Saliva and salivary components affect goat rumen fermentation in short-term batch incubations

Palma-Hidalgo, J. M.; Belanche, A.; Jiménez, E.; Martín-García, A. I.; Newbold, C. J.; Yáñez-Ruiz, D. R.

Published in:
Animal

DOI:
10.1016/j.animal.2021.100267

First published: 01/07/2021

Document Version
Publisher's PDF, also known as Version of record

Animal 15 (2021) 100267

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Short communication: Saliva and salivary components affect goat rumen fermentation in short-term batch incubations

J.M. Palma-Hidalgo [a], A. Belanche [a], E. Jiménez [a], A.I. Martín-García [a], C.J. Newbold [b], D.R. Yáñez-Ruiz [a,*]

[a] Estación Experimental del Zaidín (CSIC), Profesor Albareda 1, 18008 Granada, Spain
[b] Scotland's Rural College (SRUC), Peter Wilson Building King's Buildings, W Mains Rd, Edinburgh EH9 3JG, United Kingdom

ARTICLE INFO

Article history:
Received 18 January 2021
Revised 27 April 2021
Accepted 29 April 2021

Keywords:
Immunoglobulin
In vitro fermentation
Microbial immune modulation
Rumen microbiome
Salivary proteins

ABSTRACT

The research about the role of saliva in ruminants has been mainly focused on its buffering capacity together with facilitation of the rumination process. However, the role of salivary bioactive components on modulating the activity of the rumen microbiota has been neglected until recently. This study developed an *in vitro* approach to assess the impact of different components in saliva on rumen microbial fermentation. Four different salivary fractions were prepared from four goats: (i) non-filtrated saliva (**NFS**), (ii) filtrated through 0.25 μm to remove microorganisms and large particles (**FS1**), (iii) centrifuged through a 30 kDa filter to remove large proteins, (**FS2**), and (iv) autoclaved saliva (**AS**) to keep only the minerals. Two experiments were conducted in 24 h batch culture incubations with 6 ml of total volume consisting of 2 ml of rumen fluid and 4 ml of saliva/buffer mix. In Experiment 1, the effect of increasing the proportion of saliva (either NFS or FS1) in the solution (0%, 16%, 33% and 50% of the total volume) was evaluated. Treatment FS1 promoted greater total volatile fatty acids (**VFA**) (+8.4%) and butyrate molar proportion (+2.8%) but lower NH_3-N concentrations than NFS fraction. Replacing the bicarbonate buffer solution by increasing proportions of saliva resulted in higher NH_3-N, total VFA (+8.0%) and propionate molar proportion (+11%). Experiment 2 addressed the effect of the different fractions of saliva (NFS, FS1, FS2 and AS). Saliva fractions led to higher total VFA and NH_3-N concentrations than non-saliva incubations, which suggests that the presence of some salivary elements enhanced rumen microbial activity. Fraction FS1 promoted a higher concentration of total VFA (+7.8%) than the other three fractions, and higher propionate (+26%) than NFS and AS. This agrees with findings from Experiment 1 and supports that 'microbe-free saliva', in which large salivary proteins are maintained, boosts rumen fermentation. Our results show the usefulness of this *in vitro* approach and suggest that different salivary components can modulate rumen microbial fermentation, although the specific metabolites and effects they cause need further research.

Implications

An effective modulation of the rumen microbiome requires comprehensive understanding of the factors that drive its composition and activity. The vast amount of saliva that enters the rumen together with the presence of a range of metabolites suggest that some salivary components may play a relevant role in shaping rumen microbial activity. These initial *in vitro* tests have shown that using saliva (instead of buffer) enhances microbial fermentation and that the effect relies on the presence of large salivary proteins. This represents an important step toward identifying the specific salivary components and mechanisms involved in rumen microbial activity and the potential development of modulation tools.

Introduction

Saliva, together with drinking water, represents the main liquid input into the rumen and it has been reported that total daily saliva secretion may reach from 117 to 183 kg in cattle (Meyer et al., 1964) and from 1.2 to 10.2 L in sheep (Somers, 1957; Tomas, 1973; Piccione et al., 2006). In all domestic animals the main function of saliva is to assist mastication and deglutition. In ruminants, however, given the lack of secretions through the rumen wall, saliva also has other important roles, including facilitating the deglutition and regurgitation of the feed during the rumination process, allowing nitrogen (as urea) to be recycled into the rumen and pro-

* Corresponding author.
E-mail address: david.yanez@eez.csic.es (D.R. Yáñez-Ruiz).

https://doi.org/10.1016/j.animal.2021.100267
1751-7311/

viding a buffered medium in which the rumen microbial activity can take place. The composition and volume of saliva that goes into the gastrointestinal tract depends on a number of variables such as the type of diet, water intake, the physiological stage of the animal, the frequency of mastication and the environmental temperature (Humphrey & Williamson, 2001). An essential component of ruminant saliva are the ions (mainly bicarbonates and phosphates), whose variable secretion rates are responsible for the buffering effect that helps balance rumen pH (Nørgaard, 1993), and counters volatile fatty acids (**VFA**) accumulation. Early studies (Mcdougall, 1948; Somers, 1957) focused on the saliva mineral composition and the factors affecting the rate of secretion. Despite the potential effect that such a great amount of fluid could have on rumen physiology, in addition to the numerous studies on the mineral composition of saliva, research on the bioactive components is relatively scarce. More recent studies have shown that the function of saliva may go beyond lubrication and pH buffering, as the composition might change in response to the presence of dietary components (i.e., tannins) in the diet (Salem et al., 2013) and suggested potential interaction between some salivary components, specially proteins, and rumen fluid inoculum (i.e., microbiota) in *in vitro* incubations in sheep and goats (Salem et al., 2013). The protein components of saliva have been suggested to play a major role in the rumen activity, both buffering pH (i.e. albumin) and regulating the microbiota (i.e. lysozyme, cytokines, immunoglobulins, Fouhse et al., 2017). Salivary immunoglobulins, especially secretory immunoglobulin A (IgA), are of particular importance due to their abundance and ability to modify symbiotic microbiota proliferation (Donaldson et al., 2018) and activity in the rumen (Fouhse et al., 2017), which might help explain the individual host specificity associated with the rumen microbiome in individual animals (Weimer, 2015). Nonetheless, the extent to which the saliva exerts such modulatory control over the rumen microbiota still remains unknown. This study was designed to develop an *in vitro* approach to assess the impact of different components in saliva on rumen microbial fermentation. Preliminary results of this study have already been published in an abstract form (Palma-Hidalgo et al., 2018).

Material and methods

Animal procedures were conducted by trained personnel according to the Spanish Animal Experimentation guidelines (RD 53/2013) and protocols were approved by the Ethical Committee for Animal Research at EEZ-CSIC. Four non-lactating 3 years-old Murciano-Granadina goats were used as saliva donors. Saliva was sampled prior to providing the diet (80% oat hay, 20% commercial concentrate) for five consecutive days by swabbing the mouth with absorbent sponges (approximately 7 × 7 cm) inserted in the mouth of the animals for 5 min. Declared composition (g/kg) of the commercial concentrate (Granulado Cabras Lactación, Macob, Granada, Spain) was 220 CP, 115 crude fiber, 85 ash, 60 fat, 5 lysine, 5 phosphorus, 3 sodium and 2 methionine; and the main ingredients were wheat bran, distillates from corn fermentation, sunflower cake and wheat. Saliva was extracted from the sponges by centrifugation at 190*g* for 10 min. Saliva from each animal was separated into four fractions: (i) Non-filtrated saliva (**NFS**) that was not processed further, (ii) Filtrated saliva 1, (**FS1**) that was centrifuged at 16,300*g* for 5 min and filtrated through 0.25 μm pore size to remove microorganisms and large particles, (iii) Filtrated saliva 2, (**FS2**) that was initially processed as fraction FS1 and then centrifuged through a 30 kDa filter (Amicon@Ultra-15 Centrifugal Filter Devices) at 2 000*g* for 20 min to remove large proteins, including immunoglobulins, and (iv) Autoclaved saliva (**AS**) that consisted of NFS which was autoclaved for 30 min at 121 °C to denature active metabolites in the saliva but keeping the minerals. Each of the four fractions of saliva samples were pooled separately for each animal and stored at −80 °C until further use. Total protein concentration was determined in each saliva fraction by spectrophotometry (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific, Waltham, MA), and polyacrilamide gel electrophoresis at 4–12% gradient was performed (Invitrogen NuPAGE™ Bis-Tris Mini Protein Gels, Thermo Fisher Scientific, Waltham, MA) to assess the effectiveness of the filtering and autoclaving processes on salivary proteins (Fig. 1). Twenty μg of each fraction was loaded in wells and the electrophoresis was run for 40 min at 180 V. Bands were stained using the Colloidal blue staining kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA) and observed with a Gel Doc XR + System (BioRad Laboratories Inc., Hercules, CA, USA).

Two *in vitro* experiments were conducted to assess: (i) the effect of replacement of bicarbonate buffer solution with saliva (Experiment 1) and (ii) the impact of different saliva fractions (Experiment 2) on the rumen microbial fermentation. Both experiments used 24 h *in vitro* batch culture incubations in anaerobic conditions at 39 °C in Hungate tubes with 6 ml of total volume consisting of 2 ml of rumen fluid and 4 ml of a saliva/buffer mix.

In Experiment 1, the tubes contained 2 ml fresh rumen fluid obtained from a single goat (rumen cannulated) before the morning feeding and adapted during two weeks to a diet consisting of 50:50 commercial concentrate:oat hay. Rumen fluid was then filtrated through double layer of cheese cloth, and mixed with 4 ml of saliva and/or bicarbonate buffer (3.5 g $NaHCO_3$ + 0.4 g $(NH_4)HCO_3$ in 100 ml dH_2O). A total of 32 incubation tubes were used following a 2 × 4 factorial design including the incubation of two saliva fractions (NFS and FS1) and four increasing doses of saliva (0%, 16%, 33% and 50% of the total volume) as a replacement of bicarbonate buffer solution. These saliva samples were obtained from four donors (n = 4) which were considered as experimental units. Incubation substrate consisted of 75 mg of the aforementioned commercial concentrate and 75 mg oat hay.

In Experiment 2, 54 incubation tubes were used to analyze the effect of four saliva fractions (NFS, AS, FS1 and FS2). These saliva fractions obtained from four saliva donors were incubated with rumen fluid obtained from three goats, different from saliva donors. Saliva fractions represented 66% of the total incubation

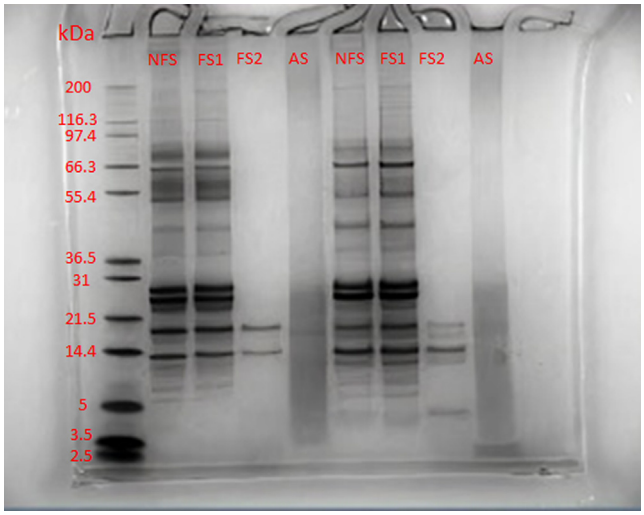

Fig. 1. Polyacrilamide gel illustrating the presence of different goat saliva metabolites. Lane 1 corresponds to Mark12™ molecular weight marker. Lanes 2–5 and 6–9 correspond to saliva fractions from the first and second saliva donor, respectively. NFS, non-filtered saliva; FS1, filtered saliva through 0.25 μm filter; FS2 filtered saliva through 30 kDa filter; AS, autoclaved saliva.

volume and six control tubes (two for each rumen fluid) were used with the aforementioned bicarbonate buffer solution instead of saliva as negative control. In this incubation the amount of substrate was 30 mg commercial concentrate and 30 mg oat hay.

In both experiments, gas pressure in the headspace of tubes was measured at 2, 4, 7, 10 and 24 h using a Wide Range Pressure Meter (SperScientific LTD, Scottsdale, AZ, USA), which then was transformed into volume units by the ideal gas law. Incubation pH was measured at the beginning and end of incubations. At 24 h, incubation was stopped by opening the bottles, then samples were taken to determine the concentration of NH_3-N by spectrophotometry (Victor X microplate reader, Perkin Elmer, Waltham, MA) and volatile fatty acids by gas chromatography (AutoSystem gas chromatograph, Perkin Elmer, Waltham, MA). In Experiment 1, results were statistically analyzed as a 2 × 4 factorial ANOVA: the effect of the saliva fraction (NFS vs FS1), the saliva proportion (0% vs 16% vs 33% vs 50%) and their interaction were considered as fixed effects, whereas the saliva donor (animal 1–4) was considered as a random effect. In Experiment 2, a one-way ANOVA was used with the saliva fraction (NFS vs FS1 vs FS2 vs AS) as the only fixed effect whereas the saliva donor (animals 1–4) and the rumen liquid donor (animals 5–7) were considered as random blocking factors. When significant effects were detected, means were compared by Fisher's protected LSD-test using the SPSS software (IBM Corp., Version 21.0, New York, USA). Effects were considered significant at $P < 0.05$ and tendency to difference at $P < 0.1$.

Results and discussion

The determination of the protein concentration using a commercial kit resulted in significant differences between the FS2 saliva (mean = 212 μg/ml) and the NFS, FS1 and AS saliva samples ($P < 0.001$). No differences were found between these three saliva samples (908, 882, 992 μg/ml; respectively). The protein concentration did not decrease in the AS saliva because the kit's first chemical reaction can be prompted with peptides comprising as few as three amino acid residues. Polyacrilamide gradient gel electrophoresis (Fig. 1) illustrated the effect of the two filtration treatments (FS1 and FS2) and autoclaving on the protein bands visualized in each saliva fraction. Saliva obtained from NFS and FS1 filtrations presented similar band patterns, given that only microbial and epithelial cells and large size feed particles were removed from FS1. In both cases, bands corresponded to proteins both larger and smaller than 30 kDa. It was assumed that these bands included transporting proteins, especially serum albumin, which comprises over 50% of the total salivary proteins and whose precursor have been previously identified within the 70 kDa and 28 kDa regions (Lamy et al., 2009). Some of those bands may also correspond to large proteins involved in immune response such as IgA and immunoglobulin G (Dietzen, 2018; Janeway et al., 2001). Indeed, Immunoglobulin heavy chain C region and Immunoglobulin gamma 2 heavy chain C region (a component of immunoglobulin G) have been previously identified in saliva from sheep and goat, respectively, around the 50 kDa region (Lamy et al., 2009) and may correspond to the bands found in the samples close to 55.4 kDa. Saliva obtained from FS2 only presented bands corresponding to proteins smaller than 25 kDa, therefore all the aforementioned high molecular weight proteins were not present. Small proteins and polypeptides such as lysozyme and most cytokines and antimicrobial peptides could pass through FS2 filtration (Stenken & Poschenrieder, 2015). Lysozyme molecular weight (14.3 kDa, Canfield, 1963) matches the profuse bands just below 14.4 kDa, whereas the rest of the bands below 30 kDa are compatible with cathelicidin antimicrobial peptides and hemoglobin subunits (Lamy et al., 2009). Despite the significant role that most of these molecules could play in the control of microorganisms entering the GIT (Fouhse et al., 2017), very few studies have explored their expression and activity in saliva from any animal other than humans. Interestingly, Lamy et al., (2009) reported that the region between 25 kDa and 35 kDa is the most discriminant for the salivary proteome across individuals and ruminant species. Unlike the other saliva fractions, no bands were detected in the AS fraction as a result of effective protein removal.

Experiment 1 (Table 1) showed that FS1 saliva promoted greater (+8.4%) total VFA and lower (−17%) NH_3-N concentrations ($P < 0.01$) than NFS fraction. More VFAs produced when incubating with FS1 saliva may be a consequence of the absence of salivary microorganisms which could potentially compete with the autochthonous rumen microbiota, hindering its fermentative activity. The removal of microbial cells in FS1 may explain the lower NH_3-N concentrations due to provision of live or dead microbial cells to extensive proteolysis of their proteins (Belanche et al., 2012). The molar proportion of both branched short-chain fatty acids (isobutyrate and isovalerate) and valerate was higher in NFS than FS1 containing incubations ($P < 0.01$), indicating greater proteolysis in the incubations containing NFS. Gas production and the concentration of the two major rumen VFA (acetate and propionate) were unchanged as a result of saliva filtration.

Replacing the bicarbonate buffer solution by increasing proportions of saliva had a substantial effect on most fermentation parameters (Table 1), such as higher NH_3-N (up to + 23%), total VFA (+8.0%) and propionate molar proportion (+11%), whereas other parameters decreased (pH, gas production and acetate molar proportion). Unlike the bicarbonate buffer, both NFS and FS1 salivary fractions contain a range of proteins that have been suggested to enhance microbial activity in the gut (Fouhse et al., 2017). NH_3-N was increased and the acetate:propionate ratio decreased as a consequence of the increasing proportion of saliva ($P < 0.05$). Butyrate molar proportion was significantly different among the four proportions of saliva used ($P < 0.01$). The increase in total VFA and N-NH_3 concentrations suggest that some salivary components might foster microbial hydrolysis activity, which may be related to the drop in the incubation pH. Indeed, a previous study in which tannins-rich substrates were pre-incubated with saliva from sheep or goats adapted to different diets and then incubated with rumen fluid showed a greater substrate degradation than when these substrates were pre-incubated with artificial saliva (Ammar et al., 2013). In order to avoid the low values in the incubation pH and potential impairment of microbial fermentation, it was decided to decrease the amount of substrate to be used in experiment 2.

Experiment 2 (Table 2) addressed the effect of the different fractions of saliva on rumen fermentation pattern. Bicarbonate buffer solution (used as control) promoted a similar pH in all four saliva fractions, suggesting that both (buffer and saliva fractions) are effective in maintaining appropriate rumen pH; however, saliva fractions led to greater total VFA and NH_3-N concentrations, which suggests that the presence of some salivary elements enhanced rumen microbial activity. Despite the initial pH being similar in all treatments, final pH after 24 h significantly diverged across salivary fractions (lower values with FS1 and FS2 compared to NFS and AS; $P = 0.001$). These pH differences partially concur with the higher NH_3-N concentrations (+35%; $P < 0.001$) found with NFS and AS saliva in comparison to the other two. On the contrary, no significant differences were found in total VFA concentration between NFS, FS2 and AS, while they were significantly higher with FS1 (+7% on average; $P < 0.001$). This agrees with findings from Experiment 1 and supports that 'microbe-free saliva', in which large salivary proteins are maintained, boosts rumen fermentation. Incubation with FS1 also resulted in the highest gas production of all treatments (+5% on average, $P < 0.001$). The fermentation patterns, represented by the relative abundances of each VFA, differed

Table 1
Effect of increasing the proportions of two goat saliva fractions to replace an artificial buffer on *in vitro* rumen fermentation (Experiment 1).

	Saliva fraction		Saliva proportion					*P*-value		
	NFS	FS1	0%	16%	33%	50%	SEM	Fraction	Proportion	Interaction
Initial pH	6.94	6.89	6.85	6.90	6.98	6.94	0.052	0.189	0.109	0.647
Final pH (after 24 h)	5.50	5.50	5.63^{a}	5.51^{b}	5.47^{b}	5.40^{c}	0.022	0.748	<0.001	0.071
Gas Production (ml)	22.4	22.8	23.9^{a}	23.9^{a}	22.3^{b}	20.2^{c}	0.423	0.217	<0.001	0.455
NH_3-N (mg/dl)	35.1	29.1	28.7^{c}	29.8^{bc}	34.3^{ab}	35.3^{a}	2.337	0.001	0.023	0.176
Total VFA (mM)	214	232	212^{b}	221^{a}	228^{a}	229^{a}	4.734	<0.001	0.001	0.882
Acetate (%)	61.4	61.0	62.6^{a}	61.9^{a}	60.8^{b}	59.7^{c}	0.495	0.303	<0.001	0.120
Propionate (%)	20.9	21.1	19.9^{c}	20.7^{bc}	21.4^{ab}	22.0^{a}	0.418	0.331	<0.001	0.750
Isobutyrate (%)	0.68	0.63	0.61	0.66	0.68	0.65	0.024	0.007	0.080	0.002
Butyrate (%)	14.3	14.7	14.4^{bc}	14.1^{c}	14.5^{b}	15.0^{a}	0.200	0.034	0.003	<0.001
Isovalerate (%)	1.09	0.97	0.96^{b}	1.05^{a}	1.08^{a}	1.03^{ab}	0.033	<0.001	0.017	0.028
Valerate (%)	1.62	1.54	1.50^{b}	1.54^{b}	1.63^{a}	1.65^{a}	0.036	0.004	0.001	0.330
Ac/Pr	2.95	2.90	3.14^{a}	2.99^{b}	2.85^{c}	2.72^{c}	0.071	0.354	<0.001	0.514

NFS, non-filtered saliva; FS1, filtered saliva through 0.25 μm pore size filter; VFA: volatile fatty acids; Ac/Pr: acetate/propionate ratio.
[a–c]Within a row means with different superscripts differ.

Table 2
Effect of using different fractions of goat saliva on *in vitro* rumen fermentation (Experiment 2).

	Saliva fraction					
	NFS	FS1	FS2	AS	SEM	*P*-Value
Initial pH	7.03	7.02	7.02	6.99	0.014	0.176
Final pH (after 24 h)	6.51^{a}	6.39^{b}	6.41^{b}	6.48^{a}	0.021	0.001
Gas Production (ml)	11.5^{c}	12.3^{a}	12.1^{b}	11.7^{bc}	0.139	<0.001
NH_3-N (mg/dl)	35.4^{a}	30.7^{b}	26.2^{c}	31.8^{ab}	1.388	<0.001
Total VFA (mM)	103^{b}	111^{a}	105^{b}	103^{b}	1.095	<0.001
Acetate (%)	61.3^{a}	56.2^{b}	57.2^{b}	61.4^{a}	0.439	<0.001
Propionate (%)	21.4^{b}	26.3^{a}	25.7^{a}	20.9^{b}	0.434	<0.001
Isobutyrate (%)	1.31^{a}	1.16^{b}	1.04^{c}	1.25^{a}	0.020	<0.001
Butyrate (%)	12.1	12.7	12.8	12.5	0.232	0.114
Isovalerate (%)	2.19^{a}	1.94^{b}	1.70^{c}	2.15^{a}	0.050	<0.001
Valerate (%)	1.70	1.71	1.59	1.71	0.044	0.202
Ac/Pr	2.98^{a}	2.25^{b}	2.35^{b}	3.06^{a}	0.052	<0.001

NFS, non-filtered saliva; FS1, filtered saliva through 0.25 μm filter; FS2 filtered saliva through 30 kDa filter; AS, autoclaved saliva; VFA: volatile fatty acids; Ac/Pr: acetate/propionate ratio.
[a–c]Within a row means with different superscripts differ. The buffer column was used as control but not included in the statistical analysis.

across fractions of saliva. NFS and AS both resulted in similar acetate and propionate proportions. As for FS1 vs. FS2, no differences were observed regarding acetate and propionate molar proportions. However, FS1 and FS2 promoted notably higher propionate (+23%) and lower (-8%) acetate molar proportions compared to NFS ($P < 0.001$).

Experiment 2 showed similar effects with the NFS and AS fractions (Table 2). This could be seen as contradictory, given that they only share the mineral component of saliva, while NFS and filtrated fractions also share the protein components. Differences in rumen fermentation when incubating with filtrated or non-filtrated saliva suggest a modulatory role of salivary microbiota and proteins on the rumen fermentation. This is supported by the significantly higher gas production (+5.1%), total VFA concentration (+7.8%) and propionate molar proportion (+26%) observed in FS1 than in AS, suggesting proteins with molecular weight over 30 kDa (including immunoglobulins) may play a key role in modulating rumen microbiota activity. Fouhse et al. (2017) and Tsuruta et al. (2012), demonstrated that salivary secretory IgA from cattle could bind to symbiotic rumen bacteria, here this observation is expanded to show that this can affect the pattern of fermentation in the rumen. Ammar et al. (2013) suggested possible interactions between saliva and rumen inoculum from sheep and goats. Given the high specificity of Ig, it would be interesting to assess their role between species or across individuals in longer term incubations periods in which salivary components and rumen microbiota can interact in a fully stabilized ecosystem.

Conclusion

Our results show that the *in vitro* model in which rumen fluid is incubated with saliva collected from animals is a useful research tool and indicates that some salivary components modulate rumen microbial fermentation. In particular, microbe-free filtrated saliva and its bioactive components have shown to increase rumen fermentative activity compared with incubation with non-filtrated or autoclaved saliva. The individual role of specific salivary metabolites and the impact across different individual animals require further research.

Ethics approval

Animal procedures were conducted by trained personnel according to the Spanish Animal Experimentation guidelines (RD 53/2013) and protocols were approved by the Ethical Committee for Animal Research at EEZ-CSIC.

Data and model availability statement

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

Author ORCIDs

J.M. Palma Hidalgo: https://orcid.org/0000-0002-6237-1822.

A. Belanche: https://orcid.org/0000-0001-5880-6021.
E. Jiménez: https://orcid.org/0000-0001-5175-2251.
A.I. Martín-García: https://orcid.org/0000-0002-5366-3938.
C.J. Newbold: https://orcid.org/0000-0001-9561-300X.
D.R. Yáñez-Ruiz: https://orcid.org/0000-0003-4397-3905.

Author contributions

AB & DRYR: Conceptualization, validation and supervision.
JMPH, AB, & EJ: Methodology, investigation, resources and formal analysis.
JMPH, AB: Data curation and software.
DRYR & AIMG: funding acquisition.
JMPH: Writing original draft.
DRYR, AB & CJN: Writing – review and editing.
All authors read and approved the final version.

Declaration of competing interest

None.

Acknowledgements

The authors thank Isabel Jiménez and Pablo González for their assistance in the sample analyses.

Financial support statement

This work was financially supported by the Spanish Research Agency (AEI) through the project AGL2017-86938-R and the Training Program for Academics Grant, Madrid, Spain (FPU16/01981).

References

Ammar, H., Bodas, R., González, J.S., Salem, A.Z.M., Giráldez, F.J., Andrés, S., López, S., 2013. Effects of pre-incubation in sheep and goat saliva on in vitro rumen digestion of tanniferous browse foliage. Journal of Agricultural Science 151, 898–906. https://doi.org/10.1017/S002185961300018X.
Belanche, A., De la Fuente, G., Moorby, J.M., Newbold, C.J., 2012. Bacterial protein degradation by different rumen protozoal groups. Journal of Animal Science 90, 4495–4504. https://doi.org/10.2527/jas.2012-5118.
Canfield, R.E., 1963. The amino acid sequence of egg white lysozyme. The Journal of Biological Chemistry 238, 2698–2707. https://doi.org/10.1016/S0021-9258(18)67888-3.
Dietzen, D.J., 2018. Amino acids, peptides, and proteins. In: Horvath, A.R., Wittwer, C.T. (Eds.), Principles and Applications of Molecular Diagnostics. Elsevier Inc., Amsterdam, Netherlands, pp. 345–380. https://doi.org/10.1016/B978-0-12-816061-9.00013-8.
Donaldson, G.P., Ladinsky, M.S., Yu, K.B., Sanders, J.G., Yoo, B.B., Chou, W.C., Conner, M.E., Earl, A.M., Knight, R., Bjorkman, P.J., Mazmanian, S.K., 2018. Gut microbiota utilize immunoglobulin a for mucosal colonization. Science 360 (6390), 795–800. https://doi.org/10.1126/science.aaq0926.
Fouhse, J.M., Smiegielski, L., Tuplin, M., Guan, L.L., Willing, B.P., 2017. Host immune selection of rumen bacteria through salivary secretory IgA. Frontiers in Microbiology 8, 1–9. https://doi.org/10.3389/fmicb.2017.00848.
Humphrey, S.P., Williamson, R.T., 2001. A review of saliva Normal composition, flow, and function. Humphrey, Williamson. 2001. Journal of Prosthetic Dentistry.pdf. The Journal of Prosthetic Dentistry 85, 162–169.
Janeway, C.A., Travers, P., Walport, M., Shlomchik, M., 2001. Immunobiology: The Immune System in Health and Disease. Garland Publishing, New York City, NY, USA.
Lamy, E., da Costa, G., Santos, R., Capela e Silva, F., Potes, J., Pereira, A., Coelho, A.V., Sales Baptista, E., 2009. Sheep and goat saliva proteome analysis: A useful tool for ingestive behavior research? Physiology and Behavior 98, 393–401. https://doi.org/10.1016/j.physbeh.2009.07.002.
Mcdougall, E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochemical Journal 43, 99–109.
Meyer, R.M., Bartley, E.E., Morrill, J.L., Stewart, W.E., 1964. Salivation in cattle. I. Feed and animal factors affecting salivation and its relation to bloat. Journal of Dairy Science 47, 1339–1345. https://doi.org/10.3168/jds.S0022-0302(64)88915-3.
Nørgaard, P., 1993. Saliva secretion and acid-base status of ruminants. A review. Acta Veterinaria Scandinavica 89, 93–100.
Palma-Hidalgo, J.M., Belanche, A., Martín-García, A.I., Yáñez-Ruiz, D.R., 2018. In vitro assessment of goat's saliva on rumen fermentation. In: Proceedings of the 10th International Symposium on the Nutrition of Herbivores. Advances in Animal Biosciences, 9, p. 396. https://doi.org/10.1017/S2040470018000146.
Piccione, G., Foà, A., Bertolucci, C., Caola, G., 2006. Daily rhythm of salivary and serum urea concentration in sheep. Journal of Circadian Rhythms 4, 2–5. https://doi.org/10.1186/1740-3391-4-16.
Salem, A.Z.M., López, S., Ranilla, M.J., González, J.S., 2013. Short- to medium-term effects of consumption of quebracho tannins on saliva production and composition in sheep and goats. Journal of Animal Science 91, 1341–1349. https://doi.org/10.2527/jas.2010-3811.
Somers, B.M., 1957. Salivary secretion and its functions in ruminants. The Australian Veterinary Journal 33, 297–301.
Stenken, J.A., Poschenrieder, A.J., 2015. Bioanalytical chemistry of cytokines – A review. Analytica Chimica Acta 853, 95–115. https://doi.org/10.1016/j.aca.2014.10.009.
Tomas, F.M., 1973. Parotid salivary secretion in sheep: Its measurement. Quarterly Journal of Experimental Physiology 58, 131–138.
Tsuruta, T., Inoue, R., Tsukahara, T., Nakamoto, M., Hara, H., Ushida, K., Yajima, T., 2012. Commensal bacteria coated by secretory immunoglobulin A and immunoglobulin G in the gastrointestinal tract of pigs and calves. Animal Science Journal 83, 799–804. https://doi.org/10.1111/j.1740-0929.2012.01026.x.
Weimer, P.J., 2015. Redundancy, resilience, and host specificity of the ruminal microbiota: Implications for engineering improved ruminal fermentations. Frontiers in Microbiology 6, 1–16. https://doi.org/10.3389/fmicb.2015.00296.