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A diet supplemented with hazelnut skin changes the microbial community composition and the biohydrogenation pattern of linoleic acid in the rumen of growing lambs

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ABSTRACT

The reuse of by-products from the agro-industry in animal feeding is desirable to increase the sustainability of the productions. Hazelnut skin is a by-product of the food industry and due to the high content in crude fat and phenolic compounds can be potentially used in ruminants' diet. In this study, we characterised and compared the microbial communities of rumen digesta (RD) from lambs fed a diet supplied with hazelnut skin vs. a control diet. Microbial DNA was extracted and high-throughput sequencing of 16S rRNA gene amplicons was performed. Six genera ([*Eubacterium*] *nodatum* group, *Acidaminococcus*, *Dialister*, *Erysipelotrichaceae* UCG-002, *Megasphaera*, and *Olsenella*) showed a positive correlation with the concentration of C18:1 t10, and their relative abundance was higher in the RD from lambs fed the control diet, compared to the RD from lambs fed the diet supplied with hazelnut skin. The results suggest that the relative abundance of microorganisms correlated to C18:1 t10 production in the rumen of animals fed a concentrate-based diet can be reduced by the administration of hazelnut skin.

HIGHLIGHTS

- A diet with hazelnut skin affected the microbial community in the rumen of lambs
- The relative abundance of the genus *Dialister* decreased with hazelnut skin
- Administration of hazelnut skin can be a strategy to reduce the C18:1 t10/t11 ratio

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Introduction

The use of alternative human-inedible feed resources is desirable for sustainable meat production. The use of agro-industrial by-products in animal feeding represents an excellent opportunity to shift towards a sustainable livestock system, with the added advantage to reduce the cost associated with their disposal (Correddu et al. 2020).

Hazelnut (*Corylus avellana* L.) kernels are a raw material widely used in the food industry, in particular for the production of sweets (Fallico et al. 2003). At the beginning of the production process, after the roasting phase, hazelnut skin is removed and represents a waste. Hazelnut skin is a by-product rich in crude fat and phenolic compounds (Caccamo et al.

2019). The main phenolic compounds in hazelnut skin are gallic acid, procyanidin dimers and trimers, flavan-3-ols, flavonols, and hydrolysable tannins, such as glansreginin A, B, and C (Del Rio et al. 2011). Phenolic compounds are plant secondary metabolites able to modulate the activity of rumen microbiota and to influence the biohydrogenation (BH) of unsaturated fatty acids in the rumen (Vasta et al. 2019). Linoleic acid (LA, C18:2 c9, c12) is a dietary fatty acid (FA) that can be biohydrogenated to stearic acid (SA, C18:0) through two different pathways: an isomerisation to rumenic acid (RA, C18:2 c9, t11) followed by a hydrogenation to vaccenic acid (VA, C18:1 t11), i.e. the t11 pathway; an isomerisation to C18:2 t10, c12 followed by hydrogenation to C18:1 t10, i.e. the t10 pathway (Meynadier et al. 2018). The presence of VA has been

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associated with health benefits (Wang et al. 2012). Conversely, the presence of C18:1 τ 10 might be linked to detrimental effects both on human health and on animal performances. Hence, a low concentration of this FA is desirable (Aldai et al. 2013; Frutos et al. 2020). Recently, the dietary administration of hazelnut skin has been investigated in dairy ewes (Campione et al. 2020) and lambs (Priolo et al. 2021). Both the studies observed a change of the BH pattern in the rumen, which reflected differences in FAs composition of the diets, but may also suggest possible effects of feeding hazelnut skin on rumen microbiota. Therefore, in this study the rumen microbiota of the lambs used in the feeding trial by Priolo et al. (2021) was characterised by high-throughput sequencing of 16S rRNA gene amplicons.

This study aimed to characterise the microbial communities and their changes in the rumen of growing lambs, fed a diet in which hazelnut skin was used as an ingredient (Priolo et al. 2021), and to correlate the presence of the main genera with the BH products.

Materials and methods

Feeding trial

The animals used in this study are the same used in the feeding trial described in Priolo et al. (2021). Full details about animals, diets, sampling, measurements at slaughter, and chemical analyses on feeds and meat were provided in Priolo et al. (2021). All the experimental procedures were accomplished following the European Union guidelines (2010/63/EU Directive).

Twenty-two Valle del Belice male lambs (2 months old, bodyweight $15.3 \pm \text{SD } 1.79$ kg) were allotted into two experimental groups. A control group (CON; 11 lambs) was fed a maize-barley based concentrate, while the other group (HS; 11 lambs) received the same diet of CON supplied with 150 g/kg dry matter (DM) of hazelnut skin, obtained after the roasting phase, as partial replacement of maize. After an adaptation period (5 days) the lambs were individually fed *ad libitum* with the respective diet. All the animals had free access to water. After 56 days of the experimental trial, all the animals were slaughtered, the whole ruminal content of each animal was collected within 10 min, instantly frozen in dry ice, and stored at -80°C until DNA extraction. Ruminal FAs profile was determined on the same samples by GC-FID analyses and data are described in Priolo et al. (2021).

DNA extraction, amplicons preparation, and sequencing

The samples for DNA extraction were thawed in ice and DNA was extracted using the PowerSoil[®] DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA, catalog number 12888-100) with the following modifications: 250 mg of rumen digesta (RD) were transferred to the PowerBead Tubes, 60 μL of Solution C1 were added, the samples were vortexed briefly and incubated at 70°C for 10 min. The samples were further homogenised with a Retsch MM300 disrupter (90 s at 30 cycles/s). The homogenisation was performed two additional times, samples were placed on ice for 1 min between homogenisation steps. Samples were centrifuged at $10,000 \times g$ for 30 s and the supernatant was transferred to a clean 2 mL tube. The next steps were performed using the Fast DNA Spin kit for soil (MP Biomedicals, Solon, OH, catalog number 116560-200): 250 μL of PPS were added and the protocol was continued using the manufacturer's instructions. DNA concentration was measured by an ND-1000 Spectrophotometer (NanoDrop Technologies, Labtech, Ringmer, UK). The DNA concentration, the 260/280 ratio, and the 260/230 ratio were 155 ± 7 ng/ μL , 2.5 ± 0.1 , 3.6 ± 0.4 (average \pm standard error), respectively. All samples were tested for amplification inhibition by sample dilution.

DNA concentration was normalised (2 ng/ μL) and the V3–V4 region of the 16S rRNA gene was amplified with Pro341F and Pro805R primers (Takahashi et al. 2014). Amplicons preparation and sequencing were performed at IGA Technology Services (Udine, Italy) by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA) using a 300 bp \times 2 paired-end protocol. Two samples from the control group were excluded from downstream analysis since $<1,000$ sequences each were obtained. The sequencing produced a total of 1,524,843 reads with an average of $76,242 \pm 6,169$ reads *per* sample (average \pm standard error).

Bioinformatic and statistic elaborations

Bioinformatic elaborations were performed in R 4.0.2 (R Core Team 2020) with the package DADA2 (Callahan et al. 2016), version 1.16.0. The first 21 bases were removed from both forward and reverse reads. Forward reads were truncated at 290 bases and reverse reads were truncated at 250 bases. The reads with expected errors higher than 1 were discarded. Specific error rates were estimated for the forward reads and the reverse reads and were used to infer the amplicon sequence variants (ASVs) on the

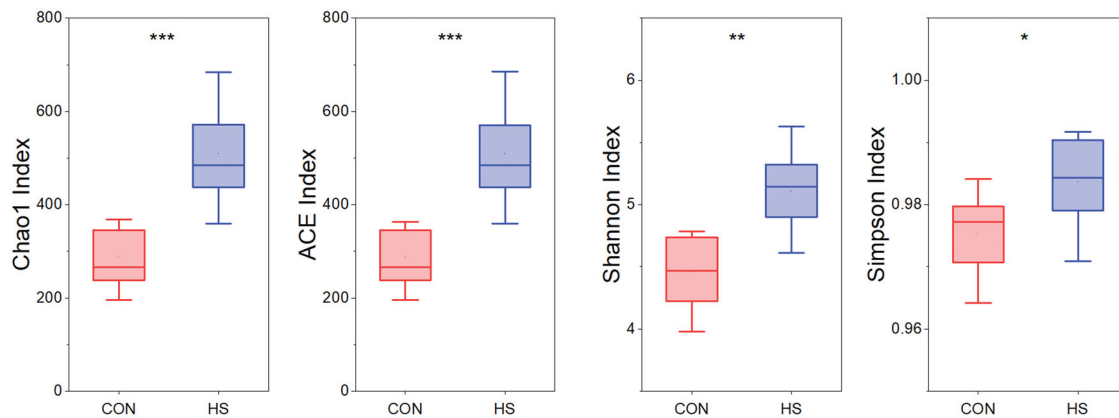


Figure 1. Diversity indexes calculated for ASV abundance. The microbial diversity was higher in the rumen of the lambs fed the diet with hazelnut skin (HS) compared to the control (CON). Significance codes: * $p < .05$; ** $p < .01$; *** $p < .001$.

dereplicated reads. The read pairs were merged with default parameters and chimeric sequences were removed. Taxonomic assignment for each ASV was performed against SILVA database v138 (Pruesse et al. 2007) with an 80% confidence. The ASVs with a relative abundance lower than 0.01% in all the samples were removed from the whole dataset. A total of 654,151 high-quality sequences were obtained with an average of $32,708 \pm 2,025$ sequences *per* sample (average \pm standard error).

The data were further processed using the vegan package, version 2.5.6 (Oksanen et al. 2019) in R 4.0.2 (R Core Team 2020). A randomly rarefied dataset (20,000 sequences *per* sample) was generated. The Chao1 index, the ACE index, the Shannon diversity index, and the Simpson index were calculated to estimate the alpha-diversity and a Kruskal-Wallis test was performed to detect significant differences between the conditions. A non-metric multidimensional scaling (NMDS) and a permutational multivariate analysis of variance (PERMANOVA) based on Hellinger transformed ASV abundance data were performed using the metaMDS and the adonis2 functions, respectively. Both the NMDS and the PERMANOVA were performed on the Bray-Curtis dissimilarity index. The taxa with a different relative abundance between the conditions were identified by a Kruskal-Wallis test. Differences were considered significant for $p < .05$ and with a trend towards significance for $.05 \leq p < .10$. Spearman correlations were performed to identify the genera correlated with the concentration of α -linolenic acid (ALA, C18:3 *c*9, *c*12, *c*15), oleic acid (OA, C18:1 *c*9), LA, and of its BH products (i.e. RA; VA; C18:2 *t*10, *c*12; C18:1 *t*10; SA; ratio C18:1 *t*10/C18:1 *t*11). Correlations were reported for $p < .1$ and were considered significant for $p < .05$. A trend towards significance was considered for $.05 \leq p < .10$.

Results

The microbial communities in the rumen of the lambs fed two different diets (with and without hazelnut skin) were characterised by high-throughput sequencing of 16S rRNA gene amplicons. Data about ruminal FAs profile and growth performance are described in Priolo et al. (2021). Rarefaction curves were obtained by plotting the number of ASVs detected vs. the number of sequences sampled and indicated that 20,000 sequences were enough to describe the biodiversity within the samples (Figure S1).

The diversity indexes calculated for the ASV abundance indicated a higher alpha-diversity in the RD of HS compared to CON (Figure 1). The difference was observed both in terms of richness (Chao1 and ACE indexes) and evenness (Shannon and Simpson indexes).

An NMDS plot showed that the microbial community in the RD of HS was different compared to the community in CON (Figure 2). This difference was further corroborated by the PERMANOVA ($R^2 = 0.17$, $p < .001$).

Eight classes represented $\sim 97\%$ of the taxa in the communities in both conditions (Figure S2). The three most abundant classes were: *Bacteroidia* ($\sim 40\%$ in CON and $\sim 43\%$ in HS), *Clostridia* ($\sim 21\%$ in CON and $\sim 25\%$ in HS), and *Negativicutes* ($\sim 16\%$ in CON and $\sim 14\%$ in HS). The classes *Bacilli* ($p < .01$) and *Saccharimonadia* ($p < .1$) were more abundant in the RD of CON (~ 6 and $\sim 2\%$, respectively) compared to HS ($\sim 1\%$ for both the classes). Conversely, the class *Clostridia* was more abundant ($p < .1$) in the RD of HS ($\sim 25\%$) compared to CON ($\sim 21\%$).

The most abundant genus in the RD in both conditions was *Prevotella* (Table 1). The second most abundant genus in CON was *Dialister* which accounted for $\sim 8\%$ of the sequences, while it was only $\sim 1\%$ in HS

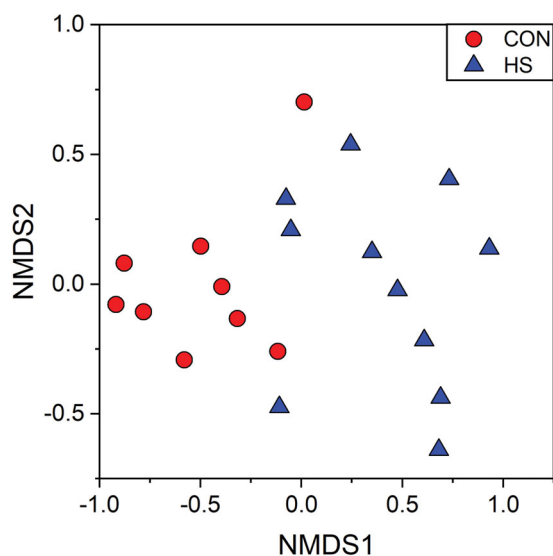


Figure 2. Non-metric multidimensional scaling plot based on Hellinger transformed ASV abundance data. The microbial community in the rumen of lambs fed the control diet (CON) was different compared to the microbial community in the rumen of lambs fed the diet with hazelnut skin (HS). Stress = 0.0862.

($p < .001$). The second most abundant genus in the RD of HS was *Succiniclasticum* (~5%), which only accounted for ~2% of the genera in CON ($p < .05$). Other 8 taxa ([*Eubacterium*] *nodatum* group, *Acidaminococcus*, *Candidatus* *Saccharimonas*, *Desulfovibrio*, *Erysipelotrichaceae* UCG-002, *Erysipelotrichaceae* UCG-007, *Megasphaera*, *Olsenella*) were more abundant in CON compared to HS (Table 1). In addition to the genus *Succiniclasticum* other 11 taxa (*Acetitomaculum*, *Anaerovibrio*, *Butyrivibrio*, *Christensenellaceae* R-7 group, *Lachnospiraceae* ND3007 group, NK4A214 group, *Prevotellaceae* UCG-003, *Pseudobutyrvibrio*, *Quinella*, *Selenomonas*, *Veillonellaceae* UCG-001) showed a higher abundance in the RD of HS compared to CON.

To detect the microorganisms potentially involved in the BH of LA in the rumen of the lambs fed the two diets, the genera with a relative abundance of 1%, or higher, in at least one sample were correlated with the concentration of ruminal LA, and its BH products, using the data provided by Priolo et al. (2021). The genera *Acetitomaculum*, *Anaerovibrio*, *Butyrivibrio*, *Kandleria*, *Pseudobutyrvibrio*, *Quinella*, *Selenomonas*, *Succiniclasticum*, the [*Ruminococcus*] *gavreauii* group, the *Christensenellaceae* R-7 group, the *Lachnospiraceae* ND3007 group, the NK4A214 group, and an uncultured member of the family *Veillonellaceae* (*Veillonellaceae* UCG-001) had a positive correlation with the concentration of VA in the rumen (Figure 3). None of the 13 genera with a positive correlation with

the concentration of VA in the rumen was correlated with the concentration of C18:1 ι 10. Nine genera ([*Eubacterium*] *nodatum* group, *Acidaminococcus*, *Dialister*, *Erysipelotrichaceae* UCG-002, *Lachnospiraceae* NK3A20 group, *Megasphaera*, *Methanobrevibacter*, *Mitsuokella*, *Olsenella*) were positively correlated with the concentration of C18:1 ι 10. Five genera ([*Eubacterium*] *nodatum* group, *Acidaminococcus*, *Dialister*, *Erysipelotrichaceae* UCG-002, and *Megasphaera*) out of the nine genera with a positive correlation with the concentration of C18:1 ι 10 were negatively correlated with the concentration of VA. Interestingly, the genus *Olsenella* and the five genera with a positive correlation with the concentration of C18:1 ι 10 and with a negative correlation with the concentration of VA, showed also a higher relative abundance in the RD of CON. Furthermore, within the genera with a positive correlation with the concentration of VA, 11 genera showed a higher relative abundance in the RD of HS. Further correlations were performed to elucidate the possible role of OA and ALA in the production of C18:1 *trans*-isomers. Eleven taxa (i.e. *Acetitomaculum*, *Anaerovibrio*, *Butyrivibrio*, *Christensenellaceae* R-7 group, *Lachnospiraceae* ND3007 group, NK4A214 group, *Pseudobutyrvibrio*, *Quinella*, *Selenomonas*, *Succiniclasticum*, and *Veillonellaceae* UCG-001) out of the 12 taxa with a higher relative abundance in the RD of the lambs fed the diet with hazelnut skin had a positive correlation with the concentration of OA. Only four genera (i.e. *Acidaminococcus*, *Dialister*, *Olsenella*, and *Roseburia*) were positively correlated with the concentration of ALA.

Discussion

The inclusion of hazelnut skin in growing lambs' diet has been evaluated (Priolo et al. 2021). The diet with hazelnut skin increased the feed conversion ratio but did not affect the dry matter intake, the final body weight, the carcass weight, and the average daily gain (Priolo et al. 2021). Furthermore, the inclusion of hazelnut skin led to the enrichment of intramuscular fat with health-promoting FAs, such as VA and polyunsaturated FAs (Priolo et al. 2021). A higher content of VA and of SA was observed in the RD of the lambs fed the diet with the inclusion of hazelnut skin (Priolo et al. 2021). In particular, Priolo et al. (2021) hypothesised that the higher content of VA in the RD of lambs given the diet with hazelnut skin was due to a higher intake of LA with the diet. Furthermore, since the content of RA, which is produced by isomerisation from

Table 1. Genera with a relative abundance of 1% (or higher) in at least one sample in the rumen of lambs fed the control diet (CON) compared to the rumen of the lambs fed the diet with hazelnut skin (HS).

Genus	Class	CON		HS		p-Value
		Average (%)	Standard error	Average (%)	Standard error	
[<i>Eubacterium</i>] <i>nodatum</i> group	<i>Clostridia</i>	0.51	0.24	0.12	0.03	.040*
[<i>Eubacterium</i>] <i>ruminantium</i> group	<i>Clostridia</i>	0.74	0.26	0.92	0.16	.362
[<i>Ruminococcus</i>] <i>gauvreauii</i> group	<i>Clostridia</i>	0.36	0.09	0.61	0.18	.518
<i>Acetitomaculum</i>	<i>Clostridia</i>	0.12	0.04	0.66	0.13	.002**
<i>Acidaminococcus</i>	<i>Negativicutes</i>	0.69	0.24	0.07	0.04	.001**
<i>Anaerovibrio</i>	<i>Negativicutes</i>	0.24	0.07	1.21	0.27	.012*
<i>Bifidobacterium</i>	<i>Actinobacteria</i>	0.01	0.01	0.23	0.23	.254
<i>Butyrivibrio</i>	<i>Clostridia</i>	0.22	0.14	1.40	0.48	.003**
<i>Candidatus Saccharimonas</i>	<i>Saccharimonadia</i>	1.54	0.36	0.83	0.22	.095
<i>Christensenellaceae</i> R-7 group	<i>Clostridia</i>	0.21	0.09	1.03	0.31	.007**
<i>Desulfovibrio</i>	<i>Desulfovibrionia</i>	0.43	0.12	0.10	0.02	.003**
<i>Dialister</i>	<i>Negativicutes</i>	7.66	1.22	1.48	0.59	<.001***
<i>Erysipelotrichaceae</i> UCG-002	<i>Bacilli</i>	1.61	0.54	0.03	0.01	.002**
<i>Erysipelotrichaceae</i> UCG-007	<i>Bacilli</i>	1.57	0.57	0.05	0.05	.001**
<i>Erysipelotrichaceae</i> UCG-009	<i>Bacilli</i>	1.51	0.54	0.46	0.12	.239
<i>Fibrobacter</i>	<i>Fibrobacteria</i>	0.84	0.48	1.53	0.58	.184
<i>Kandleria</i>	<i>Bacilli</i>	0.01	0.01	0.13	0.12	.574
<i>Lachnospira</i>	<i>Clostridia</i>	0.29	0.13	0.11	0.05	.138
<i>Lachnospiraceae</i> ND3007 group	<i>Clostridia</i>	0.02	0.01	0.89	0.46	.022*
<i>Lachnospiraceae</i> NK3A20 group	<i>Clostridia</i>	6.04	1.67	3.45	0.65	.184
<i>Megasphaera</i>	<i>Negativicutes</i>	1.71	0.38	0.08	0.03	<.001***
<i>Methanobrevibacter</i>	<i>Methanobacteria</i>	0.31	0.17	0.17	0.06	.381
<i>Mitsuokella</i>	<i>Negativicutes</i>	0.27	0.12	0.09	0.04	.135
NK4A214 group	<i>Clostridia</i>	0.43	0.16	1.49	0.29	.002**
<i>Olsenella</i>	<i>Coriobacteria</i>	0.66	0.26	0.23	0.06	.057
<i>Oribacterium</i>	<i>Clostridia</i>	0.82	0.27	0.67	0.13	.849
<i>Phascolarctobacterium</i>	<i>Negativicutes</i>	0.03	0.03	0.27	0.26	.803
<i>Prevotella</i>	<i>Bacteroidia</i>	22.79	1.57	18.87	1.53	.119
<i>Prevotellaceae</i> UCG-001	<i>Bacteroidia</i>	1.43	0.35	2.39	0.63	.271
<i>Prevotellaceae</i> UCG-003	<i>Bacteroidia</i>	0.35	0.09	0.60	0.10	.087
<i>Prevotellaceae</i> UCG-004	<i>Bacteroidia</i>	0.50	0.17	1.10	0.25	.102
<i>Pseudobutyrvibrio</i>	<i>Clostridia</i>	0.06	0.04	0.70	0.20	<.001***
<i>Quinella</i>	<i>Negativicutes</i>	N.D. ^a	N.D.	1.63	0.88	.005**
<i>Rikenellaceae</i> RC9 gut group	<i>Bacteroidia</i>	3.64	0.46	4.77	0.91	.425
<i>Roseburia</i>	<i>Clostridia</i>	0.24	0.14	0.08	0.04	.383
<i>Ruminococcus</i>	<i>Clostridia</i>	3.28	0.79	2.38	0.47	.470
<i>Schwartzia</i>	<i>Negativicutes</i>	0.21	0.10	0.39	0.14	.183
<i>Selenomonas</i>	<i>Negativicutes</i>	0.12	0.05	0.91	0.39	.066
<i>Succiniclasticum</i>	<i>Negativicutes</i>	1.72	0.62	4.99	1.32	.025*
<i>Succinivibrio</i>	<i>Gammaproteobacteria</i>	4.36	1.63	2.76	0.98	.790
<i>Succinivibrionaceae</i> UCG-001	<i>Gammaproteobacteria</i>	5.89	2.16	4.98	2.35	.271
<i>Succinivibrionaceae</i> UCG-002	<i>Gammaproteobacteria</i>	N.D.	N.D.	0.21	0.14	.189
<i>Treponema</i>	<i>Spirochaetia</i>	1.13	0.26	2.50	0.90	.425
<i>Veillonellaceae</i> UCG-001	<i>Negativicutes</i>	0.48	0.31	1.56	0.56	.042*
Other Genera		2.94	0.22	3.58	0.34	
Unclassified		21.99	1.53	27.31	1.60	

^aN.D.: not detected (i.e. relative abundance = 0%). Significance codes: ·*p* < .1; **p* < .05; ***p* < .01; ****p* < .001.

LA in the first step of BH, was similar in the rumen of the animals fed the two diets, it was hypothesised that a reduction of the saturation of VA to SA occurred when hazelnut skin was added. In the present study, the microbial communities in the RD of the lambs in the feeding trial conducted by Priolo et al. (2021) have been characterised to shed light on the microbial diversity and the possible role of the main microbial groups in the BH pathway. The aim was to understand if the changes observed by Priolo et al. (2021) in the BH pathway of LA were correlated to changes in the composition of rumen microbiota.

The presence of hazelnut skin in the diet influenced the microbial community in the rumen both in terms

of alpha- and beta-diversity. Changes in the composition of rumen microbiota have been observed both when condensed (Buccioni et al. 2017) and hydrolysable tannins (Mannelli et al. 2019) were supplied. But it has been demonstrated that the inhibitory effect on rumen metabolism and BH is stronger with condensed tannins compared to hydrolysable tannins (Costa et al. 2018). Our data showed that several bacterial genera had a different relative abundance in the RD of the lambs fed the two diets. The data reported in Priolo et al. (2021) suggested that the BH of LA occurred by 't10 shift' (the ratio C18:1 t10/C18:1 t11 was higher than 1 in both the conditions) regardless of the feeding group, but despite the content of C18:1 t10 was

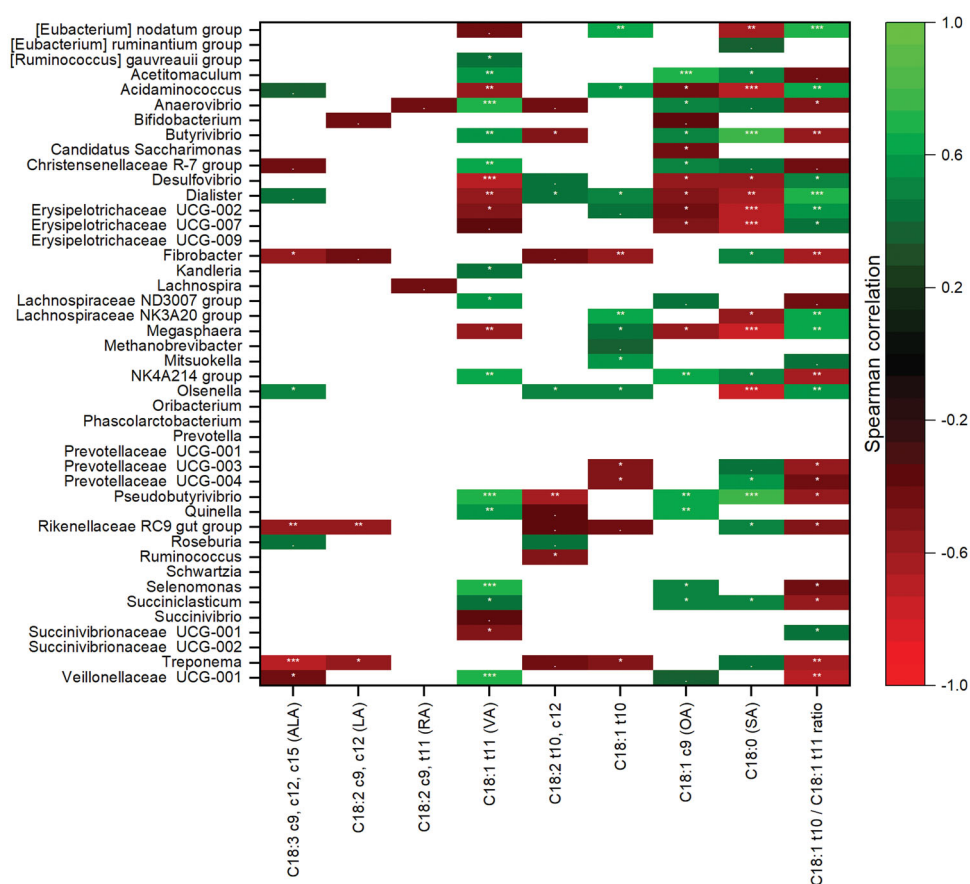


Figure 3. Spearman correlations between the detected microorganisms and the concentration of α -linolenic acid (ALA, C18:3 c9, c12, c15), oleic acid (OA, C18:1 c9), linoleic acid (LA, C18:2 c9, c12) and its BH products. Only correlations with $p < 0.1$ were reported. Only the genera with a relative abundance of 1%, or higher, in at least one sample were considered. The genera with a positive correlation with the concentration of vaccenic acid (VA, C18:1 t11) were different from the genera with a positive correlation with C18:1 t10. Significance codes: $\cdot p < .1$; $\ast p < .05$; $\ast\ast p < .01$; $\ast\ast\ast p < .001$.

not different in the RD of lambs that received the control diet (without hazelnut skin) and in the RD of lambs that received the diet with hazelnut skin, a tendency to a higher C18:1 t10/t11 ratio was observed when the control diet was supplied (Priolo et al. 2021). Our data highlighted that the relative abundance of 11 bacterial genera within the ones that positively correlated with the concentration of VA (i.e. *Acetitomaculum*, *Anaerovibrio*, *Butyrivibrio*, *Christensenellaceae* R-7 group, *Lachnospiraceae* ND3007 group, NK4A214 group, *Pseudobutyrvibrio*, *Quinella*, *Selenomonas*, *Succiniclasticum*, and *Veillonellaceae* UCG-001) was higher in the RD of HS, while the relative abundance of the genera positively correlated with the concentration of C18:1 t10 (i.e. [*Eubacterium*] *nodatum* group, *Acidaminococcus*, *Dialister*, *Erysipelotrichaceae* UCG-002, *Megasphaera*, and *Olsenella*) was higher in the RD of CON. In particular, the relative abundance of the genus *Dialister* was significantly higher in the RD of CON compared to HS. The presence of the genus *Dialister* has been

previously correlated with the production of C18:1 t10 in goats (Dewanckele et al. 2018) and dairy cows (Dewanckele et al. 2019). The same studies correlated with a higher concentration of C18:1 t10 also the presence of the genera *Megasphaera*, *Eubacterium*, *Sharpea* (family *Erysipelotrichaceae*) (Dewanckele et al. 2018), and *Acidaminococcus* (Dewanckele et al. 2019). Furthermore, also the involvement of the genus *Olsenella* in the production of C18:1 t10 has been suggested (Dewanckele et al. 2020). Despite further studies are needed to demonstrate the involvement of these genera in the BH of LA throughout the C18:1 t10 pathway, our data suggest that a change in the BH of LA occurred when hazelnut skin was supplied in the diet of growing lambs. The change in the BH pathway was driven by a change in the composition of the microbial communities in the rumen, and the genus *Dialister* seemed to be a key player. However, the involvement of OA and ALA in the C18:1 t10 production in this study cannot be ruled out. The concentration of OA in RD of HS was higher compared to

CON (Priolo et al. 2021). Oleic acid can be converted into several *trans* isomers (Mosley et al. 2002) and was thus hypothesised that VA in RD of HS could be produced also by isomerisation of OA (Priolo et al. 2021). This result was supported also by this study, since all the taxa that correlated positively with the concentration of OA, had a positive correlation also to the concentration of VA. The C18:1 t10/C18:1 t11 ratio could be influenced also by the BH of ALA, since the four genera with a positive correlation with the concentration of ALA showed also a positive correlation with the concentration of C18:1 t10 and/or with the concentration of the intermediate C18:2 t10, c12.

Conclusions

The effect of dietary administration of hazelnut skin on the microbial communities in the rumen of growing lambs was investigated by high-throughput sequencing of 16S rRNA gene amplicons. The microbial communities in the rumen of the lambs fed the diet with hazelnut skin were different compared to the microbial communities in the rumen of the lambs fed the control diet. In the rumen of the lambs fed the diet with hazelnut skin the relative abundance of microorganisms positively correlated to the concentration of VA was higher. Conversely, the relative abundance of bacterial taxa positively correlated to the concentration of C18:1 t10 was higher in the rumen of the lambs fed the control diet. The results presented in this study, together with those obtained by Priolo et al. (2021), suggest that the dietary administration of hazelnut skin may be a promising strategy to reduce the C18:1 t10/t11 ratio in the rumen of animals fed a concentrate-based diet.

Ethical approval

The experimental plan was approved by "Organismo Preposto al Benessere degli Animali (OP BA)" of the University of Catania. Protocol number: 15295.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Nucleotide sequence accession number

The sequences are available at the National Center for Biotechnology Information (NCBI), BioProject number PRJNA714514, under the following SRA BioSample accession numbers: SAMN18310697–SAMN18310716.

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