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J Vet Res 66, 411-419, 2022
DOI:10.2478/jvetres-2022-0052

Influence of direct-fed microbial blend and *Ferula elaeochytris* on *in vitro* rumen fermentation pattern and degradability during simulated ruminal acidosis

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Received: February 20, 2022 *Accepted: September 13, 2022*

Abstract

Introduction: The use of probiotics and phytobiotics has attracted interest because of their protective effect against acidosis. *Ferula elaeochytris* (FE) is considered a good source of bioactive compounds, mainly monoterpene α-pinene. This study aimed to investigate the effect of a direct-fed microbial blend (Pro) and FE on rumen fermentation parameters *in vitro* under normal and acidosis conditions. **Material and Methods:** An *in vitro* experiment using the Hohenheimer Futterwerttest (HFT) gas production system was conducted. An acidosis challenge was made to compare the effectiveness of the probiotics blend and FE extract on ruminal pH regulation. To generate different ruminal fermentation parameters, the design of the trial considered the 2 additives (Pro and FE) × 6 incubation times (2, 4, 8, 12, 24 and 48 h) × 2 conditions (acidosis and normal) × 2 incubation runs for each feedstuff (barley, alfalfa and straw). **Results:** An acidosis challenge was successfully induced. The Pro and FE additives had no impact on the observed rumen fermentation parameters such as volatile fatty acid concentration or ammonia ($P = 0.001$). The acidosis condition decreased total *in vitro* degradability (IVD) by 3.5% and 21.9% for barley and straw, respectively ($P < 0.001$). The additives had different significant effects on the IVD of nutrients during both normal and acidosis conditions. In alfalfa samples, FE supplementation significantly decreased the IVD of all observed nutrients under the ruminal acidosis condition, although it had no effect during the normal condition. **Conclusion:** An acidosis challenge was successfully induced and the effect of additives was varied on fermentation parameters and rumen degradability of different feeds either under normal or acidosis conditions.

Keywords: HFT, phytobiotic, probiotic, α-pinene.

Introduction

Mismanagment of herd feeding and the resulting nutritional diseases cause disorders in the rumen. Modern feeding practices in dairy cattle often depend on feeding energy-dense diets (19, 29), which can result in metabolic disorders. Of the metabolic digestive disorders, ruminal acidosis is considered the most prevalent animal health and welfare issue in intensive ruminant production systems. Because acidosis causes poor digestion and systemic inflammation, which affects ruminants' general health and productivity (38), there has been a surge of interest in developing natural feed additives that can regulate ruminal pH and thereby prevent or mitigate the severity of acidosis (10, 26).

Several studies have been conducted on potential feeding strategies to decrease the incidence of acidosis, such as providing additives including buffers, yeasts, plant extracts and probiotics (32). In particular, the use of probiotics has attracted interest because of their protective effect against acidosis (35). A blend of probiotics showed higher efficacy than single strain probiotics in strengthening animal health (9, 24). In addition, probiotic mix supplementation improves

in vitro rumen digestibility, fermentation parameters, and additionally, gut health by increasing cell proliferation, and modulating the population of gut microbiota (12). However, there is limited information about the specific mode of action of probiotic blends on *in vitro* fermentation and the digestibility of different feed stuffs and different conditions such as ruminal pH.

Recent studies have shown the positive effects of the use of plants rich in secondary compounds on dry matter intake, ruminal fermentation and subsequently rumen pH in cattle fed an energy-dense diet (24). *Ferula elaeochytris* (FE) is considered a good source of bioactive compounds. Due to its antimicrobial effect and strong antioxodative properties, FE has been suggested as a dietary supplement in livestock production (1, 8). However, limited data are available on the use of FE in ruminant feed. In support of supplementation of this kind, the effect of FE on ruminal fermentation *in vitro* was previously investigated. However, the authors suggested that the optimal level of FE as a nutritional supplement had not yet been determined (21). *Ferula elaeochytris* is a novel and untried feed additive to use for modulation of rumen fermentation, especially under stress conditions such as acidosis. We hypothesised that FE could alter rumen fermentation in such a way that it could help to mitigate the severity of acidosis.

Despite the evidence of plant extracts and probiotics altering rumen fermentation, to our knowledge, no research exists on the influence of FE and multispecies probiotic mixture on fermentation and degradation of different feed stuffs or on its mitigation of ruminal acidosis which offers findings achieved through use of an *in vitro* simulation model. Therefore, the objective of this study was to exploit such a model to evaluate the effects of probiotic blends and FE supplementation to different feedstuffs on ruminal pH, ammonia level, volatile fatty acids (VFA), lactate, methane and total gas production, and nutrient degradation under normal and acidosis conditions.

Material and Methods

Substrate preparation. *Ferula elaeochytris* roots were harvested at the stage of flowering in the south of Turkey at 1,000–1,200 m above sea level, dried under shelter and subsequently ground for homogenous mixing with feed. The probiotic was obtained from a commercial company (SCD Bio Livestock, SCD Probiotics, Kansas City, MO, USA). The probiotic blend (Pro) was composed of *Bacillus subtilis*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus, Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Saccharomyces cerevisiae*, and *Streptococcus thermophilus.*

Dried alfalfa hay, barley and wheat straw were used as substrates in fermenters during the experiment. A portion of all substrates were mixed to prepare total mixed rations (TMR) from them, and the mixture was ground with a centrifugal mill to pass through 1.0 mm screens before incubation. The nutritional compositions of the substrates were analysed according to AOAC International (3), and the composition of the volatile fraction of FE was used which was established in a previous study (21) (Table 1). For the *in vitro* fermentation, substrates were used as TMR consisting of the alfalfa, barley and straw in a ratio of 0.35:0.50:0.15, while for the *in vitro* degradation individual alfalfa, straw and barley substrates were used to evaluate the effect of both conditions and feed additives on each substrate.

Table 1. Chemical composition of the used feedstuffs and volatile fraction composition of *Ferula elaeochytris* (FE)

Parameter	Barley	Straw	Alfalfa
Dry matter, %	90.76	93.12	93.21
Organic matter, %	88.58	86.00	84.63
Ether extract, %	2.45	1.55	2.08
Crude protein, %	10.50	2.82	23.45
Crude fibre, %	6.38	42.18	17.8
aNDFom, %	33.94	77.93	34.22
ADF, %	7.00	51.42	24.18
ME, MJ/kg	2.34	5.72	3.80
TDN, %	23.11	40.79	30.50
Volatile composition of FE, mg/g[1]			
α-pinene		2.167	
Camphene		*	
β-pinene		0.085	
Verbenone		0.015	
Elemene		0.011	
1,4-cineole		0.012	
Cis-verbenol		0.016	
Trans-verbenol		0.048	
Limonene		*	
Myrtenol		0.010	

aNDFom – Neutral detergent fibre assayed with heat-stable amylase and expressed exclusive of residual ash; ADF – Acid detergent fibre expressed inclusive of residual ash; ME – Metabolisable energy, calculated using the prediction equations of Menke and Steingaß (27); TDN – Total digestible nutrients, calculated from the ME value as per the equation recommended by the National Research Council (30); [1] – Microdistillation; *– Below the limit of detection, $P < 0.01$

Rumen fluid collection and processing. Two cannulated Holstein cows were used as rumen fluid donors and fed straw and concentrates (70:30). Rumen fluid was collected before morning feeding using a probe from the cannula. The collected rumen fluid was mixed into a thermal flask preheated to 39°C, filtered through 4 layers of cheesecloth to eliminate feed particles, and immediately transferred to the laboratory of the Department of Animal Nutrition and Nutritional Disease in the Faculty of Veterinary Medicine at Ankara University, where the pH was measured. All procedures were performed under anaerobic conditions by flushing

with CO_2 and the time required for all treatments was less than 30 min.

***In vitro* buffer solution for fermentation and degradation.** The buffer solution for *in vitro* fermentation comprised macroelement solution including Na_2HPO_4, KH_2PO_4 and $MgSO_4.7H_2O$; microelement solution including $CaCl_2.2H_2O$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$ and $FeCl_3.6H_2O$; buffer solution including $NaHCO_3$ and NH_4HCO_3; resazurin solution; and reductant solution including $Na_2S.7H_2O$ and NaOH. In the acidosis condition, the concentration of the microelement solution was decreased to reach a lower pH. For *in vitro* degradation, the buffer solution prepared with buffer solution A (containing KH_2PO_4, 10.0 g/L; $MgSO_4.7H_2O$, 0.5 g/L; NaCl, 0.5 g/L; and $CaCl_2.2H_2O$, 0.1 g/L in distilled water for the normal condition and urea, 0.5 g/L, in distilled water for the acidosis condition) and solution B (containing Na_2CO_3, 15 g/L and $Na_2S.9H_2O$, 1.0 g/L in distilled water) was freshly prepared at 39°C and pH condition 6.8 (with A in 5:1 proportion to B) for normal conditions. To simulate acidosis, Na_2CO_3, 3,75 g/L and $Na_2S.9H_2O$, 0.43 g/L were prepared to provide a low rumen fluid pH level.

***In vitro* fermentation.** The experimental material was incubated with a modified *in vitro* Hohenheimer Futterwert Test (HFT) gas production system (36) by allocating the probiotic blend and FE mixed with each tested foodstuff to a specific syringe. Two hundred milligrams of the dried TMR consisting of barley, alfalfa and straw as substrates was incubated with 30 mL of a ruminal buffered suspension with 1×10^{10} colony-forming units per g Pro (0.1 µL/mL) and 0.05 mg/mL FE or without either supplement in simulated acidosis. The difference between normal and acidosis condition was achieved by buffer composition as previously mentioned. Thus, each feed additive–supplemented substrate was incubated separately under two different pH levels (normal and acidosis). Dosages were moderate in comparison to previous *in vitro* screening studies (21, 35). The syringes, previously heated in the incubator to 39°C, were filled with mixed buffered ruminal fluid that was immediately bubbled with CO_2. To yield ample ruminal fermentation parameters, the design of the trial considered the 2 additives (Pro and FE) × 6 incubation times (2, 4, 8, 12, 24 and 48 h) × 2 conditions (acidosis and normal) × 2 incubation runs with 6 replicates of each.

Sampling and analysis. For each incubation time, samples of fermentation fluid for pH ammonia and VFA analysis were collected and stored at –20°C. The analysis of pH was done immediately after collection of samples with a pH-meter (Hanna Instruments, Leighton Buzzard, UK). The analysis of ammonia nitrogen in rumen samples was carried out with the indophenol blue method at 546 nm using spectrophotometry according to the method described by Mickdam *et al*. (29). The VFA concentrations were determined with the procedures described by Geissler *et al.* (14) and Metzler-Zebeli *et al.* (28) using gas chromatograph (GC) and a GC-2010 chromatograph (Shimadzu Co., Kyoto, Japan). The thawed samples were centrifuged at 4,000 × *g* for 15 min at 4°C. The supernatant was mixed with ice-cold 25% metaphosphoric acid solution. Then the samples were centrifuged again at 11,000 rpm for 10 min at 4°C and the supernatant was transferred into GC vials for VFA analysis. The gas volume of the syringes was recorded in each incubation time. To determine the emission of CO_2 and CH_4 gases, the data of VFA concentration in rumen fluid samples were used. The calculation formula was adopted from Blümmel *et al.* (7) and was CO_2 = (Acetic acid/2) + (Propionic acid/4 + 1.5 × Butyric acid) and CH_4 = (Acetic acid + 2 × Butyric acid) – CO_2. The analysis of lactate was evaluated using an enzymatic assay procedure and K-DLATE (Rapid) Assay Kit 12/12 (Megazyme, Bray, Ireland).

Substrate degradation. To determine the *in vitro* degradation of barley, alfalfa and straw samples with the same feed additives in acidosis simulation, the Daisy^{II} incubator method disseminated by Ankom Technology was used (2). Ankom jars were used in this degradation process.The values of pH were measured to ensure that acidosis (5–5.5) and normal (6–7) conditions were prevailing. Samples of all substrates in 0.5 gmass amounts were ground to pass through a 1 mm screen. The products were separately transferred into Ankom F57 filter bags sized 4.5 × 4.0 mm with a pore size of 25 µm and when heat sealed, the bags were incubated in the rumen fluid for 48 h. One filter bag was also incubated for blank correction to be used in the calculation. Eight filter bags were used for *in vitro* degradation in each treatment. The incubator consisted of four cylinder jars with 25 filter bags each.

The filter bags were removed at the end of the incubation and immediately washed with cold tap water until the water ran clear, dried at 105°C for 3 h, equilibrated for 15 min in a desiccator, and processed for the determination of *in vitro* degradation (IVTD). The filter bags were then treated with boiling neutral detergent solution for 1 h in an Ankom Fiber Analyzer (Ankom Technology, Macedon, NY, USA), rinsed with cold tap water, dried, and the *in vitro* true DM degradation (IVDMD), *in vitro* organic matter degradability (IVOMD) and *in vitro* neutral detergent fibre degradability (IVNDFD) were calculated.

Statistical analysis. Data were analysed in a mixed linear model with the PROC MIXED procedure in SAS (34). Each sample was considered as a random effect in the model and experimental unit of the study. For the *in vitro* true digestibility data, the treatment (normal, acidosis, NorPro, AcidPro, NorFE, or AcidFE) was a fixed effect in the model. In rumen parameters, the data were also evaluated in a mixed model with a repeated measures manner. The treatments mentioned above, time, and two-way interactions were considered fixed effects in the mixed model for rumen parameters. Studentised residuals were calculated with all fixed effects and interactions. Then, outliers were removed from the model (<−4 or >4). For time-dependent data,

degrees of freedom were calculated using the between-within model approximation, while the Kenward–Roger approximation was used for IVTD data. Furthermore, the Shapiro–Wilk test was used to evaluate the normal distribution of the data with the PROC UNIVARIATE procedure in SAS (34). Log transformation was used for parameters that did not have normal distribution in the mixed model. For time-dependent data, the covariance structure of the model was determined according to a spatial power law because of unequal intervals of the fermentation sampling time points. Tukey–Kramer adjusted P-values were used during multiple comparisons of the data because of group numbers. All data were reported as least-square means ± pooled SEM in the tables. The significance level was considered at $P \leq 0.05$ for all data, except for Tukey–Kramer adjusted multiple comparisons.

Results

***In vitro* rumen fermentation.** As intended by the modified buffer composition, the pH values of the ruminal fluid under acidosis conditions decreased starting from the fourth hour of the incubation. The mean pH ranged from 6.6−6.9 throughout the experiment under normal incubation conditions while the pH decreased progressively and then remained at a constant value of 5.4−5.7 under acidosis conditions (Fig. 1).

The incubation period had a great impact on total VFA ($P = 0.042$) and ammonia N ($P < 0.001$), with the minimum production being observed at 2 h and the maximum production after 12 h of incubation (Fig. 2). Despite the absence of interaction between supplementation and time, there are noticeable differences in the concentration of both VFA (Fig. 2A) and ammonia (Fig. 2B) in all groups at different times. For example, the highest rate of ammonia and VFA was observed after incubation for 24 h, and the lowest rate was noted after incubation for 2 h.

The additives had no impact on VFA or ammonia concentration either under normal or acidosis conditions (Table 2). Although the pH of the rumen fluid was significantly lower in all artificial acidosis groups, supplementation did not change the situation for either group in the present study (Table 2). Even though treatment × time interactions of the rumen pH levels of the samples were observed at 4 h, 8 h, 12 h, and 24 h of the study ($P = 0.01$), there was no interaction at 2 h of the study. Over time, most of the observed rumen kinetics were significantly changed ($P \leq 0.05$), except for acetate, valerate, and CH_4 levels.

***In vitro* rumen degradation.** As expected, the acidosis condition decreased total IVD ($P < 0.001$) by 3.5% and 21.9% for barley and straw, respectively (Table 3). Ruminal acidosis had no significant effect on IVTD of nutrients in alfalfa samples. The treatments had different significant effects on IVD of nutrients during both normal and acidosis conditions. The probiotic blend and FE supplements did not affect barley IVDM, IVOM, or total IVD in normal or acidosis conditions. Furthermore, the additives did not affect the IVNDFD of barley in normal rumen conditions. However, the IVNDFD of barley was lower in the FE-supplemented group than in the unsupplemented and probiotic blend supplemented groups during acidosis conditions ($P = 0.05$). During acidosis, probiotic blend supplementation did not affect the IVNDFD of barley.

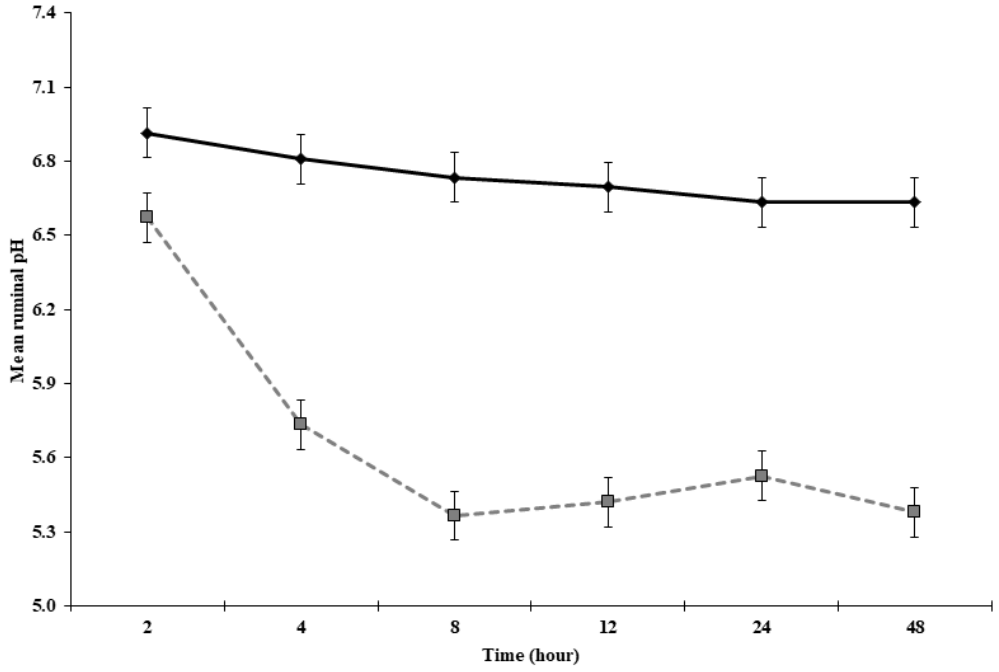

Fig. 1. Dynamics of the fermentation fluid pH (mean ruminal pH) during the experimental period under normal and acidosis condition ($P < 0.001$)

Table 2. Effects of direct-fed microbial blend and *Ferula elaeochytris* (FE) on *in vitro* rumen fermentation parameters during ruminal acidosis

Parameter	Treatment						SEM	P-value		
	Normal	Acidosis	NorPro	AcidPro	NorFE	AcidFE		Treatment	Time	Treatment × Time
pH	6.75[a]	5.59[b]	6.76[a]	5.72[b]	6.76[a]	5.74[b]	0.10	0.0001	<0. 001	0.01
Ammonia, mmol/L	16.56	9.73	14.90	11.28	16.02	11.16	5.37	0.87	<0.001	0.35
Total VFA, mmol/L	57.04	52.79	54.60	47.52	54.52	55.53	5.83	0.88	0.04	0.80
Acetate, mmol/L	35.23	30.74	33.96	27.84	33.67	32.52	3.90	0.78	0.06	0.83
Propionate, mmol/L	10.63	10.60	10.31	9.03	10.50	11.21	1.25	0.87	0.03	0.80
Butyrate, mmol/L	1.35	1.22	1.29	1.20	1.32	1.24	0.24	0.99	0.01	0.53
Isobutyrate, mmol/L	6.37	7.11	5.77	6.46	5.66	7.74	1.31	0.86	0.02	0.43
Valerate, mmol/L	1.87	1.61	1.71	1.58	1.78	1.39	0.33	0.92	0.06	0.50
Isovalerate, mmol/L	1.59	1.52	1.47	1.40	1.59	1.46	0.10	0.71	0.04	0.82
Lactate, mmol/L	3.02	3.04	3.32	3.06	3.23	3.14	0.11	0.39	0.001	0.13
GAS, mL	54.00	39.40	46.20	34.20	34.20	36.50	11.46	0.79	<0.001	0.86
CO_2, mmol/L	22.30	19.84	21.49	17.98	21.44	20.90	2.29	0.79	0.05	0.82
CH_4, mmol/L	15.63	13.33	15.04	12.26	14.87	14.09	1.94	0.82	0.07	0.82

Normal – no supplement, without acidosis; Acidosis – no supplement, with acidosis; NorPro – probiotic supplementation, without acidosis; AcidPro – probiotic supplementation, with acidosis; NorFE – *Ferula elaeochytris* supplementation, without acidosis; AcidFE – FE supplementation, with acidosis; VFA – Volatile fatty acids; GAS – Total gas volume; SEM – Standard error of means; [a,b] – Values with different superscripts in the same line are significantly different ($P \leq 0.05$). Data are represented as least square means

Table 3. Effects of direct-fed microbial and *Ferula elaeochytris* (FE) on *in vitro* true digestibility of barley, alfalfa and straw during ruminal acidosis

Parameter	Normal	Acidosis	NorPro	AcidPro	NorFE	AcidFE	SEM[4]	P-value
Degradation (% of incubated)[2] Barley								
Dry matter (DM)	86.36[ab]	83.79[c]	87.16[a]	80.96[c]	84.34[abc]	82.24[c]	0.80	<0.001
aNDFom[3]	48.48[a]	38.78[a]	51.52[a]	28.09[b]	40.87[a]	32.93[a]	3.02	<0.001
Organic matter (OM)	88.44[ab]	86.26[bc]	89.12[a]	83.86[c]	86.73[abc]	84.95[c]	0.68	<0.001
Total	87.62[a]	85.29[b]	88.35[a]	83.33[b]	85.79[ab]	83.88[b]	0.72	<0.001
Straw								
Dry matter (DM)	35.89[a]	26.35[b]	35.71[a]	20.39[c]	38.92[a]	34.69[a]	1.41	<0.001
aNDFom[3]	23.39[a]	12.00[b]	23.18[a]	14.88[b]	27.01[a]	21.97[a]	1.68	<0.001
Organic matter (OM)	45.87[a]	37.82[b]	45.72[a]	32.79[c]	48.43[a]	44.86[a]	1.19	<0.001
Total	40.30[a]	31.42[b]	40.13[a]	25.87[c]	43.12[a]	39.19[a]	1.31	<0.001
Alfalfa								
Dry matter (DM)	78.54[a]	77.23[ab]	78.89[a]	76.35[ab]	78.95[a]	75.04[b]	0.69	0.001
aNDFom	41.54[a]	37.99[ab]	42.5[a]	35.59[ab]	42.67[a]	32.02[b]	1.88	0.001
Organic matter (OM)	82.16[a]	81.07[ab]	82.45[a]	80.34[ab]	82.5[a]	79.26[b]	0.54	0.001
Total	80.00[a]	78.78[ab]	80.33[a]	77.96[ab]	80.38[a]	76.74[b]	0.64	0.001

Normal –no supplement, without acidosis; Acidosis – no supplement, with acidosis; NorPro – probiotic supplementation, without acidosis; AcidPro – probiotic supplementation, with acidosis; NorFE – FE supplementation, without acidosis; AcidFE – FE supplementation, with acidosis; [2] – Apparent disappearance – (supply−residue)/supply ×100; aNDFom – Neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; [4] – Standard error of means, [a,b,c] – Values with different superscripts in the same line are significantly different ($P \leq 0.05$). Data are represented as least square means

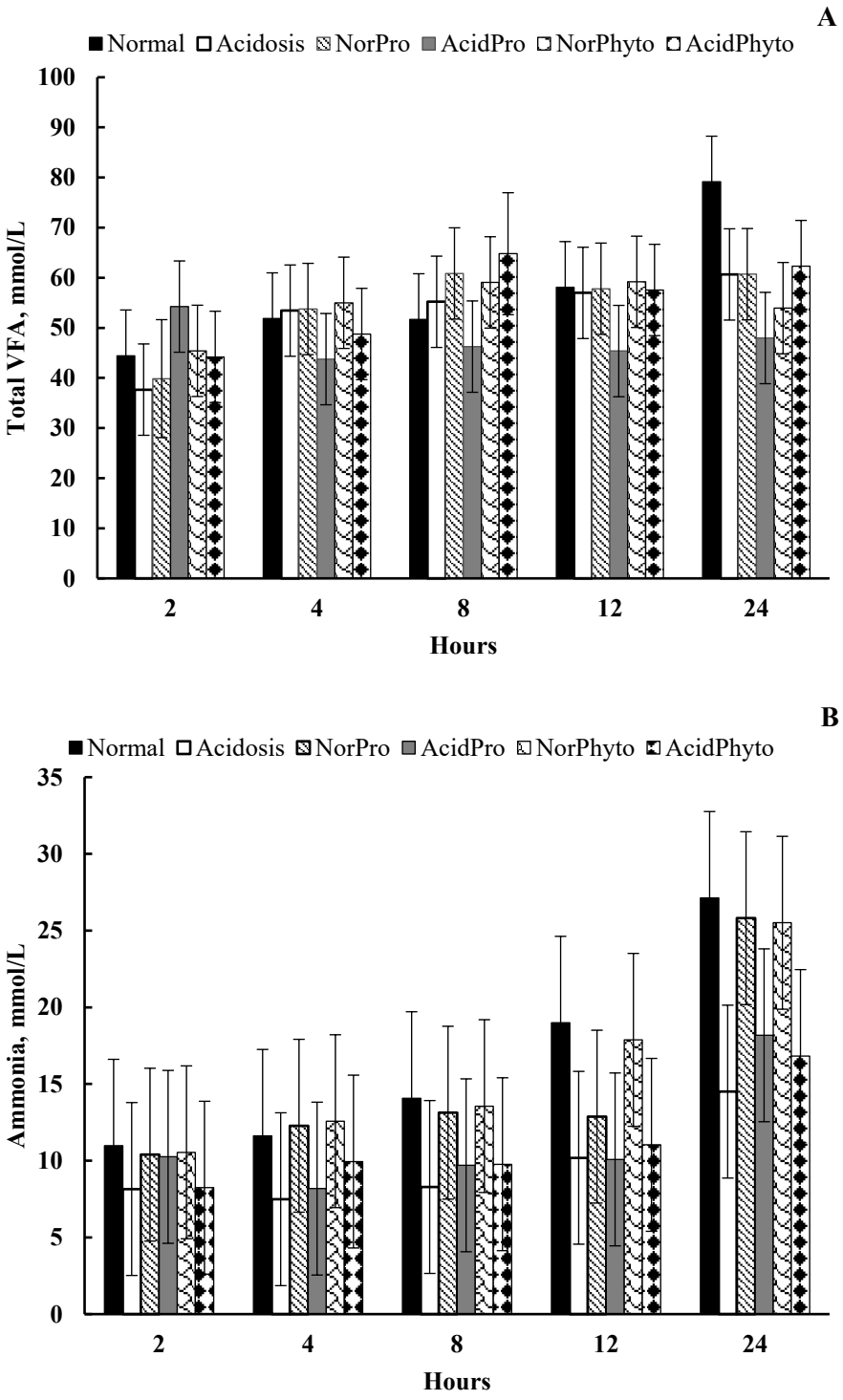

Fig. 2. Variation of the concentrations of total volatile fatty acids (A) and ammonia (B) over time under normal and rumen acidosis conditions. Normal – no supplement, without acidosis; Acidosis – no supplement, with acidosis; NorPro – probiotic supplementation, without acidosis; AcidPro – probiotic supplementation, with acidosis; NorFE – *Ferula elaeochytris* supplementation, without acidosis; AcidFE – FE supplementation, with acidosis

In straw samples, the supplementations did not affect the IVDMD under normal rumen conditions. The IVDMD was significantly lower in the acidosis group without supplements than in the normal rumen group. Furthermore, the IVDMD was lower in the probiotic blend–supplemented group than in the unsupplemented group under acidosis conditions. Interestingly, FE delivery to the rumen during acidosis raised IVDMD to levels similar to those of groups with normal rumen conditions. Moreover, FE supplementation during acidosis acted with similar effects on the IVNDFD, IVOMD and total IVTD of the straw samples.

In alfalfa samples, FE supplementation significantly decreased the IVD of all observed nutrients under

ruminal acidosis conditions, although it had no effect during normal conditions. Probiotic blend supplementation had no significant effect on alfalfa digestibility parameters regardless of ruminal acidosis status.

Discussion

The desire to develop natural feed additives with the capacity to stabilise ruminal pH and thereby prevent or lessen the severity of acidosis has significantly grown in recent years (10, 26). This is because acidosis results in suboptimal digestion and systemic inflammation, ultimately impairing ruminants' overall health and productivity (38). In this context, probiotics have a positive effect on the rumen environment by stabilising pH and providing nutrients to ruminal microbes (11). In addition, numerous studies have revealed the various effects of various phytochemicals on rumen ecology and metabolic outputs (17, 29, 31). Therefore, this study aimed to investigate the effects of probiotic blends and FE supplementation to different feeds on the fermentation profile and degradation using an *in vitro* acidosis model.

Many pH thresholds have been researched to characterise subacute ruminal acidosis (SARA); most commonly, ruminal pH decreased between 5.6 and 5.8 for several hours daily (*e.g.* 3 or 5.4 h/d) during SARA (33, 37). In this context, the acidosis challenge was successfully induced as intended by a modified buffer composition with a comparable pH range (5.4−5.7), but for a longer period of time. The aim of the study was to compare the effectiveness of a probiotics blend and FE extract on ruminal pH regulation that resembled feeding practices when acidosis affects cows over a long period of time. To the best of our knowledge, the current study is the first to report on the effect of a probiotic blend and FE on the regulation of *in vitro* ruminal fermentation under acidosis challenge. Because of their potent antibacterial and antioxidant activities, the probiotic blend and FE have been shown in other research to be interesting feed additives (1, 8); therefore, they were investigated for their impact on rumen fermentation characteristics under acidosis conditions. Terpenoid compounds made up the majority of bioactive chemicals identified in FE, with α-pinene; β-pinene, myrcene and limonene; linalool, terpineol and neryl acetate; β-caryophyllene, germacrene B, germacrene D and δ-cadinene; and caryophyllene oxide, α-cadinol, guaiol and spathulenol being the major ones. The main bioactive compound of FE was previously identified as α-pinene, a monoterpene (21). Selective antibacterial properties of this compound, a key component of the essential oils of several *Ferula* species, have been demonstrated (23).

Although the pH of the rumen fluid was significantly lower in all artificial acidosis groups, the supplementation did not change the situation either both groups in the present study, similarly to previous studies, where that probiotic blend and FE did not alter the ruminal pH, ammonia N or VFA concentrations (21, 35). This could be due to the severity of the mimicked acidosis condition (29). According to the published literature, there is a great impact of bioactive substances on VFA production and ammonia levels (6, 16). No influence on rumen fermentation was observed when a blend of linalool, p-cymene, α-pinene and β-pinene was used in dairy goats. The authors suggested that the absence of the effect was possibily due to the intensive microbial degradation of these compounds in the rumen. This theory of rapid microbial degradation was also supported by Haider (15), who observed low recovery of α-pinene when incubated with rumen fluid for 24 h. Moreover, Klevenhusen *et al.* (22) reported that responses of ammonia N and total VFA concentration were more pronounced with a dosage of active compounds exceeding 100 mg/g, which was far higher than administered in the current study.

The effect of acidosis was more obvious in the straw IVD, which could be due to the microbial dysbiosis including reduction in fibrolytic bacteria caused by acidosis conditions (13, 19, 20). According to some reports, probiotics boost the ruminant's ability to digest fibre and increase the availability of cellulose and hemicellulose as an energy source (4); however, the effect of probiotic supplementation on improving fibre degradation was not clear in the current study. This could be due to the lower adaptability of these probiotics in the ruminal environment due to the short incubation time. Moreover, it is believed that different strains have varying impacts on the rumen and not all yeasts have the ability to alter the rumen metabolism (18). Interestingly, the supplementation of FE during the acidosis condition improved the IVDNDF and IVDOM of straw, which in turn was reflected in an improvement of the IVDMD, which appears to be difficult to explain in how it transpired in the current study.

The variance in the effect of probiotic blend and FE supplementation on the degradation of different incubated substrates may indicate the difference in the effect of additives on the ecosystem in the rumen and the extent to which these additives are affected by the composition of the incubated substrates. Therefore, further investigation might be geared towards its application in an *in vivo* acidosis challenge. There are many distinct types of microorganisms involved in the intricate process of rumen fermentation, but rumen bacteria are thought to be the most significant (5). However, the effect of supplementation on the rumen microbiota was not investigated in the current study. Therefore, it is necessary to study the effect of these additives on rumen microbes under different incubation conditions to understand the extent of the activity of these supplementation as modifiers of the internal rumen environment.

In conclusion, this *in vitro* acidosis model for cows representing the severe acidosis condition revealed significant changes in rumen fermentation parameters.

The effect of additives was varied and depended on fermentation parameters and rumen degradability of different feeds either under normal or acidosis conditions. This study shows for the first time that supplementation of FE may be effective in the degradation of straw under the acidosis condition. The supplementation of FE showed promising results for the IVDNDF, IVDOM and IVDM of straw during an acidosis challenge, suggesting a benefit in the form of enhancement of the microbial degradation of fibres. However, the effect of FE on rumen microbiome under different incubation conditions needs further investigation.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The source of the funding of research and publication was the authors' own money.

Animal Rights Statement: None required.

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