Animal 18 (2024) 101149

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Rumen fermentation parameters and papillae development in Simmental growing bulls with divergent residual feed intake

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ARTICLE INFO

Article history:
Received 22 November 2023
Revised 22 March 2024
Accepted 25 March 2024
Available online 3 April 2024

Keywords:
Beef cattle
Feed efficiency
Fermentative traits
Histo-morphometry
Ruminal microbiota

ABSTRACT

Residual feed intake (**RFI**), a widespread index used to measure animal feed efficiency, is influenced by various individual biological factors related to inter-animal variation that need to be assessed. Herein, 30 Simmental bulls, raised under the same farm conditions, were divided on the basis of RFI values into a high efficient group (**HE**, RFI = − 1.18 ± 0.33 kg DM/d, n = 15) and a low efficient group (**LE**, RFI = 0.92 ± 0.35 kg DM/d, n = 15). Subsequently, bulls were slaughtered at an average BW of 734 ± 39.4 kg. Their ruminal fermentation traits were analysed immediately after slaughtering and after 24 h of *in vitro* incubation. Furthermore, ruminal micro-biota composition and ruminal papillae morphology were examined. The LE group exhibited a higher propionate concentration as a percentage of total volatile fatty acids (17.3 vs 16.1%, $P = 0.04$) in the rumen fluid collected during slaughtering, which was also confirmed after *in vitro* fermentation (16.6 vs 15.4% respectively for LE and HE, $P = 0.01$). This phenomenon resulted in a significant alteration in the acetate−to−propionate ratio (**A:P**) with higher values for the HE group, both after slaughter (4.01 vs 3.66, $P = 0.02$) and after *in vitro* incubation (3.78 vs 3.66, $P = 0.02$). Methane production was similar in both groups either as absolute production (227 vs 218 mL for HE and LE, respectively) or expressed as a percentage of total gas (approximately 22%). Even if significant differences ($P < 0.20$) in the relative abundance of some bacterial genera were observed for the two RFI groups, no significant variations were observed in the alpha (Shannon index) and beta (Bray–Curtis index) diversity. Considering the papillae morphology, the LE subjects have shown higher length values (6.26 vs 4.90 mm, $P < 0.01$) while HE subjects have demonstrated higher papillae density (46.4 vs 40.5 n/cm^2, $P = 0.02$). Histo-morphometric analysis did not reveal appreciable modifications in the total papilla thickness, boundaries or surface between the experimental groups. In conclusion, our results contribute to efforts to analyse the factors affecting feed efficiency at the ruminal level. Propionate production, papillae morphology and a few bacterial genera certainly play a role in this regard, although not a decisive one.

Implications

Given the significant impact of animal efficiency in the livestock farming sector from an economic and environmental viewpoint, the investigation of the main causes that influence this important characteristic plays a fundamental role. Alteration of the fermentative pathways at the ruminal level could be one of the possible factors. The outcomes suggest that propionate production and the relative abundance of some bacterial genera are involved. Moreover, variation in papillae morphology suggests that end-product absorption ability is another aspect related to animal feed efficiency.

Introduction

In growing animals, the residual feed intake (**RFI**) is an index of feed efficiency calculated by the difference between the actual and predicted intake from BW and daily gain (Koch et al., 1963). Efficient animals are characterised by a negative RFI value because they consume less feed compared with that estimated from their performance. Economically, feed consumption represents the greatest cost item, significantly affecting the livestock sector. Furthermore, feed consumption is closely related to environmental concerns (excretion, land use and water pollution) and, consequently, to climate change issues (Makkar and Beeve, 2013). Despite moderate hereditability (Berry and Crowley, 2012), RFI is an attractive trait to investigate in response to the challenges of the agricultural sector.

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https://doi.org/10.1016/j.animal.2024.101149
1751-7311/

Overall, a relevant fraction of inter-subject variability in RFI could be related to the biological processes that occur during digestion (Herd et al., 2004) and, in ruminant species, to rumen fermentation. Changes in ruminal micro-biota and the adsorption capacity of end-products, e.g. volatile fatty acids (**VFAs**), are key factors in feed efficiency. End-product uptake is closely associated with the fermentation process, and several studies (Bannik et al., 2008; Malhi et al., 2013; Lam et al., 2018; Johnson et al., 2019) have demonstrated a correlation between VFAs, particularly butyrate, and the development of the rumen papillae. These affect the uptake capacity of the rumen, representing the surface available for substance exchange (Gäbel et al., 2002). The production of VFA influences not only rumen morphology but also other biological processes such as methanogenesis. Methane production causes a significant energy loss during the digestive process in cattle, approximately from 6 to 12% (Johnson and Johnson, 1995), and consequently leads to a reduction in animal feed efficiency, as shown in previous *in vivo* studies conducted on dairy cows (Yan et al., 2010; Olijhoek et al., 2018).

RFI is the result of various metabolic processes and, at the ruminal level, the most important factors are the type of fermentation and strength of absorption. Each of them makes a substantial contribution; therefore, it is useful to assess these factors simultaneously. In the current study, we focused on multiple aspects, including fermentation patterns, methane production, rumen bacteria composition and ruminal papillae morphology, in two groups of growing Simmental bulls with divergent RFI to concurrently identify the factors affecting RFI.

Material and methods

Animals and experimental design

The study was conducted on young Simmental bulls between February 2021 and March 2022 at the Italian Simmental Breeders Association Genetic Centre, Fiume Veneto (45° 55′ 8.654″ N, 12° 43′ 23.32″ E). In a study conducted in the same centre, Romanzin et al. (2021) accurately described how the animals were managed before the performance test. All bulls received the same total mixed ratio composed of ground corn (28.2% DM), corn silage (27.2% DM), sunflower and rapeseed meals (14.6% DM), wheat straw (8.7% DM), barley (5.9% DM), dried beet pulp (5.8% DM), wheat bran (5.8% DM), soybean meal (1.9% DM) and a mix of minerals and vitamins (1.9% DM) twice a day. Animal RFI was assessed as previously detailed in Romanzin et al. (2021). In brief, young bulls with an average age of 9 months were moved to pens (six animals for each) equipped with two electronic feeders (RIC system; Hokofarm Group, Marknesse, The Netherlands) to record the individual daily feed intake. The animals were weighed every 42 days. The feed conversion (**FC**) rate was calculated as dry matter intake (**DMI**) divided by the average daily gain (**ADG**). RFI was calculated as the difference between actual DMI and expected DMI (**eDMI**) calculated using the following multiple linear regression:

$$\text{eDMI} = \alpha + \beta\ (\text{ADG}) + \gamma\ \left(\text{BW}^{0.75}\right) + \varepsilon$$

where BW is the mid-test BW; α, β and γ are parameters of the regression equation; ε is the error term.

A total of 230 animals were evaluated according to their RFI. Excluding animals used for reproductive purposes (approximatively 35%), 30 healthy bulls with extreme RFI values were chosen to create two groups with divergent RFI values: high efficient group (**HE**, RFI = − 1.18 ± 0.33 kg DM/d, n = 15) and low efficient group (**LE**, RFI = 0.92 ± 0.35 kg DM/d, n = 15). Subsequently, bulls were raised under the same conditions before being slaughtered at an average BW and age of 734 ± 39.4 kg and 17.1 ± 0.85 months. Animals were last fed 12 h before being transported to the slaughterhouse and had free access to fresh water until slaughter.

Measurements and sample collection at the slaughterhouse

The weights of the hot carcasses were recorded approximately 1 h *postmortem*, and the dressing percentage was calculated as (hot carcass weight/slaughter weight) × 100. Immediately after slaughter, rumen fluid samples were collected from each bull for the determination of fermentative parameters (pH, protozoa counts and VFA concentration) and micro-biota evaluation (DNA sequencing). Simultaneously, samples of rumen wall tissues were taken from the dorsal sac for histo-morphometric analysis. Finally, 500 mL of ruminal fluid was collected from each animal for the *in vitro* trial, stored separately in airtight glass bottles refluxed with CO_2 and maintained at 39 °C until use.

In vitro fermentation experiment

To measure methane production and fermentative characteristics, an *in vitro* experiment was set up as reported by Braidot et al. (2023a). In brief, the rumen fluid collected from each bull was filtered using a cheesecloth layer and diluted with the buffer solution proposed by Menke et al. (1979) (1:2 ratio, v/v). The buffered rumen fluid was divided (500 mL) into two fermenters for each animal and incubated for 24 h in a water bath at 39 °C. The same total mixed ratio used for bull feeding was adopted as the substrate (3 300 mg as DM) in all fermentation trials. Before use, the substrate was dried at 60 °C for 48 h and subsequently ground to a particle size of approximately 0.5 mm. The gas generated during fermentation was quantified using a gas counter (Ritter Apparatebau GmbH & Co. KG), and the methane concentration was detected using an infra-red gas analyser sensor (RI. Sens mono IR1, Ritter Apparatebau GmbH & Co. KG). The final pH was directly measured in the fermenters using a pH-metre (GLP 22, Crison Instruments, S. A. Barcelona, Spain), while fermented fluid samples were collected for NH_3 (10 mL) and VFA (5 mL added with H_2SO_4 0.1 N, 1:1 ratio v/v) determination. Samples were preserved at −20 °C until use.

Analysis of samples collected from the rumen or at the end of the fermentation trial

The NH_3 concentration in samples from the *in vitro* trial was assessed using an Ammonia Gas Sensing Combination Electrode (Hach Company, Colorado, USA). The VFA concentration in rumen fluid collected during slaughter or after the *in vitro* experiment was determined as reported by Braidot et al. (2023b). Briefly, samples were centrifuged at 20 000 g for 20 min at 4 °C, and the supernatant was filtered using polypropylene filters (pore diameter, 0.45 μm). The filtrate was injected into HPLC, and the chromatogram was cross-checked with the outcomes of a standard mixture.

Rumen microbial population determination

Immediately after collection, 5 mL of ruminal fluid was mixed with 18.5% formaldehyde (1:1 ratio, v/v) to quantify protozoa, as described by Dehority (2003). Ruminal micro-biota were determined using 16 s genomic sequencing. The DNA extraction procedure, primer used for the amplification procedure and the protocol used to obtain the final raw sequence, were reported by Spanghero et al. (2023). Microorganisms were identified using the Greengenes (v13-8) and Silva (v.138) databases. Finally, after microorganism determination, the indices that describe alpha (Shannon) and beta (Bray–Curtis) diversity for the RFI groups were calculated in the R

environment using the appropriate function of the Vegan package (v. 2.5–7; Oksanen et al., 2015).

Tissue sampling and histo-morphometric analysis

During the slaughtering process, a tissue sample of the rumen (approximately 40 cm^2) was collected from the dorsal sac of each bull. The sample was divided into two parts: one was kept in a physiological solution and the other was pinned to a polystyrene stand to avoid tissue shrinkage while being preserved in 4% (v/v) buffered formaldehyde solution.

The first portion of the tissue sample was used to macroscopically determine the length, width and density of the papillae. This last aspect was evaluated in three different points of ruminal tissue (1 cm^2 each), and from each one, 15 papillae (excluding damaged ones) were randomly taken. After collection, their length and width were measured using a stereomicroscope (Wild M8, Leica). The second tissue sample was used to assess the thickness of the rumen epithelial layer. Two portions were trimmed from each fixed rumen sample, taking care not to damage the papillae. These portions were processed automatically (Tisbe, DiaPath) and embedded in paraffin (Canova, Diapath). Paraffin sections, obtained using a rotary microtome (Leica Biosystems 2135), were stained with haematoxylin–eosin, and two digital slides were acquired using an Aperio CS scanner (Leica, model AT2) at 5X magnification.

For each digital slide, the first three papillae were chosen, starting from the left edge, resulting in six papillae for each bull. Within each papilla, three different regions (apical, middle and basal) with a length of 1 000 µm were identified (Fig. 1A). At four different points (250, 500, 750 and 1 000 µm from the distal edge, Fig. 1B) the overall thickness and that of the *lamina propria* and epithelium + *lamina propria* were measured (adapted from Dieho et al., 2016). The *stratum corneum* and epithelium thicknesses were calculated by subtracting the other measurements. In the apical region, the *stratum corneum* luminal boundary length (total papilla) and *stratum basale* boundary length (*lamina propria*) were measured (Fig. 1C). Subsequently, the ratio between the two boundaries was calculated. Finally, the total papillae and *lamina propria* surface were assessed. The epithelium + *stratum corneum* surface was derived from other measurements. All histo-morphometric measurements were performed using the Aperio Imagescope software (version 12.4.0.05043).

Statistical analysis

The data were statistically analysed using the GLM procedure of SAS software (version 9.4; SAS Institute) using a factorial model that considered RFI as a fixed factor. For the *in vitro* fermentative traits, the ruminal inoculum collected from each bull was tested twice (analytical replicates) within each fermentation trial, and the outcomes were averaged before statistical analysis (statistical replicates). The models adopted for data analysis have been carefully reported in Supplementary Material (S1). The data derived from genome sequencing were analysed using the R environment (v. 4.1.3) and the vegan package (v. 2.5–7; Oksanen et al., 2015).

Results

Feed intake and animal performance

Table 1 reports the BW, DMI and growth performance of the animals. At the end of the feed control trial, bulls were slaughtered at an average age of 17.0 and 17.3 months with a final BW of 742 and 724 kg for HE and LE, respectively, with no significant differences between the groups. In addition, ADG was similar in both groups. As expected, RFI was significantly different ($P < 0.01$) between the two experimental groups, with values of −1.18 and 0.92 kg DM/d for HE and LE, respectively. Furthermore, the LE group showed higher DMI (11.0 vs 9.40 kg DM/d, $P < 0.01$) and FC (8.07 vs 6.15 kg DM/kg, $P < 0.01$) values. Considering the post-mortem performance, no significant differences were observed between the groups regarding carcass weight (401 and 389 kg,

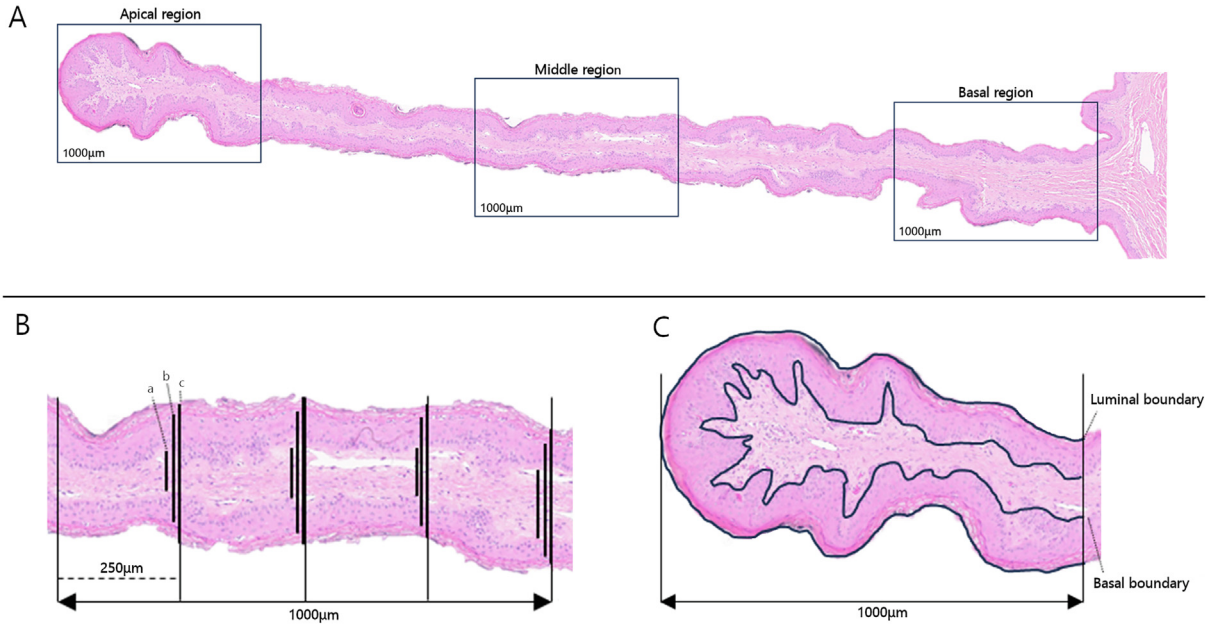

Fig. 1. Micrograph showing the different regions identified in the ruminal papilla of Simmental bulls (A). The *lamina propria* (a), epithelium + *lamina propria* (b), and the overall thickness (c) were measured for each region at different points (B). Basal boundary and luminal boundary lengths were measured in the apical region of the ruminal papilla (C).

Table 1
Growth performance, feed intake, and slaughter performance of 30 Simmental bulls with high and low feed efficiency.

	Feed efficiency			
Item	High	Low	RMSE	*P*-value
Age at slaughter (month)	17.0	17.3	0.63	0.35
Final BW (kg)	742	724	38.4	0.40
ADG (kg/d)	1.57	1.40	0.25	0.13
DMI (kg DM/d)	9.40	11.0	0.66	<0.01
FC (kg DM/kg)	6.15	8.07	0.99	<0.01
RFI (kg DM/d)	−1.18	0.92	0.34	<0.01
Carcass weight (kg)	401	389	21.3	0.12
Dressing out (%)	54.5	53.8	1.83	0.32

Abbreviations: ADG = average daily gain; DMI = DM intake; FC = feed conversion ratio; RFI = residual feed intake.

for HE and LE, respectively) or dressing-out proportion (54.5 and 53.8%, for HE and LE, respectively).

Rumen liquor and in vitro fermentation results

The main characteristics of the rumen fluid collected at the slaughterhouse are reported in Table 2. The pH and total protozoa number between the two groups did not differ. Similarly, total VFA production did not differ between the groups, whereas propionate concentration as a percentage of total VFAs was higher in the LE group (17.3 vs 16.1%, P = 0.04). Consequently, the acetate−to−propionate ratio (**A:P**) increased for the HE group (4.01 vs 3.66, P = 0.02), and no other modifications were observed in the VFA profile.

Table 3 shows the results achieved after the *in vitro* incubation of the rumen liquid collected at slaughter from each bull. After 24 h of fermentation, the total gas recorded in the *in vitro* fermentation was similar for both groups (1 044 vs 983 mL). Overall, both RFI groups generated approximately 22% of methane in total gas, with no variation in its absolute production (227 vs 218 mL, for HE and LE respectively). Furthermore, the feed efficiency of bulls did not influence ammonia and pH levels after the incubation period. The total amount of VFAs produced during fermentation was similar in the two experimental groups, but their composition differed accordingly with variations in the rumen fluids collected during slaughter. There was a significant difference in propionate production, with a lower value for the HE group than that for the LE group (15.4 vs 16.6%, P < 0.01). As a result, the A:P ratio increased for the HE group (3.78 vs 3.66, P = 0.02).

Meta-genomic analysis

A total of 150 genera were identified in the rumen fluid samples, with 87 being present in both groups and in at least 50% of the samples analysed. The 10 most abundant genera dominated the rumen microbial community, accounting for 64.6% of the detected genera (data not shown). *Prevotella* was the most abundant genus in the rumen community, with a mean relative abundance of 33.6%. Other abundant genera included Rikenellaceae_RC9_gut_group (4.85%), Bacteroidales_RF16_group (3.92%) and *Methanobrevibacter* (3.88%).

To assess the alpha diversity, the Shannon index was calculated for both groups, and the results are shown in Fig. 2. Both classes of feed efficiency exhibited a high richness in microbial species, but no significant difference was observed between the groups with values of 5.56 and 5.62 for HE and LE, respectively. For beta diversity analysis, the Bray–Curtis dissimilarity was tested, and the outcomes of the PCoA analysis are shown in Fig. 3. No differentiation between groups was observed without sample clustering, suggesting no significant variation in taxonomic composition. The volcano plot in Fig. 4 illustrates the differences between the predominant genera across the HE and LE categories. Significant differences were observed in the relative abundance of certain bacterial genera. The HE group displayed a higher relative abundance for the genera Veillonellaceae_UCG-001, *Methanosphaera and* Bacterioidales_BS11_gut_group, whereas the LE subjects exhibited significantly higher relative abundance for Absconditabacteriales_(SR1), *Endomicrobium, Moryella, Succinivibrio* and *Treponema.*

Rumen papillae

Table 4 summarises the results of the analysis of rumen papillae size. For the macroscopic measurements, a statistically significant difference was found in the papillae length and density. The LE subjects have shown higher length values (6.26 vs 4.90 mm, P < 0.01) while HE subjects have demonstrated higher papillae density (46.4 vs 40.5 n/cm^2, P = 0.02). No appreciable modifications

Table 2
pH, protozoa count, and VFA concentrations in the rumen fluid collected during the slaughtering of 30 Simmental bulls with divergent residual feed intake.

	Feed efficiency			
Item	High	Low	RMSE	*P*-value
pH	6.34	6.24	0.23	0.31
Total protozoa (10^3Cell/mL)	3 345	3 299	1 061	0.92
Entodinia (10^3Cell/mL)	3 292	3 233	1 024	0.89
Holotrica (10^3Cell/mL)	52.5	65.9	54.8	0.58
Total VFA (mM)	113	118	13.4	0.44
Acetate (%)	64.0	63.1	1.41	0.16
Propionate (%)	16.1	17.3	1.35	0.04
Isobutyrate (%)	1.16	1.06	0.23	0.29
Butyrate (%)	14.1	13.6	2.30	0.59
Isovalerate (%)	3.18	3.33	0.53	0.54
Valerate (%)	1.39	1.54	0.23	0.14
A:P	4.01	3.66	0.32	0.02

Abbreviations: VFAs = volatile fatty acids; A:P = acetate/propionate ratio.

Table 3
Fermentative traits obtained after 24 h of *in vitro* incubation of the rumen fluid collected from the Simmental bulls with high and low feed efficiency.

	Feed efficiency			
Item	High	Low	RMSE	*P*-value
Total gas (mL)	1 044	983	108	0.21
Methane (mL)	227	218	26.9	0.45
Ammonia (mg/dL)	42.8	40.2	6.46	0.35
pH	6.65	6.65	0.07	0.95
Total VFA (mM)	104	101	12.6	0.61
Acetate (%)	57.9	57.9	1.43	0.86
Propionate (%)	15.4	16.6	0.98	0.01
Isobutyrate (%)	1.96	2.00	0.42	0.83
Butyrate (%)	18.7	17.9	1.78	0.29
Isovalerate (%)	4.18	3.89	0.66	0.33
Valerate (%)	1.68	1.73	0.38	0.79
A:P	3.78	3.50	0.25	0.02

Abbreviations: VFAs = volatile fatty acids; A:P = acetate/propionate ratio.

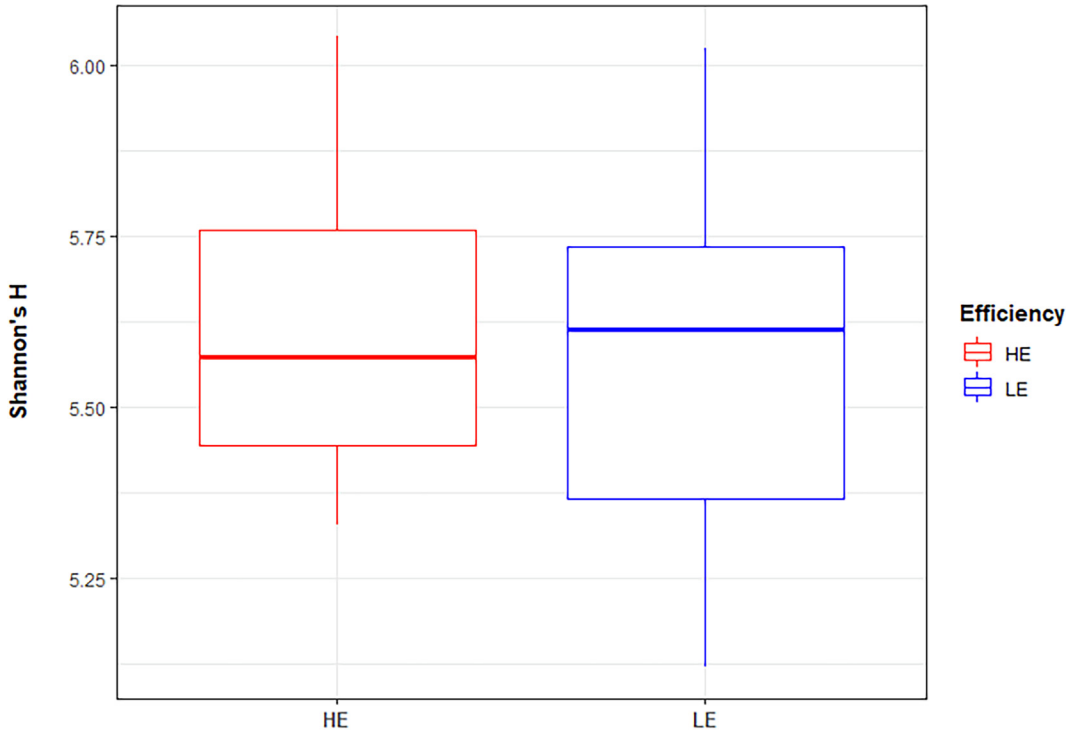

Fig. 2. Boxplot of the alpha-diversity expressed as Shannon diversity index (H) calculated for the two groups of Simmental bulls which differ in their residual feed intake, high efficiency (HE) in red and low efficiency (LE) in blue, respectively.

between the groups in terms of total *papilla* width, thickness, boundaries or surface were registered.

Discussion

Animal performance

Animals considered in the trial were bulls selected from the Italian Simmental population and subjected to a performance test under feeding and rearing conditions similar to those used in fattening farms. Growth and slaughter performance were comparable to those reported in other studies on the Simmental breed (Spanghero et al., 2017; Romanzin et al., 2021 and 2022). The HE Simmental bulls showed lower DMI (approximately 15%) but similar final BW and ADG than LE bulls, as found in another trial on growing Charolais bulls (Guarnido-Lopez et al., 2022). Recently, in a similar experiment with crossbreed animals, the HE bulls showed greater ADG and final BW despite a DMI comparable to that of the LE bulls (Idowu et al., 2023). These findings demonstrate the importance of farm feed efficiency in reducing feed consumption while maintaining or improving growth performance (Herd et al., 2003).

Different DMI levels may influence rumen and microbial development, ruminal feed transit time and digestive ability. The entire digesting process accounts for approximately 10% of RFI variance (Cantalapiedra-Hijar et al., 2018), and several studies have demonstrated that HE animals had higher DM digestibility (Kenny et al., 2018; Costa-Roura et al., 2021). However, Coppa et al. (2023) did not show any significant differences in digestibility among bulls with different RFIs. To explain the discrepancy in feeding efficiency, they proposed a novel hypothesis based on the observed

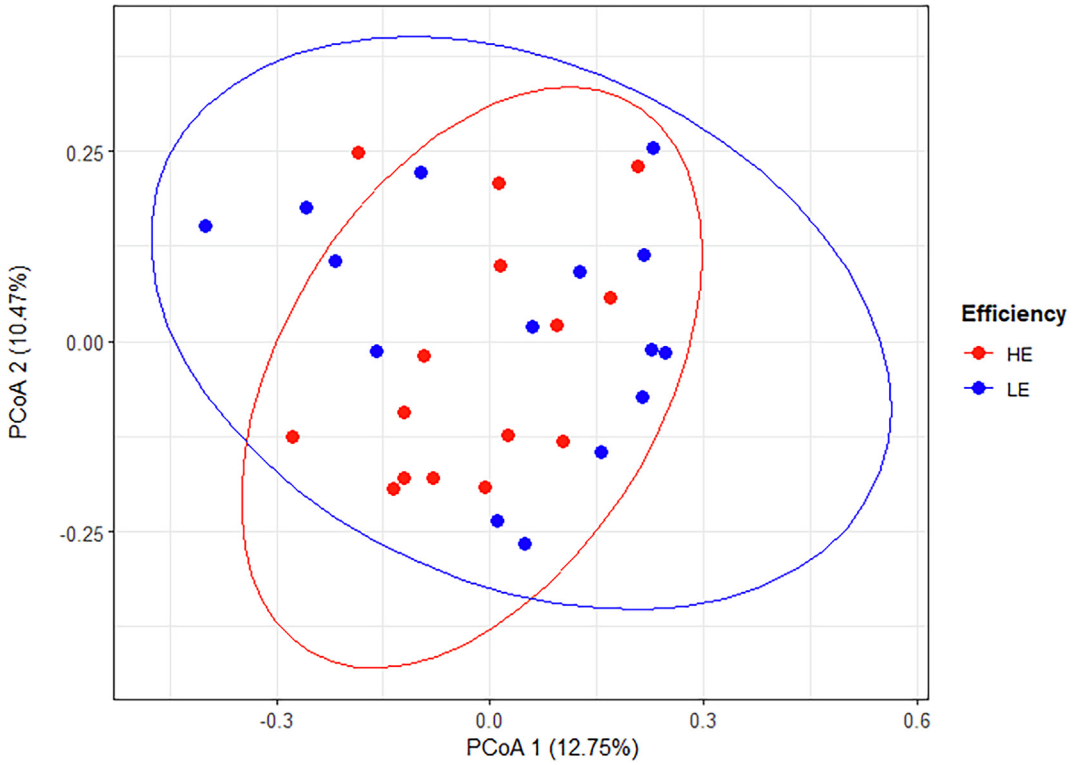

Fig. 3. Principal Coordinate Analysis (PCoA) plot of beta-diversity between the two feed efficiency groups of Simmental bulls obtained using the Bray-Curtis dissimilarity index. High-efficiency group (HE) is represented in red while the low-efficiency group (LE) is represented in blue.

larger rumen size in less efficient growing bulls, where rumen size plays a predominant role. In fact, visceral organs require a great amount of energy for their maintenance; therefore, a reduction in rumen development in more efficient bulls results in a lower metabolic energy requirement. In the present investigation, the overall rumen dimension was not considered, and it was not possible to confirm the data from the abovementioned study, despite the efficient animals having a somewhat higher carcass weight (not significantly) and presumably visceral organs with a lower mass.

Fermentative traits

Improving animal feed efficiency is considered a potential strategy to reduce the impact of greenhouse gas emissions in the livestock sector (Basarab et al., 2013; Beauchemin et al., 2020). However, our results did not show significant differences in methane production between groups with different feed efficiency. Fregulia et al. (2021) evaluated the association between feed efficiency and ruminal parameters, and found a greater variability among the outcomes from scientific literature when methane production was considered. This result suggests that the relationship between these two factors is not fully understood. Digestive processes and passage rate have a substantial effect on inter-animal variance (Herd et al., 2004) and methane output (Cabezas-Garcia et al., 2017). In general, methane yield differences between HE and LE bulls can be attributed to a variety of factors, and some authors have identified the difference in DMI between animals as one of the potential factors influencing methane production (Nkrumah et al., 2006; Fitzsimons et al., 2013; McDonnell et al., 2016). In this study, methane production was evaluated using an *in vitro* system in which the same substrate amount was used for both groups, thereby reducing the difference due to different DMI. In their study that considered 49 diets, Danielsson et al. (2017) showed a very high correspondence (R^2 = 0.96) between *in vivo* and *in vitro* outcomes. Despite this, *in vitro* systems are not perfect replications of *in vivo* processes, but they can reduce variability due to differences in the digestive process. In this study, no difference was found in the amount of methane generated between the two efficiency groups, which supports the previous hypothesis that differences in DMI are one of the potential causes of the discrepancy in methane production.

Kenny et al. (2018) reviewed the relationship between feed efficiency and the VFA profile by comparing data from several scientific studies and obtaining varying results. In the present study, the feed efficiency did not affect the total VFA concentration in the rumen liquid, which is consistent with previous studies (Hernandez-Sanabria et al., 2012; Lawrence et al., 2013; Fitzsimons et al., 2014). However, a difference in their composition was observed for the two efficiency groups with a higher propionate concentration as a proportion of total VFAs for the LE animals, which is in agreement with previous findings (Lawrence et al., 2011; McGovern et al., 2018; Johnson et al., 2019; Lage et al., 2020).

Higher propionate concentrations without significant acetate modification led to a higher A:P ratio in the HE group, which is in accordance with previous investigations (Krueger et al., 2009; Carberry et al., 2012; Fitzsimons et al., 2013). Lage et al. (2020) suggested that this phenomenon could be related to differences in the passage rate between animals, which would modify the amount of substrate available for fermentation and alter VFA production. The outcomes achieved *in vitro* confirm the VFA profile observed in the rumen liquor collected at the abattoir, showing similar differences in the VFA profile. Because there was no difference in the substrate amount or the passage rate between fermenters in the *in vitro* system, we hypothesised that these two aspects were not the main causes of the VFA discrepancy between the groups. Other authors (McGovern et al., 2018; Johnson et al.,

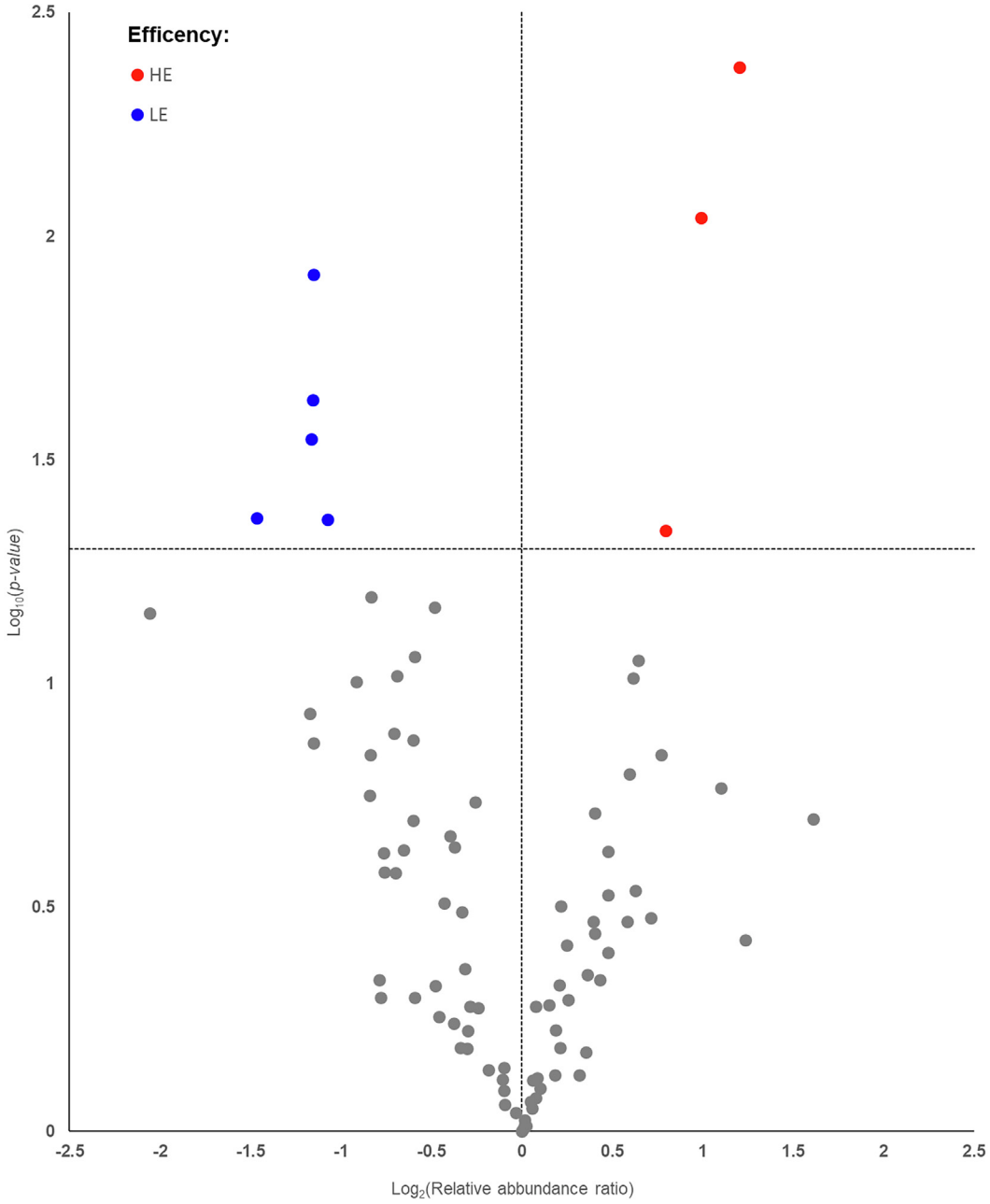

Fig. 4. Volcano plot of the relative abundance for the predominant genera present in both feed efficiency groups of Simmental bulls. The coloured dots represent genera that are significantly different ($P < 0.20$) between groups. Blue dots corresponds to Absconditabacteriales_SR1, Moryella, Endomicrobium, Treponema, and Succinivibrio while the red dots are Veillonellaceae_UCG-001, Methanosphaera, and Bacteroidales_BS11_gut_group. High-efficiency group (HE) is represented in red while the low-efficiency group (LE) is represented in blue.

2019) hypothesised that differences in VFA composition could be attributable to the micro-biome of animals with different feed efficiency. Possible alterations in microorganism populations in the two groups are discussed in the appropriate section.

Rumen microbiota

The protozoa population in the rumen environment is known to play a significant role in ruminal fermentation, including methane emission and ammonia production, as reported in several recent reviews and *meta*-analyses of *in vivo* (Dai and Faciola, 2019; Firkins et al., 2020) and *in vitro* (Spanghero et al., 2022) experiments. In the present study, the protozoa number did not differ between the two groups. In addition, in agreement with previous findings (Guan et al., 2008; Carberry et al., 2012; Zhang et al., 2022), no variation in rumen ammonia and methane yields were detected.

The feed efficiency of donor bulls did not affect the alpha diversity of the ruminal bacterial micro-biota, as measured by the Shannon index. Similar findings were reported by McGovern et al. (2018) in Simmental bulls fed with concentrates and grass silage with Shannon indices of 4.35 and 4.53 for high and low RFI, respectively. Some recent reviews on feed efficiency in ruminants have argued that the ruminal micro-biota is genetically less diverse

Table 4
Macroscopic parameters and histomorphometric measurements of the rumen *papillae* collected from the Simmental bulls with high and low feed efficiency.

Item	Feed efficiency		RMSE	*P*-value
	High	Low		
Papillae				
Length (mm)	4.90	6.26	1.95	<0.01
Width (mm)	2.54	2.47	0.57	0.99
Density (n/cm^2)	46.4	40.5	11.6	0.02
Thickness (μm)				
Lamina propria	133	129	52.6	0.72
Epithelium	158	156	35.3	0.77
Stratum corneum	40.4	41.2	8.12	0.88
Total papilla	337	332	68.3	0.85
Boundary (μm)				
Lamina propria	3 435	3 268	927	0.33
Total papilla	2 789	2 687	264	0.19
Boundary ratio	0.82	0.84	0.10	0.51
Surface (μm^2)				
Lamina propria	93 833	93 920	16 717	0.84
Epithelium + *Stratum corneum*	221 216	208 809	79 988	0.28
Total papilla	324 689	300 399	96 706	0.14

and rich in efficient cattle than in inefficient ones (Cantalapiedra-Hijar et al., 2018; Fregulia et al., 2021). However, several studies on beef cattle have found no significant differences between divergent RFI groups (McCann et al., 2014; Li and Guan, 2017; McGovern et al., 2018; McLoughlin et al., 2020; Costa-Roura et al., 2021; Lopes et al., 2021; Liu et al., 2022). The relationship between feed efficiency and rumen micro-biota is likely to be much more complex than expected. The rumen micro-biota affects the host's nutritional absorption and energy metabolism, and its composition is determined by various factors that can be grouped into macro-categories: genetics, diet and the environment of the host (Newbold and Ramos-Morales, 2020; Wang et al., 2021; Lin et al., 2023). In this study, we considered bulls of the same age and breed, reared under the same conditions and fed the same diet. Despite these controlled conditions, high variability between subjects was observed, as shown in Fig. 3.

A higher relative abundance of the *Absconditabacteriales* genus was found in LE bulls, and to the best of our knowledge, this is the first study to find a link between this genus and the feed efficiency of ruminants. Previous studies have linked the abundance of *Absconditabacteriales* with deteriorating udder health in mastitic cows (Zhong et al., 2018) and yaks (Liu et al., 2019) fed a forage-based diet. In contrast, in a study using a continuous culture system, Arce-Cordero et al. (2022) showed a decrease in *Absconditabacteriales* as NDF increased.

Hernandez-Sanabria et al. (2012) found a greater abundance of *Moryella* in HE calves fed a low-energy density diet, but no differences were observed in those on a high-energy density diet. *Moryella* is considered a core member of the rumen micro-biota in diets with fast rumen passages, and Qiu et al. (2019) found *Moryella* to be positively associated with DMI. It has been known that a different DMI can alter the bacterial composition of the rumen by modulating the abundance of fast- or slow-growing microbes. This study's findings suggest that higher DMI in LE bulls may have favoured the development of *Moryella*.

Information about *Endomicrobia* is limited because they have been ignored for a long time, but Levy and Jami (2018) found a high percentage of this genera in bacterial communities associated with protozoa, suggesting an association also in the rumen. Alves et al. (2021) reported an important role for *Endomicrobium* in the regulation of urinary nitrogen excretion in beef cattle. Nevertheless, in this study, no changes were recorded in the protozoan count or in the concentration of nitrogenous compounds (such as ammonia).

A higher relative abundance of the *Treponema* genus was observed in LE bulls. Earlier studies reported no significant differences between groups with different feed efficiencies (Costa-Roura et al., 2021), a negative association between *Treponema* and ADG but not with the FC ratio (McLoughlin et al., 2020) and a higher abundance in the HE groups (Auffret et al., 2020). According to the latter authors, some species belonging to this genus produce more acetate, which is a lesser source of energy for ruminants than butyrate, thus lowering feed efficiency. However, Welch et al. (2021) identified a positive association between *Treponema* and butyrate concentrations.

Succinivibrio is believed to be negatively linked to rumen methane production because its metabolic pathways require hydrogen, limiting its availability for methanogenesis (Pope et al., 2011). Auffret et al. (2020) found a significantly greater abundance of this genus in HE beef cattle. However, some authors, including the present study, found an opposing link in lambs (Perea et al., 2017), *Bos indicus* steers (Lopes et al., 2021) and dairy cows (Jewell et al., 2015).

To the best of our knowledge, no other studies have linked ruminal Veillonellaceae_UCG-001 with feed efficiency. However, it seems that this genus plays a certain role in the production of VFAs, which goes beyond that of propionate production typical of *Veillonellaceae*, but there are disagreements between the conclusions. Several studies have found an association between this genera and rumen fermentation parameters, such as acetate (Zhang et al., 2023), butyrate, ammonia (Rehemujiang et al., 2023), valerate, isobutyrate and methane production (Yu et al., 2020).

Methanosphaera (order Methanobacteriales), a genus of methanogenic bacteria, produces methane only via methanol reduction. This very restricted metabolism links this bacterial genus to the dietary availability of pectin, which is hydrolysed to produce methanol (Hobson and Stewart, 1997). In turn, some specific ingredients, such as beet pulps, influence the presence of dietary pectin. Given the same proportion of beet pulps in the diet given to bulls in this study, it can be assumed that a greater rumen degradation of by-products in HE animals would have increased pectin availability, promoting *Methanosphaera* growth. *Methanobrevibacter* and *Methanosphaera* are the main genera of methanogens present in ruminants. Zhou et al. (2009) found an abundance of *Methanobrevibacter* spp. AbM4 and *Methanosphaera stadtmanae*, but similar total methanogens, in the rumen of efficient animals. Carberry et al. (2014) did not find these differences in a study on Limousin × Friesian heifers selected for divergent RFI. In this study,

the significant difference found in *Methanosphaera* was not sufficient to determine a change in methane production. This may be partly due to the limited presence of *Methanosphaera* (0.22%) compared with other genera of methanogenic bacteria identified in the rumen liquid of bulls (*Methanobraevibacter* 3.88%).

Liu et al. (2019) found a greater richness of the Bacteroidales_BS11_gut_group in ruminants that receive diets rich in forage, which was associated with higher concentrations of acetate and total VFAs. Bacteroidales_BS11_gut_group may improve the degradation of the hemicelluloses, resulting in an increase of acetate and butyrate (which, in the present study, did not occur significantly) and thus methane. In a study on the rumen micro-biota of Nellore steers (Lopes et al., 2021), among OTUs attributable to the Bacteroidales_BS11_gut_group OTUs, two were significantly higher in the low RFI steers and two were significantly higher in the high RFI steers.

This last aspect, added to the greater presence of *Methanosphera* and the lower presence of *Succinivibrio* in the HE group, was not able to significantly alter ruminal methane production. Although the eight genera that differed between the experimental groups could have influenced several parameters at the ruminal level, they represented only 3.87% of the total identified genera. Other microorganisms, such as the genus *Prevotella* (average presence 33.6%), constituted a greater part of the overall micro-biota, but they did not differ significantly between the two efficiency groups. Therefore, it is difficult to presume that these eight genera play a significant role in feed efficiency.

Papillae

This study relates macroscopic and microscopic morphological alterations of the rumen papillae to feed efficiency. The dorsal sac region was selected for tissue sampling because it is one of the most representative regions in terms of papilla development (Lesmeister et al., 2004; Steele et al., 2014). The papillae length was identified as the best indicator to detect ruminal modification (Lesmeister et al., 2004), and our measurements showed a significant difference with longer papillae in LE animals ($P < 0.01$). Previous studies have shown different results when the correlation between papillae morphology and animal performance was evaluated. Ragionieri et al. (2016) found a correlation between calf ADG and papillae length, whereas Kern et al. (2016) observed no association in beef steers. Pereira et al. (2016) did not observe any correlation between papillae characteristics and bull feed efficiency. This finding was confirmed by Coppa et al. (2023), who pointed out the absence of differentiation in rumen histological traits between bulls with divergent RFI. Some scientific studies (Montanholi et al., 2013; Kenny et al., 2018) have linked feed efficiency differences to variations in intestinal adsorption capacity rather than ruminal functions, potentially explaining the lack of papillae morphology differentiation between HE and other animals.

However, other studies based on the genomic approach (Elolimy et al., 2018 and 2019) suggest that variation in feed efficiency may be associated with differences in the expression of genes that regulate the adsorption/transport capacity in papillae. On the basis of this, we can speculate that longer papillae in LE animals may be a compensation mechanism for lower transport capacity. Moreover, papillae density is different in the efficiency groups, but the total adsorption surface calculated by Malhi et al. (2013) revealed similar values (data not presented). This situation corroborates the previous hypothesis of a possible difference in adsorption/transport capacity between the two efficiency groups. Lam et al. (2018) proposed a possible association between HE animals and epithelial thickness; however, no significant variations were found at a microscopic level (different papillae *strata*) for the tissue sample examined.

The primary causes of papilla formation are related mainly to feeding techniques that modify pH and short-chain VFAs (Bannik et al., 2008), with butyrate being the primary development factor (Mentschel et al., 2001; Malhi et al., 2013). No change in pH or butyrate concentration was observed for the two groups considered. Consequently, lacking the main sources of variability, the absence of variations is in agreement with previous studies.

In conclusion, our results provide a small contribution to efforts in the analysis of factors affecting feed efficiency. Propionate production, rumen papillae morphology and a few bacterial genera play a role, probably not decisively, in this regard. Several studies in recent decades have attempted to analyse the ruminal metabolism affecting this trait, but to date, no issues have been identified that can substantially explain the variability present in cattle populations. Innovative approaches that consider, for example, the joint action between the host genome and micro-biome or *meta*-transcriptomic analysis may provide new interesting insights.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101149.

Ethics approval

The experimental procedures followed EU Directive 2010/63/EU, Italian legislation on animal care (DL n. 26-4 March 2014), and the guidelines of the University of Udine. This study was approved by the Ethics Committee of the University of Udine (prot. no. 11/2018).

Data and model availability Statement

None of the data were deposited in an official repository. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

Acknowledgements

The authors would like to thank the staff of the Genetic Centre of ANAPRI for their excellent work. Special thanks go to Dr. F. Antoniazzi and Prof. C. Di Loreto (Department of Medicine, University of Udine) for their collaboration.

Financial Support Statement

This research was financed by the Dual Breeding project (PSRN 2014/2020 - Sottomisura 10.2); Ministero delle Politiche Agricole Alimentari e Forestali.

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