frontiers in Animal Science

TYPE Original Research
PUBLISHED 24 April 2026
DOI 10.3389/fanim.2026.1783759

Check for updates

OPEN ACCESS

EDITED BY
Markku Saastamoinen,
Natural Resources Institute Finland (Luke), Finland

REVIEWED BY
Consiglia Longobardi,
University of Naples Federico II, Italy
Sarah-Alica Dahl,
Technical University of Munich, Germany

*CORRESPONDENCE
Vincenzo Tufarelli
vincenzo.tufarelli@uniba.it

†PRESENT ADDRESS
Francesco Giannico,
Link Campus University, Rome, Italy

‡These authors have contributed equally to this work

RECEIVED 08 January 2026
REVISED 26 February 2026
ACCEPTED 30 March 2026
PUBLISHED 24 April 2026

CITATION
Colonna MA, Mancini L, Giannico F, Tarricone S, Carbonara C, Tedone L, Ceci E, Tufarelli V, Cosentino C, Chiarini M, Bernardi M, Risoli F, Apa C, Calasso M, Celano G, De Angelis M and Calabrese FM (2026) Grazing on *Camelina sativa* pasture: effects on mare body condition, metabolic profile, and fecal microbiota. *Front. Anim. Sci.* 7:1783759. doi: 10.3389/fanim.2026.1783759

COPYRIGHT
© 2026 Colonna, Mancini, Giannico, Tarricone, Carbonara, Tedone, Ceci, Tufarelli, Cosentino, Chiarini, Bernardi, Risoli, Apa, Calasso, Celano, De Angelis and Calabrese. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Grazing on *Camelina sativa* pasture: effects on mare body condition, metabolic profile, and fecal microbiota

Maria Antonietta Colonna[1], Leonardo Mancini[1‡], Francesco Giannico[2†‡], Simona Tarricone[1‡], Claudia Carbonara[3‡], Luigi Tedone[1], Edmondo Ceci[2‡], Vincenzo Tufarelli[4*], Carlo Cosentino[5], Margherita Chiarini[1], Matteo Bernardi[1], Fabiola Risoli[1], Carmen Apa[1], Maria Calasso[1], Giuseppe Celano[1‡], Maria De Angelis[1] and Francesco Maria Calabrese[1]

[1]Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy, [2]Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Italy, [3]Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy, [4]Department of Precision and Regenerative Medicine and Jonian Area, Section of Veterinary Science and Animal Production, University of Bari Aldo Moro, Valenzano, Bari, Italy, [5]Department of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Potenza, Italy

This study evaluated the potential of *Camelina sativa* as a forage crop for extensive horse breeding focusing on body condition, serum biochemical parameters, 16S rRNA gene sequencing, and metabolomic profiling of mares' feces. The aim was to characterize microbiota composition, predict microbial functions, and identify volatile organic compounds (VOCs). Six mares underwent a longitudinal study over 12 weeks: in the first 6 weeks, the mares grazed on a control pasture; afterwards, they were shifted to a pasture containing camelina for a further 6 weeks. At the end of each grazing period, blood and feces were individually collected for analysis. Grazing on camelina pasture increased the contents of ash, acid detergent lignin (ADL), and acid insoluble ash (AIA) ($p < 0.01$), while it decreased the fecal concentration of N-free extracts ($p < 0.05$), crude fiber, neutral detergent fiber (NDF) ($p < 0.01$), serum alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), non-protein nitrogen (NPN), cholesterol, chlorides, calcium, and potassium ($p < 0.05$). Alpha- and beta-diversity metrics clearly distinguished pre- and post-diet collected fecal samples, clustering into two different microbiota subclusters. A beneficial effect on the gut microbial community was observed, with an increase in the relative abundance of specific taxa acting as homeostasis biomarkers, i.e., the genera *Lysinibacillus* and *Planococcaceae* indicate an enhanced bile acid biotransformation capacity. Although limited by the small sample size, our statistically significant VOCs and predicted metabolic pathways suggest biosynthetic flexibility in response to fiber-rich diets, showing possible microbial adaptation to maintain redox balance with an increased energy yield under fermentative conditions and improved metabolic efficiency in producing short chain fatty acids (SCFAs).

KEYWORDS
16S rRNA gene sequencing, drought-tolerant pasture, gut microbiota, hematological profile, Murgese mares, volatile organic compounds

1 Introduction

Camelina (*Camelina sativa* L. Crantz) cultivation has been spreading both as a grazing crop and for fresh forage production (Tedone et al., 2022), as seed pods (Jolazadeh et al., 2025), or for oil extraction from its seeds. Camelina is tolerant of cold and drought and can grow in semiarid regions (Mondor and Hernández-Álvarez, 2022; Piergiovanni et al., 2025). Moreover, camelina is considered a low-input plant due to its minimal nutrient requirements and lower dependency on water, pesticides, and fertilizers, in comparison with traditional oilseed crops such as canola and sunflower (Zając et al., 2020). Its seeds are rich in oil (30–49%) and protein (24–31%) and contain fatty acids of the n-3 and n-6 series, tocopherols, phytosterols, and phenolic compounds (Ibrahim and El Habbasha, 2015). Previous studies have focused on the nutritional value of camelina pasture for milk production in goats (Colonna et al., 2021), ewes (Cais-Sokolińska et al., 2011), and cows (Riaz et al., 2022). While camelina oil supplementation has been thoroughly investigated in the horse diet (Hartwig et al., 2025a; 2025b), there is still little information on the use of camelina as a pasture crop for horses.

The gastrointestinal system of horses has a very delicate balance; one of the main risk factors for digestive disorders in horses is related to a diet rich in starch and low in fiber. Starch digestibility in horses is limited due to the low production of pancreatic α-amylase. Starch consumption should not exceed 1 g of starch/kg body weight (BW)/meal, because higher amounts could lead to the occurrence of metabolic pathologies such as hindgut acidosis, dysbiosis, and colic (Raspa et al., 2024).

Though grazing is a cost-effective method, horse breeders face several issues, such as overgrazing and unproductive pastures. The application of rotational grazing systems may improve forage efficiency and contribute to prolonging the grazing season by allowing pastures to rest and re-grow between grazing periods (Kenny et al., 2024). Identifying forage species resistant to summer drought and characterized by a good nutritional value may delay the sowing of grasslands, thus extending the period in which pasture is available to horses, meeting the animals' needs.

The connection between horse breed welfare and gut microbiota has been investigated in different horse subspecies (Zaitseva et al., 2023; Carrillo Heredero et al., 2024), and specific priming for welfare has been dedicated to horse athletes animals (Mach et al., 2020; 2021). A forage-based, fiber-enriched diet (Muhonen and Julliand, 2023), along with low-starch levels (Irving et al., 2025), has been shown to modulate the microbial commensal community in athletic horses.

The "Murgese" is an autochthonous horse breed native to Apulia, in Southern Italy, well known for its vivid black or blue roan coat and a convex rostrum called "Roman-nose" (Figure 1) (Bramante et al., 2022; Alhaddad et al., 2024). Traditionally, this breed is reared extensively in farms mainly located in the Apulian Murgia area, characterized by rocky soil. The etymological origin of the word "Murgia" derives from the Latin "murex", which means "murice, sharp rock". In the past, the Murgese horse was used as a working draft horse and for mule production, but after the agricultural mechanization boom in the 70s, this breed experienced a severe population decrease to the point of being considered at risk of genetic erosion (Bramante et al., 2022). Nowadays, Murgese horses are experiencing renewed interest and notoriety, also at an international level, especially for equestrian tourism purposes, dressage sport activities, and for the mounted surveillance activities of the Italian and German police forces. The breed is characterized by good temperament, robustness, sure step, strong limbs and hooves, and a natural ability to cope with infectious diseases, notably endemic tick-borne diseases (TBDs) (Rubino et al., 2006; Selvaggi et al., 2010). In 2008, the Murgese horse studbook was established to improve the effectiveness of breeding strategies (Bramante et al., 2022), and nowadays, the Murgese population



FIGURE 1
Miscellaneous Murgese horses. **(A)** mares grazing on pasture; **(B)** mare evaluated for its Body Condition Score (BCS); **(C)** free exercise in the paddock area; **(D)** lactating mare with its foal.

accounts for 5,823 animals bred in Italy, among which 3,879 are in Apulia (Italian Veterinary Information System). Because of its rustic nature, this breed is well adapted to harsh climates (cold in the winter, hot and dry in the summer) and low-quality pastures of the Apulian Murgia environment.

To our knowledge, the scientific literature lacks evidence regarding the microbiota features of the Murgese horse; moreover, the impact of grazing on Camelina sativa pasture on serum parameters, Body Condition Score (BCS), and fecal features has never been investigated. Thus, the aim of this study was to evaluate the influence of grazing on a pasture containing Camelina sativa on body condition, serum biochemical profile, and the fecal microbiota metataxonomic and related metabolomic profiles of Murgese mares.

2 Materials and methods

2.1 Animals and management

This study was conducted with the consent of the horses' owner, who exclusively handled the animals, in compliance with current regulations regarding animal husbandry and welfare (Legislative Decree no. 146 of March 26, 2001). No ethical approval was required (Regulation 2019/1010 of the European Parliament and of the Council), because the blood samples were collected by the local health authority veterinarian during periodic animal control.

The study was conducted during the spring of 2025, at the farm "Allevamento delle Lame" located in Casamassima (province of Bari, Apulia Region, Italy; N: 40.917991, E: 16.953245, 223 m above sea level). Six black-coated Murgese mares, born from parents registered in the relevant herd books of the breed and unrelated to each other, were used. Mares were on average 5.5 years old (range 4.5 – 6.5) and had lived in this farm since birth. They were reared in an extensive system with free access to pasture during the day, while they were penned in a fenced paddock at night, provided with straw *ad libitum* and a mineral salt lick containing sodium (34.8%), calcium (1.45%), magnesium (1.5%), phosphorus (0.5%), plus biotin and vitamins (EquiVital PLUS; Imima GmbH, Puchheim, Germany). Mares were in good health and in a good body condition, devoid of any signs of lameness, injury, intestinal disorders, or infectious disease.

The farm is located in an area characterized by a Mediterranean climate, with precipitation concentrated mainly from October to April and a very dry summer. During 2024, the mean seasonal temperature and rainfall were 14.3 °C and 151.3 mm in autumn, 10.7 °C and 317.1 mm in winter, and 20.8 °C and 93.5 mm in spring, respectively (Supplementary Figure 1).

The trial was conducted in a grassland sown in a medium-textured soil with outcropping stone. The grassland was 10 hectares wide, flat, with exposure to the East, and without trees. The grassland was confined to the farm to enable free access for the mares. Half of this grassland was sown with a mixture of seeds composed of oat (70%, *Avena sativa*), vetch (20%, *Vicia sativa*), and ryegrass (10%, *Lolium perenne*) (control pasture), while the other half was sown with a mixture of seeds composed of oat (70%, *Avena sativa*), vetch (20%, *Vicia sativa*), and camelina (10%, *Camelina sativa*) (camelina pasture). The control pasture was sown on October 10, 2024, while the camelina pasture was sown on October 25, 2024.

Six mares were used in a longitudinal study over 12 weeks: in the first 6 weeks, mares grazed on the control pasture (T0), while afterwards, they were shifted to the camelina pasture where they grazed for a further 6 weeks (T1). Mares had *ad libitum* access to water.

At the end of each grazing period, blood and fecal samples were individually collected from mares to carry out the related analyses.

2.2 Pasture sampling, nutritive value, and metabolized energy

During the trial, every 2 weeks, pasture samples were collected according to the methods described by Cosentino et al. (2021). The samples were first dried in an oven at 65 °C, then weighed to calculate the dry matter; afterwards, they were analyzed in triplicate according to the procedures suggested by the Association of Official Analytical Chemists (AOAC) (2000).

The combined method between the Menke and Steingass (1988) *in vitro* gas production technique and the modified first-stage technique of Tilley and Terry (1963) was used to analyze the organic matter digestibility (OMD) and net gas production (GP, ml/g DM), as thoroughly described by Abdouli and Attia (2007). As described by Alvarado et al. (2022), the metabolizable energy (ME, MJ/kg DM) was calculated according to the following formula: ME = 2.20 + 0.136 GP + 0.0057 CP ($g \times KG^{-1}$ DM), where GP is net gas production in mL from 200 mg dry sample after 24 h of incubation and CP is the crude protein value.

2.3 Body weight, body condition score, and coat color

Body weight (BW) was calculated using the following formula: BW (kg) = [(heart girth)^2 x body length]/11877 as proposed by Carroll and Huntington (1988), where HG is the heart girth and BL is the body length expressed in cm. Body condition score (BCS) was assessed at the end of each grazing period, using a 9-point scale to assess physically and visually some anatomical points as described by Henneke et al. (1983), where: 1 = extremely thin, 2 = very thin, 3 = thin, 4 = moderately thin, 5 = ideal, 6 = moderately fleshy, 7 = fleshy, 8 = fat, and 9 = extremely fat. The score was assigned following bilateral visual inspection and palpation of the crest of the neck, spinous processes of the lumbar region, tail head, withers, ribs, and the scapula.

At the same time as the BCS evaluation, the mares' coats were evaluated. Three breed experts, authorized by the "National Association of the Murgese Horse and Martina Franca Ass Breeders" (A.N.A.M.F.), judged the mares' coats, assigning scores for three parameters: color, shine, and uniformity. Each judge could give a value from 0 to 3 points for each parameter, and the coat was evaluated as the sum of the three parameters (from 0 to 9).

2.4 Feces collection and chemical composition

At the end of each grazing period, feces were individually collected from the mares for three consecutive days; fecal samples were collected upon deposition and immediately stored in individual sterile plastic bags and temporarily placed on ice for no more than 2 h. Upon arrival at the laboratory, the central part of each fecal sample was collected and transferred into a sterile container for microbiological analyses. The remaining feces were first dried in an oven at 103 °C for 48 h and then weighed to determine the dry matter (DM), as described by Cappai et al. (2020).

Chemical composition was determined according to the procedures described by Giannico et al. (2024); briefly, all the samples were analyzed in triplicate using the procedures suggested by the Association of Official Analytical Chemists (AOAC, 2000). Analyses to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were carried out according to Van Soest et al. (1991). All the fiber fractions were expressed as ash-corrected values.

2.5 Blood sampling collection and biochemical serum analyses

At the end of the two grazing periods (T0, T1), blood was collected in the morning (between 8.00 and 8.30) from each mare before leaving the paddock. Straw and water were not restricted before blood collection. Vacuette 9 mL tubes with CAT serum clot activator were used to draw blood from the *vena jugularis* (7 mL from each horse) and samples were transported in a portable refrigerator to the laboratory. The serum was separated from blood cells by centrifugation (4000 rpm for 20 min), and stored at -18 °C before analysis. The biochemical parameters and the electrolytes of the serum were determined by the automatic analyzer Alinity ci-series (Abbott, IL, USA).

2.6 Sample collection and DNA extraction

Approximately 500 mg of each fecal sample was used for DNA extraction. To ensure sufficient DNA yield and minimize extraction bias, each sample underwent two independent extractions, and the resulting DNA fractions were pooled. Genomic DNA was isolated using the FastDNA® Pro Soil-Direct Kit (MP Biomedicals, Irvine, CA, USA), adapted with three sequential pre-washing steps including PBS and EDTA. The concentration and purity of the extracted DNA were then measured with a Qubit® 3.0 fluorometer (Thermo Fisher Scientific, Milan, Italy).

2.7 DNA amplification and sequencing

The V3–V4 hypervariable region of the bacterial 16S ribosomal RNA (rRNA) gene was targeted for amplification via PCR. Amplicons were obtained by using the universal Illumina primer pair (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Each 25 µl PCR reaction contained 12.5 µl of 2× Kapa HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA, USA), 5 µl of each primer (at 1 µM concentration), and 2.5 µl of template DNA. Thermal cycling conditions consisted of an initial denaturation, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension step at 72 °C for 5 min.

After amplification, Agencourt AMPure beads (Beckman Coulter, Milan, Italy) were used to remove any residual primers and impurities from PCR amplicons. Single libraries were indexed using the Nextera XT Index Kit Set A (Illumina Inc., San Diego, CA, USA). DNA libraries were assessed in terms of fragment length and concentration on an Agilent TapeStation 4200 using a D1000 ScreenTape assay, confirming the expected amplicon size (630 bp) and the absence of primer-dimer artifacts.

Following quality control and quantification, the indexed libraries were normalized individually to 4 ng/µl. Equal volumes of these normalized libraries were pooled to achieve the required molarity for sequencing, and a PhiX Control v3 library (Illumina) was used as a spike-in for the pool at 30% of the total volume, serving as an internal sequencing control to increase diversity. The final pooled library, along with the appropriate buffers and sequencing reagents, was loaded onto an Illumina MiSeq2 platform (at the Department of Soil, Plant, and Food Sciences, University of Bari). Sequencing was performed using a MiSeq Reagent Kit v3 in a 2 × 300 bp paired-end run.

2.8 16S metabarcoding and bioinformatics analyses

Post-sequencing bioinformatics analyses were conducted using the QIIME2 (Quantitative Insights Into Microbial Ecology 2) pipeline and the relevant nested packages useful to obtain alpha- and beta-diversity estimates. To avert any contamination occurrence, a negative control consisting of all the reagents and without the sample was also included in the workflow of 16S amplification and library preparation. Samples were analyzed as biological duplicates. Libraries were quantified using a Qubit fluorometer (Invitrogen Co., Carlsbad, CA, USA) and pooled, including the PhiX control library, to an equimolar amount (4 nM final concentration). FastQ file quality was assessed using FastQC software (Bolyen et al., 2019) and analyzed using the QIIME2 dedicated pipeline (https://qiime2.org) microbiome platform (version 2020.8). Denoising was computed with the q2-deblur QIIME plugin (https://github.com/qiime2/q2-deblur). Taxonomy was inferred with the QIIME-compatible database SILVA 138 SSU database, using an amplicon sequence variant (ASV) table based on error-corrected reads. Alpha diversity metrics, including Shannon entropy and Faith's PD, were also computed by using QIIME2 nested plugins (Chao and Bunge, 2002). Functional metabolic profile prediction starting from the 16S abundance matrix was carried out by using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) QIIME2 nested pipeline, version 2.0. Pathway prediction was inferred on the basis of the.biom file. Further clustering of the metabolic pathways was carried out by using the Biocyc database for pathway assignation. The 16S rRNA fastQ raw files from the study were deposited at the NCBI Sequence Read Archive (SRA) under the following accession number: PRJNA1356358.

2.9 Untargeted metabolomics

An aliquot (1–3 g) of each fecal sample was used to perform untargeted metabolomics analysis and to obtain mass spectral profiles. Approximately 2 g of each sample was transferred into 20 mL headspace vials and sealed with polytetrafluoroethylene (PTFE)-coated silicone rubber septa. A 10 µl aliquot of 4-methyl-2-pentanol (final concentration, 4.97 µg/g) was added as an internal standard. Volatile compounds were extracted using a CombiPAL system injector autosampler (CTC Analytics, Zwingen, Switzerland). After equilibration at 60 °C for 15 min, volatiles were adsorbed onto a conditioned 50/30 µm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA) for 30 min. The fiber was then thermally desorbed in the heated injection port (230 °C) of the gas chromatograph operating in splitless mode, with helium as the carrier gas at a flow rate of 1 mL/min. Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a Clarus 680 gas chromatograph (PerkinElmer, Beaconsfield, UK) equipped with an Rtx-WAX column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Restek, Bellefonte, PA, USA) coupled with a Clarus SQ8 mass spectrometer (PerkinElmer). The ion source and transfer line temperatures were set at 250 °C and 230 °C, respectively. The oven temperature program was as follows: initial temperature 35 °C for 8 min; ramped to 60 °C at 4 °C/min; to 160 °C at 6 °C/min; and finally to 200 °C at 20 °C/min, where it was held for 15 min. Electron ionization spectra were acquired at 70 eV over an m/z range of 34–350. Compound identification was performed using the National Institute of Standards and Technology (NIST) 2008 mass spectral library, applying a peak area threshold >1,000,000 and a match probability ≥85% for volatile organic compound (VOC) identification.

2.10 Statistical analysis, pairwise group tests, and multiple correction

Statistical analyses of the data regarding the mares' feces composition and the serum biochemical profile were performed using Python software (version 3.11.13) (Python, n.d). The data obtained were analyzed using ANOVA for repeated measures, with the animal as a repeated factor and the type of pasture as a non-repeated factor. Results were reported as least squares means and pooled standard error of the mean (SEM). Significance was declared at $p < 0.05$.

Pairwise group statistics were run in the R environment using the MetaboAnalyst package (Pang et al., 2021). The non-parametric test (Wilcoxon rank-sum test) was corrected for multiple testing, and only statistically significant differences with an adjusted p-value (FDR) cut-off lower than 0.05 were kept and further analyzed. The non-parametric test and fold change were combined and graphically rendered as a volcano plot.

3 Results

3.1 Chemical composition of pastures

The chemical and fatty acid composition of the two pastures is shown in Table 1. No significant difference between pastures was observed for the chemical composition. The pasture containing camelina showed a markedly ($p < 0.05$) lower concentration of palmitic acid (C16:0) and of total SFAs as compared to the control pasture. On the other hand, the concentration of C18:1 n-9, C20:1, and total MUFA was markedly ($p < 0.05$) higher in the camelina pasture. Among PUFAs, the camelina pasture contained a lower ($p < 0.05$) amount of C18:3 n-6 and a higher ($p < 0.05$) concentration of C18:3 n-3. Neither the organic matter digestibility nor the *in vitro* gas production differed between pastures.

3.2 Body weight, body condition score, and coat color

Table 2 shows the evolution of body weight, body condition score, and coat color of mares after grazing on the control and camelina pastures. No differences were observed between pastures for any of the observed parameters. Although feed intake was not assessed individually, mares showed sorting activity at the beginning of grazing on the camelina pasture, likely due to their unfamiliarity with this pabular species essence and to an initial wariness of camelina. However, after a week of grazing on this pasture, the mares gradually increased their intake of camelina grass.

3.3 Feces quality and serum biochemistry profile

Table 3 shows the chemical composition of the mares' feces collected after the two grazing periods. No differences in feces appearance and consistency were detected between the two grazing periods. Grazing on the camelina pasture led to an increase in ash, ADL, and AIA contents ($p < 0.01$), while it determined a decrease in dry matter ($p < 0.059$ and in the concentration of N-free extracts ($p < 0.01$), crude fiber ($p < 0.05$), and NDF ($p < 0.01$) as compared to the control.

Table 4 shows the effect of pasture on the serum biochemical profile of Murgese mares. Grazing on the camelina pasture resulted a significant decrease in the serum concentration of alkaline phosphatase (ALP, $p < 0.01$), gamma-glutamyl transferase (GGT, $p < 0.01$), glutamate pyruvate transaminase (GPT, $p < 0.05$), cholesterol ($p < 0.05$), chlorides ($p < 0.05$), calcium ($p < 0.01$), and potassium ($p < 0.01$).

3.4 16S metabarcoding taxa annotation (presence and relative abundances) at different taxonomic levels

Qiime2 metabarcoding analyses based on demultiplex sample files ranged between 97K and 142K passing-filter reads, with a fraction of artifacts below 1% as shown in Supplementary Table 1. Only one of the samples was discarded because of too low DNA concentration during the extraction phase, which resulted in a low read number. The diversity matrix indices were thus based on 12 pre-diet *versus* 11 post-diet samples.

Metabarcoding annotation against the SILVA 138 database returned a total of 19 phyla, 34 classes, 75 orders, 124 families, 220 genera, and 322 species.

TABLE 1 Chemical and fatty acid composition and nutritive value of the two pastures.

Parameters	Control pasture	Camelina pasture	SEM	p - value
Dry matter (%)	35.90	35.60	0.061	0.087
Protein (%)	10.61	10.44	0.007	0.073
Ether extract (%)	2.99	3.39	0.038	0.082
Ash (%)	9.76	9.50	0.044	0.094
Crude fiber (%)	30.83	30.20	0.063	0.081
N-free extracts (%)	45.81	46.71	0.103	0.096
NDF (%)	55.07	56.12	0.032	0.112
ADF (%)	30.82	31.25	0.029	0.084
ADL (%)	4.10	4.57	0.094	0.066
AIA (%)	0.46	0.48	0.021	0.087
GP (ml/g DM)	223.35	234.15	0.134	0.104
OMD (MJ/kg DM)	8.47	8.68	0.069	0.069
ME (MJ/kg DM)	8.335	8.628	0.057	0.074
Fatty acids (% on FAME)				
C12:0	0.08	0.08	0.002	0.087
C14:0	0.89	0.81	0.013	0.103
C15:0	0.19	0.17	0.009	0.088
C16:0	17.76[a]	16.59[b]	0.057	0.031
C17:0	0.24	0.22	0.066	0.074
C18:0	4.06	4.00	0.011	0.058
C20:0	0.24	0.22	0.039	0.062
C22:0	0.32	0.33	0.007	0.069
Total SFA	23.38[a]	22.24[b]	0.017	0.033
C16:1	0.74	0.67	0.051	0.077
C18:1 n-9	3.94[b]	4.89[a]	0.034	0.047
C20:1	0.33[b]	0.56[a]	0.017	0.044
Total MUFA	4.79[b]	5.94[a]	0.023	0.046
C18:2 n-6	17.08	17.70	0.004	0.091
C18:3 n-6	40.49[a]	39.08[b]	0.023	0.047
C18:3 n-3	0.11[b]	1.42[a]	0.034	0.039
C20:3 n-6	0.01	0.08	0.004	0.065
C20:4 n-6	0.02	0.26	0.001	0.059
C20:3 n-3	0.16	0.21	0.012	0.071
C20:5 n-3	0.10	0.12	0.003	0.067
Total PUFA	57.82	58.78	0.034	0.077
Other acids	14.00	13.05	0.086	0.088

NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin; AIA, Acid Insoluble Ash; GP, gas production (ml/g DM); OMD, organic matter digestibility (MJ/kg DM); FAME, fatty acid methyl ester; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values within a row with different superscripts differ significantly: a, b: $p < 0.05$.

From a taxonomic point of view, the multivariate analyses we performed to understand whether a linear separation of groups occurred were based on the inspection and usage of the genus matrix. The partial least squares discriminant analysis (PLS-DA), supported by the prediction model (high Q2 value), allowed for a clear separation of T1 and T0 fecal samples, meaning a different presence and possible contribution of taxa within each group.

In detail, both axes in the PLS-DA plot describe approximately 30% of the distribution, with a higher impact of *Phoenicibacter*, *Breznakia*, *Colidextribacter*, and *Anaerofilum*. *Pygmaiobacter*, the *Family XIII AD3011 group of Anaerovoracaceae*, *Anaeroplasma* genera, and unresolved genera scaled to the upper family level of Christensenellaceae (all having a VIP score greater than 2).

It is worth noting that the genera resolved at the Christensenellaceae family level, together with *Anaeroplasma* and *Anaerofilum*, among the

TABLE 2 Effect of grazing on *Camelina sativa* pasture on body weight, body condition score (BCS), and coat color.

Parameters	Control pasture	Camelina pasture	p - value
Body weight	447 ± 45	452 ± 38	0.134
BCS	5.5 ± 0.5	6.0 ± 0.5	0.087
Coat color	7.0 ± 0.5	9.0 ± 1.0	0.102

major contributing vectors, were much more abundant in samples after the *Camelina sativa* consumption, whereas all the other six aforementioned genera decreased in this group.

3.5 Alpha and beta diversity estimates

Metabarcoding taxa profiles were inspected by pairwise statistics and returned statistically significant variables at different taxonomic levels. The complete taxonomy (.biom file) was inspected in terms of alpha- and beta-diversity metrics estimates. More precisely, the Kruskal-Wallis corrected test returned a significant difference in Faith's pd metric (q-value = 0.009) between untreated (T0) and treated (T1) fecal horse microbiota samples (Supplementary Figure 2).

At the same time, the unifrac emperor statistics used for the beta diversity metric allowed to clearly distinguish fecal samples into two different subclusters (Supplementary Figure 3). As reported in Figure 2 and Supplementary Table 2, pairwise corrected (FDR) non-parametric tests between T0 and T1 groups returned a total of 34 statistically significant genera, 13 of which (*Rummeliibacillus*, *Lysinibacillus, Anaerofilum*, *LD1-PB3*, *Planococcaceae, Streptococcus*, *Brevibacterium, Coriobacteriales Incertae Sedis, Anaeroplasma, Lactobacillaceae*, *Bacteroidales UCG-001, Lachnospiraceae ND3007 group*, and *Treponema)* increased as a consequence of the treatment, whereas 21 decreased (*Erysipelotrichaceae UCG-003*, *Aeromicrobium*, *Phoenicibacter*, *Clostridiaceae*, *Bacteroidaceae*, *Selenomonadaceae*, *Catenibacterium, Prevotellaceae, Ga6A1 group*, *Paraprevotella*, *Family XIII AD3011 group of Anaerovoracaceae*, *UCG-004*, *Defluviitaleaceae* uncultured, *Blautia*, *Negativicutes*, *Breznakia*, *Victivallaceae*, *Selenomonadaceae*, *Prevotella*, *Clostridium sensu stricto*, and *Succinivibrionaceae*).

Looking at the relative abundance, the statistically significant increased genera at T1 with percentage values greater than 0.5% were: unresolved genera clustered in the Lactobacillaceae family, *Lysinibacillus*, and *Planococcaceae*. Conversely, the *Family XIII AD3011 group of Anaerovoracaceae*, *Phoenicibacter*, *Prevotellaceae Ga6A1 group*, *Paraprevotella*, and unresolved genera belonging to the Bacteroidaceae and Clostridiaceae families decreased at T1.

3.6 Statistically significant 16S predicted biochemical pathways and VOCs

The Picrust2 biochemical pathway prediction was useful to detail the profile of putatively increased and decreased functions ascribable to the microbiota members. Based on the matrix of metacyc pathways, the non-parametric Wilcoxon rank-sum test (corrected by Benjamini-Hochberg multiple test), combined with fold change analyses, returned a total of 14 statistically significant pathways. Specifically, the superpathway of glyoxylate bypass and TCA, S-methyl-5-thio-α-D-ribose 1-phosphate degradation, L-methionine salvage cycle III, allantoin degradation to glyoxylate III, superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass, L-glutamate degradation VIII (to propanoate), glyoxylate cycle, TCA cycle IV (2-oxoglutarate decarboxylase), enterobactin biosynthesis, catechol degradation I (meta-cleavage pathway), and fatty acid salvage were increased after *Camelina sativa*-enriched pasture. Four biocyc pathways decreased, namely, the superpathway of hexitol degradation (bacteria), lactose and galactose degradation I, superpathway of glycerol degradation to 1,3-propanediol, and L-glutamate_degradation_VIII_(to_propanoate) (Figure 3).

The untargeted GC/MS metabolomics, inspected by the Wilcoxon corrected test combined with fold change analysis,

TABLE 3 Effect of grazing on *Camelina sativa* pasture on the chemical composition (% DM) of Murgese mares' feces.

Parameters	Control pasture	Camelina pasture	SEM	p - value
Dry matter	98.41^{a}	97.91^{b}	0.6276	0.025
Protein	6.35	6.52	0.6408	0.390
Ether extract	3.03	3.20	0.4485	0.370
Ash	12.05^{B}	16.24^{A}	2.1779	0.001
Crude fiber	39.53^{A}	37.40^{B}	0.7138	0.001
N-free extracts	39.02^{A}	36.64^{B}	2.2904	0.008
NDF	77.76^{A}	75.48^{B}	0.9275	0.001
ADF	49.86	48.68	2.8160	0.320
ADL	15.13^{B}	18.17^{A}	1.8956	0.001
AIA	3.73^{B}	8.47^{A}	2.7541	0.001

SEM, Standard Error Mean; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; ADL, Acid Detergent Lignin; AIA, Acid Insoluble Ash. Values within a row with different superscripts differ significantly: A, B: $p < 0.01$; a, b: $p < 0.05$.

TABLE 4 Effect of grazing on *Camelina sativa* pasture on serum biochemical profile of Murgese mares.

Parameters	Control pasture	Camelina pasture	Horse reference interval	SEM	p - value
Total protein (g/dl)	6.73	6.48	5.5–7.3	0.213	0.197
Albumin (g/d)	2.68	2.62	2.7–4.2	0.105	0.447
Total bilirubin (mg/dl)	0.73	0.73	0.50–3.0	0.063	1.000
Direct bilirubin (mg/dl)	0.24	0.34	0.09–0.68	0.068	0.072
ALP (U/l)	358.50^{A}	261.17^{B}	160–380	42.380	0.002
GPT (U/l)	14.83^{a}	7.67^{b}	7–40	6.041	0.043
AST (U/l)	298.00	275.83	170–330	21.478	0.258
Amylase (U/l)	<2.50	<2.50	<3.0	0.775	1.000
NEFA (mmol/l)	0.537	0.556	0.02–0.43	0.068	0.799
BHA (mg/dl)	1.265	1.278	1.00–3.10	0.060	0.826
GGT (U/l)	36.83^{A}	14.67^{B}	8.25	6.118	0.001
CK (U/l)	513.17	409.33	100.6–315.2	62.280	0.176
Creatinine (mg/dl)	0.90	0.79	0.9–2.0	0.277	0.648
NPN (mg/dl)	22.50	13.33	21.0–38.9	5.584	0.100
Cholesterol (mg/dL)	81.50^{a}	65.83^{b}	55–100	8.065	0.033
Triglycerides (mg/dL)	14.17	14.33	5–45	3.439	0.957
Chlorides (mmol/L)	97.33^{a}	89.17^{b}	90–100	3.637	0.012
Calcium (mg/dL)	11.05^{A}	9.88^{B}	11.0–13.5	0.531	0.006
Iron (g/dL)	156.50	119.33	75–200	18.941	0.052
Potassium (mmol/L)	5.05^{A}	4.05^{B}	2.5–5.2	0.209	0.001

SEM, Standard Error Mean; ALP, Alkalin phosphatase; GPT, Glutamate Pyruvate Transaminase; AST, Aspartate aminotransferase; NEFA, Non-Esterified Fatty Acids; BHA, Beta-Hydroxy-butyric Acid; GGT, Gamma-glutamyl transferase; CK, Creatine kinase; NPN, Non-protein Nitrogen.
Values within a row with different superscripts differ significantly: A, B: $p < 0.01$; a, b: $p < 0.05$.

returned a different profile of VOCs in the two subsets of samples. Butanoic acid,2-methyl-; Butanoic acid; Decanal; 2-Dodecanol; 1-Octanol,2-butyl-; Hexane,2,3,4trimethyl; Furan,3-methyl-; Decane,4-methyl-; Phenol,3,5-dimethyl-; Phenol,2-ethyl-, and Phenol increased as a consequence of the camelina diet, whereas Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-,(3R-trans)-; Trichloromethane; Tridecane; 1H-Pyrrole-2,5-dione,3-ethyl-4-methyl-; 5,9-Undecadien-2-one,6,10-dimethyl-,(E)-; and 5-Hepten-2-one,6-methyl- significantly decreased (Figure 4).

3.7 Correlation analysis between statistically significant metabolites and biochemical pathways

After obtaining the sets of statistically significant VOC metabolites and pathways, we proceeded with a correlation analysis (Spearman test). In addition, in this case, only statistically significant correlations with an R value greater than 0.5 were kept. The range of correlation values was not large, and the majority of the over-threshold R values fell within the interval 0.53-0.56. Two VOCs, namely, 1-Octanol, 2-butyl- and Furan, 3-methyl-, positively correlated with various metabolic pathways, including lactose and galactose degradation and fatty acid salvage (Supplementary Figure 4).

4 Discussion

In the attempt to identify herbaceous species that are well adapted to the drought conditions of Southern Italy, this study aimed to test whether *Camelina sativa* could be included in crop pastures for horses reared in a semi-extensive environment. In support of the biochemical profile of mares' serum, the improved health status was assessed by means of fecal metataxonomics, identified taxa, volatile organic compounds (VOCs), and predicted metabolism.

Grazing is more than an active food search activity for horses; the need to fulfill the requirement of the "3Fs" (Forage, Freedom, and Friends) formulated by Fraser (1992) is essential to ensure their welfare (Sauer et al., 2019; Krueger et al., 2021). Unlimited consumption of forage is necessary to satisfy the horse's physiological requirements, mainly the need to assume sufficient feed and fiber intake to ensure normal gastrointestinal function, but it also has a pivotal role in the prevention of abnormal behaviors. The active search for food and its consumption are driven by the horse's internal motivation, which also depends on the breed's attitude (Janczarek et al., 2018; Greppi et al., 2024). Since horses reared in wild/semi-wild environments graze up to 18 hours per day, they may have access to numerous species of grasses and herbs, being able to express a sorting activity in relation to appetite and preference (Nicholls, 2018). The searching activity may be very

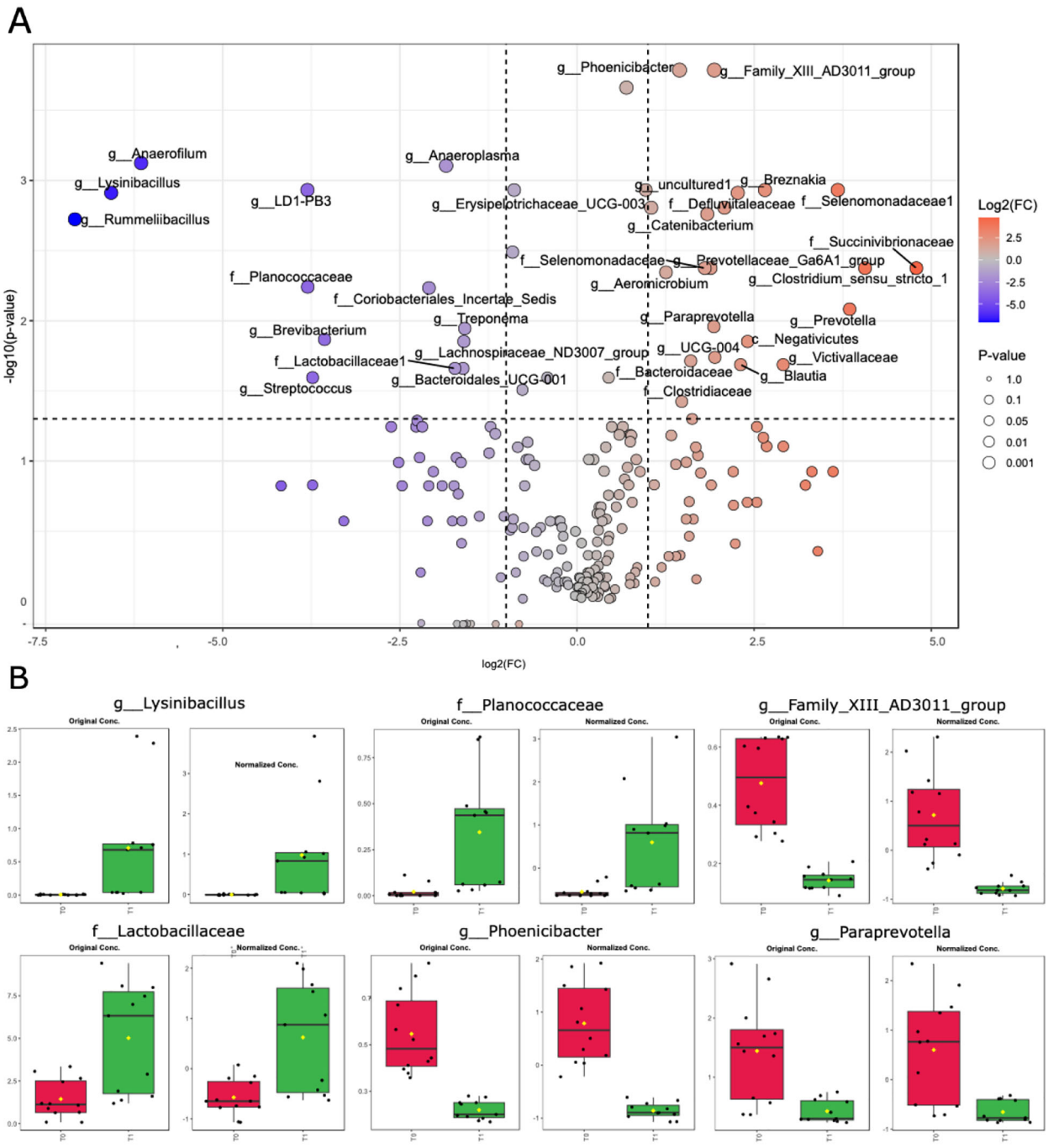


FIGURE 2
Statistically significant taxa at the genus level. **(A)** Statistically significant genera emerged from a non-parametric Wilcoxon rank-sum test combined with fold change (FC) analysis. Blue and pink dots mark increased and decreased genera at T1, respectively. The -log10 (*p* values) are meaningful of statistical significance and have been plotted versus the log2 fold change. **(B)** Box plots relative to genera with a relative abundance greater than 0.5%. The bigger the dot, the lower the p - value.

different when performed on a natural, spontaneous pasture or on a grassland with few species sown in defined amounts, while still being able to satisfy the horse's nutritional requirements. Therefore, the introduction of a "new" species in the grassland composition, such as the introduction of *Camelina sativa*, needs thorough investigation under behavioral aspects and animal metabolic perspectives. Changes in the daily pattern of grazing and time spent grazing are behavioral responses of the animal to the amount and variety of pasture (Bertolucci et al., 2008). Several studies reported that in horses fed *ad libitum*, no statistical differences in the total and daily amount of food ingestion were observed (Giannetto et al., 2025). Likewise, in our study, we did not observe differences in the time spent grazing and the amount of feed intake between the two pastures. According to earlier reports, when given free-choice nutrition, adult horses maintain a constant body weight (Houpt, 1990), as also found in our study. According to previous studies (Ermers et al., 2023), horses have fiber requirements that range from 30% to 40% of acid detergent fiber (ADF). Higher levels of

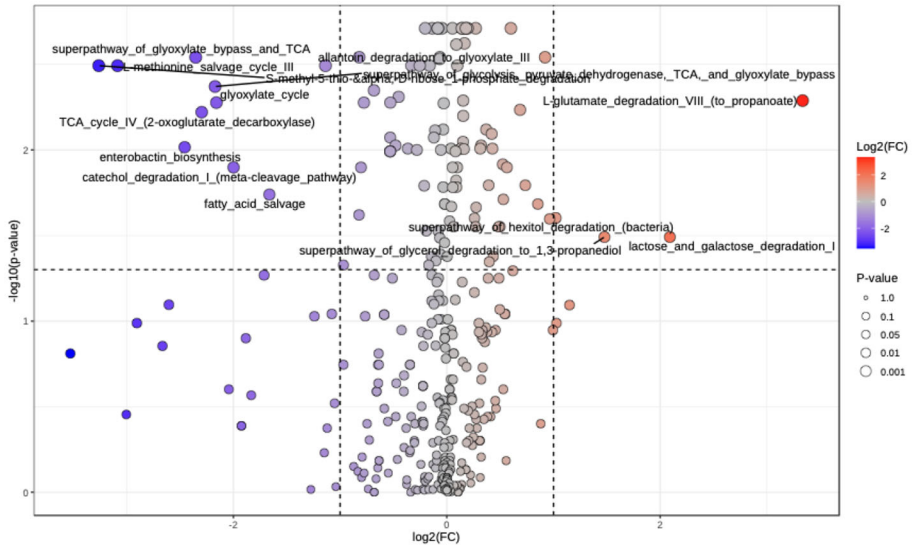


FIGURE 3
Statistically significant predicted metabolic pathways. Statistically significant predicted pathways from a non-parametric Wilcoxon rank-sum test combined with fold change (FC) analysis. Blue and pink dots mark increased and decreased altered metabolic pathways at T1, respectively. The -log10 (p values) are meaningful of statistical significance and have been plotted versus the log2 fold change. The bigger the dot, the lower the p - value.

ADF determine a decrease in energy levels because of the low digestibility of this fiber fraction. Conversely, 50–70% of the fiber should be neutral detergent fiber (NDF), since it contains the same components as ADF plus hemicellulose, which helps guarantee proper gastrointestinal function (Prigge et al., 2021). The two pastures studied during this trial showed adequate contents of ADF and NDF, supporting the adaptability of camelina as a forage species essence for horses.

In this study, the coat color was positively influenced by the consumption of the camelina pasture, appearing more vivid and bright, probably because of camelina's high content of polyunsaturated fatty acids (Warren and Vineyard, 2013).

The chemical composition of feces collected from Giara horses grazing in the wild showed a low concentration of crude protein and a high amount of ADL and crude ash (Cappai et al., 2020), probably because of the lower consumption of green foliage due to the seasonal climatic conditions during the experiment. In our study, the Murgese mares grazed on green pasture, and their feces contained a higher amount of protein and a lower amount of ash and ADL as compared to Giara horses.

Serum biochemistry is an essential tool for the evaluation of the animal's health status. ALP levels, along with other parameters, such as ALT, AST, GGT, and bilirubin (Öztürk and Mamak, 2024), are important markers of liver and bone health and, more generally, of

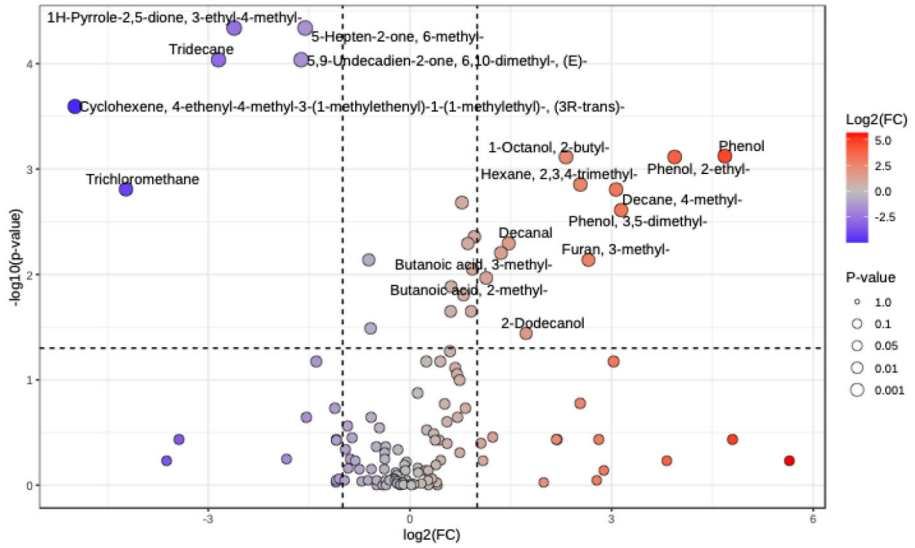


FIGURE 4
Statistically significant VOCs from GC/MS. Statistically significant genera emerged from a non-parametric Wilcoxon rank-sum test combined with fold change (FC) analysis. Blue and pink dots mark increased and decreased VOCs at T1, respectively. The -log10 (p values) are meaningful of statistical significance and have been plotted versus the log2 fold change. The bigger the dot, the lower the p - value.

the animal's overall clinical status. Therefore, lower values of these parameters indicate a reduced inflammatory state. Popova et al. (2025) hypothesized that liver-associated enzymes, such as ALP and total bilirubin, show consistent seasonal patterns; reduced level of ALP implies diminished hepatic or osteoblastic activity under heat stress. In our study, ALP decreased at the end of the grazing period on the camelina pasture, in correspondence with an increase in environmental temperature as compared to the previous months.

Chlorine, calcium, and potassium are essential electrolytes that play important roles in equine physiology. Chlorine, along with sodium, is essential for regulating water balance and thermoregulation, while potassium is crucial for nerve transmission and muscle contraction. Calcium, on the other hand, plays an important role in muscle contraction, blood clotting, and bone health. Horses can lose electrolytes, particularly chloride and potassium, through sweat, especially during intense exercise or in hot conditions (Popova et al., 2025).

The 16S metabarcoding and metabolomics analyses allowed us to investigate specific differences in terms of taxa, predicted metabolism, and VOC abundances. Both the PLS-DA based on the matrix composed of the assigned genera and the diversity distance metrics highlighted the presence of two subpopulations, indicating an effect of the treatment.

The presence of *Lysinibacillus* (in the *Firmicutes* phylum) after camelina consumption is an important marker of homeostatic condition, as observed when comparing healthy horses versus those with colitis (Costa et al., 2012). Its presence confers an increased bile acid biotransformation capacity (Yan et al., 2021). Together with *Planococcaceae*, *Lysinibacillus* has been identified as a biomarker of Mongolian horses when these were compared with thoroughbred and hybrid horses (Wen et al., 2022). The *Lysinibacillus* genus was also found to be absent in Anglo-Arabian horses compared to Hanoverian and French Saddle breeds (Massacci et al., 2020). Although these subspecies do not share geographical, climatic, and cultural contexts with our Murgese mares, the presence of this genus may be related to their exceptional characteristics of rusticity and resistance. In terms of protein production, as reported by Qin and colleagues, the presence of *Lysinibacillus* is crucial for lipid and amino acid metabolisms in Mongolian horses (Qin et al., 2025).

Another observed increase involved the increased concentration of the *Planococcaceae* genus. It is worth highlighting that *Planococcaceae* density has been noted to increase with time at room temperature (Beckers et al., 2017); i.e., it is not detected in fresh samples. Therefore, we cannot exclude that the time elapsed between feces sample collection and storage at freezing temperature may have affected the genus overgrowth.

As with our study cohort, another study reports that the *Planococcaceae* genus decreases in equine fecal samples following a sudden dietary change in Mangalarga Marchador horses (Franzan et al., 2022).

In addition to the necessary considerations related to the diet, *Planococcaceae* appeared as a discriminant disease risk predictor in gut bacterial community from athletic horses bred for racing; in fact, the genus covaries with the microbial community structure, thus potentially driving changes within differently identified Shannon diversity groups (Leng et al., 2024).

Moreover, among detected taxa with a relative abundance greater than 0.5%, the *Family XIII AD3011 group of Anaerovoracaceae* decreased after camelina intake. In a grazing study of horses on warm- vs cool-season grass pastures, a higher abundance of this taxon was linked to lower non-structural carbohydrate to water-soluble carbohydrate ratio (NSC/WSC); i.e., more soluble sugars relative to total NSC. We can explain this effect by considering lipid *vs* carbohydrate content and carbohydrate quality (soluble *vs* total NSC) as key determinants of the *Family XIII AD3011* abundance. Thus, high-fat supplements (likely those enriched in camelina) may shift mare hindgut fermentation toward other microbial groups.

Another detected metataxonomic indicator of horse health status was the statistically significant decrease in *Phoenicibacter*, a bacterium found in diarrhea-affected Przewalski's horses (Li et al., 2024).

The *Prevotellaceae Ga6A1* group, rarely described in horse fecal microbiota, is less abundant in aged horses (Baraille et al., 2024) and is reported to impact the fermentation of plant polysaccharides and contribute to volatile fatty acid (VFA) production, namely, acetate, propionate, and butyrate. A recent study reports how the taxon increased in cashmere goats receiving a total mixed ration of flaxseed meal, compared with a basal diet with added flaxseed oil and with a basal diet with added heated flaxseed grain (Guo et al., 2023). Most of the fermentable energy from camelina comes from residual protein and fat, rather than from soluble fiber (Riaz et al., 2022).

In agreement with the findings reported by Raspa and colleagues, lower abundances of *Paraprevotella* were found in diets showing lower contents of soluble fiber (Raspa et al., 2024); accordingly, we detected a decreased concentration of fiber in T1 fecal samples.

This taxon decreases when high-starch diets are administered instead of high-fiber diets, and this evidence suggests compensatory shifts toward fiber utilization by other microbes (e.g., *Fibrobacter*, *Ruminococcus*).

The Prevotellaceae family (including the Prevotellaceae Ga6A1 group and Paraprevotella genus) are reported in equine hindgut ecosystems as saccharolytic members contributing to the fermentation of plant carbohydrates and to volatile fatty acid production. In our trial, grazing on the camelina pasture resulted in a significant decrease in fecal NDF and N-free extracts, together with an increase in ADL. According to Kauter and colleagues, the reduction in NDF—especially when accompanied by higher lignin—supports a shift in the carbohydrate pool toward a less fermentable, more lignified fiber profile, plausibly reducing the availability of hemicellulose/soluble carbohydrates for hindgut saccharolytic taxa. Equine/pony feeding studies show that hindgut bacterial communities respond rapidly to marked diet transitions, with detectable shifts within a few days and subsequent recovery over longer periods, where carbohydrate quality (forage vs concentrate/high-starch) is a major driver of Bacteroidetes/Prevotellaceae dynamics (Kauter et al., 2019; Collinet et al., 2021; Fernandes et al., 2021).

Under a pathological point of view, increased concentration of this taxon was found in horses affected by *Chlamydia*, with an observed strong negative correlation with ALT (Jin et al., 2023). Although only indirectly inferred from 16S sequencing, our pathway predictions showed an increase in fatty acid salvage metabolism after camelina consumption. The increased lipid oxidation is also

supported by the increase in decanal and 2-dodecanol metabolites from our metabolomics experiment. The detection of increased 1-Octanol,2-butyl- concentrations is indicative of the presence of another substrate for β–oxidation.

The diet altered the predicted metabolic pathways, and the increase in the glyoxylate shunt highlighted the possibility of using two-carbon compounds, i.e., acetate or fatty acids, for growth, bypassing the CO_2 production in the classical tricarboxylic acid cycle (TCA). This indicates an enhanced central energy metabolism and carbon reutilization, supporting the horses by recycling acetate and maintaining energy balance in low-carbohydrate conditions. Moreover, glyoxylate generated from allantoin (another increased predicted pathway) can directly enter the glyoxylate shunt.

Importantly, this altered metabolic pathway implies biosynthetic flexibility in response to fiber-rich diets or low simple-sugar availability, which also shows: i) possible microbial adaptation to maintain redox balance and energy yield under fermentative, anaerobic conditions; and ii) improved metabolic efficiency in producing short-chain fatty acids beneficial for host energy metabolism. Consistent with this evidence, in connecting nitrogen and carbon metabolism, we found an increase in the L-glutammate degradation pathway, a source of succinate and other short-chain fatty acids (SCFAs), indicating a microbiota process of compensation for low carbohydrate availability, using amino acids for energy.

Another important finding of the camelina diet comes is the reduction in lactose and galactose degradation, which in adult horses reflects dietary specialization for fibrous feeds, where a forage-adapted hindgut microbiome is dominated by fiber-degrading bacteria (Boshuizen et al., 2021).

On the counterpart, our GC/MS analyses revealed a specific metabolite decrease in Butanoic acid,2-methyl-, a metabolic marker of protein and amino-acid fermentation, whose presence implies enhanced microbial proteolytic activity. Butanoic acid increase indicates a healthier and fiber-rich fermentation produced by a more balanced *Camelina sativa* fiber-driven microbiota, which in turn impacts anti-inflammatory gene expression (Christodoulou et al., 2022). The fermentation increase after *Camelina sativa* consumption is also supported by the increase in Furan,3-methyl- in Murgese mares, whereas the increase of phenol-derived compounds (Phenol,3,5-dimethyl-; Phenol,2-ethyl- and Phenol) indicates amino acid catabolism, especially via degradation of aromatic amino acids such as tyrosine and phenylalanine.

In conclusion, our study highlighted the potential benefits of feeding *Camelina sativa* pasture to Murgese mares. Our analyses showed that energy, protein, and bioactive compound content increased, along with an improved serum lipid profile and anti-inflammatory and antioxidant effects. Beneficial fermenters among commensal microbiota gut members act synergically by increasing short-chain fatty acids (SCFAs), thus leading to an improved nutrient utilization.

Among limitations, this study is limited by the small number of horses included in the experimental groups. Although this is due to the low number of available Murgese broodmares in Apulian farms, we plan to adopt a multi–farm sampling approach in the future.

Another limitation concerns the conclusions inferred based on metabolic pathway predictions. Based on this preliminary evidence, we will extend our findings by considering a more robust evaluation of gene functions, inspecting metatranscriptomics sequencing data.

Among the strengths, we highlight the multidisciplinary approach based on biochemical serum profiles and multiomics results.

It is also worth noting that the presented results rely only on statistically significant multiple test corrected data.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA1356358.

Ethics statement

No ethical approval was required for this study because the blood samples were collected by the local health authority veterinarian during periodic animal control. The owner exclusively handled the animals, in compliance with current regulations regarding animal husbandry and welfare. Written informed consent was obtained from the owner for the participation of the animals in this study.

Author contributions

MAC: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. LM: Investigation, Methodology, Writing – original draft. FG: Funding acquisition, Project administration, Writing – original draft. ST: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. CCa: Conceptualization, Data curation, Methodology, Writing – original draft. LT: Investigation, Writing – original draft. EC: Investigation, Methodology, Writing – original draft. VT: Methodology, Visualization, Writing – original draft, Writing – review & editing. CCo: Methodology, Visualization, Writing – original draft. MCh: Investigation, Writing – original draft. MB: Investigation, Writing – original draft. FR: Investigation, Writing – original draft. CA: Investigation, Writing – original draft. MCa: Investigation, Writing – original draft. GC: Investigation, Writing – original draft. MDA: Conceptualization, Funding acquisition, Writing – original draft. FC: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This study was carried out within the Agritech National Research Center and received funding from

the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022, Spoke 7).

Acknowledgments

The authors express their gratitude to Serafino Romanazzi of the "Allevamento delle Lame" farm for animal care and handling and to the technicians of the Department of Soil, Plant, and Food Sciences, Massimo Lacitignola, Nicolò De Vito, and Domenico Gerardi, for their laboratory assistance.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Author disclaimer

This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2026.1783759/full#supplementary-material

References

Abdouli, H., and Attia, S. B. (2007). Evaluation of a two-stage *in vitro* technique for estimating digestibility of equine feeds using horse faeces as the source of microbial inoculum. *Anim. Feed Sci. Technol.* 132, 155–162. doi: 10.1016/j.anifeedsci.2006.03.005

Alhaddad, H., Powell, B. B., Del Pinto, L., Sutter, N., Brooks, S. A., and Alhajeri, B. H. (2024). Geometric morphometrics of face profile across horse breeds and within Arabian horses. *J. Equine Vet. Sci.* 132, 104980. doi: 10.1016/j.jevs.2023.104980

Alvarado, T. D., Elghandour, M. M., Ekanem, N. J., Alcala-Canto, Y., Velázquez, A. E., Pacheco, E. B. F., et al. (2022). Influence of Azadirachta Indica and Cnidoscolus Angustidens dietary extracts on equine fecal greenhouse gas emissions. *J. Equine Vet. Sci.* 116, 104049. doi: 10.1016/j.jevs.2022.104049

AOAC (2000). *Official Methods of Analysis, 17th Edition of The Association of Official Analytical Chemists* (Arlington, VA: Oxford University Press).

Baraille, M., Buttet, M., Grimm, P., Milojevic, V., Julliand, S., and Julliand, V. (2024). Changes of fecal bacterial communities and microbial fibrolytic activity in horses aged from 6 to 30 years old. *PloS One* 19, e0303029. doi: 10.1371/journal.pone.0303029

Beckers, K. F., Schulz, C. J., and Childers, G. W. (2017). Rapid regrowth and detection of microbial contaminants in equine fecal microbiome samples. *PloS One* 12, e0187044. doi: 10.1371/journal.pone.0187044

Bertolucci, C., Giannetto, C., Fazio, F., and Piccione, G. (2008). Seasonal variations in daily rhythms of activity in athletic horses. *Animal* 2, 1055–1060. doi: 10.1017/s1751731108002267

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37, 852–857. doi: 10.1038/s41587-019-0209-9

Boshuizen, B., Vidal Moreno de Vega, C., De Maré, L., de Meeûs, C., de Oliveira, J. E., Hosotani, G., et al. (2021). Effects of aleurone supplementation on glucose-insulin metabolism and gut microbiome in untrained healthy horses. *Front. Vet. Sci.* 8. doi: 10.3389/fvets.2021.642809

Bramante, G., Pieragostini, E., and Ciani, E. (2022). Genetic variability within the Murgese horse breed inferred from genealogical data and morphometric measurements. *Diversity* 14, 422. doi: 10.3390/d14060422

Cais-Sokolińska, D., Majcher, M., Pikul, J., Bielińska, S., Czauderna, M., and Wójtowski, J. (2011). The effect of Camelina sativa cake diet supplementation on sensory and volatile profiles of ewe's milk. *Afr. J. Bio/Technol.* 10, 7245–7252. doi: 10.4314/ajb.v10i37

Cappai, M. G., Pudda, F., Wolf, P., Accioni, F., Boatto, G., and Pinna, W. (2020). Variation of haematochemical profile and vitamin E status in feral Giara horses from free grazing in the wild to hay feeding during captivity. *J. Equine Vet. Sci.* 94, 103220. doi: 10.1016/j.jevs.2020.103220

Carrillo Heredero, A. M., Sabbioni, A., Asti, V., Ablondi, M., Summer, A., and Bertini, S. (2024). Fecal microbiota characterization of an Italian local horse breed. *Front. Vet. Sci.* 11. doi: 10.3389/fvets.2024.1236476

Carroll, C. L., and Huntington, P. J. (1988). Body condition scoring and weight estimation of horses. *Equine Vet. J.* 20, 41–45. doi: 10.1111/j.2042-3306.1988.tb01451.x

Chao, A., and Bunge, J. (2002). Estimating the number of species in a stochastic abundance model. *Biometrics* 58, 531–539. doi: 10.1111/j.0006-341x.2002.00531.x

Christodoulou, C., Mavrommatis, A., Simoni, M., Righi, F., Prandi, B., Tedeschi, T., et al. (2022). The amino acid profile of Camelina sativa seeds correlates with the strongest immune response in dairy ewes. *Animal* 16, 100621. doi: 10.1016/j.animal.2022.100621

Colonna, M. A., Giannico, F., Tufarelli, V., Laudadio, V., Selvaggi, M., De Mastro, G., et al. (2021). Dietary supplementation with Camelina sativa (L. Crantz) forage in autochthonous Ionica goats: Effects on milk and caciotta cheese chemical, fatty acid composition and sensory properties. *Animals* 11, 1589. doi: 10.17582/journal.pjz/2018.50.4.1421.1433

Cosentino, C., Colonna, M. A., Musto, M., Dimotta, A., Freschi, P., Tarricone, S., et al. (2021). Effects of dietary supplementation with extruded linseed and oregano in autochthonous goat breeds on the fatty acid profile of milk and quality of Padraccio cheese. *J. Dairy Sci.* 104, 1445–1453. doi: 10.3168/jds.2020-18805

Costa, M. C., Arroyo, L. G., Allen-Vercoe, E., Stämpfli, H. R., Kim, P. T., Sturgeon, A., et al. (2012). Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. *PloS One*. doi: 10.1371/journal.pone.0041484

Ermers, C., McGilchrist, N., Fenner, K., Wilson, B., and McGreevy, P. (2023). The fibre requirements of horses and the consequences and causes of failure to meet them. *Animals* 13, 1414. doi: 10.3390/ani13081414

Franzan, B. C., da Silva Coelho, I., de Souza, M. T., de Moraes Santos, M. M., de Almeida, F. Q., and Silva, V. P. (2022). Fecal microbiome responses to sudden diet change in mangalarga marChador horses. *J. Equine Vet. Sci.* 108, 103803. doi: 10.1016/j.jevs.2021.103803

Fraser, A. F. (1992). *The behavior of the horse* (Wallingford, Oxon, UK: CAB International), ISBN: .

Giannetto, C., Aragona, F., Fazio, F., Piccione, G., Giudice, E., Arfuso, F., et al. (2025). Investigation of the impact of seasonal climate conditions on feed intake and body weight in horses. *Int. J. Biometeorol.* 69, 1101–1110. doi: 10.1007/s00484-025-02881-z

Giannico, F., Karatosidi, D., Carbonara, C., Ragni, M., Tarricone, S., Caputi Jambrenghi, A., et al. (2024). Effects of grazing on grassland or wood-pasture, slaughtering age and ageing time on meat production and quality in Podolian young bulls. *Vet. Quart.* 44, 1–12. doi: 10.1080/01652176.2024.2381544

Greppi, M., Bordin, C., Raspa, F., Maccone, E., Harris, P., Ellis, A. D., et al. (2024). Feeding behaviour related to different feeding devices. *J. Anim. Physiol. Anim. Nutr.* 108, 1393–1404. doi: 10.1111/jpn.13977

Guo, Y., Liu, S., Li, Y., Guo, X., Zhao, Y., Shi, B., et al. (2023). Intestinal microbiota community and blood fatty acid profiles of albas cashmere goats fed with flaxseed oil and whole flaxseed. *Animals* 13, 3531. doi: 10.3390/ani13223531

Hartwig, S., Burron, S., Richards, T., Rankovic, A., Ma, D. W., Pearson, W., et al. (2025a). The effect of dietary camelina, flaxseed, and canola oil supplementation on skin fatty acid profile and immune and inflammatory responses in healthy adult horses. *J. Anim. Sci.* 103, skaf025. doi: 10.1093/jas/skaf025

Hartwig, S., Rankovic, A., McCrae, P., Gagliardi, K., Burron, S., Ellis, J., et al. (2025b). The effects of a plant-based and a plant-and marine-based n-3 oil supplement on behavioral reactivity, heart rate variability, and plasma fatty acid profile in young healthy horses. *J. Anim. Sci.* 103, skaf117. doi: 10.1093/jas/skaf117

Henneke, D. R., Potter, G. D., Kreider, J. L., and Yeates, B. F. (1983). Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet. J.* 15, 371–372. doi: 10.1111/j.2042-3306.1983.tb01826.x

Houpt, K. (1990). Ingestive behavior. *Vet. Clin. North. Am. Equine Pract.* 6, 319–337. doi: 10.1016/s0749-0739(17)30544-8

Ibrahim, F., and El Habbasha, E. S. (2015). Chemical composition, medicinal impacts and cultivation of camelina (Camelina sativa). *Review* 8, 114–122. Available online at: https://www.researchgate.net/profile/El-Sayed-El-Habbasha/publication/298073329_Chemical_composition_medicinal_impacts_and_cultivation_of_camelina_Camelina_sativa_Review/links/57153acd08ae0f1a39b1a1de/Chemical-composition-medicinal-impacts-and-cultivation-of-camelina-Camelina-sativa-Review.pdf

Irving, J., Pineau, V., Shultz, S., ter Woort, F., Julien, F., Lambey, S., et al. (2025). Impact of low-starch dietary modifications on fecal microbiota composition and gastric disease scores in performance horses. *Animals* 15, 1908. doi: 10.3390/ani15131908

Italian Veterinary Information System. Available online at: https://www.vetinfo.it/j6_statistiche/#/ (Accessed 21 September 2025).

Janczarek, I., Wilk, I., Pietrzak, S., Liss, M., and Tkaczyk, S. (2018). Taste preferences of horses in relation to their breed and sex. *J. Equine Vet. Sci.* 64, 59–64. doi: 10.1016/j.jevs.2018.02.010

Jin, Y., Li, W., Ba, X., Li, Y., Wang, Y., Zhang, H., et al. (2023). Gut microbiota changes in horses with Chlamydia. *BMC Microbiol.* 23, 246. doi: 10.1186/s12866-023-02986-8

Jolazadeh, A., Azizi, A., Sharifi, A., Memarzadeh, F., Fallali, N., and Imani-Rad, E. (2025). Evaluation of camelina seed pods as a novel feed ingredient for ruminants: nutritional value, fermentation characteristics and nutrient digestibility. *Vet. Anim. Sci.* 29, 100477. doi: 10.1016/j.vas.2025.100477

Kenny, L. B., Weinert-Nelson, J., Burk, A., and Williams, C. (2024). "Managing equine grazing for pasture productivity," in *Horse pasture management*. Ed. P. H. Sharpe (Academic Press Elselvier, Oxford, UK), 161–179.

Krueger, K., Esch, L., Farmer, K., and Marr, I. (2021). Basic needs in horses?—a literature review. *Animals* 11, 1–16. doi: 10.3390/ani11061798

Leng, J., Moller-Levet, C., Mansergh, R. I., O'Flaherty, R., Cooke, R., Sells, P., et al. (2024). Early-life gut bacterial community structure predicts disease risk and athletic performance in horses bred for racing. *Sci. Rep.* 14, 17124. doi: 10.1038/s41598-024-64657-6

Li, Z., Luo, Z., and Hu, D. (2024). Assessing fecal microbial diversity and hormone levels as indicators of gastrointestinal health in reintroduced Przewalski's horses (Equus ferus przewalskii). *Animals* 14, 2616. doi: 10.3390/ani14172616

Mach, N., Lansade, L., Bars-Cortina, D., Dhorne-Pollet, S., Foury, A., Moisan, M. P., et al. (2021). Gut microbiota resilience in horse athletes following holidays out to pasture. *Sci. Rep.* 11, 5007. doi: 10.1038/s41598-021-84497-y

Mach, N., Ruet, A., Clark, A., Bars-Cortina, D., Ramayo-Caldas, Y., Crisci, E., et al. (2020). Priming for welfare: gut microbiota is associated with equitation conditions and behavior in horse athletes. *Sci. Rep.* 10, 8311. doi: 10.1038/s41598-020-65444-9

Massacci, F. R., Clark, A., Ruet, A., Lansade, L., Costa, M., and Mach, N. (2020). Inter-breed diversity and temporal dynamics of the fecal microbiota in healthy horses. *J. Anim. Breed. Genet.* 137, 103–120. doi: 10.1111/jbg.12441

Menke, K. H., and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.* 28, 7–55.

Mondor, M., and Hernández-Álvarez, A. J. (2022). Camelina sativa composition, attributes, and applications: a review. *Eur. J. Lipid Sci. Technol.* 124, 2100035. doi: 10.1002/ejlt.202100035

Muhonen, S., and Julliand, V. (2023). Fibre composition and maturity of forage-based diets affects the fluid balance, fecal water-holding capacity and microbial ecosystem in French Trotters. *Animals* 13, 328. doi: 10.3390/ani13030328

Nicholls, L. *Environmental enrichment for the horses: The need to forage*. (2018). Available online at: https://journal.iaabcfoundation.org/the-need-to-forage/#:~:text=Internal%20stimuli%20may%20also%20cause,hay%20net%20in%20the%20stable (Accessed December 15, 2025).

Öztürk, M., and Mamak, N. (2024). "Importance and areas of use of biomarkers in veterinary medicine clinical practice," in *Current Research and Treatment in Animals*. Eds. M. Kabu and A. C. Tunc (Livre de Lyon, France), 1–12.

Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., et al. (2021). MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res.* 49, W388–W396. doi: 10.1093/nar/gkab382

Piergiovanni, A. R., Alberghini, B., Zanetti, F., Ponzoni, E., Brambilla, I. M., and Galasso, I. (2025). The effect of year and variety on the nutritional value of Camelina sativa meal. *EFoRT* 251, 841–849. doi: 10.1007/s00217-025-04672-7

Popova, M., Petrov, R., Pastir, E., and Todorova, M. (2025). Influence of age and season on blood biochemical parameters of free-roaming Karakachan horses. *Bulg. J. Vet. Med.* doi: 10.15547/bjvm.2025-0021

Prigge, J. L., Sheaffer, C. C., Jungers, J. M., Jaqueth, A. L., Lochner, H. L., and Martinson, K. L. (2021). Forage characteristics and grazing preference of cover crops in equine pasture systems. *J. Equine Vet. Sci.* 103, 103663. doi: 10.1016/j.jevs.2021.103663

Python. *The Python Language Reference*. Available online at: https://docs.python.org/3/reference/index.html (Accessed September 23, 2025).

Qin, X., Xi, L., Zhao, L., Han, J., Qu, H., Xu, Y., et al. (2025). Exploring the distinctive characteristics of gut microbiota across different horse breeds and ages using metataxonomics. *Front. Cell. Infect. Microbiol.* 15. doi: 10.3389/fcimb.2025.1590839

Raspa, F., Chessa, S., Bergero, D., Sacchi, P., Ferrocino, I., Cocolin, L., et al. (2024). Microbiota characterization throughout the digestive tract of horses fed a high-fiber vs. a high-starch diet. *Front. Vet. Sci.* 11. doi: 10.3389/fvets.2024.1386135

Riaz, R., Ahmed, I., Sizmaz, O., and Ahsan, U. (2022). Use of Camelina sativa and by-products in diets for dairy cows: A review. *Animals* 12, 1082. doi: 10.3390/ani12091082

Rubino, G., Cito, A. M., Lacinio, R., Bramante, G., Caroli, A., Pieragostini, E., et al. (2006). Hematology and some blood chemical parameters as a function of tick-borne disease (TBD) signs in horses. *J. Equine Vet. Sci.* 26, 475–480. doi: 10.1016/j.jevs.2006.08.005

Sauer, F. J., Hermann, M., Ramseyer, A., Burger, D., Riemer, S., and Gerber, V. (2019). Effects of breed, management and personality on cortisol reactivity in sport horses. *PloS One* 14, 1–19. doi: 10.1371/journal.pone.0221794

Selvaggi, M., Pesce Delfino, A. R., and Dario, C. (2010). Exon 1 polymorphisms in the equine CSN3 gene: SNPs distribution analysis in Murgese horse breed. *Anim. Biotechnol.* 21, 252–256. doi: 10.1080/10495398.2010.509646

Tedone, L., Giannico, F., Tufarelli, V., Laudadio, V., Selvaggi, M., De Mastro, G., et al. (2022). Camelina sativa (L. Crantz) fresh forage productive performance and quality at different vegetative stages: Effects of dietary supplementation in Ionica goats on milk quality. *Agriculture* 12, 91. doi: 10.3390/agriculture12010091

Tilley, J. M. A., and Terry, R. A. (1963). A two stage technique for *in vitro* digestion of forage crops. *J. Br. Grassland Soc* 18, 104–111. doi: 10.1111/j.1365-2494.1963.tb00335.x

Van Soest, P. V., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583–3597. doi: 10.3168/jds.s0022-0302(91)78551-2

Warren, L. K., and Vineyard, H. R. (2013). "Fat and fatty acids," in *Equine applied and clinical nutrition*. Eds. R. J. Geor, P. A. Harris and M. Coenen (Sounders Elsevier, Philadelphia, PA), 136–155.

Wen, X., Luo, S., Lv, D., Jia, C., Zhou, X., Zhai, Q., et al. (2022). Variations in the fecal microbiota and their functions of Thoroughbred, Mongolian, and Hybrid horses. *Front. Vet. Sci.* 9. doi: 10.3389/fvets.2022.920080

Yan, Q., Zhang, S., Li, S., Wang, G., Zhang, A., Jin, T., et al. (2021). Cultivation and genomic characterization of the bile bacterial species from cholecystitis patients. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.739621

Zaitseva, S., Dagurova, O., Radnagurueva, A., Kozlova, A., Izotova, A., Krylova, A., et al. (2023). Fecal microbiota and diet composition of buryatian horses grazing warm-and cold-season grass pastures. *Microorganisms* 11, 1947. doi: 10.3390/microorganisms11081947

Zając, M., Kiczorowska, B., Samolińska, W., and Klebaniuk, R. (2020). Inclusion of camelina, flax, and sunflower seeds in the diets for broiler chickens: apparent digestibility of nutrients, growth performance, health status, and carcass and meat quality traits. *Animals* 10, 321. doi: 10.3390/ani10020321