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Effect of feeding *Yucca schidigera* extract and a live yeast on the rumen microbiome and performance of dairy cows fed a diet excess in rumen degradable nitrogen

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ABSTRACT

Nitrogen (N) loss from livestock agriculture via ammonia and nitrous oxide can reduce feed efficiency, production and negatively affect the environment. One option to reduce N loss is to add dietary supplements such as *Yucca schidigera* extract which has ammonia-binding properties and contains antimicrobial steroidal saponins, or *Saccharomyces cerevisiae* yeast, which can stabilise rumen pH and promote fibre degradation, increasing microbial growth and demand for degradable N. To determine the effect of *Yucca schidigera* extract when fed alone or in combination with a live yeast on the performance, rumen metabolism, microbiome and N balance, six rumen cannulated dairy cows were fed a mixed ration (**C**), mixed ration with *Y. schidigera* extract (De-Odorase®, Alltech®; 5 g/cow/day; **D**), or mixed ration with *Y. schidigera* extract (5 g/day) and *Saccharomyces cerevisiae* (Yea-Sacc®, Alltech®, 1 g/cow per day; DY), in a 3 × 3 Latin rectangle design study with three periods of 49-day duration. Digesta samples were collected via the ruminal cannula during the final week of each period and separated into liquid (**LPD**) and solid (**SPD**) phases for microbiome analysis using 16S rRNA amplicon sequencing. DM intake was 0.8 kg/d lower ($P < 0.05$) in cows fed DY than C or D, with milk protein concentration 1.7 g/kg higher in C than D or DY. There was a beta diversity (Bray Curtis) clustering of the LPD in cows fed D or DY compared to C ($P < 0.05$), driven by an increase in *Prevotella ruminicola*-related operational taxonomic units (OTUs), and a decrease in *P. brevis* and *P. bryantii* OTUs. A methanogen OTU, *Methanobrevibacter olleyae*, was decreased in cows fed D or DY and an unclassified species of Gammaproteobacteria was increased in DY (LDA > 2.0, $P < 0.05$) compared to C. Rumen pH, ammonia and total VFA concentration were not affected by treatment ($P > 0.05$) but the concentration of propionate and iso-butyrate were lower at 1700 and 2000 h in cows fed DY compared to C ($P < 0.05$). Measurements of N balance were unaffected by supplementation with D or DY, and there was no effect of treatment on slurry pH. In conclusion, supplementing with an extract of *Yucca schidigera* either alone or in combination with a live yeast had only a small effect on performance, with *Yucca schidigera* altering species associated with carbohydrate and protein metabolism, and reduced *Methanobrevibacter olleyae* which is involved in methanogenesis.

Implications

Feeding an extract from Yucca plants either alone or in combination with a live yeast in a diet that is excess in rumen degradable nitrogen alters rumen metabolism and the diversity of the microbial community, including species involved in carbohydrate and nitrogen metabolism. A bacterial species related to methanogenesis is also reduced, which may decrease the environmental impact of milk production.

Introduction

Ammonia (NH_3) emissions contribute to the formation of fine particles in the atmosphere (<2.5 μm; $PM_{2.5}$), which is associated with several adverse health conditions in humans (Giannakis et al., 2019). The World Health Organisation has recently implemented an upper threshold of $PM_{2.5}$ at 10 μg/m^3, with the UK government setting a target to reduce ammonia emissions by 16% by 2030 compared to 2005 (Defra, 2019). Agriculture contributes 88% of NH_3 emissions in the UK, with dairy cows producing 28% from slurry application, cattle housing, slurry storage and grazing (Defra, 2019). In dairy systems, the efficiency of capture of dietary

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N into milk N is comparatively low at approximately 25%, with some 30% of dietary N excreted in the faeces and a further 35–45% in the urine (Sinclair et al., 2014). Excess NH_3 is produced in the rumen when dairy cow diets are oversupplied with rumen degradable N (**RDN**), or graze grass, which is high in CP and RDN (Totty et al., 2013). The excess NH_3 is absorbed across the rumen epithelium and transported to the liver where it is converted into urea, which can then either be recycled back to the rumen directly across the rumen or in the saliva, or excreted in the urine (Nolan, 1975; Getahun et al., 2019). When urea in urine comes into contact with the enzyme urease present in faecal material, NH_3 is released. This, along with other forms of N in cattle slurry, such as nitrous oxide and nitrate, has negative implications for the environment (Hynes et al., 2016).

Yucca schidigera contains saponins, which are a group of high molecular weight glycosides, with the saccharide chain units (1–8 residues) linked to a steroidal aglycone moiety (Patra and Saxena, 2009). The glycofraction has been demonstrated to bind NH_3 (Headon et al., 1991), while the steroidal components have been recognised to have antiprotozoal and anti-bacterial properties in the rumen, which may assist in the regulation of the release of NH_3 within the digestive tract (Wallace et al., 1994). While studies have been undertaken *in vitro* and *in vivo* on the effects of *Y. schidigera* extract on protozoal numbers (Benchaar et al., 2008; Hristov et al., 1999), rumen fermentation (Holtshausen et al., 2009; Lovett et al., 2006), or a limited range of bacterial species (Wallace et al., 1994), there is no information using more recently developed analytical techniques to specifically investigate the effect on microbial community diversity in the rumen. Furthermore, the NH_3 binding capacity of *Y. schidigera* extract may reduce the rate and extent of release of NH_3 from cattle slurry, independent of its effects in the rumen (Wallace et al., 1994), but few studies have been conducted in this area.

Inclusion of live yeast in cattle rations has been reported to reduce dissolved oxygen, particularly at the interface between bacteria and fibre, thereby stimulating cellulolytic bacterial growth (Chaucheyras-Durand et al., 2008). An increase in microbial growth may subsequently increase the utilisation of NH_3 for microbial protein synthesis (Jouany, 2006), improve N capture and enhance performance, changes that may be synergistic to the effect of *Y. schidigera* extract.

The hypothesis was that the addition of *Y. schidigera* extract to the diet would alter the rumen microbiome to reduce the degradation of protein and decrease rumen ammonia concentration, and that the addition of a live yeast when fed alongside *Y. schidigera* extract would increase the abundance of fibre digesting bacteria, further increasing the utilisation of ammonia in the rumen and improving N use efficiency in dairy cows fed when diets excess in degradable N. The objectives of the current study were to determine the effect of *Y. schidigera* extract when fed alone or in combination with *Saccharomyces cerevisiae* on the rumen microbiome and metabolism, and N excretion in dairy cows when fed a diet excess in rumen degradable N.

Material and methods

Animals, diets and experimental design

The study was conducted over a 21-week period from November 2020 to April 2021 at the Harper Adams University Dairy Cow Metabolism Unit (Shropshire, UK) and was undertaken in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986 (amended 2012), and received local ethical approval.

Six Holstein-Friesian dairy cows in their third lactation that had previously been fitted with a permanent rumen cannula (10 cm diameter; Bar Diamond, Idaho, USA) were used. The cows were 76 days (SE ± 24.8) postcalving, weighed 650 kg (±26.2), and yielded 37 kg (±1.9) of milk per day at the start of the study. Each cow was randomly assigned to one of three dietary treatments in a 3 × 3 Latin rectangle design (Mead et al., 1993), with three periods of seven weeks duration, with measurements undertaken during the final 7 days of each period. For the first six weeks of each period, the cows were group-housed in a pen bedded with sawdust with the area in front of the feed barrier scraped out twice a day. In the seventh week of each period, the cows were restrained in individual metabolism stalls fitted with mattresses for five days for rumen sampling and the total collection of urine and faeces. Cows had continuous access to water at all times.

All cows received a basal total mixed ration (**TMR**) that was formulated to meet their metabolisable energy and metabolisable protein requirements and supply excess rumen degradable N (511 g/cow/d or 21% excess metabolisable protein derived from effective degradable nitrogen and digestible undegradable protein; MPN; Thomas, 2004; Table 1). The three dietary treatments were: **C**: TMR without supplementation; **D**: TMR plus *Yucca schidigera* extract (De-Odorase® Alltech®, KY, D, fed at the manufacturer recommended rate of 5 g per cow per day), or DY: TMR plus De-Odorase® (5 g per cow per day) and *Saccharomyces cerevisiae* CBS 493.94 yeast (Yea-Sacc®, Alltech®, KY, containing 1 × 10^9 colony forming units/g of product), fed at the manufacturers recommended rate of 1 g per cow per day. De-Odorase® is a Mojave yucca pulverised product obtained from stems of *Yucca Schidigera* Roezl containing a mix of saponins and glycofractions. The supplements were mixed with 100 g of ground barley as a carrier prior to manual mixing into the TMR, with cows fed C also receiving 100 g of ground barley. During the adaptation period, all cows received their diets via individual Calan gates (American Calan, Northwood, NH, USA) at approximately 0800 h and a rate of 105% of the

Table 1
Dietary formulation (g/kg DM) of the basal total mixed ration (TMR) fed to dairy cows fed the Control TMR (C), TMR with De-Odorase® (D) or TMR with De-Odorase® and Yea-Sacc® (DY).

Ingredient	g/kg DM
Grass silage	550
Barley	211
Sugar beet	72
Soypass[1]	64
Wheat distillers dark grains	31
Rapeseed meal	31
Soya bean meal (Hipro)	21
Palm kernel meal	9
Minerals/vitamins[2]	4
Megalac[3]	4
Molasses	3
Predicted composition	
ME[4], MJ/kg DM	12.1
MPE[5], g/kg DM	103
MPN[6], g/kg DM	125
MPB[7], g/kg DM	52
MPE[5], % of requirements	100
MPN[6], % of requirements	121

[1] A rumen-protected source of soybean (KW Alternative Feeds, Leeds, UK).
[2] Minerals/Vitamins premix (KW Alternative Feeds, Leeds, UK), major minerals g/kg: Ca 220, P 30, Mg 80, Na 80, trace minerals mg/kg: Cu 1 000; I 400, Mn 4 000; Se 160, Zn 3 000; Vitamins (IU): A 1 000 000; D_3 300 000; E 4 000; B_{12} 135.
[3] A rumen-protected source of fat (Volac, Royston, UK).
[4] ME, Metabolisable energy.
[5] MPE, Metabolisable protein-rumen energy limited.
[6] MPN, Metabolisable protein-rumen nitrogen limited.
[7] MPB, Metabolisable protein from bypass protein.

previously recorded intake, with refusals collected three times a week (Monday, Wednesday and Friday).

Performance

During the sampling week of each period, intake was recorded daily. The cows were milked twice daily using a portable milking machine (Milkline, London, UK) at 0600 and 1600 h, with the yield recorded and samples collected on four occasions (two AM and two PM) during the sampling week for subsequent composition analysis. Animals were weighed (Tru Test, Auckland, New Zealand) at the beginning and end of each period. Grass silage and TMR samples were collected daily during the sampling period and stored at −20 °C prior to subsequent analysis.

Rumen metabolism

Rumen fluid samples were collected on day three of each sampling week at 0800 (immediately before morning feeding), 1100, 1400, 1700, 2000, and 0200 h. Four grab samples of digesta were collected from the ventral region of the rumen approximately 50 cm through the cannula, removing a sample of digesta and then placing into a bucket. Rumen fluid was collected by inserting a 250 ml glass bottle into the same area. The rumen fluid and digesta were then strained through four layers of muslin cloth to separate the solid digesta from the liquid. The pH of the strained rumen fluid was recorded immediately after sampling (Bibby Scientific Limited, Staffordshire, UK) and liquid samples of 45 ml were added to 5 ml of 25% w/v HPO_3 solution and stored at −20 °C for subsequent analysis of volatile fatty acids (VFA), lactate and NH_3. Rumen fluid and digesta samples collected at 1400, 1700 and 2000 h were also stored in 15% glycerol solution at −20 °C for subsequent microbial community analysis of the liquid phase (LPD) and solid phase (SPD) digesta.

Faecal and urine collection

On days two to seven of each sampling period, total urine output was collected for five days using a modified catheter bag secured over the vulva of the cow with Velcro® straps and connected to a 25 L barrel. One litre of 20% sulphuric acid was added to each 25 L barrel to maintain urinary pH below pH 3.0, and after each 24 h period, a 1% subsample was taken and stored at −20 °C for subsequent analysis. Faecal samples were collected daily for the same five consecutive days by collecting all deposited material on three occasions over a 24 h period. Faecal material was then weighed and a 1% subsample of the daily output was stored at −20 °C prior to bulking and subsequent analysis. On day seven of each sampling week, subsamples of the urine and faeces were collected and mixed in a 10 L bucket per cow at a ratio of 2:1, respectively (i.e., 1.5 kg faeces with 750 g urine), and incubated in 2.5 L plastic bottle for 24 h. For the first six hours, pH was recorded at hourly intervals, and then at 24 h. Subsamples (approximately 100 g) were also collected at each time point and stored at −20 °C for subsequent analysis.

Chemical analysis

Forage, TMR, and faecal samples were bulked between days within each sampling week of each period, and the subsamples analysed according to Association of Official Analytical Chemists (2012) for DM (943.01), CP (990.03; intra-assay CV of 2.48%) and ash (942.05), with NDF (intra-assay CV of 1.36%) determined according to Van Soest et al. (1991) using heat-stable α-amylase (Sigma, Gillingham, UK) and sodium sulphite, and expressed exclusive of residual ash. Milk composition was analysed by National Milk Laboratories (NML; Wolverhampton, UK) for fat, CP, lactose and urea using near midinfrared spectroscopy (MIR; Foss, Denmark).

The VFA content of the grass silage and rumen fluid was analysed using gas chromatography (GC) using a DB-FFAP column (30 m × 0.250 mm × 0.2 μm; Agilent J and W, GC columns, Cheadle, UK) and a flame ionization detector (Agilent Inc. Wilmington, DE). Conditions were: carrier gas nitrogen; flow rate 2.7 ml/min; column pressure 0.8 bar; split ratio 30:1; oven temperature 235 °C; injector temperature 250 °C; detector temperature 300 °C. Rumen lactate concentration was determined by high-performance liquid chromatography (Agilent 1100, Germany) using a 300 × 7.8 mm column (Rezex ROA-Organic Acid, Phenomenex, Macclesfield, UK), with a mobile phase of 0.005 N H_2SO_4, flow rate of 0.5 ml/min, pressure at 32 bar, detector temperature of 40 °C, and wavelength set at 210 nm. Rumen and slurry NH_3 concentrations were determined using an auto-titrator (FOSS 1030 auto-titrator, FOSS, Warrington, UK; Buchi Labortechnik AG CH-9230, Flawil, Switzerland).

Solid digesta phase samples were washed twice in 300 ml of 0.9% w/v saline solution and homogenised in a stomacher in 100 ml of 0.9% w/v saline (Seward, West Sussex, UK) at 230 rpm for 5 mins to detach fibre-associated microbes (Ramos et al., 2009). Both the supernatant from this process and rumen liquid samples (5 ml) were centrifuged for 20 min at 10 000*g*, and 0.25 g of the microbial pellet was transferred to a 2 ml screw top tube for DNA extraction. The DNA was extracted following the protocol of Yu and Morrison (2004) by repeated bead-beating followed by precipitation, elution and purification using columns and reagents from the QIAamp ® DNA Stool Mini Kit, (Qiagen Ltd, Manchester, UK).

The PCR amplification was carried out in triplicate 25 μl reactions following the protocol by Kozich et al., (2013). The PCR products were cleaned and quantitated using the Quant-It PicoGreen high sensitivity dsDNA assay kit (Fisher Scientific UK Ltd., Loughborough, UK), pooled in equimolar quantities and 80 μl run on a 1% w/v agarose/TBE gel to separate residual primers and dNTPs. The band at the expected size containing the amplicons was excised and purified using a Promega Wizard® SV Gel purification kit (Promega UK, Southampton, UK). The libraries were quality assessed using an Agilent 2100 Bioanalyzer System (Agilent Technologies. Santa Clara, CA, US) and sequenced using the Illumina MiSeq v2 250 paired-end reagent kit (Illumina UK, Cambridge, UK.) at Edinburgh Genomics (University of Edinburgh, UK).

Sequence analysis

Sequence data were analysed using mothur 1.44.0 (Schloss et al., 2009) with steps to assemble paired-end reads and remove low-quality and chimeric sequences. Sequence counts in each library were normalised by subsampling to 38 500 sequences per sample in the liquid phase digesta, and 10 500 sequences per sample in the solid phase digesta. An operational taxonomic unit (**OTU**)-based approach was selected to describe the microbial community diversity with sequences clustered at 97% identity, and taxonomic classification using the SILVA 132 SEED reference database (Yilmaz et al., 2014). Taxonomic classification of selected OTUs were also carried out using the BLASTn against type material from the NCBI reference database (Altschul et al., 1990).

Statistical analysis

Performance and rumen metabolism parameters were evaluated by repeated measures analysis of variance as a 3 × 3 Latin rectangle design (Mead et al., 1993) using GenStat Release 18.1 (VSN International Ltd). Results are reported as treatment means

Table 2
Nutritional composition (g/kg DM) and fermentation characteristics of grass silage (GS) and the total mixed ration (TMR).

	GS	TMR
DM, g/kg	301	394
CP	131	168
Ash	90.9	79.9
Organic matter	909	920
NDF	487	420
Fermentation characteristics, g/kg		
pH	3.89	
NH_3-N, g/kg of total N	19.8	
Acetate	36.5	
Propionate	0.780	
Iso-butyrate	0.481	
Butyrate	0.328	
Iso-valerate	0.127	
Lactic acid	19.1	

with SED, with the level of significance set at $P < 0.05$, and a tendency stated at $P < 0.10$. Tukeys test was conducted posthoc to determine treatment means that differed.

After subsampling, depth of coverage and alpha diversity were summarised using the number of observed OTU's (**OBS**), Chao1, Shannon (H′) and inverse Simpson indices, respectively. Beta diversity was calculated using the Bray Curtis dissimilarity metric with the resulting distance matrix used to generate a Non-metric Multi-Dimensional Scaling plot with significant differences determined using analysis of molecular variance with 10 000 iterations. Taxonomic biomarkers associated with respective treatment groups were determined using Linear Discriminant Analysis (**LEfSe**; Segata et al., 2011) with values of $P < 0.05$ and cut-off effect size set at LDA > 2.0.

Results

Forage, diet and animal performance

The grass silage had a low pH and NH_3-N content at 3.9 and 19.8 g/kg DM, and a lower DM, CP and organic matter content than the TMR, but was 67 g/kg DM higher in NDF (Table 2). Intake of DM was 0.8 kg/day lower ($P = 0.015$) in cows when fed DY compared to C (Table 3). Milk performance was not affected ($P > 0.05$) by dietary treatment, with mean values of 33.9, 1.50, and 1.15 kg/day, for milk, milk fat and protein yield, respectively. In contrast, milk protein content was decreased by 2.1 g/kg ($P = 0.020$) when cows were fed D compared to C. Milk urea concentration was not affected by treatment ($P > 0.05$), with a mean value of 26.3 mg/dl. Similarly, feed conversion efficiency was not affected ($P > 0.05$) by dietary treatment with a mean value of 1.78 kg milk/kg DM intake.

Rumen metabolism

Mean rumen pH and mean NH_3 concentration were not affected ($P > 0.05$) by dietary treatment, with mean values of pH 6.08 and 51.5 mg/L, respectively (Table 4), but varied with time ($P < 0.001$), with rumen pH being highest prior to feeding at 0800 h and then decreasing to a nadir at 1700 h where it remained until 0200 h, while the concentration of NH_3 was highest at 1400 h. Dietary treatment also had no effect ($P > 0.05$) on the total or individual VFA concentration, with mean values of 82.4, 53.5, 15.7, 10.7, 0.41, 1.21, 0.86 μM, for total VFA, acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate respectively. At 1700 h, however, rumen propionate concentration was 5.8 μM higher in cows when fed C than DY, and at 2000 h was 8.2 μM higher in cows fed C than either of the supplemented diets ($P = 0.028$). Iso-butyrate concentration was also 0.26 μM lower ($P = 0.016$) at 1700 and 2000 h when cows were fed D or DY than C. Mean lactate concentration was not affected ($P > 0.05$) by dietary treatment with a mean value of 0.171 μM, but varied during the day, being highest at 1400 h.

Rumen microbiome

Low abundance OTUs (total number of reads per OTU < 10) were removed from the dataset, resulting in 2630 OTUs in the LPD dataset and 1647 OTUs in the SPD dataset. Coverage, measured using Good's statistic, was between 98.2% and 99.0% for LPD samples and between 95.9% and 98.0% for SPD samples. Relative phylum abundance within the LPD samples were Bacteroidetes (47%), Firmicutes (23%), Euryarcheota (8%), Spirochaetes (7%), unclassified bacteria (5%), Proteobacteria (5%); with the remaining 5% consisting of low abundance phyla. Relative phylum abundance within the SPD samples were Bacteroidetes (39%), Firmicutes (32%), Spirochaetes (15%), Euryarcheota (8%), Fibrobacteres (3%); the remaining 3% consisted of unclassified bacteria and low abundance phyla (Supplementary Table S1).

Supplementation with D or DY had no effect ($P > 0.05$) on observed OTU (OBS) and Chao 1 in both LPD and SPD samples (Table 5). However, the inverse Simpson index tended to decrease in both LPD samples ($P = 0.053$), and SPD samples ($P = 0.060$) when cows were fed diets supplemented with D or DY compared to C. Supplements had no effect ($P > 0.05$) on the Shannon index for LPD samples, while the index decreased ($P = 0.040$) in samples from cows fed D compared to C. Taxon ratios (Archaea:Bacteria

Table 3
Performance of dairy cows fed a Control total mixed ration (TMR; C), TMR with *Yucca schidigera* (De-Odorase®) (D) or TMR with *Yucca schidigera* (De-Odorase®) and *Saccharomyces cerevisiae* (Yea-Sacc®) (DY).

	Treatment			SED	*P*-value
	C	D	DY		
DM intake, kg/d	21.9[a]	21.7[a]	21.1[b]	0.23	0.015
Milk yield, kg/d	34.0	33.8	33.8	0.68	0.949
Milk fat, g/kg	44.1	44.0	43.9	1.98	0.992
Milk fat, kg/d	1.51	1.52	1.48	0.080	0.904
Milk protein, g/kg	34.9[a]	32.8[b]	33.6[b]	0.52	0.020
Milk protein, kg/d	1.19	1.13	1.14	0.033	0.194
Milk lactose, g/kg	46.4	46.8	46.1	0.34	0.191
Milk urea, mg/dl	27.1	26.5	25.3	1.26	0.373
Feed conversion efficiency[1]	1.76	1.74	1.86	0.090	0.422
Live weight change, kg/d	0.35	0.64	0.69	0.298	0.503

[1] kg of energy corrected milk/kg of DM intake.

Table 4
Rumen metabolism and volatile fatty acid content (μM) of dairy cows fed a Control total mixed ration (TMR; C), TMR with *Yucca schidigera* (De-Odorase®) (D) or TMR with *Yucca schidigera* (De-Odorase®) and *Saccharomyces cerevisiae* (Yea-Sacc®) (DY).

	Treatment			SED	*P*-value		
	C	D	DY		Tr	Ti	Tr × Ti
Rumen pH							
0800 h	6.39	6.45	6.48	0.106	0.588	<0.001	0.973
1100 h	6.25	6.21	6.24				
1400 h	5.93	5.95	6.05				
1700 h	5.95	5.91	5.95				
2000 h	5.92	5.91	6.01				
0200 h	5.93	5.92	5.97				
Rumen NH_3, mg/L							
0800 h	36.6	35.0	37.3	12.91	0.262	<0.001	0.263
1100 h	52.4	60.2	55.5				
1400 h	68.0	84.0	54.7				
1700 h	74.7	78.8	47.1				
2000 h	57.3	35.5	52.5				
0200 h	27.1	38.9	30.7				
Total volatile fatty acids							
0800 h	81.4	77.8	69.6	16.25	0.449	0.782	0.115
1100 h	67.5	85.9	91.6				
1400 h	82.9	97.3	69.4				
1700 h	95.0	76.6	65.4				
2000 h	114	76.3	73.6				
0200 h	81.8	88.1	88.8				
Acetate							
0800 h	54.5	52.5	46.0	11.11	0.523	0.762	0.180
1100 h	43.8	57.1	61.2				
1400 h	52.8	60.8	44.6				
1700 h	60.4	46.4	41.5				
2000 h	72.7	49.6	47.5				
0200 h	55.1	57.5	59.5				
Propionate							
0800 h	14.3	13.6	12.7	2.95	0.410	0.237	0.028
1100 h	12.7	15.4	16.6				
1400 h	16.4	20.4	13.8				
1700 h	18.8	16.5	13.0				
2000 h	22.4	14.4	14.1				
0200 h	14.5	16.9	16.3				
Butyrate							
0800 h	10.5	9.75	8.89	1.920	0.158	0.616	0.084
1100 h	9.00	10.8	11.0				
1400 h	11.0	13.0	8.65				
1700 h	12.7	10.9	8.77				
2000 h	15.3	10.0	9.68				
0200 h	10.2	11.3	10.7				
Iso-butyrate							
0800 h	0.39	0.41	0.38	0.090	0.280	0.213	0.016
1100 h	0.34	0.42	0.44				
1400 h	0.48	0.49	0.42				
1700 h	0.60	0.38	0.31				
2000 h	0.61	0.32	0.35				
0200 h	0.33	0.35	0.35				
Valerate							
0800 h	0.98	0.91	0.89	0.278	0.515	0.040	0.090
1100 h	0.94	1.18	1.34				
1400 h	1.26	1.51	1.00				
1700 h	1.46	1.41	1.02				
2000 h	1.90	1.23	1.17				
0200 h	1.09	1.28	1.28				
Iso-valerate							
0800 h	0.69	0.67	0.71	0.197	0.951	0.013	0.097
1100 h	0.76	0.98	0.98				
1400 h	0.94	1.12	0.88				
1700 h	1.00	0.96	0.81				
2000 h	1.19	0.72	0.83				
0200 h	0.68	0.77	0.81				
Lactate							
0800 h	0.00	0.00	0.00	0.2446	0.618	0.001	0.350
1400 h	0.183	0.651	0.423				
2000 h	0.00	0.129	0.154				

Tr = Treatment, Ti = Time.

and Firmicutes:Bacteroidetes) were unaffected by D and DY inclusion in both LPD and SPD samples.

Dietary supplement had an effect on the beta diversity (Bray Curtis dissimilarity) between microbial communities. In both LPD and SPD samples, the microbial communities from cows fed D clustered from those receiving C ($P = 0.001$) and DY clustered from C (LPD: $P = 0.001$, SPD: $P = 0.025$). Weaker microbial community clustering was found between samples from cows DY or D (LDP: $P = 0.014$, SPD: $P = 0.118$).

LEfSe identified a number of OTU biomarkers associated with D and Y supplementation in the diet (Table 6). In LPD samples, reduced relative abundance of OTUs 00013 and 00030 classified as *P. brevis* and *P. bryantii* respectively was associated with D and DY supplementation. A concurrent increase was found in the relative abundance of OTUs 00006 and 00011, both classified as *P. ruminicola*. In both LPD and SPD phases, there was a decrease in OTU00014 classified as *Methanobrevibacter olleyae* associated with D and DY supplementation. An unclassified Gammaproteobacteria (OTU00021) increased in abundance with DY supplementation only and an OTU classified as an uncultured candidate phyla radiation was identified as a discriminant rumen microbe associated with D and DY supplementation.

Nitrogen balance and slurry nitrogen

Nitrogen intake was 30 g/day lower ($P = 0.006$) and the amount of N digested 31 g/day lower ($P < 0.04$) in cows when fed DY than C, but there was no effect of treatment ($P > 0.05$) on daily faecal N output or N digestibility, with mean values of 175 g and 0.666 kg/kg, respectively (Table 7). Dietary treatment also had no effect ($P > 0.05$) on urinary N output or the proportion of urinary N of total faecal N, with mean values of 195 g and 522 g/kg, respectively. Dietary treatment also had no effect ($P > 0.05$) on daily milk N yield content or N use efficiency (calculated as daily milk N as a proportion of N intake), with mean values of 181 g and 340 g/kg, respectively. Slurry NH_3 concentration, total N or N loss in the first six hours of measurement was similar across all treatments with mean values of 2.45, 5.68, and 0.062 g/l. The initial slurry pH was 7.62 and increased until 6 h of incubation but there was no effect ($P > 0.05$) of treatment (Fig. 1).

Discussion

The current study was conducted to determine the effect of dietary supplementation with *Y. schidigera* extract alone or in combination with *Saccharomyces cerevisiae* on the performance, rumen metabolism and microbiome in high-yielding dairy cows when fed excess RDN. The basal ration fed to all cows was predicted to oversupply MPN by 21%, which reflects the high degradability of N often found in grazed perennial ryegrass swards, particularly in the early grazing season (Atkins et al., 2020). The chemical analysis of the grass silage was consistent with other studies conducted in the UK, although the NH_3-N content was lower than some others (Sinclair et al., 2015; Tayyab et al., 2018). A number of previous studies have reported that *Saccharomyces cerevisiae* and *Y. schidigera* extract supplementation have no effect on DM intake or performance of dairy cows (Dias et al., 2018, Wilson et al., 1998), although a meta-analysis undertaken by Desnoyers et al., (2009) reported a small but significant increase in intake and milk yield when dairy cows were supplemented with yeast. There was, however, a decrease in DM intake when cows were supplemented with DY in the current study, although the milk yield and milk fat content were unaffected. Lovett et al. (2006) reported similar results when an extract of *Y. schidigera* was fed to high-yielding dairy cows and it was suggested that performance was maintained

Table 5
Alpha diversity of the rumen microbial community in liquid phase digesta (LPD) and solid phase digesta (SPD) in dairy cows fed a Control total mixed ration (TMR; C), TMR with *Yucca schidigera* (De-Odorase®) (D) or TMR with *Yucca schidigera* (De-Odorase®) and *Saccharomyces cerevisiae* (Yea-Sacc®) (DY).

	Treatment			SED	*P*-value
	C	D	DY		
LPD					
OBS	1 392	1 418	1 428	32.6	0.548
Chao1 Index	1 961	2 028	1 937	73.1	0.468
Inverse Simpson Index	41.3	34.4	35.1	2.57	0.053
Shannon Index	4.90	4.79	4.83	0.056	0.187
Archaea:Bacteria	0.079	0.094	0.085	0.007	0.318
Firmicutes:Bacteriodetes	0.488	0.488	0.519	0.015	0.278
SPD					
OBS	823	814	852	33.9	0.534
Chao1 Index	1 246	1 257	1 337	88.4	0.559
Inverse Simpson Index	32.2	24.2	29.7	2.88	0.060
Shannon Index	4.71	4.50	4.69	0.075	0.040
Archaea:Bacteria	0.081	0.083	0.084	0.013	0.986
Firmicutes:Bacteriodetes	0.837	0.835	0.822	0.039	0.956

Abbreviations: OBS = number of observed operational taxonomic units.

Table 6
Linear discriminant analysis (LEfSe) of mean filtered sequence counts in dairy cows fed a Control total mixed ration (TMR; C), TMR with *Yucca schidigera* (De-Odorase®) (D) or TMR with *Yucca schidigera* (De-Odorase®) and *Saccharomyces cerevisiae* (Yea-Sacc®) (DY) (LDA > 2.0; P < 0.05). LPD: Liquid phase digesta samples. SPD: Solid phase digesta samples.

Phase	Mean Filtered Sequence Counts			LDA	*P*-value	Taxonomic Classification	
	C	D	DY			SILVA 132 SEED	BLASTn Type Strain best hit (% Identity)
LPD							
OTU00021	327	283	819	2.43	0.002	Unclassified *Gammaproteobacteria*	*Chelonobacter oris* (87%)
OTU00006	701	1 120	1 034	2.32	0.013	*Prevotellaceae*	*Prevotella ruminicola* (96%)
OTU00013	865	610	553	2.2	<0.001	*Prevotellaceae*	*Prevotella brevis* (90%)
OTU00014	476	168	258	2.19	0.003	*Methanobacteriaceae*	*Methanobrevibacter olleyae* (98%)
OTU00011	465	569	680	2.04	0.018	*Prevotellaceae*	*Prevotella ruminicola* (94%)
OTU00024	301	511	400	2.02	0.002	Patescibacteria (Candidate phyla radiation)	*Gimesia aquarii* (83%)
OTU00030	368	159	288	2.02	0.002	*Prevotellaceae*	*Prevotella bryantii* (91%)
SPD							
OTU00014	294	93.1	205	2.01	0.002	*Methanobacteriaceae*	*Methanobrevibacter olleyae* (98%)

Abbreviations: OTU = operational taxonomic unit.

Table 7
Nitrogen (N) balance and slurry composition[1] of dairy cows fed a Control total mixed ration (TMR; C), TMR with *Yucca schidigera* (De-Odorase®) (D) or TMR with *Yucca schidigera* (De-Odorase®) and *Saccharomyces cerevisiae* (Yea-Sacc®) (DY).

N, g/d	Treatments			SED	*P*-value
	C	D	DY		
Intake	539[a]	532[a]	509[b]	6.9	0.006
Faecal output	174	176	174	8.7	0.986
Digested	365[a]	357[ab]	334[b]	10.0	0.040
Digestibility, g/g	0.673	0.669	0.656	0.0166	0.578
Urine	204	187	194	17.5	0.643
Urine N of total excreted N, g/kg	534	507	525	19.9	0.411
Milk N	187	177	178	5.2	0.194
N use efficiency, g/kg[2]	345	330	350	8.8	0.130
Slurry pH	8.52	8.34	8.37	0.098	0.201
Slurry ammonia, g/L	2.49	2.55	2.30	0.355	0.283
Slurry total N, g/L	5.72	5.63	5.68	0.276	0.949
Volatile N loss[3], g/L	−0.016	0.028	0.176	0.1155	0.351

[1] Slurry consisted of faeces: urine at 2:1 ratio.
[2] (Milk N/Intake N) × 100.
[3] N loss in first six hours.

by an increase in digestibility and improved microbial growth efficiency. In the current study, however, neither DM (data not presented) nor N digestibility was affected by treatment. Milk protein content (g/kg) in the current study decreased in cows when fed D or DY compared to C, although milk protein yield was unchanged. The dietary inclusion of yeast has previously been reported to have little effect on milk protein content (Ambriz-Vilchis et al., 2017; Desnoyers et al., 2009), and the reduction in milk protein was, therefore, more likely to be a result of supplementation with *Y. schidigera*. This result is similar to Wilson et al. (1998) who reported that a minor decrease in milk protein content may have been due to the antimicrobial properties of the *Y. schidigera* saponins, which may have reduced the synthesis of microbial protein in the rumen.

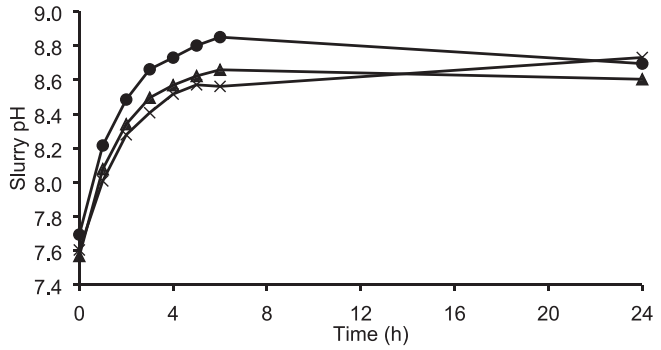

Fig. 1. Slurry pH of dairy cows fed a Control total mixed ration (TMR; ●), TMR with De-Odorase® (■) or TMR with De-Odorase® and Yea-Sacc® (▲). (SED = 0.111; Time, $P < 0.001$; Treatment, $P = 0.201$; Treatment × Time, $P < 0.130$).

Rumen metabolism and microbiome

In the present study, supplementation of *Y. schidigera* or *Y. schidigera* with *S. cerevisiae* had only a small effect on the rumen microbiome functional activity measured by pH or total VFA concentration. Yeast has been reported to increase rumen pH and promote the growth of fibrolytic and lactate-utilising bacteria due to its capacity to scavenge excess oxygen from the rumen (Chaucheyras-Durand et al., 2008). In the current study, the addition of *S. cerevisiae* in combination with *Y. schidigera* increased the abundance of an unclassified Gammaproteobacteria previously classified as a possible succinate producer (Snelling et al., 2019), which was associated with an increase in lactate concentration at 2000 h in cows fed DY compared to C, where no lactate was detected. A decrease in the concentration of propionate at 1700 and 2000 h was also associated with supplementation with *S. cerevisiae*, although the direct effect of yeast on rumen metabolism was not possible to determine as it was fed in combination with *Y. schidigera*.

Rumen NH_3 concentration was also not affected in the current study with an inclusion rate of *Y. schidigera* of 5 g per cow per day, with previous studies reporting similar results despite considerably higher inclusion rates of 20 and 60 g per cow per day (Hristov et al., 1999). Conversely, *in vitro* studies with *Y. schidigera* extract have reported a decrease in NH_3 concentration, which was suggested to be due to increased ammonia-binding or reduced proteolysis in the rumen caused by the antiprotozoal activity of the saponins (Wallace et al., 1994).

In the current study, there was a reduction in OTU diversity in both the LPD and SPD samples in cows when fed D, and *Y. schidigera* extract has previously been reported to have antimicrobial properties (Wang et al., 2000). If the effect of supplementation was detrimental to some microbial groups and beneficial to others, this may be reflected in the change in the diversity of the rumen microbiome. For example, cellulolytic bacteria have been reported to be more susceptible to lysis from *Y. schidigera* extract (Wang et al., 2000).

Bray Curtis clustering of microbial communities between D/DY samples and C was driven by discriminant OTUs in both the liquid and solid phases. Reduced relative abundance of OTU00014 classified as *Methanobrevibacter olleyae* was associated with the inclusion of D and DY supplements. This species, as with other related rumen methanogens, relies on the availability of H_2 to reduce carbon compounds to methane. In ciliate protozoa, as well as rumen bacteria and fungi, hydrogen is a product of energy metabolism (Newbold et al., 2015) and Yucca saponins have previously been reported to have antiprotozoal activity (Wallace et al., 1994). Therefore, a decrease in H_2 availability could explain the decrease in the prevalence of *Methanobrevibacter olleyae* in cows fed *Y. schidigera* extract in the current study.

Prevotellaceae were also discriminant for D and DY inclusion, with decreased abundance of OTUs classified as *P. brevis* and *P. bryantii* and increased abundance of OTUs classified as *P. ruminicola* with D/DY inclusion, respectively. The genus *Prevotella* is one of the most abundant genera in the rumen and cultured strains, such as *P. ruminicola* 23, are known to be able to utilise ammonia and peptides as a N source for growth (Kim et al., 2017). However, it was not possible to directly measure the functional activity of the rumen microbial community as metagenomic analysis was not undertaken in the current study.

Nitrogen balance and slurry analysis

Ammonia is a basic compound with an approximate pK of 8.95 at 35 °C (Martinelle and Häggström, 1997), when it is an equal proportion of NH_3 molecules and NH_4+ ions. At pH values below pH 9.3, a higher proportion will be present in the aqueous solution (NH_4+), therefore, reducing the amount of volatile NH_3 lost to the environment (Sigurdarson et al., 2018). When urea present in urine and the urease in faeces react, NH_3 is produced (Huntington and Archibeque, 2000) and the glycofraction of *Y. schidigera* extract has been reported to contain NH_3 binding properties that can reduce the amount of NH_3 that is volatilised (Wallace et al., 1994; Kavanagh et al.; 2019). Dietary treatment had no significant effect on either mean slurry pH or total slurry N or NH_3-N concentration. After six hours, slurry pH was numerically lower in cows fed D (pH 8.55) than C (pH 8.85), which is calculated to reduce the loss of NH_3 at this time point to 0.312 mg/l in D compared to 0.553 mg/l in C. However, further work is required with a greater number of animals to determine whether this difference is significant, and if it would have a meaningful impact at a farm level.

Conclusion

Supplementing the diet of dairy cows with an extract of *Y. schidigera* reduced DMI compared to unsupplemented cows or those fed *Y. schidigera* extract along with *Saccharomyces cerevisiae,* but did not affect milk yield, although there was a decrease in milk protein concentration in cows when fed diets containing *Y. schidigera*. The inclusion of *Y. schidigera* extract alone or in combination with *Saccharomyces cerevisiae* had only a small effect on rumen fermentation products. There was a reduced alpha diversity of the rumen microbiome in cows fed *Y. schidigera* extract alone, although the ratios of higher taxonomic groups were not affected. However, in both the solid and liquid phase digesta, there was a lower relative abundance of *Methanobrevibacter olleyae* in cows when fed *Y. schidigera* either alone or in combination with a live yeast, which may reduce methane production. The balance of *Prevotella* species was also affected by the inclusion of *Y. schidigera* and *Saccharomyces cerevisiae* but there was no effect on N excretion, while slurry pH tended to decrease in cows fed *Y. schidigera* after six hours, although volatile N loss was not affected.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100967.

Ethics approval

The procedures for the animals used in this experiment were conducted in accordance with the Animals (Scientific Procedures

Act) 1986 (amended 2012) and were approved by the local ethics committee at Harper Adams University.

Data and model availability statement

Sequence data are available from the European Nucleotide Archive (https://www.ebi.ac.uk/ena) under project accession number PRJEB59717 and sample accession numbers ERR10854553 – ERR10854660. Supplementary Material is available via https://doi.org/10.6084/m9.figshare.23999013. No other data have been deposited in an official repository but is available from the corresponding author upon reasonable request.

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

The authors did not use any artificial intelligence-assisted technologies in the writing process.

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Declaration of interest

The authors declare that there is no conflict of interest.

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