were identified and their distribution was different among samples investigated (Fig. 1 panel C). Particularly, among Holstein samples, the presence of *Faecalibacterium, Gastranaerophilales, Lachnoclostridium*, and *Roseburia* genera was detected only in treated samples (TRT_H). Among Modicana samples, *Bifidobacterium, [Eubacterium]_brachy_group, Faecalibacterium, Pseudobutyrivibrio, UCG-004, Butyrivibrio,* and *Solobacterium* were not detected in TRT_M samples. In addition, the absence of *Saccharofermentans* in both Holstein control (CTRL_H) and treated (TRT_H) samples as well as of *Faecalitalea* and *Sharpea* in both Modicana control (CTRL_M) and treated (TRT_M) samples was revealed (Fig. 1 panel C).

3.2. Alpha and beta diversities and differential analysis

Shannon indices are plotted in Supplementary Fig. S1 panel A-D and based on the Wilcoxon test no difference in richness was detected within breeds (Supplementary Fig. S1 A-B) and between the two dietary regimes (CTRL and TRT) (Supplementary Fig. S1 C-D).

The results of the beta-diversity, based on Bray-Curtis distance method, depicted in Fig. 2, measuring the dissimilarity among samples in relation to breeds (Holstein or Modicana) and dietary regime (CTRL or TRT), revealed a different behaviour of Holstein and Modicana breads towards the diet. As reported in Table 3, based on PERMANOVA results, both diet and breed, significantly affected the faecal microbial community (p < 0.001).

The models (Table 2) used for the differential analysis allowed to detect the genera showing significant differences, in terms of occurrence percentage, between Holstein breeds allocated to control and treated groups (CTRL_H vs TRT_H) (Table 4) as well as between Holstein and Modicana breeds allocated to control (Table 5) or treated (Table 6) group. At the genus level, no difference was detected between control and treated Modicana groups. In detail, as reported in Table 4, among Holstein, the genera RF39, *Clostridia_UCG-014, Pseudobutyrivibrio*, and *Prevotellaceae_UCG-001* prevailed in treated group (TRT_H) while *Saccharofermentans, Acetitomaculum, Negativibacillus, Paraclostridium*, Family_XIII_AD3011_group, *Alloprevotella, Christensenellaceae_R-7_group*, and *Bacteroidales_RF16_group* were mainly detected in control samples.
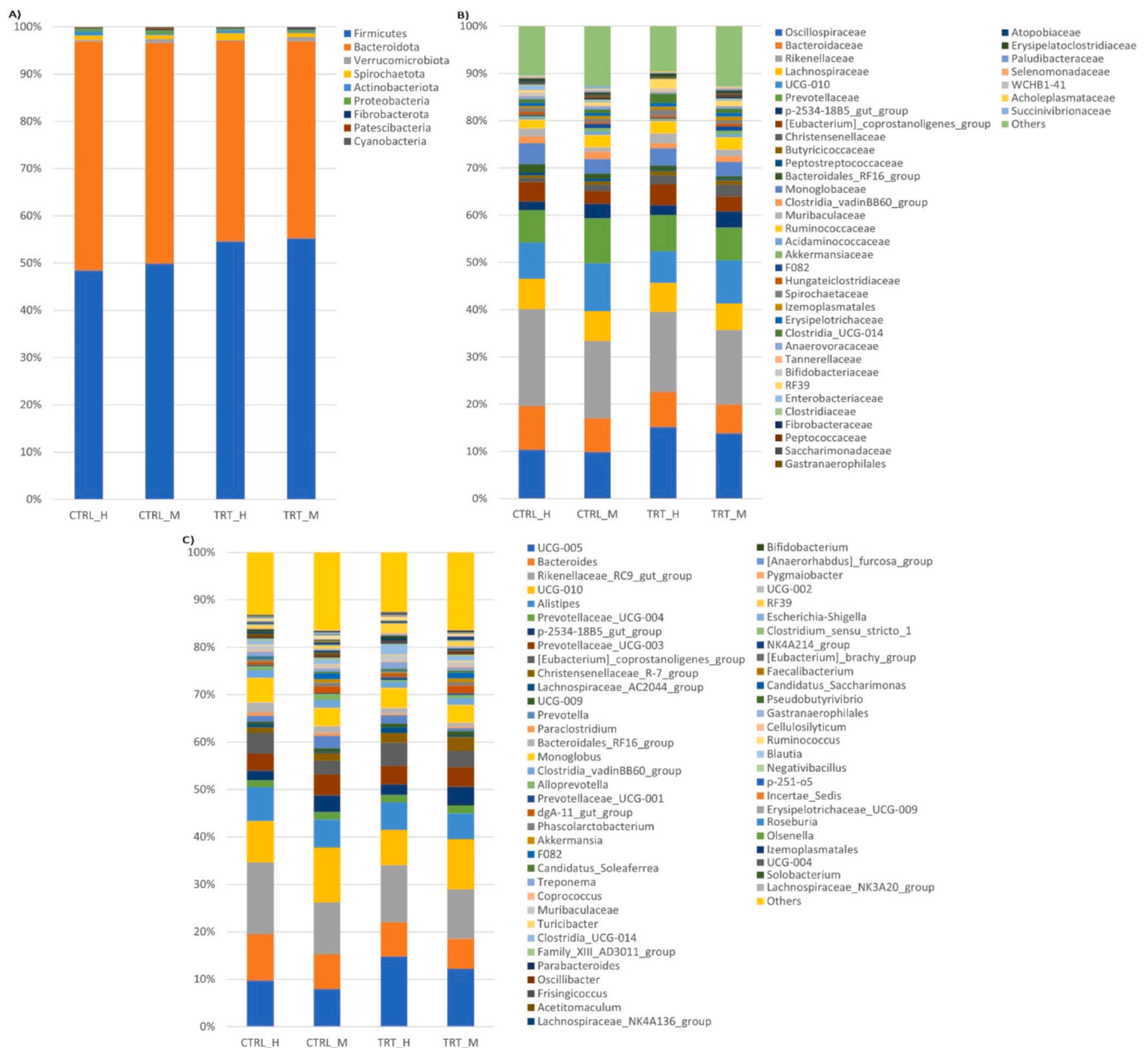
Table 5 shows the genera detected with different occurrence in Holstein and Modicana control groups (CTRL_H and CTRL_M). In particular, the genera *F082, UCG-002, dgA-11_gut_group*, and *p-2534–18B5_gut_group* were more prevalent in the Modicana control group (CTRL_M), while *Bifidobacterium, Lachnospiraceae_AC2044_group*, and *Pygmaiobacter* prevailed in Holstein control group (CTRL_H).

Concerning Holstein and Modicana treated groups (Table 6), *Akkermansia, F082, Phascolarctobacterium, Acetitomaculum, dgA-11_gut_group, NK4A214_group, Family_XIII_AD3011_group, Oscillibacter*, and *p-2534–18B5_gut_group* showed high occurrence in Modicana (TRT_M) whereas *Bifidobacterium, Lachnospiraceae_AC2044_group, Pseudobutyrivibrio, Prevotella, UCG-004, Pygmaiobacter, Ruminococcus, Clostridia_UCG-014, RF39, Treponema*, and *Prevotellaceae_UCG-001* prevailed in Holstein treated group (TRT_H).

Fig. 1. Relative abundance at phylum (A), family (B), and genus (C) level, detected in faecal samples of Holstein and Modicana cows allocated to control (CTRL_H and CTRL_H) and treated (TRT_H and TRT_M) groups.

4. Discussion

The composition and dynamics of the gut microbiota of cattle are significantly affected by several variables, and, among these, the composition of the diet plays a central role in influencing both the structure and abundance of this community (Kim and Wells, 2016).

Up to now, the effect of the diet on the welfare and performance of cattle was mainly evaluated by studying the diversity and structure of the rumen microbiota, since responsible for feed fermentation. Nevertheless, recent studies demonstrated that is possible to understand the effect of the diet, in particular the diet composition or the content of the fiber, by evaluating the composition and dynamics of the faecal microbiota, avoiding invasive and traumatic procedures (Kotz et al., 2021; Hagey et al., 2022).

Overall, our previous studies clearly demonstrated that the use of olive cake, as feed supplement, resulted in positive effects on phenotypic traits of the animal host. In fact, in beef cattle, metabolic, endocrine, as

well as performance responses (Chiofalo et al., 2020a; Bionda et al., 2022) improved after the integration of olive cake. In addition, the olive cake supplementation positively impacted on both milk composition profile (Chiofalo et al., 2020b) and faecal microbiota (Russo et al., 2023).

According to that, the present study aimed to in-depth evaluate, in Holstein and Modicana dairy cows, the changes in the faecal bacterial biota combined to the supplementation of olive cake to understand how the breeds can adapt to changes in the composition of the diet.

The differences observed in the taxonomic composition allowed us to classify the two breeds into distinct groups indicating a distinctive profile of the faecal microbiota. Corroborating previously reported data, Firmicutes and Bacteroidota were the phyla mainly detected, accounting for more than 90% of the bacterial biota of both Holstein and Modicana breed (De Oliveira et al., 2013; Williamson et al., 2022). It is well known that members of the Bacteroidota phylum carry out several functions in the gut, such as the degradation of carbohydrates, whereas Firmicutes,
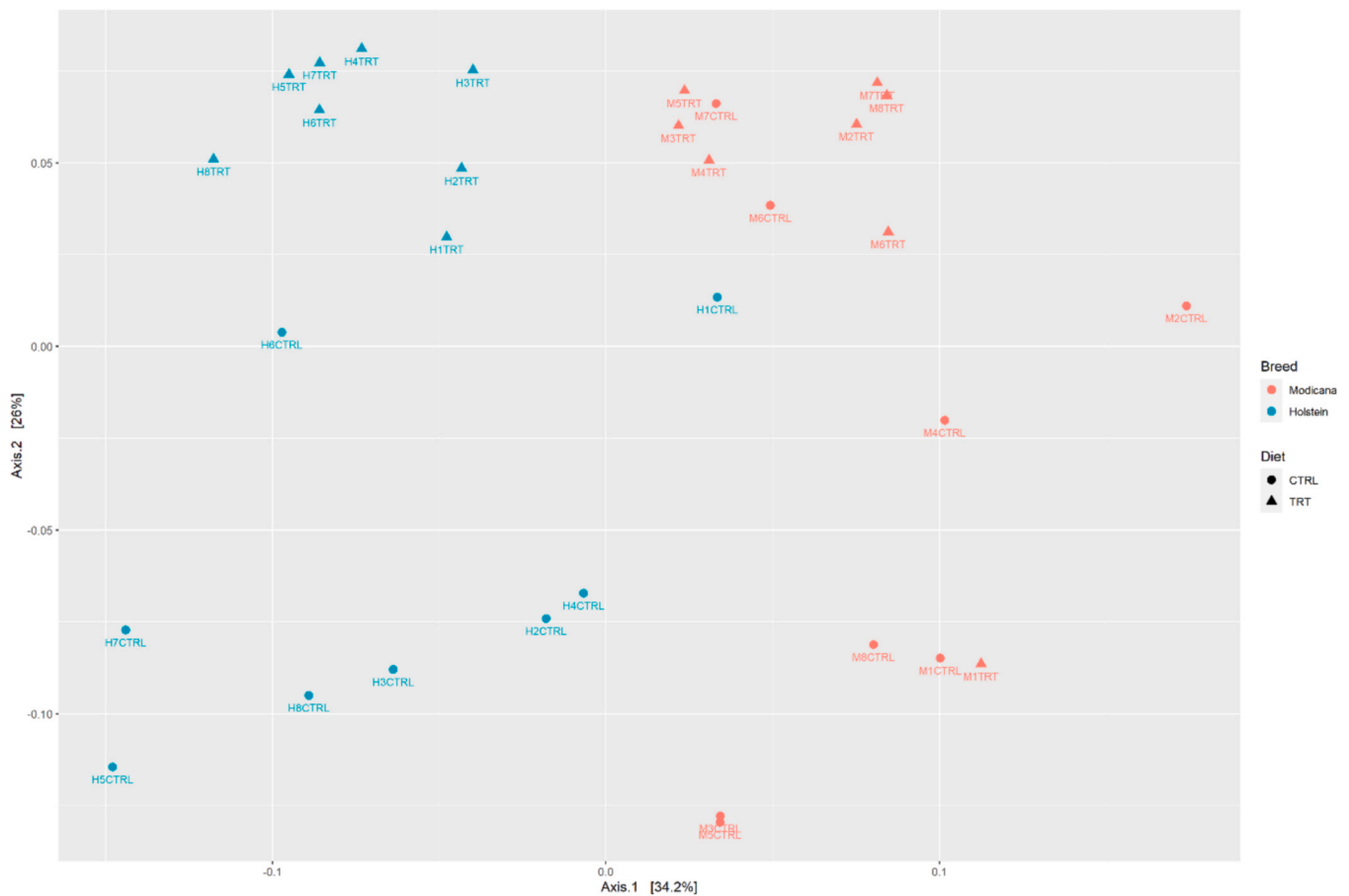
Fig. 2. PCoA, in according to Bray-Curtis distance, depicting the distribution of samples.

Table 3
PERMANOVA statistics.

Variable	F statistics	R²	p-value
Diet	7.12	0.15	0.001 *
Breed	12.32	0.26	0.001 *
* p < 0.05			

Table 4
Genera most differentiated between conditions of diet (TRT vs CTRL) within Holstein breed.

Genus	log2FC	p-value	p-adj
RF39	1.714712815 * *	0.001	0.001
Clostridia_UCG-014	1.229261753 * *	0.001	0.001
Pseudobutyrivibrio	1.092800996 * *	0.009	0.039
Prevotellaceae_UCG-001	0.800440537 * *	0.001	0.007
Christensenellaceae_R-7_group	0.843548153 *	0.001	0.001
Saccharofermentans	-3.401373718 *	0.001	0.007
Acetitomaculum	-2.673572756 *	0.001	0.001
Negativibacillus	-1.533946763 *	0.002	0.013
Paraclostridium	-1.26537121 *	0.001	0.001
Family_XIII_AD3011_group	-1.165998508 *	0.001	0.003
Alloprevotella	-0.935218006 *	0.002	0.013
Bacteroidales_RF16_group	-0.74425463 *	0.001	0.001
* *most prevalent in TRT; * most prevalent in CTRL.			

Table 5
Genera most differentiated between the CTRL groups of breeds (Modicana vs Holstein).

Genus	log2FC	p-value	p-adj
F082	1.807250903 * *	0.001	0.001
UCG-002	1.336467381 * *	0.004	0.039
dgA-11_gut_group	0.998667574 * *	0.001	0.001
p-2534–18B5_gut_group	0.678263659 * *	0.005	0.043
Bifidobacterium	-3.182300201 *	0.001	0.001
Lachnospiraceae_AC2044_group	-1.280598676 *	0.001	0.009
Pygmaiobacter	-1.134411722 *	0.002	0.019
*most prevalent in CTRL of Modicana; * * most prevalent in CTRL of Holstein			

high content of fiber present in the experimental diet.

In the present study, mainly in Holstein cows, the olive cake supplementation determined the upregulation of bacterial genera recognized as health-promoting, such as *Pseudobutyrivibrio* and *Christensenellaceae_R7-group*, as well as taxa associated with feed efficiency. Members of the *Pseudobutyrivibrio* genus, along with producing volatile fatty acids by the degradation of complex plant polysaccharides, are recognized as probiotics in the rumen, enhancing the intestinal mucosal immune barrier and in turn preventing inflammation (Kopečný et al., 2003; Wang et al., 2021). Similarly, the *Christensenellaceae_R7-group*, recognized as potentially beneficial bacteria, can maintain the structure of the gut (Jenkins et al., 2015; Fan et al., 2021a; Kim et al., 2021; Tardón et al., 2021). Previous studies demonstrated the ability of the aforementioned microbial group to inhibit the colonization of pathogens as well as the aptitude to alter the host gene expression, reducing the inflammation status during *Escherichia coli* infection (Fan et al., 2021a; Tardón et al., 2021). Interestingly, *Akkermansia* and

along with degrading fiber and starch, are involved in the production of butyrate which plays a central role in the rumen energy metabolism and is linked with the health of the gut (Thomas et al., 2011; Miguel et al., 2019; Kim et al., 2021). The high abundance of Firmicutes in both Holstein and Modicana cows treated with olive cake was related to the

Table 6

Genera most differentiated between the TRT groups of breeds (Modicana vs Holstein).

Genus	log2FC	p-value	p-adj
Saccharofermentans	5.075845931 * *	0.001	0.001
Akkermansia	2.238518359 * *	0.001	0.001
F082	2.169875579 * *	0.001	0.001
Phascolarctobacterium	1.402681928 * *	0.001	0.003
Acetitomaculum	1.363884142 * *	0.003	0.012
dgA-11_gut_group	1.148040671 * *	0.001	0.001
NK4A214_group	1.068305089 * *	0.002	0.008
Family_XIII_AD3011_group	1.01121559 * *	0.006	0.018
Oscillibacter	1.008497186 * *	0.001	0.001
p-2534–18B5_gut_group	0.731864714 * *	0.001	0.007
Sharpea	-9.669200043 *	0.001	0.001
Bifidobacterium	-8.31114493 *	0.001	0.001
Lachnospiraceae_AC2044_group	-1.659015315 *	0.001	0.001
Faecalitalea	-1.640396814 *	0.006	0.018
Pseudobutyrivibrio	-1.62755804 *	0.004	0.014
Prevotella	-1.528283653 *	0.001	0.001
UCG-004	-1.211847205 *	0.001	0.003
Pygmaiobacter	-1.160439526 *	0.001	0.001
Ruminococcus	-1.018782737 *	0.003	0.011
Clostridia_UCG-014	-0.964943345 *	0.001	0.001
RF39	-0.97298215 *	0.001	0.001
Treponema	-0.943960734 *	0.006	0.018
Prevotellaceae_UCG-001	-0.730910638 *	0.005	0.017

* *most prevalent in TRT of Modicana; * most prevalent in TRT of Holstein

Bifidobacterium genera showed high occurrence in treated groups of Modicana and Holstein, respectively. Among gut-associated microbes, *Akkermansia* was recently recognized as a member of the faecal core microbiota of cows (Dowd et al., 2008; Mtshali et al., 2022). Based on the health-promoting properties associated with *A. muciniphila*, such as the ability to contribute to a healthy mucus-associated microbiota and exert a wide range of probiotic features (Liu et al., 2014; Zhou et al., 2019; Mtshali et al., 2022) a positive association between the abundance of the *Akkermansia* genus and the health of cows was supposed (Mtshali et al., 2022). It is well known that several strains ascribed to the *Bifidobacterium* genus are widely recognized as probiotics and, in cattle, bifidobacteria can produce short-chain fatty acids, such as acetate, propionate, and butyrate, which can prevent various systemic inflammatory disease (e.g. arthritis, spondylitis, mastitis, etc.) (Shively et al., 2018; Wang et al., 2021). In addition, in a recent study, evaluating the rumen microbiome structure of 60 lactating Holstein dairy cows, with clinical and subclinical mastitis, the reduction of bifidobacteria was associated with a decline in immunity and antiinfection ability (Wang et al., 2021). Among taxa notoriously associated with feed efficiency, the unclassified members of the RF39 order as well as *Prevotellaceae_UCG-001* greatly varied by diet mainly in Holstein cows. This evidence is corroborated by several studies which recognized the RF39 order as a member of the rumen of lactating Holstein cows (Jami and Mizrahi, 2012; Jami et al., 2014; Jewell et al., 2015). In this regard, Jami and co-workers (2014), correlated the rumen bacterial biota of 15 dairy cows with both production parameters and milk composition. Although no significant correlation was detected between the bacterial community and residual feed intake (RFI), the authors postulated the involvement of the RF39 taxon in feed efficiency, contributing to host physiological variation. However, the data did not confirm the positive association between RF39 and feed efficiency in cattle (Jami et al., 2014). Different results were obtained by McGovern and co-workers (2018). In fact, by studying the microbiota of both the rumen solid and liquid digesta of four cohorts of Simmental bulls divergent for RFI phenotype, the authors demonstrated that the RF39 taxon was negatively correlated to RFI suggesting a highly efficient rumen microbiome (McGovern et al., 2018). In addition, in a cohort of 14 Holstein cows, monitored through the first two lactation cycles, RF39 correlated with increased gross feed efficiency (GFE), a widely used method to measure the yield of milk produced (Jewell et al., 2015).

It is well known that members of the Prevotellaceae family act by degrading protein, starch, and hemicellulose (Rubino et al., 2017) and are involved in the metabolism of glutathione, phenylalanine, starch, sucrose, and galactose as well as in the metabolism of amino acids and carbohydrate (Xue et al., 2020). As reported by Liu et al. (2019), Prevotella effectively utilizes forages to produce succinate and acetate as the end-product of glucose catabolism reducing nitrogen loss.

In the present study, evalutating the microbial community of Modicana breed, a similar faecal microbiota profile between control and treated groups was revealed, even Firmicutes phylum was most abundant in treated group than control one. Its notheworthy that Firmicutes phylum exerts an important effect in the rumen energy metabolism and in the health of the gut (Kim et al., 2021; Thomas et al., 2011; Miguel et al., 2019), reflecting a higher production of butyrate. As revealed by the beta diversity plot, the Modicana breed showed a higher inter-individual diversity that Holstein breed, which could explain its higher adaptability to the dietary intervention. In addition, based on the concept of "permissive" and "restrictive" gut microbiotas, or "responders" and "non-responders", host genetics seem to play a key role on the microbial resilience of the host's gut microbiota (Weimer, 2015; Roehe et al., 2016; Camarinha-Silva et al., 2017; Fan et al., 2021b). The involvement of host genetics in the definition of the faecal microbiota community, already demonstrated in animals with different genetic backgrounds (Fan et al., 2021b), could be postulated also for Modicana breed.

5. Conclusions

The present study showed that olive cake supplementation in the diet increased the abundance of taxa recognised as favourable to health or associated with feed efficiency. The increase of the Firmicutes phylum in Holstein breed reflects the digestive capacities of fibrous sources. However, the integration of the olive cake had a different effect on Holstein and Modicana breeds, emphasising the importance of the host genetic profile in determining the faecal bacterial biota composition.

CRediT authorship contribution statement

Vincenzo Lopreiato, Luigi Liotta, and Cinzia Lucia Randazzo: Conceptualization. **Enrico D'Alessandro, Luigi Liotta, Alessandra Pino, and Cinzia Lucia Randazzo:** Methodology. **Viviana Floridia, Annalisa Amato, and Nunziatina Russo:** Formal analysis. **Viviana Floridia and Alessandra Pino:** Data curation. **Viviana Floridia, Vincenzo Lopreiato, and Alessandra Pino:** Writing – original draft. **Luigi Liotta, Cinzia Caggia, and Cinzia Lucia Randazzo:** Supervision. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

Nunziatina Russo, Alessandra Pino, Cinzia Caggia and Cinzia Lucia Randazzo declare that they are members of ProBioEtna, a spinoff of the University of Catania, Italy. In addition, the authors declare that they do not have any personal, financial, professional, political, or legal interests with a significant chance of interfering with the perfor-mance of their ethical or legal duties.

Data Availability

Data will be made available on request.

Acknowledgments

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.micres.2023.127510.

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