

THE EFFECT OF (QUEBRACHO) TANNINS ON METABOLICAL PROCESSES IN THE RUMEN DURING SIMULATED DIGESTION IN BIOREACTOR (*IN VITRO*)

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ABSTRACT

The present study investigated the effect of dietary tannins on ruminal fermentation parameters using a continuous *in vitro* bioreactor system (Sartorius Biostat A plus Bioreactor System). The experiment was carried out in the form of a cross-over design and compared a control diet with a tannin-enriched diet supplemented with 5 % of quebracho tannins. The incubation lasted 16 days, during which each diet was fed for 8 days, consisting of 5 days of adaptation followed by 3 days of sampling. The incubations were repeated three times. The results demonstrated that tannin supplementation increased pH, altered redox potential, and significantly reduced dry matter digestibility. Furthermore, a marked decrease in volatile fatty acid (VFA) concentrations was observed, although the acetate-to-propionate ratio remained unaffected. These findings indicate that tannins primarily suppress rumen fermentation by limiting substrate degradation and microbial activity, thereby influencing overall fermentation efficiency.

Keywords: *in vitro* rumen fermentation; gas chromatography; pH; redox potential; digestibility; volatile fatty acids.

INTRODUCTION

Rumen fermentation represents a key metabolic process in ruminants, where complex plant materials are converted by a diverse microbial community into VFAs that serve as the principal energy source for the host animal (Ding et al., 2023). *In vitro* fermentation systems are widely utilized to study ruminal processes because they allow precise control of experimental conditions, reduce variability and reduce the need for animal usage compared with *in vivo* experiments



(Scicutella et al., 2025). Tannins are polyphenolic secondary metabolites that are abundant in many plant species and can significantly influence digestive processes through interactions with proteins, carbohydrates, and microbial enzymes. Their effects on ruminal fermentation are complex and concentration-dependent, ranging from inhibition of microbial activity and reduced digestibility to potential benefits such as improved nitrogen utilization or reduced methane emissions (Frutos et al., 2004). Despite extensive research, the overall impact of tannins on rumen metabolism remains incompletely understood due to variability in tannin structure, concentration, and experimental conditions. The aim of this study was therefore to evaluate the effect of tannin supplementation at 5% of dry matter on selected fermentation parameters, including pH, redox potential, dry matter digestibility, and VFA production, using a continuous *in vitro* bioreactor system.

MATERIAL AND METHODS

The experiment was carried out using continuous incubations *in vitro* in a bioreactor (Sartorius Biostat A plus Bioreactor System) that simulated ruminal conditions, including a constant temperature of 39 °C, strict anaerobic environment maintained by continuous CO₂ flow, homogeneity using a metallic stirrer rotating at 20 rpm, and a steady inflow of artificial saliva (McDougall, 1948) dosed into the bioreactor vessel every 15 minutes using a peristaltic pump, averaging a flow of 1038 ml per day. The working volume of incubation media was maintained at 1 L. Each experimental run lasted sixteen days and consisted of two phases of equal duration. In the first phase, the bioreactor was fed using a control diet (Table 1), whereas in the second phase the same diet was supplemented with quebracho tannins using tannin concentrate Tannivin® SR (Erbslöh), at a concentration corresponding to 5 % of dry matter. The bioreactor was fed once daily with 10 g of the respective diet, administered via nylon bags. Each bag was incubated in the bioreactor for 48 h and then replaced with a fresh one. Each phase included a five-day adaptation period followed by a three-day sampling period during which measurements and sample collections were conducted (Table 2). The entire experiment was repeated three times.

Tab. 1: Composition of the feed ration used during fermentation in the bioreactor.

Components	Unit	Composition
Maize silage	%	40,5
Grass haylage 1 st cut	%	21,3
Sugar beet cake	%	14,2
Molasses	%	2,4
Supplemental mixture ¹	%	19,9
Rapeseed meal	%	1,7
Total	%	100

¹ Supplemental mixture - composition (%): wheat (29.5), barley (12.5), corn (19.0), soybean meal (17.5), rapeseed meal (14.0), mineral-vitamin premix SM Production U (3.5), limestone (1.5), salt (0.5), sodium bicarbonate (2.0)

Tab. 2: *Timeline of a single bioreactor run*

Bioreactor run	
16 days	
8 days – control diet	8 days – tannin-enriched diet
5 days – adaptation period	5 days – adaptation period
3 days – sample collection	3 days – sample collection

Rumen fluid formed the basis of the digestive environment and served as the inoculum for cultivation in the bioreactor. It was collected on the first day of each bioreactor run from the rumen of two dairy cows using an esophageal tube (a private farm Lenka Klíčová, Božice, 1st and 3rd run) or from cannulated cows (Agrovýzkum Rapotín Ltd., 2nd run). This change was due to restrictions imposed due to the risk of foot-and-mouth disease transmission. After collection, rumen fluid was transferred to the laboratory in a heat-stable box, homogenized and filtered through four layers of gauze. The feed retained on the gauze was used as an initial nutrient source to support adaptation and initiate fermentation processes.

During the sampling period, 2 ml of liquid inoculum was collected from the bioreactor for subsequent VFA analysis. For the determination of dry matter digestibility, part of the feed was incubated in six F57 filter bags (O.K. SERVIS BioPro, Czech Republic), 0.5 g per bag, plus one blank bag. After 48 hours of incubation, filter bags were removed, rinsed externally, dried, and weighed. pH and redox potential were measured using an InPro3253i/SG/225 electrode (METTLER TOLEDO) with values being recorded every 20 minutes. Buffer consumption and effluent volumes were recorded daily at 8:45. Digestibility was calculated from feed and residue weights using equation below.

$$\text{Dry matter digestibility (\%)} = \left(100 - \left(W3 - (W1 \times C1)\right) \times 100\right) / W2$$

Where:

- W1 = weight of empty bag
- W2 = feed sample weight
- W3 = dried bag weight after incubation
- C1 = blank correction factor

The VFA profile analysis was performed using gas chromatography with an Agilent Technologies 8860 GC System equipped with a DB FATWAX UI column (30 m × 0.32 mm × 0.25 µm) and a flame ionization detector. The profile consisted of the three most abundant VFAs: acetic acid, propionic acid, and butyric acid.

Inoculum samples were first homogenized, and subsequently centrifuged using a Microspin 12 (Biosan) for 10 minutes at a relative centrifugal force of 4000× g. After centrifugation, 1000 µl of supernatant was collected and mixed with 200 µl of 25% metaphosphoric acid. The samples were then incubated at 4 °C for 30 minutes and centrifuged again for 10 minutes at 12,000× g. For gas chromatographic analysis the resulting solution was diluted 1:9.

Gas chromatography parameters:

- Injection split: 20:1; 250 °C
- Carrier gas: Helium
- Column flow rate: 1.5 ml/min
- Oven temperature: 60 °C with a temperature gradient of 20 °C/min to a final temperature of 180 °C
- Detector temperature: 250 °C
- Fuel gas: Hydrogen flow of 30 ml/min
- Oxidizing gas: Compressed air flow of 400 ml/min
- Injection volume: 1 µl

Data was analyzed using software Statistica. A general linear model was used with treatment (control, tannin-enriched diet) as a fixed effect and experimental run (Run 1, 2, 3) as a random effect. Graphs were created in Microsoft Excel. Outliers in box plots were defined using the interquartile range (IQR), with values outside $1.5 \times \text{IQR}$ considered as outliers.

RESULTS

Chart 1 shows pH values from three measurement days for the control group and the tannin-enriched group across all three bioreactor runs. Chart 2 presents redox potential values in the same manner.

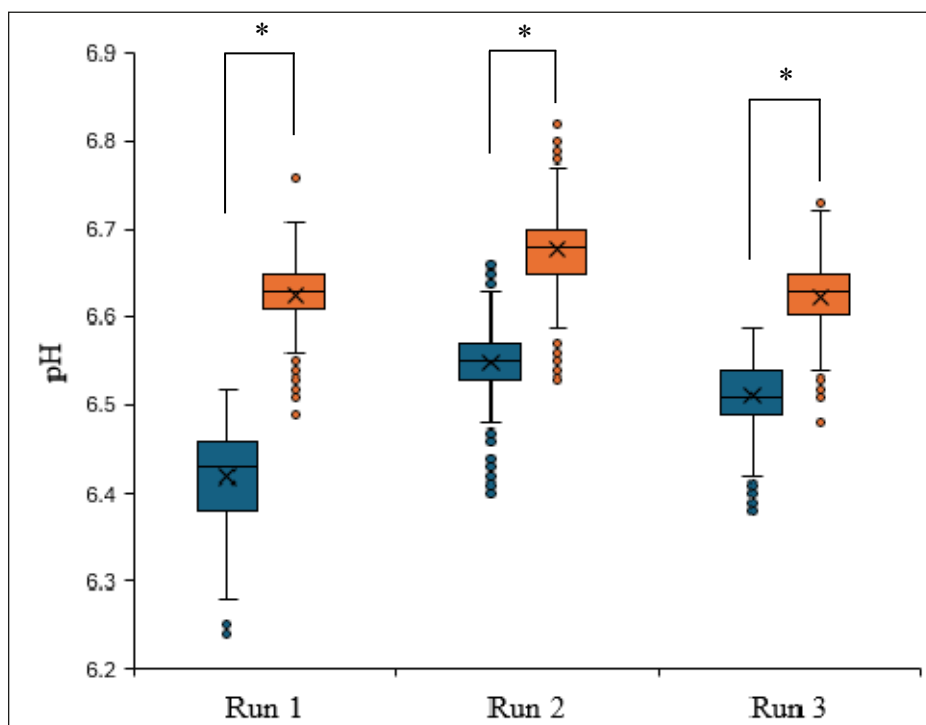


Chart 1: Box plot showing pH values for individual bioreactor runs. The control group is shown in blue, while the tannin-enriched group is shown in orange. Data points of the same colour represent outliers. Whiskers represent the maximum and minimum values excluding outliers. The box boundaries represent the first and third quartiles. The line represents the median, and the cross indicates the mean. * indicates a significant difference ($P < 0.05$) between groups.

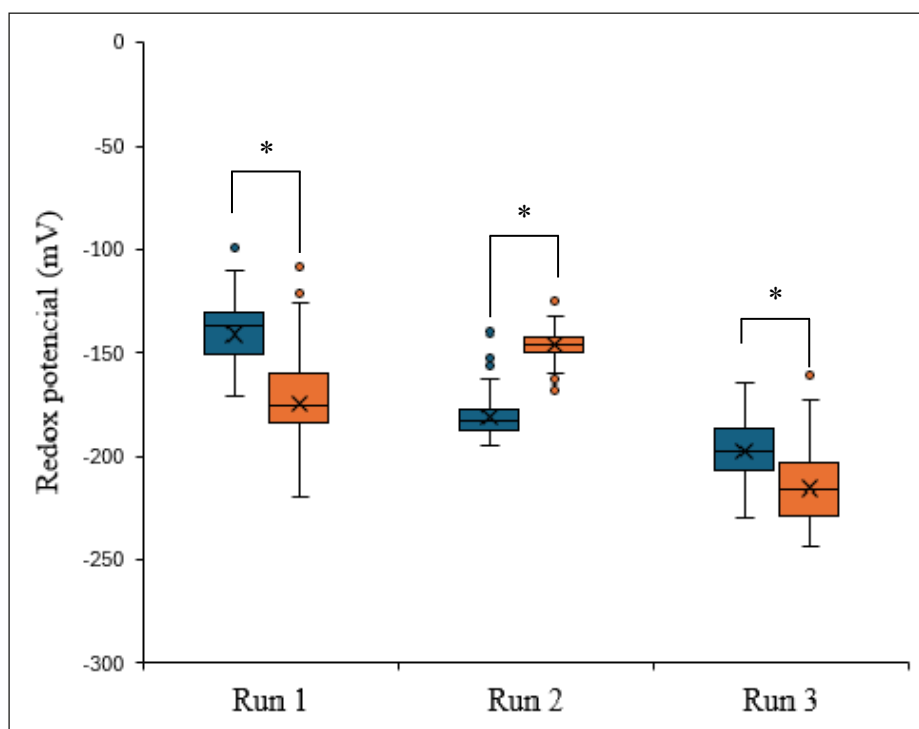


Chart 2: Box plot showing redox potential values (mV) for individual bioreactor runs. The control group is shown in blue, while the tannin-enriched group is shown in orange. Data points of the same colour represent outliers. Whiskers represent the maximum and minimum values excluding outliers. The box boundaries represent the first and third quartiles. The line represents the median, and the cross indicates the mean. * indicates a significant difference ($P < 0.05$) between groups.

The supplementation of tannins significantly influenced several fermentation parameters. The pH of the fermentation medium increased consistently across all experimental runs following tannin addition, although the values remained within the physiological range typical for ruminal conditions. Changes in redox potential were also statistically significant; however, the direction of these changes differed between experimental runs, indicating a possible effect of variations in microbial composition of the inoculum.

During the 1st run the mean pH increased from 6.42 to 6.63 and redox potential decreased from -141 to -174 mV; for the 2nd run from 6.55 to 6.68 for pH and from -197 to -216 mV for redox potential; and for 3rd run pH increased from 6.51 to 6.62 and redox potential decreased from -197 to -216 mV.

A pronounced reduction in dry matter digestibility was observed after tannin supplementation. The digestibility decreased from an average value of 55.2% in the control group to 45.8% in the tannin-enriched group, representing a substantial decline in nutrient availability.

The analysis of VFAs revealed a significant decrease in the concentrations of all major VFAs in response to tannin supplementation. Acetic acid concentration decreased from 982 to 574 µg/mL, propionic acid from 1034 to 558 µg/mL, and butyric acid from 476 to 206 µg/mL. The total concentration of VFAs was similarly reduced, indicating an overall suppression of fermentation activity. Despite these decreases, the ratio of acetate to propionate was not significantly affected.

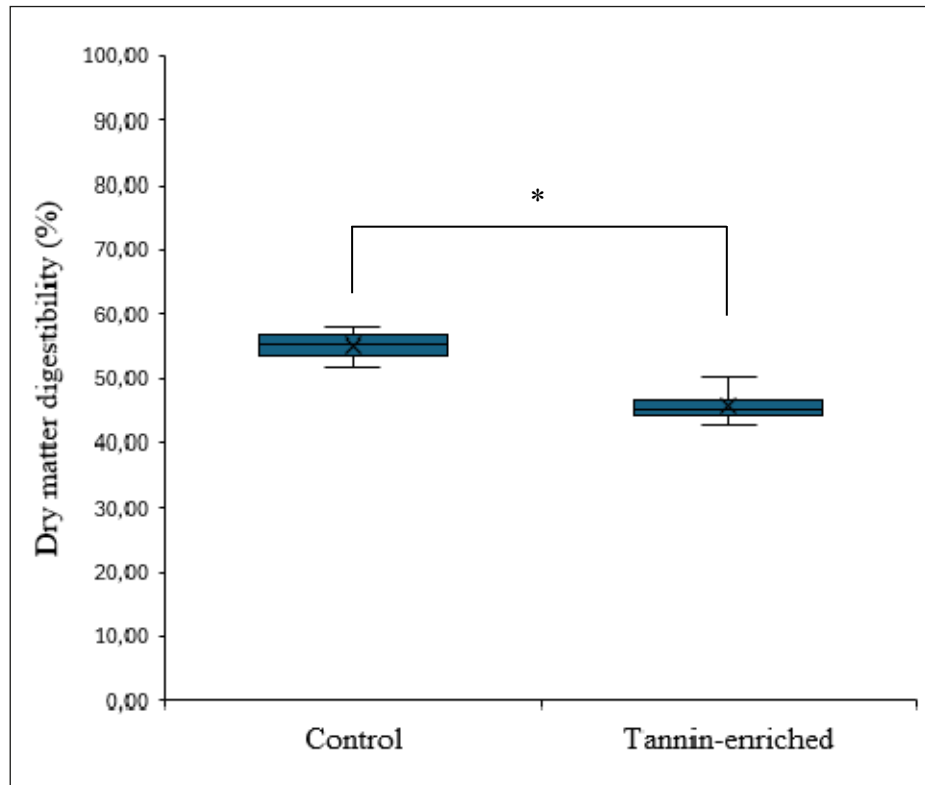


Chart 3: Box plot showing dry matter digestibility (%) for the control and the tannin-enriched group. Whiskers represent maximum and minimum values. The box boundaries represent the first and third quartiles. The line indicates the median, and the cross indicates the mean. * indicates a significant difference ($P < 0.05$) between groups.

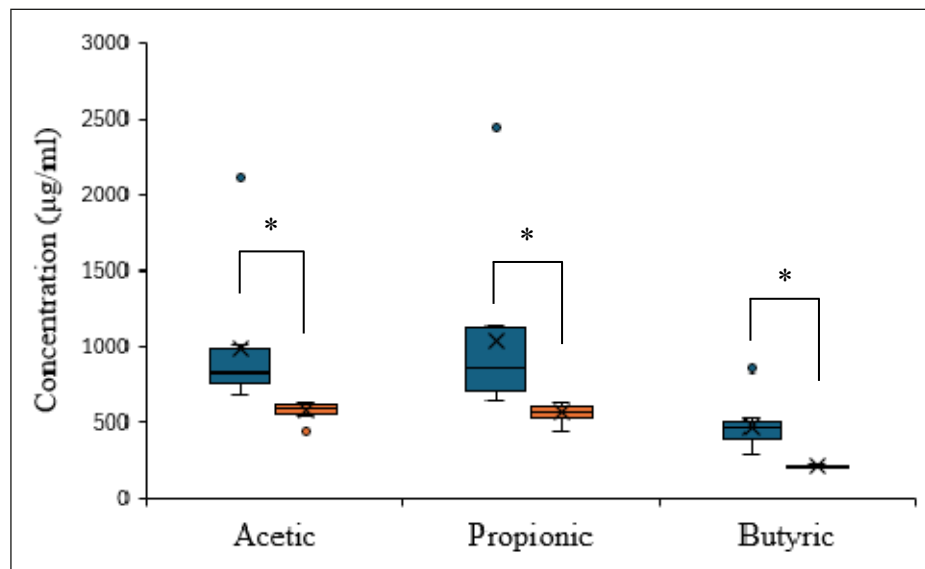


Chart 4: Box plot showing concentrations (µg/mL) of individual VFAs. The control group is shown in blue, while the tannin-enriched group is shown in orange. Points in the same colours represent outliers. Whiskers indicate the maximum and minimum values excluding outliers. The box boundaries represent the first and third quartiles. The line indicates the median. The cross marks the mean. * indicates a significant difference ($P < 0.05$) between groups.

DISCUSSION

During experimentation a significant increase in ruminal pH was observed after the supplementation of tannin into the diet. The elevated pH was most likely a consequence of reduced production of VFAs by the fermentation system. Tannins affect feed digestibility and substrate availability for processes involved in the formation of these metabolites. A reduced concentration or degradation rate of these metabolites in the rumen may therefore influence not only pH but also the ruminal microbiome (Díaz Carrasco et al., 2017). An increase in ruminal pH associated with a higher tannin intake was also observed by (Olafadehan et al., 2016) in goats fed a diet containing 6.8 % tannins in dry matter and by (Díaz Carrasco et al., 2017) using a tannin extract in Holstein cattle.

The effect of tannins on redox potential varied across experimental runs. The simplest explanation for the discrepancy between runs 1 and 3 and run 2 is the use of different inoculum for fermentation initiation in the second run. During preparation of this inoculum, rumen fluid was not mixed with previously digested feed before the experiment, as the rumen fluid had already been supplied pre-filtered. Although the rumen fluid was obtained from the same breed in a different location, this difference may have led to a variation in microbial composition and subsequently influence redox potential during fermentation. These findings slightly differ from those reported by (Dias Batista et al., 2021), who did not observe a significant change in redox potential across different tannin concentrations, however this study measured redox potential only once and thus our significant difference can be caused by superior continuous measurement system. Alongside the present study, this appears to be one of the very few studies investigating the effect of tannins on redox potential.

Dry matter digestibility is primarily influenced by the ability of tannins to bind dietary proteins, thereby protecting them from degradation processes in the rumen. Tannins also reduce cellulolytic and proteolytic activity by affecting the ruminal microbiome, which further contributes to decreased dry matter digestibility (Valcl & Lavrenčič, 2025). The effect of tannins on digestibility is consistent with previous studies. (Valcl & Lavrenčič, 2025) reported decreases in both dry matter and crude protein digestibility depending on tannin type and concentration (5 % and 10 % DM supplementation) in cannulated rams. (Foggi et al., 2022) observed a decrease in organic matter digestibility after feeding cattle with quebracho tannin extract, while (Norris et al., 2020) confirmed a reduction in dry matter digestibility in bulls even at a 3 % tannin supplementation level.

VFAs are a key component of animal metabolism, primarily serving as an energy source (up to 70 % of metabolizable energy) (Bergman, 1990). Their profile is associated with fermentation quality, nutrient partitioning, variation in methane production, and the chemical composition of animal products such as milk (Dijkstra, 1994). A statistically significant decrease in their concentrations indicates a reduction in available energy substrates, which may have a substantial

impact on animal performance *in vivo*. This decrease is again linked to the effects of tannins on digestibility and rumen microbiome. Reduced VFA synthesis may reflect the effect of tannins on feed degradation, as supported by the observed decrease in dry matter digestibility. Our results are not fully consistent with other studies like (Dschaak et al., 2011), who observed a significant effect of tannins *in vivo* only on total VFA concentration and not on individual VFAs or (Norris et al., 2020) who also did not observe significant changes in VFA concentrations *in vivo* following tannin supplementation. These discrepancies may be explained by differences in experimental conditions, including tannin dose and diet composition. The effect of tannins on the acetate-to-propionate ratio was not confirmed in this study, which is consistent with the findings of (Dschaak et al., 2011) and (Norris et al., 2020).

CONCLUSION

The results of this study demonstrate that tannin supplementation at a level of 5 % of dry matter significantly affected ruminal fermentation under *in vitro* conditions. The addition of tannins increased pH, altered redox potential, reduced dry matter digestibility, and decreased the production of VFAs. However, the relative proportions of major fermentation products, expressed as the acetate-to-propionate ratio, remained unchanged.

These findings indicate that tannins suppress fermentation primarily through a reduction in microbial degradation of feed substrates. The study contributes to a better understanding of how plant secondary metabolites modulate rumen fermentation and highlights the potential application of tannins in animal nutrition strategies.

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