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RESEARCH ARTICLE

Impact of diet supplemented with microencapsulated condensed tannins on cow milk nutritional profile

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Feed production systems should consider more sustainable solutions to reduce the pressure on earth's finite resources. In this work, we investigated the effect of microencapsulated condensed tannins (MCTs)-enhanced diet on the cow milk characteristics. The diet reduced the urea content, while preserved and the n-3 and n-6 portion of fatty acids. Aside from the rising of vitamin C level, MCTs had a negative effect on the amount of B group vitamins. MCTs may be considered a promising feed supplement that offers a good trade-off in terms of milk chemical quality while increasing the environmental sustainability of cattle livestock.

Keywords Microencapsulation, Fortification, Raw milk, Fatty acids, Vitamins, Milk proteins.

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INTRODUCTION

The resilience of modern food chains is seriously questioned due to the current anthropogenic demand on the earth's finite resources (Godfray *et al*. 2010). Given that farmed animals' use of natural resources including land, soil and water—many of which are required to produce animal feed—contributes to climate change, livestock environmental impact is substantial (Matassa *et al*. 2016).

Due to an existing precarious position regarding the availability of fertile soils and freshwater, feed production systems should take alternate, sustainable and less expensive sources into consideration in order to reduce the dependence of feed supply chain from extra countries.

Despite vegetable residues and food industry by-products being an attractive feed solution, in turn, their exploitation is challenging due to the seasonal production, the reduced stability of the biocomponents and poor knowledge around animal welfare and feeding behaviour.

Amongst vegetable products, grain legumes for food are the highest-ranked cause of food waste and loss worldwide, accounting for up to 60% of the production in the last decade (FAOSTAT 2022). The underlying causes of losses vary up to the production area and supply chain step (e.g. during and/or postharvest; in processing; and over the distribution), and in many cases, the end-use follows the quality standard required by each market.

Legumes are used for either food or feed (Bouchenak and Lamri-Senhadji 2013) as proteins, fibres and micronutrient sources (Rochfort and Panozzo 2007; Dellavalle *et al*. 2013). However, the bioavailability of specific

micronutrients is impaired due to the presence in seed coat of different antinutritional factors such as tannins and dietary fibres (raffinose, stachyose and verbascose; Wang *et al*. 2009).

Tannins are diverse and complex water-soluble phenolic compounds widespread in the plant kingdom, derived from the plant secondary metabolism. They are recognised for their ability to bind proteins and function as a component of the plant's chemical defence mechanism against pathogen invasion and herbivore attack (Mueller-Harvey *et al*. 2019).

Tannins are conventionally divided into two main categories: hydrolysable (HTs) and condensed tannins (CTs; Khanbabaee and van Ree 2001).

The HTs are intricate molecules with a polyol at their centre that can hydrolyse when exposed to acids, bases or esterases to produce polyol and its constituent phenolic acids (Patra and Saxena 2011).

The CTs are mainly polymers of the flavan-3-ol units, which are linked by C4:0–C8:0 and C4:0–C6:0 interflavonoid linkages. At pH 3.5–7.0, CTs and plant proteins can react by forming stable and soluble CT–protein complexes (Jones and Mangan 1977).

Depending on the chemical composition, concentration, amount consumed, species of consumer animals and basic diet, tannins in feed may be harmful, harmless or beneficial to animal health (Makkar 2003).

Intake of tannins at a modest amount benefits cattle's ability to grow and/or utilise nutritional energy (Rivera-Méndez *et al*. 2016) and lambs (Rojas-Román *et al*. 2017).

Because of their antimicrobial and protein-binding activities, tannins are known to modify ruminal biohydrogenation and nitrogen metabolism (Patra and Saxena 2011), with positive effects on milk and cheese quality. In fact, as a consequence of the impairment of ruminal biohydrogenation, dietary tannins are often reported to reduce the saturated fatty acids (SFAs) content and increase the amount of unsaturated fatty acids (UFAs), namely C18:1 trans-11 and C18:2 cis-9, trans-11 in milk (Frutos *et al*. 2020). Since tannins bind to proteins, they can increase the ratio of ruminal protein leakage (Waghorn 2008), increasing both the nonammonia nitrogen (NAN) outflow from the rumen and the essential amino acids (EAA) plasma content (Min *et al*. 2002).

Dietary tannins could exert different effects on cow milk quality and composition when supplemented with a green herbage-based diet or a dry forage-based diet, depending on the chemical structure and the amount of dietary tannins.

On the contrary, the inclusion of CTs in animal diets may reduce feed intake by lowering tasting or imposing an astringency perception in the animal mouth by forming complexes with salivary proteins (Landau *et al*. 2000). In addition, tannins, like other polyphenolic compounds, are usually sensitive to oxygen, light, heat and enzymes, and exposure to these factors determines their loss in activity. To overcome these drawbacks, several approaches have been proposed, such as microencapsulation which is widely used in food and pharma industry to increase the stability and bioavailability of certain medical compounds and has found large application in the animal farming industry for biofortification of animal feed (Konkol and Wojnarowski 2018).

Fang and Bhandari (2010) observed that the administration of MCTs rather than free tannins allowed for overcoming the issue linked to their oxidative instability and bitter taste while simultaneously enhancing the bioavailability of these compounds on the desired site.

The active substances are microencapsulated to achieve a variety of goals, including disguising tastes or disagreeable colours, extending the duration of conservation, protecting against oxidation and achieving slow or controlled release in a particular place (García and Garrigós 2016).

The type of animal to be fed, the chemistry of the bioactive molecule and the goal of its microencapsulation all play a role in the formulation and production methods for microencapsulation.

This paper aims to compare the effect of MCTs on cow milk overall quality; the practical effect of the diet was checked in Italian Friesian dairy cows reared in a farm located in Basilicata Region, Italy. The proximate composition, fatty acids, water- and fat-soluble vitamin pool, whey proteins and aromatic profile of the milk were assessed.

MATERIALS AND METHODS

Feeding

All procedures were approved by the Body responsible of Animal Welfare inside the Council for Agricultural Research and Economics (authorisation prot. no. 65632 dated 10 September 2020). The experiment was performed in the period between December 2020 and February 2021, in a commercial private farm, run by Dr Rocco Bochicchio, located in the municipality of Potenza, Italy. Twenty lactating Italian Friesian multiparous dairy cows were used. Animals were divided into two equivalent groups (n = 10 cows/group), control group (C) and microencapsulated tannins group (MCTs), balanced for age (4.5 ± 1.21 years C group, 4.2 ± 1.81 T group), live body weight (743.1 ± 62.2 kg C group, 732.9 ± 51.8 kg MCT group), average milk yield (32.5 ± 5.15 kg/day C group, 32.4 ± 4.77 kg/day MCT group), parity (2.1 ± 1.20 C group, 1.9 ± 1.79 MCTs group), days in lactation (230.1 ± 10 days C group, 226.1 ± 9 days MCT group), average milk protein content (3.3%) and milk average fat content (4.0%). MCT group received 600 g/head per day of condensed tannins, corresponding to 2.5% of daily dry matter intake (Table 1). MCTs were prepared as a 60:40 mixture of maltodextrins and Arabic gum, plus 600 g of condensed tannins, for a total amount of 2600 g/day of microcapsules of tannins,

Table 1 Dry matter daily intake for dairy cows.

Animal feed	Fresh (kg)	Dry Matter (kg)
Forages (alfalfa hay, barley silage, oat hay, vetch, ryegrass)	14.0	10.9
Concentrate (maize, soybean meal 45%, maize corn, dry beet pulps, wheat meal, mix vitamins and minerals supplement)	15.0	13.2
Total diet intake	29.0	24.1

included in concentrate (Table 2). The microcapsules were prepared according to the procedure described by Tolve *et al*. (2021). The ratio forage/concentrate feed was 45/55; considering a total daily dry matter intake of 24.1 kg/day, condensed tannins were added as a total amount of 600 g/day, corresponding to 2.5% of dry matter intake. The two diets were administered as *unifeed*, consisting in a mixture of all the feeds planned in the diet. The ingredients were chopped and mixed in such a way that animals could not choose what to eat. C and MCTs diets were isoenergetic and isoproteic (Table 2): chemical analysis was performed to determine dry matter, crude protein, ether extract and ash (AOAC 1995) using methods 920.39, 976.06 and 942.05, respectively. The analyses of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were performed following the method of Van Soest *et al*. (1991). During the trial, milk production was reported for all the cows in both the experimental groups from Days 0 to 35. Aliquots of the individual milk from both C and MCT group were collected at Day 0 and after 35 days from the beginning of the diet.

Chemical analyses

Fifty millilitre of milk samples was obtained from each cow in the control (n = 10) and treated (n = 10) groups and used for the analyses. Sampling was done on Day 0 and after 35 days of trial. The milk samples were quickly chilled before being delivered to the laboratory, where they were kept at −80°C pending chemical analyses.

Table 2 Proximate analyses (%) of unifeed diets for control (C) and microencapsulated tannin (MCTs) group.

Parameter	C group (n = 10)	MCTs group (n = 10)
Dry matter (%)	55.00	55.80
Crude Protein (%)	14.85	14.15
Ether extract (%)	4.00	4.00
Ash (%)	10.50	9.00
Starch (%)	18.00	20.00
NDF (%)	46.80	46.30
ADF (%)	31.65	29.40

Proximate analyses of milk samples

The fresh milk samples were analysed for pH, urea, protein, casein, fat and lactose content by mid-infrared spectroscopy (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark), according to ISO 9622 (2013). Each sample was analysed in duplicate.

GC analysis of fatty acids

Milk lipids were extracted according to the method of Bligh and Dyer (1959). Extracts were methylated adding hexane (1 mL) and 2 N methanolic KOH (0.05 mL). Afterwards, fatty acid methyl esters (FAME) were separated and quantified as reported by Di Trana *et al*. (2004) using a gas chromatograph (Varian model 3800) fitted with an automatic sampler (CP 8410) for a multiple injection and equipped with a flame ionisation detector. Through a cyanopropyl polysiloxane (DB 23, J & W) fused silica capillary column (60 m × 0.25 mm i.d.), fatty acid methyl ester (FAME) was separated. The operating parameters were 1.2 mL/min of low-pressure helium, 250°C for the FID detector and 230°C for the split–splitless injector with a split ratio of 1:100. Following the injection of the sample (1 μL), the column temperature was maintained at 60°C for 5 min, then increased by 2°C/min to 225°C and held at 225°C for 20 min. The retention times of a combination of CLA isomer standards (cis-9, trans-11 97% and trans-10, cis-12 3%; Larodan, Malmö, Sweden) and a known mixture of standard (F.A.M.E. Sigma-Aldrich C4-C24 18,919-1AMP, Lot: LRAC7954) were used to identify the different fatty acid peaks. Fatty acids were expressed as percentage of total FAME.

Whey protein fractions analyses

RP-HPLC was used to separate milk proteins (Vincenzetti *et al*. 2008). Briefly, skimmed milk was produced by centrifuging 20 mL of fresh milk at 3000 *g* for 30 min at 15°C. Skim milk was clarified by adding two volumes of buffer CL (0.1 M bis-tris, pH 8.0 containing 8 M urea, 1.3% trisodium citrate, 0.3% DTT), and 100 μL was loaded into the reversed-phase column (RP-HPLC Agilent 1260 Infinity; Agilent Technologies). The 300 Å C4 Prosphere (5 m, 4.6 mm I.D., 150 mm; Alltech) was the reversed-phase column. Trifluoroacetic acid (TFA)/H_2O 1:1000 v/v (buffer A) was used to equilibrate the column, and the following step gradient with TFA/H_2O/acetonitrile 1:100:900 v/v (buffer B) was used to achieve elution: time 10 min, %B = 0; time 10 min, %B = 20; time = 0.1 min, %B = 40; time 40 min, %B = 60. Fractions of 0.5 mL were collected at a flow rate of 1 mL/min. At 280 nm, the proteins that were eluted from the RP-HPLC columns were seen. The standard

solutions of lysozyme, bovine milk β-lactoglobulin (β-Lg); bovine milk α-lactalbumin (α-La) and human lactoferrin (LF) were prepared in buffer CL.

Lactoperoxidase activity

Before lactoperoxidase (LPO) activity determination, centrifugation at 3000 *g* for 30 min at 15°C was used to skim each milk sample. LPO activity was evaluated on each sample by a continuous spectrophotometric rate determination using as substrate 2,2′-Azinobis (3-Ethylbenzothiazoline-6-Sulfonic Acid; ABTS; Sigma Chemical Co) according to Pruitt and Kamau (1994).

Water-soluble vitamin determination

The determination of the vitamin C content was performed using a spectrophotometric method (L-Ascorbic Acid kit; Boehringer, Mannheim, Germany) in which the ability of L-ascorbic acid to reduce the tetrazolium salt MTT [3-(4,5-dimethylthiazol−2)-2,5-diphenyltetrazolium bromide] in the presence of the electron carrier PMS (5-methylphenazinium methosulfate) to formazan. The MTT–formazan complex was determined at λ = 578 nm.

Using an RP-HPLC method modified by Albalà-Hurtado *et al*. (1997), for both sample preparation procedures and chromatographic conditions, the B vitamin pool was quantified. Ten gram of milk and 1 g of solid TCA were thoroughly mixed for 10 min using a magnetic stirrer plate. Thereafter, the solution was centrifuged at 1250 *g* for 10 min to separate the two phases. The supernatant was gathered and put into a 10-mL volumetric flask. The acquired solid residue was then mixed with 3 mL of TCA 40 mg/L (w/v) and centrifuged as before. The supernatant was gathered and added to a 10 mL volumetric flask with TCA at a concentration of 40 mg/L (w/v). Acid extracts were filtered *via* a 0.45 m membrane filter before analysis (Millipore, Bedford, MA, USA). An HPLC Agilent 1260 Infinity LC System was used to separate the molecules (Agilent Technologies), using a C18 HiQSil HS column 5 µm, 4.6 mm i.d, 250 mm (Kya Tech Corporation, Tokyo, Japan). The mobile phase contained glacial acetic acid at a concentration of 24 g/L (w/v), octane sulfonic acid at a concentration of 5 mmol/L, and trimethylamine at a concentration of 85:15 in methanol (pH 3.6). Flow rates of 0.9 mL/min and 25°C were used for the analyses. One hundred microlitre of injection volume was utilised, and both 254 and 270 were used to detect the eluent. The quantification of the vitamins was accomplished by connecting the peaks to the standard curves and measuring the area of each standard peak using the valley-to-valley integration mode.

Fat-soluble vitamin determination

The method was based on the protocol of Albalá-Hurtado *et al*. (1997) with some modifications. Before performing the analysis, milk was prepared by the following protocol: to 5 mL of milk in a graduated flask, 0.1 g of ascorbic acid was added followed by the addition of 10 mL of absolute ethanol and 2 mL of 60% potassium hydroxide solution. The graduated flask was closed hermetically and kept it in the dark, and the mixture was incubated at 60°C for 30 min under shaking and cooled for 5 min in cold water (water and ice), following that 5 mL of n-hexane was added and the mixture was shaken thoroughly for 1 min. The content was transferred into a 50-mL separatory funnel, and the phase is left to separate for 5 min; this procedure was repeated three times. All organic phases (transparent upper part after separation in the funnel) are combined. The collected solvent (approximately 15 mL) was evaporated by a speedvac evaporator. To facilitate solvation, a volume of 2, 5 and 5 mL of absolute ethanol were successively added to each flask. Each flask was then filled with methanol to the specified level. For no longer than 1 week for vitamins A and D3 and 2 weeks for vitamin E, the standard stock solutions were kept in the dark at a temperature of −20°C. An HPLC Agilent 1260 Infinity LC System was used to carry out the chromatographic runs (Agilent Technologies). The column used was a C18 HiQSil HS, 5 µm, 4.6 mm i.d, 250 mm (Kya Tech Corporation). The optimised mobile phase was an isocratic mixture of methanol and water (95:5, v/v) with a flow rate of 1 mL/min, monitoring at 280 nm and a total analysis time of 15 min.

Acidifying capability of starter cultures

Seventeen natural starter cultures (i.e. lattoinnesti; Table S1) were tested in this study. At the Culture Collection of Industrial Microbiology Laboratory (University of Basilicata), cultures were kept as freeze-dried stocks made from skim milk with an ascorbic acid content of 0.1% by weight (11% w/v) and propagated (2% v/v, 16 h, 42 °C) in skim milk (11% w/v) before each assay. To assess the acidifying capability, starter cultures were inoculated (5% v/v) in bulk pasteurised milk (from Dr Bochicchio farm) and incubated at 42°C for 7 h; every 1 h, pH values were measured (Double Pore Slim electrode, Hamilton Company, Reno, NV, USA). AVCL7-5 and AVCL7-2 cultures, showing the fast acidification rate, were selected and used for acidification kinetics (5% v/v inoculum, incubation at 42°C for 7 h) in both C and MCT pasteurised milks, collected at Day 0 and after 35 days from the beginning of the diet. At 1-h intervals, pH values were measured. The commercial starter culture TLC-45 (Santamaria S.r.l., Burago di Molgora, MB, Italy) was used for comparison. Two biological replications of each experiment were run.

Electronic nose analysis

Electronic nose (EN) used (AIRESENSE Analytics GmbH, Schwerin, Germany) was made with an array of 10 sensors into a small chamber (Caputo *et al*. 2015). Detection limit

of the hot sensors was in the range of 1 ppm. The individual and bulk milk samples, stored at −80°C, were thawed at 4°C overnight and heated in a water bath at 30°C. To perform the assay, 5 mL of milk was placed in a 50-mL vial. Measurements were conducted at a constant temperature (30°C). The sample run lasted 60 s and was followed by 300 s flushing time.

Statistical analyses

Using Matlab™ software (Matlab R2021a, Natick, MA, USA), proximate, fatty acids, proteins and vitamins analysis data were examined. Every measurement was made in triplicate. The average of three values and their standard deviations were used to represent the results. To compare replicates and find the significant influence of factors on chemical composition, a two-way analysis of variance (ANOVA) with interaction was conducted using time (0, 35 days) and diet (Control, Microencapsulated tannins) as fixed factors. The relationships between the most important chemical properties were determined using Pearson correlation. To determine the link between several variables and identify the primary sources of variability, standard simple corresponding analyses (SCA) and principal component analyses (PCA) were applied. In these models only, the parameters showing significant differences were considered as variables. To verify that the variables selected could discriminate milk samples according to diet, linear discriminant analysis (LDA) was performed. The data gathered from the EN were processed with principal component analysis (PCA) and linear discriminant analysis (LDA) using WinMuster Version 1.6.2.2 software in order to distinguish the experimental group.

RESULTS AND DISCUSSION

Proximate analyses of the milk

Proximate composition of the samples is shown in Table 3. Neither diet nor time affected milk's proximate composition, while MCT-enhanced diet reduced both milk yield and urea content. The average urea content in the control group was 31.81 ± 7.63 mg/dL at Day 0 and remained statistically unvaried after 35 days (30.67 ± 4.41). In contrast, the urea concentration decreased significantly ($P < 0.05$) by 27% from 0 to 35 days with MCTs supplementation. This might be due to the reduction of nitrogen (N) absorption, which is affected by dietary tannins, by lowering both the rate and extent of ruminal protein degradation and NH_3 concentrations in rumen and blood (Naumann *et al*. 2017). Being dietary crude protein the dominant nutritional factor influencing milk urea nitrogen (Nousiainen *et al*. 2004), a reduced urea content in milk from cows fed with MCTs was expected. Our results are comparable to those of other authors (Cabiddu *et al*. 2009; Kälber *et al*. 2011) that observed a reduction of ovine and bovine milk urea content after feeding with CTs for a period ranging between 56 and 21 days, respectively. In another study (Anantasook *et al*. 2015), dietary CTs mixed with saponins did not affect the milk urea nitrogen after 21 days exposure to the experimental diet, but milk yield and fat content increased.

The evolution of milk yields over the experiment is shown in Figure 1. From 0 to 15 days, both the C and MCT groups showed a very similar trend. Afterwards, the MCT group's yields fall while increasing in C. The diet's effect was assessed using one-way ANOVA. Data analysis indicates that MCTs had an effect on milk yield ($P < 0.05$), in particular after 15 days of exposure to the diet the average milk yield falls from 29.78 to 28.45 L/day, whereas the control group showed increasing in milk production from 31.05 to 32.12 L/day (averages values for group). Grainger *et al*. (2009) reported that milk yield, fat and protein percentage of milk reduced when dairy cows were dosed daily with 163–326 g CTs from *Acacia mearnsii*. Similar results were found by Henke *et al*. (2017a, 2017b), who observed a reduction in milk yield after 21 days of testing dietary Quebracho tannin extract at 30 g/kg of dry matter on cow's wellness and milk quality (13-days adaptation phase

Table 3 Effect of the diet (C, control; MCTs, microencapsulated tannins) on the chemical composition of the milk at Day 0 (T0) and Day 35 (T35).

	C		MCTs		
Parameter	T0	T35	T0	T35	*P*-value diet
Fat (%)	3.50 ± 0.51	3.88 ± 0.26	3.69 ± 0.85	3.54 ± 0.88	NS
Protein (%)	3.21 ± 0.23	3.24 ± 0.31	3.10 ± 0.36	3.36 ± 0.6	NS
Lactose (%)	4.77 ± 0.08	4.76 ± 0.07	4.67 ± 0.24	4.65 ± 0.38	NS
Casein (%)	2.49 ± 0.19	2.54 ± 0.27	2.41 ± 0.33	2.64 ± 0.53	NS
Urea (mg/dL)	31.81 ± 7.63	30.67 ± 4.41	23.67 ± 5.90	17.24 ± 4.43	<0.001
pH	6.61 ± 0.06	6.58 ± 0.08	6.60 ± 0.04	6.62 ± 0.12	NS

Values are means ± standard deviation ($n = 10$); NS, not significant.

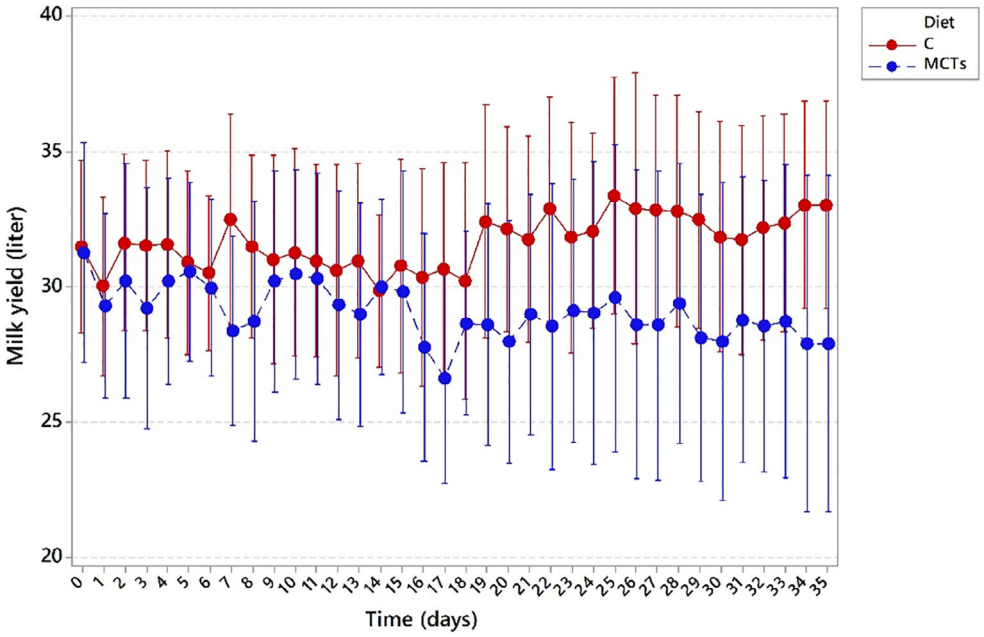

Figure 1 Evolution of milk's yields over the experiment in control (C) and microencapsulated tannin (MCT) groups.

followed by an 8-days sampling phase feed). Also, they did not find any significant difference in protein evolution but observed a significant reduction of milk urea content in the test group. On the contrary, milk yield was increased in dairy cows fed with other CTs sources, such as peanut skins, *Hedysarum coronarium* (Italian sainfoin) and *L. corniculatus* (birdsfoot trefoil), confirming as the type of tannins plays a key role in the treatments (Patra and Saxena 2011).

Fatty acid profile

Fatty acid composition of milk from different diets is shown in Table 4. The most prevalent FAs were palmitic acid (C16:0), oleic acid (C18:1 n-9), stearic acid (C18:0) and myristic acid. (C14:0). MCTs had limited but interesting effects on the FA composition. The effect of the diet was evident for medium and long-chain FA evolution. Short-chain FA concentration was not affected either by diet or time. Concerning the nutritional evaluation of the fat fraction of the milk sampled after 35 days, the n-6 and n-3 portions were more stable and higher in the test group (MCTs), whereas were decreased in the control group. The concentration of beneficial FAs such as α-linolenic acid (18:3 n-3) and linoleic acid (18:2 n-6) slightly risen as a result of microencapsulated tannins supplementation ($P < 0.05$), whereas decreased in the control group. The level of stearic acid (C18:0) increased significantly in the control group whereas was stable in the test group, confirming the capability of tannins to reduce the extent of the last step of the ruminal biohydrogenation (Frutos *et al*. 2020). Diet and time impacted the evolution of vaccenic acid (C18:1 trans-11), but the extent of its reduction was larger in the control group. The concentration of the docosanoic acid (C22:0) was found twofold increase in the control group, but it remained constant in the test one. Furthermore, the portion of total polyunsaturated fatty acids was unvaried in the milk from the test group ($P < 0.001$), resulting in a higher P/S ratio. Also, the level of eicosapentaenoic acid (C20:5 n-3) decreased in the control group from 0.12 to 0.02 g/100 g total FAs, whereas the test group did not show any variation. Our results agree with those of other authors (Toral *et al*. 2011), for who tannin supplementation did not impact the concentration of the major classes of FAs in milk (i.e. saturated, short and medium chain FAs) but had effects on the minor portion of long-chain and polyunsaturated and beneficial FAs, such as 18:2 n-6 and 18:3 n-3. Increasing of these FAs in milk has been reported in response to CT- or HT-enriched diet (Buccioni *et al*. 2015; Henke *et al*. 2017a, 2017b; Focant *et al*. 2019).

Whey protein content

The results of LPO activity and whey protein content in milk samples are presented in Table 5. Lysozyme was not detected in these experiments; in the literature, it is reported that lysozyme is present at trace level in bovine milk (about 10 ng/mL; Król *et al*. 2010). A significant increase in LPO activity after 35 days of treatment with the T was observed ($P < 0.001$). Although many studies concerning the effect of CT-based feed on the bovine milk proteome have been conducted, the effect on LPO activity has not been largely studied, making challenging the discussion. LPO is a member of the peroxidase-

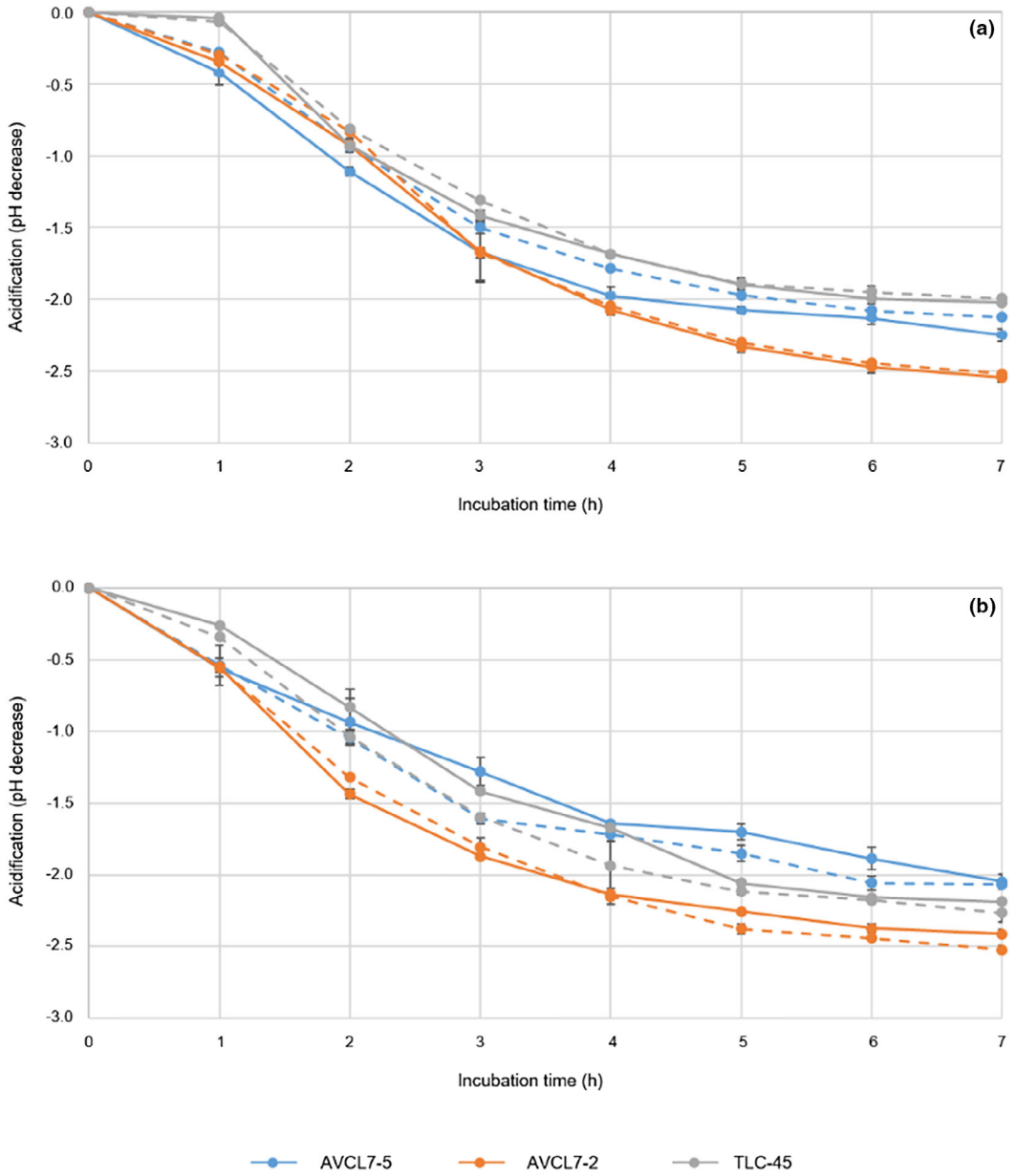

Figure 2 Acidification kinetics of AVCL7-5 (blue lines), AVCL7-2 (orange lines) and TLC-45 (grey lines) starter cultures in pasteurised milks, collected at Day 0 (panel a) and after 35 days (panel b) from the beginning of the diet. Control feed (C): continuous lines; feed with microencapsulated tannin supplementation (MCTs): dotted lines.

cyclooxygenase superfamily and one of the most abundant enzymes in bovine milk, constituting about 1% of whey proteins. LPO is an antioxidant enzyme and is a natural antimicrobial system that eliminates the harmful effects of microorganisms in milk, increasing the shelf life of raw milk. In a recent work, it was shown that the dietary tannin supplementation on cow's diet improved both the reducing power and the radical scavenging capacity of milk probably because they have an indirect effect in preserving antioxidants (Menci *et al*. 2021).

The amount of β-Lg found in this work at T0 (3.68–3.90 mg/mL) is close to that found by other authors (Konrad *et al*. 2000; Andersson and Mattiasson 2006; Indyk *et al*. 2017). The concentrations of α-La we have detected, and the ratio β-Lg/α-La differs from many works found in the literature, but our results are similar to those found by Turner *et al*. (2005).

β-Lg and α-La rose in both groups, but the differences were not due to diet. It is known from literature that β-Lg concentration increases with the stage of lactation; therefore,

Table 4 Milk fatty acid composition in relation to the diet (C, control; MCTs, microencapsulated tannins) and time (*t*) at the beginning (Day 0) and the end of the trial (Day 35).

	C				MCTs						
	Day 0		Day 35		Day 0		Day 35		*P*-value		
Fatty acids	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*D*	*t*	*D* × *t*
C4:0	5.32	±1.14	4.82	±1.76	4.30	±1.38	4.74	±0.78	NS	NS	NS
C6:0	3.68	±0.23	3.37	±1.14	3.23	±0.68	3.26	±0.54	NS	NS	NS
C8:0	2.21	±0.30	2.01	±0.66	1.93	±0.50	1.97	±0.44	NS	NS	NS
C10:0	4.55	±1.16	4.02	±1.21	4.04	±1.22	4.17	±1.18	NS	NS	NS
C11:0	0.52	±0.16	0.49	±0.16	0.44	±0.19	0.49	±0.15	NS	NS	NS
C12:0	4.48	±1.32	3.94	±1.04	4.03	±1.16	3.71	±1.73	NS	NS	NS
C12:1	0.11	±0.04	0.10	±0.03	0.10	±0.04	0.11	±0.72	NS	NS	NS
C13:0	0.12	±0.02	0.10	±0.02	0.11	±0.03	0.10	±0.03	NS	NS	NS
C14:0 ISO	0.13	±0.02	0.12	±0.02	0.12	±0.02	0.12	±0.02	NS	NS	NS
C14:0	11.75	±2.04	11.00	±1.5	11.13	±1.85	11.32	±1.17	NS	NS	NS
C14:1 CIS	1.16	±0.37	1.12	±0.31	1.12	±0.37	1.22	±0.35	NS	NS	NS
C15:0 ISO	0.25	±0.04	0.24	±0.04	0.25	±0.03	0.25	±0.03	NS	NS	NS
anteiso C15:0	0.52	±0.09	0.49	±0.08	0.51	±0.1	0.58	±0.09	NS	NS	NS
C15:0	0.96	±0.14	0.93	±0.05	0.94	±0.14	0.92	±0.08	NS	NS	NS
C15:1	0.07	±0.02	0.07	±0.02	0.08	±0.01	0.08	±0.01	NS	NS	NS
C16:0 ISO	0.21	±0.04	0.21	±0.02	0.23	±0.03	0.22	±0.03	NS	NS	NS
C16:0	24.26	±3.52	26.52	±6.3	23.39	±2.05	23.09	±2.02	NS	NS	NS
C16:1 trans	0.15	±0.01	0.12	±0.03	0.14	±0.03	0.15	±0.01	NS	NS	NS
C16:1 cis	1.16	±0.12	0.88	±0.23	0.95	±0.39	1.08	±0.30	<0.05	<0.01	<0.01
C17:0 ISO	0.26	±0.07	0.27	±0.05	0.26	±0.1	0.30	±0.05	NS	NS	NS
anteiso C17:0	0.36	±0.04	0.34	±0.06	0.38	±0.06	0.40	±0.07	NS	NS	NS
C17:0	0.43	±0.06	0.47	±0.1	0.46	±0.05	0.44	±0.04	NS	NS	NS
C17:1	0.16	±0.04	0.14	±0.04	0.15	±0.05	0.14	±0.02	NS	NS	NS
C18:0 iso	0.04	±0.02	0.03	±0.01	0.03	±0.02	0.03	±0.01	NS	NS	NS
C18:0	9.28	±1.01	11.92	±0.89	11.54	±2.5	11.41	±2.94	<0.05	NS	NS
C18:1 trans-9	0.29	±0.17	0.19	±0.02	0.27	±0.16	0.22	±0.04	NS	NS	NS
C18:1 trans-11	0.78	±0.38	0.22	±0.28	0.35	±0.55	0.28	±0.42	<0.05	<0.05	<0.05
C18:1 n-9	19.16	±4.67	18.97	±3.92	19.18	±8.07	20.50	±2.37	NS	NS	NS
C18:1 n-7	0.58	±0.11	0.50	±0.07	0.60	±6.46	0.56	±0.06	NS	NS	NS
C18:2 trans-9, trans-12	0.57	±0.07	0.54	±0.12	0.61	±0.09	0.61	±0.07	NS	NS	NS
C18:2 trans-9, cis-12	0.66	±0.12	0.64	±0.1	0.76	±0.09	0.70	±0.07	NS	NS	NS
C18:2 cis-9, trans-12	0.21	±0.05	0.21	±0.05	0.25	±0.06	0.25	±0.06	NS	NS	NS
C18:2 n-6	3.66	±0.43	3.18	±0.67	3.84	±0.43	4.02	±0.46	<0.05	NS	NS
C18:3 n-6	0.03	±0.01	0.03	±0.01	0.04	±0.02	0.04	±0.01	NS	NS	NS
C18:3 n-3	0.67	±0.09	0.60	±0.13	0.72	±0.1	0.77	±0.12	<0.01	NS	NS
C18:2 cis-9, trans-11	0.59	±0.06	0.49	±0.11	0.58	±0.16	0.56	±0.14	NS	NS	NS
C20:0	0.13	±0.03	0.15	±0.06	0.17	±0.04	0.13	±0.05	NS	NS	NS
C20:1	0.10	±0.03	0.09	±0.03	0.12	±0.03	0.12	±0.03	NS	NS	NS
C21:0	0.03	±0.01	0.03	±0.01	0.03	±0.02	0.03	±0.03	NS	NS	NS
C20:2 n-6	0.01	±0.01	0.02	±0.01	0.02	±0.02	0.03	±0.01	<0.001	<0.001	NS
C20:3 n-6	0.01	±0.01	0.01	±0.01	0.01	±0.01	0.02	±0.01	NS	NS	NS
C20:4 n-6	0.03	±0.03	0.09	±0.03	0.10	±0.03	0.11	±0.02	NS	NS	NS
C20:3 n-3	0.09	±0.02	0.12	±0.02	0.12	±0.02	0.12	±0.01	NS	NS	NS
C20:5 n-3	0.12	±0.05	0.02	±0.01	0.06	±0.02	0.03	±0.02	<0.001	<0.01	<0.001
C22:0	0.04	±0.04	0.09	±0.02	0.09	±0.04	0.10	±0.02	<0.01	<0.01	<0.01
C22:2n-6	0.02	±0.04	0.01	±0.01	0.00	±0.01	0.01	±0.01	NS	NS	NS
C22:4 n-6	0.01	±0.01	0.01	±0.02	0.01	±0.02	0.01	±0.02	NS	NS	NS

(continued)

Table 4 (Continued).

	C				MCTs						
	Day 0		Day 35		Day 0		Day 35		*P*-value		
Fatty acids	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*D*	*t*	*D* × *t*
C22:5 n-3	0.02	±0.03	0.02	±0.02	0.02	±0.03	0.02	±0.02	NS	NS	NS
C22:6 n-3	0.03	±0.02	0.04	±0.01	0.04	±0.02	0.05	±0.01	NS	<0.05	NS
SFA	69.54	±4.67	71.57	±5.42	67.62	±4.64	67.79	±3.10	NS	NS	NS
UFA	30.45	±4.67	28.43	±5.42	32.38	±4.64	32.21	±3.10	NS	NS	NS
MUFA	23.73	±4.32	22.41	±4.36	25.23	±4.29	24.87	±2.49	NS	NS	NS
PUFA	6.72	±0.71	6.03	±1.2	7.14	±0.81	7.34	±0.87	<0.01	NS	NS
n-3	0.93	±0.09	0.79	±0.18	0.92	±0.11	0.99	±0.15	<0.05	NS	NS
n-6	3.75	±0.45	3.36	±0.72	4.02	±0.46	4.24	±0.50	<0.01	NS	NS
P/S	0.12	±0.01	0.07	±0.02	0.11	±0.02	0.11	±0.02	<0.01	NS	NS
n-6/n-3	4.03	±0.37	4.26	±0.33	4.37	±0.25	4.29	±0.27	NS	NS	NS
Total trans	1.80	±0.70	1.06	±0.42	1.37	±0.59	1.26	±0.35	NS	<0.01	NS

MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; n-3, C18:3 n-3 + C20:2 n-3 + C20:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3; n-6, C18:2 n-6 + C18:3 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:4 n-6. Values (in % of total fatty acids) are means± standard deviation (n = 10); NS, not significant.

Table 5 Whey protein content and LPO activity in milk samples.

	C				MCTs						
	0		35		0		35		*P*-value		
Proteins	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*D*	*t*	*D* × *t*
LPO (U/mL)	2.76	±0.54	3.26	±1.35	3.10	±0.59	7.31	±2.66	<0.001	NS	NS
β-Lg (mg/mL)	3.90	±0.88	5.54	±1.05	3.68	±1.33	5.30	±0.56	NS	<0.001	NS
α-La (mg/mL)	0.35	±0.16	0.65	±0.18	0.53	±0.20	0.82	±0.32	<0.05	<0.001	NS
LF (mg/mL)	0.05	±0.02	0.00	±0.00	0.06	±0.02	0.00	±0.00	NS	<0.001	NS

Values are means ± SD (n = 10); NS, not significant; LPO, Lactoperoxidase activity; *β-Lg*, β-Lactoglobulin; *α-La*, α-Lactalbumin; *LF*, Lactoferrin. Values (in % of total fatty acids) are means ± SD (n = 10); NS, not significant.

the slightly higher level of this protein at T35 may be due to this effect. Unlike β-Lg, the concentration of α-La in bovine milk decreases at the end of lactation (Deeth and Bansal 2019). According to reports, the α-La concentration decreasing is favourably connected with the decrease in milk's lactose content (Farrell *et al*. 2004), but in our experiment, we did not experienced either lactose or α-La decline. Both groups experienced a sharp decline in LF content, indicating that time ($P < 0.001$) rather than diet is to blame for this decline. It is difficult to explain the decrement of LF content found at T35 in the present study; however, Cheng *et al*. (2008) reported that lactoferrin concentration depends on several factors that may also interact amongst them. These authors found that LF amount in milk is positively correlated with the lactation stage but negatively correlated with the daily milk production and that LF and lactose showed a negative correlation mainly because of the elevated SCC that reduced the synthetic activity of the mammary gland. Other authors found that LF concentration is affected also by milking frequency (Gedye *et al*. 2020). Whey proteins are synthesised in the mammary glands, from different nitrogen sources. About 60% of the amino acids absorbed in the small intestine come from bacterial proteins, and the remaining 40% come from undegraded food proteins in rumen. Strong acids released by the abomasum halt all microbial activity and trigger the breakdown of proteins into AA by digestive enzymes. Most of those AA absorbed by the mammary glands are used to synthesise milk protein. The ability of CTs to form complexes with protein forages may protect these proteins from degradation in the rumen and may lead to a large amount of AA available for milk protein synthesis (Min *et al*. 2003). On this ground, it seems obvious that the test group had more LPO activity, but it is unclear why diet had so little of an impact on the whey protein fraction. The topic is hampered by the paucity of evidence in the literature regarding the effects of supplemented CTs, particularly when it is microencapsulated, on the whey protein. Most of the studies

present in the literature investigated the effect of different CT source plants. For instance, Chedea *et al*. (2017) found that milk from cows fed a diet including grape pomace (2.7 kg DM/day per 3 months) significantly increased in β-Lg but not α-La, whereas Bennato *et al*. (2022) did not detect any differences after cows were fed for 70 days with grape pomace (1 kg DM/day). On the contrary, the milk's individual protein composition was unaffected by feeding dairy cows birdsfoot trefoil (*L. corniculatus*), with the exception of a rise in LF after 14 days of trefoil-based feeding (Turner *et al*. 2005).

Vitamin content in milk

Water-soluble vitamins found in bovine milk are a good source of the minimal amount of vitamins that both children and adults should consume each day. In Table 6, the vitamin content for each milk control sample and each milk sample obtained from cows fed by microcaps is shown. Vitamin B_3 content was significantly reduced ($P < 0.01$) in the samples collected from the MCTs group. The decrease of vitamin B_3 content in the sample collected on day 35 refers both for C and T groups ($P < 0.05$). While the control group showed a regular trend of vitamin B_3 content and the variation was not significant, in MCTs group this value collapsed. At day 35, nicotinamide content was reduced in both groups. Vitamin B_6 mean content was 1.52 and 2.37 μM for control and test group, respectively. In the samples collected at the end of the treatment, we detected a significant reduction only in MCTs group, suggesting as this effect can be mainly attributed to the diet rather than the stage of lactation. No significant differences have been found within or between the groups, indicating that neither feed nor lactation had an impact on the vitamin B_9 content. Throughout the study, vitamin C concentration behaved very differently amongst the groups; its beginning levels were significantly different across the C and MCTs, and over the experiment, it increased in MCTs while decreasing in C group. The larger increase in vitamin C content can be due to the diet ($P < 0.01$). Vitamin A showed a significant decrease ($P < 0.05$) in MCTs group, but was quite constant in C. Vitamin D_3 content has not been affected by the treatment, nor lactation stage affected its content in milk samples. Vitamin E contents varied in both groups and showed a treatment-dependent trend, as rose in C whereas decreased in MCTs group. Vitamin K showed a significant ($P < 0.05$) increase in C group, whereas in T the level of this vitamin remained stable. To our knowledge, there is a lack of information regarding how CTs affects vitamin content in bovine milk, or its metabolism and absorption (Naumann *et al*. 2017). Tannic acid has been demonstrated to have a deleterious impact on vitamin A status in rats, and it may interact with thiamine to decrease the absorption of vitamin B. Overall, the research on the effect of CTs on the vitamin content of bovine milk is limited and somewhat inconsistent. Further research is needed to fully understand the impact of CTs on the vitamin content of milk and the potential implications for human nutrition.

Acidifying capability

The results of acidification screening showed that most of natural cultures decreased the pH values to 4.6 (need for milk coagulation) in about 6–7 h. AVCL7-5 and AVCL7-2 showed the fastest reduction of pH (up to pH 4.6 in 4 h). AVCL7-2 culture had the greatest acidification ability in all milk samples, regardless of the type of feeding (pH lowering to 4.6 after 4 h of incubation in milk collected at 0 days, Figure 2 (a); pH 4.6 after 3 h of incubation in milks collected at the end of feeding, i.e. 35 days, Figure 2 (b). The type and duration of feeding did not significantly affect the acidification ability of starter cultures. The major effect was related to the type of starter culture; as expected, the natural milk starter cultures (lattoinnesti) showed a higher

Table 6 Water- and fat-soluble vitamin content in control (C) and microencapsulated tannin (MCT) groups.

	C				MCTs						
	0		35		0		35		*P*-value		
Vitamins	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*D*	*t*	*D* × *t*
Vit. B_3 μM	35.35	±13.03	28.59	±6.77	27.52	±4.92	11.40	±4.29	<0.01	NS	NS
Nicotinamide μM	8.56	±4.74	6.13	±1.51	7.16	±2.51	5.06	±2.91	NS	NS	NS
Vit. B_6 μM	1.52	±0.61	1.37	±1.05	2.37	±0.91	1.09	±1.53	<0.05	NS	NS
Vit. B_9 μM	2.20	±0.45	2.33	±0.33	2.34	±0.52	2.26	±0.47	NS	NS	NS
Vit. C μM	52.33	±15.12	43.17	±12.7	28.34	±6.1	83.92	±12.46	<0.01	<0.05	<0.01
Vit. A μM	0.77	±0.31	0.75	±0.28	1.23	±0.71	0.82	±0.15	<0.05	NS	NS
Vit. D_3 μM	0.09	±0.01	0.11	±0.02	0.13	±0.02	0.11	±0.01	NS	NS	NS
Vit. E μM	0.35	±0.16	1.10	±0.35	1.23	±0.73	0.87	±0.20	<0.05	NS	<0.001
Vit. K μM	0.10	±0.02	0.25	±0.19	0.29	±0.09	0.35	±0.03	<0.01	NS	NS

Values are means ± SD ($n = 10$); NF, not found; NS, not significant.

acidification rate compared with the commercial culture. These results suggest that the biofortified milks can be used in dairy production because they did not affect the viability and functionality of starter cultures.

Aromatic fingerprint of milk

In Figure 3 are depicted the results of PCA (a, c) and LDA (b, d) of individual (a, b) and bulk milk (c, d) data from EN data. The results have shown that the diet affected footprint olfactory of the milk. Amongst two algorithms, LDA was more sensitive to the effect of samples and PCA effective to discriminate both the individual and bulk milk samples. However, bulk milk's PCA discriminated between samples more effectively than individual milk (87.8% of variance vs 97.5%). The aromatic profile of milk sample from MCT group was characterised by the reduction ($P < 0.05$) of the signal from sensors S1 (aromatic compounds), S3 (ammonia), S4 (hydrogen), S5 (alkene) and increasing ($P < 0.05$) from sensors S7 and S9 (sulphur compounds).

Multivariate analyses

In Figure 4 are depicted the results of SCA using data of those parameters affected by diet. Analyses of contingent table indicate as two components account for 96.2% and may be sufficient to explain the total inertia. In the obtained raw contribution table (Tables S2 and S3), the highest

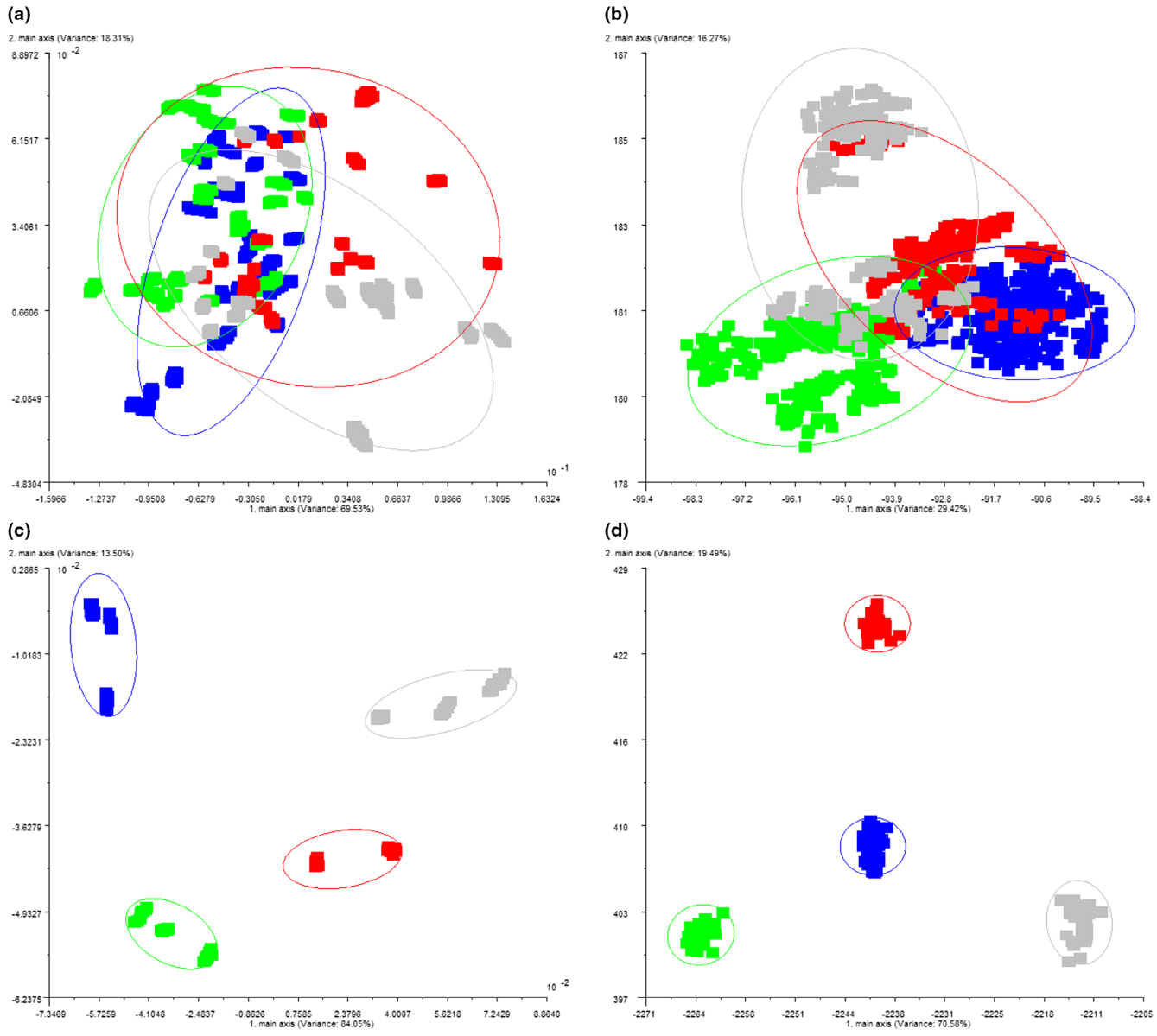

Figure 3 Principal component analysis (a, c) and linear discriminant analysis (b, d) of individual (a, b) and bulk milk (c, d) data from EN analysis. Blue = control group at Day 0; green = microencapsulated tannin group at Day 0; red = control group at Day 35; grey = treated group at Day 35.

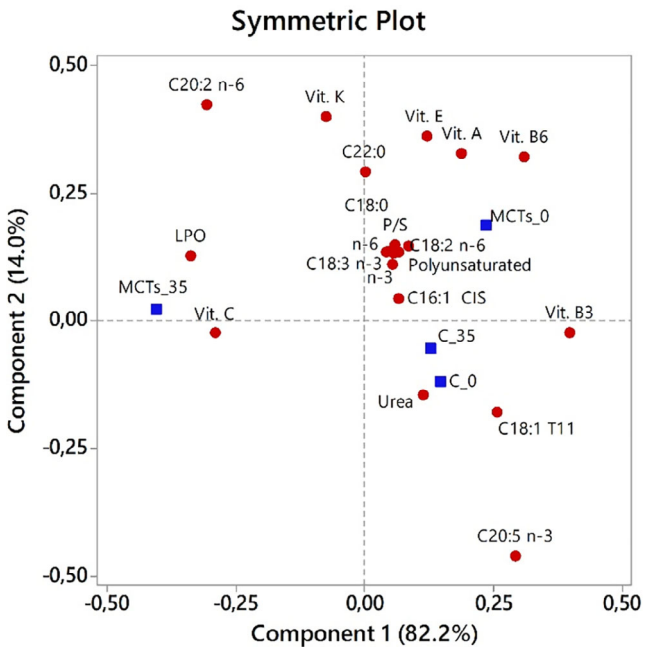

Figure 4 Symmetric plot obtained from SCA obtained from the relationship matrix with the parameters that were significantly affected by the diet. C: Control group; MCTs: microencapsulated tannin groups; 0: sampling at Day 0; 35: sampling at Day 35. LPO: Lactoperoxidase activity.

quality values account for LPO (0.992), vitamin B3 (0.991) and vitamin C (0.996); therefore, these data are best represented by the two components, whereas C18:1 t11 has the worst representation (0.371). Vitamin B3 and vitamin C contribute the most to the variability of component one, whereas urea, vitamin B6, and C18:0 contribute the most for component two. In raw contribution table, the column labelled 'inertia' is the proportion of the total inertia contributed by each raw; thus, vitamin B3 (35.7%) and vitamin C (37.2%) had a stronger association with the column categories (treatment) than the other row categories. This is represented quite clearly by symmetric plot (Figure 4) wherein is shown as the control groups, before and after diet, are depicted in the fourth square, while the experimental groups (before and after diet) are clearly separated and discriminated by vitamin C content and LPO activity. PCA was lower effective than SCA to discriminate the groups based on the selected parameters (first, second and third components of PCA explain just 60% of the variance), providing unsatisfactory results (Figure S1). Linear discriminant analysis was used to evaluate whether milk before diet could be discriminated from milk produced after feeding with microencapsulated tannins. A stepwise procedure resulted in a model in which responses (fatty acids that were affected by diet) were able to correctly predict the milk group for 77.8% of the cases (66.7% % for milk before diet and 88.9% for milk after diet). A significant negative correlation was found between vitamin B3 and C18:0 ($r = -0.55$; $P = 0.001$), vitamin B3 and C20:2 n-6 ($r = -0.625$; $P = 0$) and C20:2 n-6 and C18:1 T11 ($r = -0.48$; $P = 0.05$); whereas the correlations were positive between C22:0 and C22:2 n-6 ($r = 0.672$; $P = 0$), C18:3 n-3 and C18:2 n-3 ($r = 0.944$; $P = 0$).

CONCLUSION

A wealth of information is available in the current literature regarding the impact of tannin-based feed on cow milk characteristics. In this study, we evaluated the effect of a short-term diet enriched with MCTs using a comprehensive approach. The experimental diet marginally reduced the milk yield, but severely reduced its urea concentration by 25%. A larger unsaturated:saturated ratio was seen in the treated group due to the preservation of the unsaturated FA fraction, particularly the polyunsaturated. The milk proximate analyses did not detect any variation over the experiment. Amongst protein analyses, a twofold increase in LPO was detected in the tested group. Aside from the large increasing of vitamin C level in the treated group, diet had a detrimental impact on the B vitamins' group content. The influence of the MCTs-based diet was highlighted by multivariate analysis techniques (SCA and LDA) of the major parameters affected, which were utilised to differentiate between the groups. While additional study is required to determine the long-term impact of adding MCTs to cattle diet, evidence from this study suggests that this approach could offer farmers a viable way to enhance the unsaturated fatty acid profile (n-3, n-6) and vitamin C content of the milk, while also save costs and provide environmental benefits.

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AUTHOR CONTRIBUTIONS

Attilio Matera: Formal analysis; investigation; writing – original draft. **Lucia Sepe:** Conceptualization; funding acquisition; methodology; supervision. **Silvia Vincenzetti:** Data curation; investigation; methodology; supervision; writing – original draft. **Roberta Tolve:** Conceptualization; methodology. **Nicola Condelli:** Formal analysis; supervision; visualization. **Salvatore Claps:** Conceptualization; funding acquisition. **Francesco Genovese:** Conceptualization; project administration; supervision. **Annamaria Ricciardi:** Investigation; project administration; supervision. **Teresa Zotta:** Investigation; methodology; supervision. **Teresa Scarpa:** Formal analysis; investigation. **Maria**

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest in this article.

DATA AVAILABILITY STATEMENT

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

REFERENCES

Albalá-Hurtado S, Rodriguez S N, Veciana-Nogués T and Mariné-Font A (1997) Determination of vitamins a and E in infant milk formulae by high-performance liquid chromatography. *Journal of Chromatography A* **778** 243–246.

Albalà-Hurtado S, Veciana-Nogue's M T, Izquierdo-Pulido T and Mariné-ont A M (1997) Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. *Journal of Chromatography A* **778** 247–253.

Anantasook N, Wanapat M, Cherdthong A and Gunun P (2015) Effect of tannins and saponins in Samanea saman on rumen environment, milk yield and milk composition in lactating dairy cows. *Journal of Animal Physiology and Animal Nutrition* **99** 335–344.

Andersson J and Mattiasson B (2006) Simulated moving bed technology with a simplified approach for protein purification: Separation of lactoperoxidase and lactoferrin from whey protein concentrate. *Journal of Chromatography A* **1107** 88–95.

AOAC International, ed (1995) *Official Method of Analysis*, 16th edn Gaithersburg, MD: AOAC International.

Bennato F, Ianni A, Florio M, Grotta L, Pomilio F, Saletti M A and Martino G (2022) Nutritional properties of Milk from dairy ewes fed with a diet containing grape pomace. *Food* **11** 1878.

Bligh E G and Dyer W J (1959) A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37** 911–917.

Bouchenak M and Lamri-Senhadji M (2013) Nutritional quality of legumes, and their role in cardiometabolic risk prevention: A review. *Journal of Medicinal Food* **16** 185–198.

Buccioni A, Serra A, Minieri S, Mannelli F, Cappucci A, Benvenuti D, Rapaccini S, Conte G and Mele M (2015) Milk production, composition, and milk fatty acid profile from grazing sheep fed diets supplemented with chestnut tannin extract and extruded linseed. *Small Ruminant Research* **130** 200–207.

Cabiddu A, Molle G, Decandia M, Spada S, Fiori M, Piredda G and Addis M (2009) Responses to condensed tannins of flowering sulla (*Hedysarum coronarium* L.) grazed by dairy sheep. Part 2: Effects on milk fatty acid profile. *Livestock Science* **123** 230–240.

Caputo A R, Morone G, Di Napoli M A, Rufrano D, Sabia E, Paladino F, Sepe L and Claps S (2015) Effect of destoned olive cake on the aromatic profile of cows' milk and dairy products: Comparison of two techniques for the headspace aroma profile analysis. *Italian Journal of Agronomy* **20** 15–20.

Chedea V S, Pelmus R S, Lazar C, Pistol G C, Calin L G, Toma S M, Dragomir C and Taranu I (2017) Effects of a diet containing dried grape pomace on blood metabolites and milk composition of dairy cows. *Journal of the Science of Food and Agriculture* **97** 2516–2523.

Cheng J B, Wang J Q, Bu D P, Liu G L, Zhang C G, Wei H Y, Zhou L Y and Wang J Z (2008) Factors affecting the lactoferrin concentration in bovine milk. *Journal of Dairy Science* **91** 970–976.

Deeth H and Bansal N (2019) Chapter 1 – whey proteins: An overview. In *Whey Proteins*, pp. 1–50. Deeth H C and Bansal N, eds. Brisbane, Australia: Academic Press.

Dellavalle D M, Vandenberg A and Glahn R P (2013) Seed coat removal improves iron bioavailability in cooked lentils: Studies using an in vitro digestion/Caco-2 cell culture model. *Journal of Agricultural and Food Chemistry* **61** 8084–8089.

Di Trana A, Cifuni G F, Fedele V, Braghieri A, Claps S and Rubino R (2004) The grazing system and season affect CLA, ω-3 and trans fatty acid contents in goat milk. *Progress in Nutrition* **6** 109–115.

Fang Z and Bhandari B (2010) Encapsulation of polyphenols – A review. *Trends in Food Science & Technology* **21** 510–523.

FAOSTAT (2022) [Internet document] URL. https://www.fao.org/platform-food-loss-waste/flw-data/en/. Accessed 23/11/2022.

Farrell H M, Jimenez-Flores R, Bleck G T, Brown E M, Butler J E, Creamer L K, Hicks C L, Hollar C M, Ng-Kwai-Hang K F and Swaisgood H E (2004) Nomenclature of the proteins of Cows' Milk—Sixth revision. *Journal of Dairy Science* **87** 1641–1674.

Focant M, Froidmont E, Archambeau Q, Dang Van Q C and Larondelle Y (2019) The effect of oak tannin (*Quercus robur*) and hops (*Humulus lupulus*) on dietary nitrogen efficiency, methane emission, and milk fatty acid composition of dairy cows fed a low-protein diet including linseed. *Journal of Dairy Science* **102** 1144–1159.

Frutos P, Hervás G, Natalello A, Luciano G, Fondevila M, Priolo A and Toral P G (2020) Ability of tannins to modulate ruminal lipid metabolism and milk and meat fatty acid profiles. *Animal Feed Science and Technology* **269** 114623.

García A and Garrigós M (2016) Microencapsulation of natural antioxidant compounds obtained from biomass wastes: A review. *Materials Science Forum* **875** 112–126.

Gedye K, Notcovich S, Correa-Luna M, Ariyarathne P, Heiser A, Lopez-Lozano R and Lopez-Villalobos N (2020) Lactoferrin concentration and expression in New Zealand cows milked once or twice a day. *Animal Science Journal* **91** e13331.

Godfray H C J, Beddington J R, Crute I R, Haddad L, Lawrence D, Muir J F, Pretty J, Robinson S, Thomas S M and Toulmin C (2010) Food security: The challenge of feeding 9 billion people. *Science* **327** 812–818.

Grainger C, Clarke T, Auldist M J, Beauchemin K A, McGinn S M, Waghorn G C and Eckard R J (2009) Potential use of *Acacia mearnsii* condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. *Canadian Journal of Animal Science* **89** 241–251.

Henke A, Dickhoefer U, Westreicher-Kristen E, Knappstein K, Molkentin J, Hasler M and Susenbeth A (2017a) Effect of dietary quebracho tannin extract on feed intake, digestibility, excretion of urinary purine derivatives and milk production in dairy cows. *Archivies of Animal Nutrition* **71** 37–53.

Henke A, Westreicher-Kristen E, Molkentin J, Dickhoefer U, Knappstein K, Hasler M and Susenbeth A (2017b) Effect of dietary quebracho tannin extract on milk fatty acid composition in cows. *Journal of Dairy Science* **100** 6229–6238.

Indyk H E, Hart S, Meerkerk T, Gill B D and Woollard D C (2017) The β-lactoglobulin content of bovine milk: Development and application of a biosensor immunoassay. *International Dairy Journal* **73** 68–73.

ISO (2013) *Milk and Liquid Milk Products—Guidelines for the Application of Mid-Infrared Spectrometry*. Method no. 9622. Geneva, Switzerland: International Organization for Standardization. [Internet document] URL https://www.iso.org/standard/56874.html. Geneva, Switzerland.

Jones W T and Mangan J L (1977) Complexes of the condensed tannins of sainfoin (*Onobrychis viciifolia* Scop.) with fraction 1 leaf protein and with submaxillary mucoprotein and their reversal by polyethelene glycol and pH. *Journal of the Science of Food and Agriculture* **28** 126–136.

Kälber T, Meier J S, Kreuzer M and Leiber F (2011) Flowering catch crops used as forage plants for dairy cows: Influence on fatty acids and tocopherols in milk. *Journal of Dairy Science* **94** 1477–1489.

Khanbabaee K and van Ree T (2001) Tannins: Classification and definition. *Natural Product Reports* **18** 641–649.

Konkol D and Wojnarowski K (2018) The use of nanominerals in animal nutrition as a way to improve the composition and quality of animal products. *Journal of Chemistry* **2018** 1–7.

Konrad G, Lieske B and Faber W (2000) A large-scale isolation of native β-lactoglobulin: Characterization of physicochemical properties and comparison with other methods. *International Dairy Journal* **10** 713–721.

Król J, Litwińczuk Z, Brodziak A and Barłowska J (2010) Lactoferrin, lysozyme and immunoglobulin G content in milk of four breeds of cows managed under intensive production system. *Polish Journal Veterinary Science* **13** 357–361.

Landau S, Perevolotsky A, Bonfil D, Barkai D and Silanikova N (2000) Utilisation of low quality resources by small ruminants in Mediterranian agro-pastoral system: The case of browse and aftermath cereal stubble. *Livestock Production Science* **64** 39–49.

Makkar H P S (2003) Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. *Small Ruminant Research* **49** 241–256.

Matassa S, Boon N, Pikar I and Verstraete W (2016) Microbial protein: Future sustainable food supply route with low environmental footprint. *Microbial Biotechnology* **9** 568–575.

Menci R, Natalello A, Luciano G, Priolo A, Valenti B, Farina G, Caccamo M, Niderkorn V and Coppa M (2021) Effect of dietary tannin supplementation on cow milk quality in two different grazing seasons. *Scientific Reports* **11** 19654.

Min B R, Attwood G T, Reilly K, Sun W, Peters J S, Barry T N and McNabb W C (2002) *Lotus corniculatus* condensed tannins decrease in vivo populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. *Canadian Journal of Microbiology* **48** 911–921.

Min B R, Barry T N, Attwood G T and McNabb W C (2003) The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. *Animal Feed Science and Technology* **106** 3–19.

Mueller-Harvey I, Bee G, Dohme-Meier F *et al*. (2019) Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. *Crop Science* **59** 861–885.

Naumann H D, Tedeschi L O, Zeller W E and Huntley N F (2017) The role of condensed tannins in ruminant animal production: Advances, limitations and future directions. *Revista Brasileira de Zootecnia* **46** 929–949.

Nousiainen J, Shingfield K J and Huhtanen P (2004) Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *Journal of Dairy Science* **87** 386–398.

Patra A K and Saxena J (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture* **91** 24–37.

Pruitt K M and Kamau D N (1994) Quantitative analysis of bovine lactoperoxidase system components and of the effects of the activated system on bacterial growth and survival. In *Indigenous Antimicrobial Agents of Milk*, pp. 73–87. Brussels: International Dairy Federation.

Rivera-Méndez C, Plascencia A, Torrentera N and Zinn R A (2016) Influence of tannins supplementation on growth performance, dietary net energy and carcass characteristics of yearling steers fed finishing diet containing dried distillers grains with solubles. *Indian Journal of Animal Science* **86** 108–111.

Rochfort S and Panozzo J (2007) Phytochemicals for health, the role of pulses. *Journal of Agricultural and Food Chemistry* **55** 7981–7994.

Rojas-Román L A, Castro-Pérez B I, Estrada-Angulo A, Angulo-Montoya C, Yocupicio-Rocha J A, López-Soto M A, Barreras A, Zinn R A and Plascencia A (2017) Influence of long-term supplementation of tannins on growth performance, dietary net energy and carcass characteristics: Finishing lambs. *Small Ruminant Research* **153** 37–141.

Tolve R, Galgano F, Condelli N, Cela N, Lucini L and Caruso M C (2021) Optimization model of phenolics encapsulation conditions for biofortification in fatty acids of animal food products. *Food* **10** 881.

Toral P G, Hervás G, Bichi E, Belenguer Á and Frutos P (2011) Tannins as feed additives to modulate ruminal biohydrogenation: Effects on animal performance, milk fatty acid composition and ruminal fermentation in dairy ewes fed a diet containing sunflower oil. *Animal Feed Science and Technology* **164** 199–206.

Turner S-A, Waghorn G C, Woodward S L and Thomson N A (2005) Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) affect the detailed composition of milk from dairy cows. *Proceedings-New Zealand Society of Animal Production* **65** 283.

Van Soest P J, Robertson J B and Lewis B A (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74** 3583–3597.

Vincenzetti S, Polidori P, Mariani P, Cammertoni N, Fantuz F and Vita A (2008) Donkey milk protein fractions characterization. *Food Chemistry* **106** 640–649.

Waghorn G (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Animal Feed Science and Technology* **147** 116–139.

Wang N, Hatcher D W, Toews R and Gawalko E J (2009) Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). *LWT-Food Science and Technology* **42** 842–848.

SUPPORTING INFORMATION

The following supporting information is available for this article:

Figure S1 Score Plot and Loading plot obtained from PCA.

Table S1 Starter cultures used in this study.
Table S2 Simple corresponding analyses results
Table S3 Analysis of contingency.
Table S4 Pearson's correlation coefficient.