



OPEN

Modulation of rumen bacterial community and feed utilization in camel and sheep using combined supplementation of live yeast and microalgae

Alaa Emara Rabee^{1✉}, Boshra R. Younan¹, Khalid Z. Kewan¹, Ebrahim A. Sabra² & Mebarek Lamara³

The combination of live yeast and microalgae as feed supplementation could improve rumen fermentation and animal productivity. This study aimed to investigate the impact of a mixture of (YA) yeast (*Saccharomyces cerevisiae*) and microalgae (*Spirulina platensis* and *Chlorella vulgaris*) as feed supplementation on feed intake, rumen disappearance of barley straw, bacteria, and fermentation, blood parameters of camels and sheep. Three fistulated camels and three fistulated rams were fed a concentrates mixture and ad libitum barley straw as a basal diet alone or supplemented with YA mixture. The dietary supplementation improved the feed intake, rumen disappearance of barley straw nutrients, and the blood immunity parameters. The YA supplementation affected rumen fermentation as well as the composition and diversity of rumen bacteria; however, the response to the supplementation varied according to animal species. Principle Coordinate Analysis (PCoA) separated bacterial communities based on animal species and feeding treatment. Phylum Bacteroidetes and Firmicutes dominated the bacterial community; and the dominant genera were *Prevotella*, *RC9_gut_group*, *Butyrivibrio*, *Ruminococcus*, *Saccharofermentans*, *Christensenellaceae_R-7_group*, and *Succiniclasticum*. Our results suggest positive impacts of YA supplementation in rumen fermentation and animal performance.

The low productivity of animals in the arid regions is attributed mainly to feed deficiency, wherever animal feeding relies on the low-protein and high-fiber roughages¹. A huge amount of crop straw is generated globally every year². Barley is grown in a wide range of adverse environmental conditions, including drought and soil salinity; thus, it is considered the main crop in desert regions³. Barley provides large quantities of lignocellulosic straw that could be used in the feeding of desert ruminants^{4,5}. However, higher lignocellulose represents a barrier towards the efficient microbial fermentation of barley in the animal rumen⁶.

The fermentation in the rumen depends on the interactions between the microbial groups, consisting of bacteria, archaea, protozoa, and fungi⁷. Rumen microbiota convert the components of animal diets such as cellulose, hemicellulose, starch, and protein to microbial proteins and volatile fatty acids that provide the host animal with about 70% of daily protein and energy requirements^{7,8}. Therefore, any modulations in the rumen microbiota and fermentation could have a significant impact on the physiology and efficiency of the host-animal. Rumen bacteria represent the majority of the microbial communities in the rumen, approximately 88% of microbial populations, with more than 10¹⁰ cells/ml^{7,9}. The composition of the animal diet and animal species are the main drivers of the composition of rumen microbial groups^{8,9}. Understanding the alterations of the rumen microbial ecosystem in different animal species and under different dietary supplementation offers the possibility to design suitable feeding strategies to optimize the rumen microbial fermentation, including fiber digestion of desert ruminants⁷⁻⁹. Desert dwelling ruminants such as camels and Barki sheep contribute significantly to food security in arid countries by providing milk and meat under harsh conditions¹⁰. These animals have been adapted to live in adverse desert conditions, including heat stress, scarcity of water, and availability of poor-quality feedstuff¹¹.

¹Animal and Poultry Nutrition Department, Desert Research Center, Cairo, Egypt. ²Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat City, Egypt. ³Forest Research Institute, University of Quebec in Abitibi-Temiscamingue, Rouyn-Noranda, Canada. ✉email: rabee_a_m@yahoo.com

Camel is multipurpose animal that is adapted to desert harsh conditions by the unique feeding behavior, physiological mechanisms, and the functional structure of the digestive tract¹². Barki sheep is the main sheep breed in the arid regions in North Africa and the Middle East, as this breed is well adapted to desert conditions¹³. The differences in the anatomy and physiology of the digestive tract as well as feeding behavior between camels and other ruminants resulted in differences in the feeding requirements, rumen microbial fermentation, and metabolism¹⁴. Gihad et al.¹⁵ indicated that camels have high efficiency than sheep in dry matter and crude fiber digestion, and nitrogen utilization. Moreover, a comparative study on camels and sheep¹⁶ showed some discrepancies in the composition of the rumen bacterial community; for instance, camels showed higher *Ruminococcus* and *Butyrivibrio*, and *Prevotella*, while sheep have higher *RC9_gut_group*. Therefore, the response of animal species to dietary intervention could be varied¹⁷.

Furthermore, intensification of animal production and restriction of antibiotics use in animal production are the main drivers of the transition to the safe and novel feed additives^{18,19}. Probiotic and prebiotic feed additives or their combinations have gained interest to manipulating rumen fermentation^{20,21}. However, feed additives need to be studied continuously to examine their efficacy, which depends on the nature of compounds, the composition of animal diet, and the response of the rumen microbiome²². Supplementation of the animal diet with probiotics such as live yeast (*Saccharomyces cerevisiae*) alone or with phytogetic substrates has been shown to affect the rumen microbiome^{20,22}. Previous studies^{23,24} indicated that the supplementation of the cattle and camel with live yeast improved the total VFA production and stabilized rumen pH, and affected the abundance of some bacterial groups, including fibrolytic bacteria. Moreover, in recent years, algae or their extracts have gained interest as feed additives in livestock diets¹⁹.

Chlorella and *Spirulina* are well-known microalgae worldwide; they are rich sources of proteins, essential amino acids, vitamins, pigments, fatty acids, minerals, and other natural bioactive compounds^{25,26}. Therefore, microalgae supplementation can boost the rumen microbiota to enhance diet fermentation. Previous studies indicated that microalgae feed additives improved animal health, growth, and fertility²⁷. Tsiplakou et al.²⁸ reported that the inclusion of *Chlorella* in goats' diet affected the abundance of rumen bacteria and milk fatty acids profile. The availability of polysaccharides and other bioactive compounds is the main reason to use of microalgae or their derivatives as a prebiotic to promote gut health and modulate gut microbiota composition²⁹. Therefore, it was hypothesized that using mixtures of prebiotics such as microalgae, and probiotics such as live yeast, as feed additives is an effective strategy to improve animal health and productivity by promoting the activities of rumen microbiota to enhance rumen fermentation and stimulate the immunity^{21,30,31}. Grimm et al.²¹ supplemented the horses with a combination of live yeast and microalgae (*Aurantiochytrium limacinum*) and found that the supplementation affected the abundance of some fibrolytic gut bacteria.

Very limited information is available regarding the supplementation of the combination of live yeast and microalgae to animal diet. This study aimed to determine the effect of a mixture of *S. cerevisiae* and microalgae mixture (*Spirulina platensis* and *Chlorella vulgaris*) on feed intake, rumen fermentation and bacteria, blood parameters, and ruminal disappearance of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of barley straw in two different animal species, camels and sheep.

Methods

Animals and ethics. The present experiment was conducted at Maryout Research Station, Desert Research Center, Alexandria, Egypt. Three fistulated adult camels (*Camelus dromedaries*, Maghraby breed; age, 6 years; average body weight, 450 ± 5.5 kg) and three fistulated adult rams (*Ovis aries*, Barki breed; age, 5 years; average body weight, 47 ± 1.7 kg) were assigned to receive two dietary treatments successively. This study was conducted under guidelines set by the Department of Animal and Poultry Production, Desert Research Center, Egypt. Moreover, the project was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Sadat City, Egypt (Project Reference: VUSC00008). All methods were performed in compliance with the ARRIVE guidelines. In addition, the project does not include euthanasia of the experimental animals. The sample size was decided based on the availability of similar animals with similar physical and physiological status.

Experimental diets. Throughout the experiment, all animals were offered ad libitum barley straw. Moreover, concentrates mixture was offered to camels at 1400 g/head and rams at 450 g/head. The concentrates mixture was either unsupplemented (control) or supplemented with yeast and algae combination (YA) (25% *S. cerevisiae*, 50% *S. platensis*, and 25% *C. vulgaris*). The YA combination was offered gradually and was supplied to the camels at 48 g/head and for sheep at 12 g/head, which represented 1% of daily total dry matter intake. The experiment was conducted for two periods, in the first period, the animals were offered the control diet for 21 days before the sampling period. Subsequently, all animals were switched to the second period with the supplemented concentrate mixture for 21 days before the sampling period. Thus, the experimental groups were (1) camels fed the control diet without supplementation (CC); (2) Rams fed the control diet (CR); (3) camels supplemented with YA mixture (TC); and (4) rams supplemented by YA mixture (TR). All animals were housed individually in well-ventilated pens and offered free drinking water and received the experimental diet as per treatment twice daily at 08:00 and 14:00 h. All refused feed was quantified to determine the feed intake. Concentrate feed mixture consisted of corn 57.5%, soybean meal 23%, wheat bran 19%, limestone 2.5%, salt 1.5%, sodium bicarbonate 0.5%, premix 0.4%, and antitoxins 0.1%. The chemical analysis of barley straw and concentrates feed mixtures are presented in Table 1. After the adaptation period, the disappearances of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) of barley straw were conducted.

	% DM	% OM	EE %	CP%	NDF %	Ash %
Barely straw	88	77.2	3.1	4.75	67	10.8
Control concentrates (C)	90	79.5	4.5	14.5	53	10.5
Treatment concentrates (T)	91	82	5.4	17.5	46	9

Table 1. The chemical composition (%) of control (C) and treatment (T) concentrates mixtures and barely straw. DM dry matter, CP crude protein, EE ether extract, NDF neutral detergent fiber.

Rumen disappearance of barley straw. Barley straw was oven-dried at 50 °C and then ground to pass a 1 mm sieve; then a 2.5 g was weighted into nylon bags (10 × 20 cm; pore size = 50 μm). One nylon bags was prepared for each of four times for each animal (3, 6, 12, 48 h). A total of four bags were placed into the rumen of each camel or ram before morning feeding. The bags were retrieved from the animal rumen after 3, 6, 12, 48 h. After the removal from the rumen, the bags were rinsed with water until the water ran clear; then squeezed and dried at 60 °C for 48 h to determine the disappearance of dry matter (DMD), neutral detergent fiber (NDFD), and crude protein (CPD) of barley straw when animal fed the control diet (C) and treatment diet (YA).

Rumen and blood samples. At the end of every experimental period, rumen and blood samples were collected before morning feeding. Rumen contents were collected from the animal's fistula before feeding and strained by two layers of cheesecloth. The rumen pH was recorded using a digital pH meter (WPA CD70). The rumen liquid was used in the analysis of rumen fermentation parameters and DNA isolation. Blood samples were collected from the jugular vein, and serum was separated by centrifuging at 10,000 × g for 5 min and then was frozen for further analysis.

Chemical analysis. The live yeast was a commercial product containing *S. cerevisiae* at the concentration of 2×10^7 colony-forming units (CFU)/g. All the *Chlorella* and *Spirulina* that were used in the experiment were obtained in the form of green dried powder from Algal Biotechnology Unit, National Research Centre, Cairo, Egypt, and their chemical composition was previously reported in El-Sayed and El-Sheekh; and El-Feky et al.^{32,33}. The contents of dry matter (DM), neutral detergent fiber (NDF), and crude protein (CP) were determined in experimental diets and barley straw before and after incubation in the rumen. DM was measured by drying the material for 48 h at 60 °C. NDF was determined by the method of Van Soest et al.³⁴ without sodium sulfite. CP was determined according to AOAC³⁵. The rumen ammonia (NH₃-N) and total VFA concentrations were determined by steam distillation in Kjeldahl distillation equipment^{35,36}. In addition, individual VFAs were measured using high-performance liquid chromatography (HPLC) and the VFA were separated using C18 column with a mobile phase containing 0.3% phosphoric acid³⁷. Serum metabolites were analyzed after thawing using a spectrophotometer with commercial reagents (Biodiagnostic-diagnostic and research reagents, Giza, Egypt) according to manufacturer's recommendations and immunoglobulin IgA, IgG, and IgM were determined using Enzyme-Linked Immunosorbent Assay (ELISA).

Bacterial community analysis. *DNA extraction, PCR amplification, and sequencing.* One milliliter of every rumen sample was centrifuged at 13,000 rpm, and the remained precipitate was used for DNA extraction by i-genomic Stool DNA Extraction Mini Kit (iNtRON Biotechnology, Inc.) according to the manufacturer's instructions. DNA was eluted in 50 μL elution buffer, and DNA quality and quantity were checked by agarose gel electrophoresis and Nanodrop spectrophotometer, respectively. The V4 region of the bacterial 16S rDNA gene was amplified using 515F and 926R primers³⁸. PCR amplification was conducted under the following conditions: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 50 °C for 60 s, and 72 °C for 90 s; and 72 °C for 10 min. PCR products purification, preparation for sequencing using Illumina MiSeq system were conducted according to the protocol described by Comeau et al.³⁹ in Integrated Microbiome Resource (IMR, Dalhousie University, Halifax, NS, Canada). Briefly, PCR-amplicons were cleaned up and normalized using the high-throughput Invitrogen SequelPrep 96-well plate kit. Then, the samples were finally pooled to make one library for the sequencing.

Determination of copy number of bacterial 16S rDNA by using qPCR. Quantitative real-time PCR (qPCR) was used to determine the total bacterial 16S rDNA copy number in the rumen samples. Standards were generated using serial dilutions of DNA isolated from *Prevotella sp*, *Ruminococcus albus*, *Butyrivibrio hungatei* purchased from DSMZ (Braunschweig, Germany). Serial dilutions of the standards ranging from 10¹ to 10⁶ copies of the 16S rDNA gene were used. The qPCR was performed using the Applied Biosystems StepOne system (Applied Biosystems, Foster City, USA). Bacterial primers F (5'-CGGCAACGAGCGCAACCC-3') and R (5'-CCATTG TAGCACGTGTGTAGCC-3')⁴⁰ were applied. The 10-μL reaction consisted of 1 μL genomic DNA, 1 μL of each primer, and 7 μL SYBER Green qPCR- master mix (iNtRON Biotechnology, Inc.). The PCR conditions were as follows 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The linear relationship between the threshold amplification (Ct) and the logarithm of 16S rDNA copy numbers of the standards was used to calculate the copy numbers of rumen bacteria per μL of DNA.

Bioinformatics analysis. All the paired-end (PE) Illumina raw sequences were processed in R (version 3.5.2) using the DADA2 (version 1.11.3) pipeline as described by Callahan et al.⁴¹. Paired-end fastq files were

Feed intake	Control (C)				Treatment (T)				P values		
	Camel (CC)		Sheep (CR)		Camel (TC)		Sheep (TR)		A	T	A × T
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Roughage feed intake											
DMI g/kg ^{0.75}	33.1 ^c	0.5	14.5 ^a	1.6	37.2 ^d	0.3	25.6 ^b	1.8	< 0.0001	0.004	0.052
OMI g/kg ^{0.75}	29.03 ^c	0.46	12.72 ^a	1.42	32.65 ^c	0.28	22.42 ^b	1.59	< 0.0001	0.004	0.052
CPI g/Kg ^{0.75}	1.6 ^c	0.02	0.7 ^a	0.07	1.8 ^d	0.015	1.2 ^b	0.08	< 0.0001	0.004	0.052
NDFI g/Kg ^{0.75}	22.2 ^c	0.35	9.7 ^a	1.1	25 ^d	0.2	17.1 ^b	1.2	< 0.0001	0.004	0.052
Total feed intake											
DMI g/kg ^{0.75}	46.2 ^b	0.5	37 ^a	1.6	50.5 ^b	0.3	48.3 ^b	1.8	0.01	0.003	0.052
OMI g/kg ^{0.75}	40.67 ^b	0.46	32.59 ^a	1.42	44.66 ^b	0.28	42.92 ^b	1.59	0.011	0.003	0.046
CPI g/Kg ^{0.75}	3.5 ^a	0.02	3.95 ^b	0.08	4.1 ^b	0.01	5.15 ^c	0.08	< 0.0001	< 0.0001	0.008
NDFI g/Kg ^{0.75}	29.2 ^c	0.35	21.6 ^a	1.1	31.1 ^c	0.2	25.8 ^b	1.2	0.002	0.026	0.26

Table 2. Effect of YA supplementation on roughage and total feed intake expressed as g/Kg^{0.75} of camel and sheep. A, animal species; T, feeding treatment; A × T, interaction between animal species and feeding treatment. DMI dry matter intake, OMI Organic matter intake, CPI crude protein intake, NDFI neutral detergent fiber intake, SE standard error, SEM standard error of the mean. ^{a,b,c,d}; Means in the same row with different superscripts differ significantly ($P < 0.05$).

demultiplexed and quality checks of forward and reverse reads were conducted based on the quality scores. Then sequence reads were quality filtered, trimmed, and dereplicated followed by merging the forward and reverse reads together to obtain the full denoised sequences. The reads were inspected for chimeras that were removed, and Amplicon Sequence Variants (ASVs) were generated. Taxonomic assignment of sequence variants was performed using a combination of the functions assign Taxonomy and assignSpecies and was compared using the SILVA reference database. Various alpha diversity indices, Chao1, Shannon, and InvSimpson were obtained. Beta diversity was assessed as the principal coordinate analysis (PCoA) based on bray–curtis dissimilarity.

Statistical analysis. The results of the relative abundance of bacteria were tested for normality using the Shapiro–Wilk test, and non-normal values were then arcsine transformed. The effect of animal species (A), feeding treatment (T), and A × T interactions on the differences in feed intake, rumen fermentation parameters, bacterial copy numbers, blood biochemical and immunological parameters, microbial diversity, and relative abundances of bacterial groups were studied by Mixed ANOVA model using Repeated Measures function in IBM SPSS software v. 20.0⁴². The between-subjects factor was the animal species, and the within-subjects factor was feeding treatment. The Duncan test through One-Way ANOVA was carried out for mean separation between all experimental groups. For all statistical tests, a $P < 0.05$ was used as a threshold of statistical significance. Pearson correlation analysis was used to identify correlation relationships between feed intake, rumen fermentation parameters, blood parameters, nutrients disappearance, and relative abundances of bacterial genera. The correlation scores were visualized as a heatmap using PAST software⁴³. All the sequences were deposited to the sequence read archive (SRA) under the accession number: PRJNA767400.

Results

Feed intake. The chemical compositions of barley straw and concentrates mixtures are presented in Table 1. The data shows that barley straw has lower crude protein (CP) and higher neutral detergent fiber (NDF) than concentrate mixtures. Additionally, concentrates mixtures of treatment (T) had higher CP and lower NDF compared to the control concentrates mixture (C). The feed intake results expressed as g/ Kg^{0.75} (Kilogram metabolic body weight) are described in Table 2. The inclusion of YA mixture in animals' diets increased the feed intake significantly (Table 2). Furthermore, species' feed intake was affected significantly, and the interaction between animal species and treatment was significant ($P < 0.05$). Camel group TC showed higher dry matter intake (DMI), organic matter intake (OMI), crude protein intake (CPI) from roughage, and neutral detergent fiber feed intake (NDFI). In contrast, sheep group TR showed a higher total CPI (Table 2).

Rumen disappearance of nutrients. The disappearances of barley nutrients (DM, CP, and NDF) at 3, 6, 12, 48 h are described in Table 3. The inclusion of the YA mixture in animals' diets improved the disappearance of DM, CP, and NDF in the rumen of camel and sheep with a significant difference at 48 h ($P < 0.05$); and animal species did not affect the disappearance. Supplemented camel group (TC) showed the highest DMD at 48 h. Moreover, supplemented sheep group (TR) showed higher NDFD at all incubation times except for 48 h, whenever TC group showed the highest NDFD. Also, sheep group TR showed greater CPD at 48 h.

Rumen fermentation parameters. The effect of the inclusion of YA supplementation on rumen fermentation parameters is shown in Table 4. Neither YA supplementation nor animal species affected rumen pH. Animal species affected rumen ammonia and total VFA concentration significantly ($P < 0.05$) (Table 4). The supplementation of the YA mixture affected the molar proportions of acetic, propionic, and isobutyric acids sig-

	Control (C)				Treatment (T)				P values		
	Camel (CC)		Sheep (CR)		Camel (TC)		Sheep (TR)		A	T	A × T
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
DMD%											
3 h	19.5	0.9	18.2	1.0	21.0	0.5	21.0	1.0	0.49	0.07	0.52
6 h	20.2	0.75	19.4	0.77	22	0.7	20.2	1.7	0.16	0.42	0.76
12 h	29.3	3.9	34	1.9	31.7	1.7	33.7	1.5	0.28	0.75	0.717
48 h	46.5 ^a	1.6	44.9 ^a	3.7	53.1 ^b	0.4	49.5 ^b	2.8	0.46	0.009	0.18
CPD%											
3 h	12.4	2.0	12.8	0.7	15.7	0.6	15.0	0.2	0.9	0.07	0.6
6 h	15.24	2.2	15.2	0.8	18.4	0.5	17	1	0.62	0.08	0.54
12 h	25.8	4.7	20.7	1	28.05	1.8	30.2	2.2	0.67	0.06	0.18
48 h	40.8 ^{ab}	5.8	32.4 ^a	0.6	47.4 ^b	3.4	50.3 ^b	1.5	0.48	0.02	0.17
NDFD%											
3 h	4.1 ^a	1.1	5.3 ^a	0.7	5.8 ^{ab}	0.1	8.4 ^b	1.1	0.14	0.02	0.34
6 h	5.6	1	7.2	0.4	7.6	0.6	9.2	2	0.13	0.25	0.9
12 h	16.25	3.1	21.7	4.9	18.4	1.96	23.3	1.2	0.14	0.60	0.94
48 h	39.1 ^a	2.05	37.2 ^a	3.7	45.8 ^b	0.34	42.5 ^b	3.1	0.50	0.006	0.55

Table 3. Disappearance of dry matter (DMD %), crude protein (CPD %), neutral detergent fiber (NDFD%) of barley straw at 3, 6, 12 and 48 h of incubation for in the rumen of supplemented and non-supplemented camels and sheep. ^{a,b}: Means in the same row with different superscripts differ significantly ($P < 0.05$).

	Control (C)				Treatment (T)				P values		
	Camel (CC)		Sheep (CR)		Camel (TC)		Sheep (TR)		A	T	AxT
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
pH	6.6	0.06	6.4	0.24	6.3	0	6.4	0.1	0.8	0.07	0.17
Ammonia, mg/dl	10.3 ^a	1.7	16.3 ^{ab}	4	13.06 ^{ab}	0.9	19.1 ^b	2.5	0.02	0.42	0.1
VFA, meq/dl	13 ^c	0.8	8.9 ^{ab}	0.3	10.2 ^b	0.3	8 ^a	0.4	0.04	0.002	0.12
Acetic, %	63 ^b	0.6	69.4 ^b	0.4	65.2 ^b	1.3	52.7 ^a	4.2	0.24	0.03	0.01
Propionic, %	26.2 ^b	0.56	19.6 ^a	0.3	21.4 ^{ab}	0.8	36.8 ^c	3.6	0.06	0.04	0.006
Butyric, %	10.1 ^b	0.1	9.8 ^b	0.4	10 ^b	0	8.2 ^a	0.7	0.1	0.08	0.1
isobutyric, %	0.73 ^a	0.08	1.1 ^a	0.25	3.4 ^b	0.63	2.3 ^b	0.15	0.36	0.006	0.12
Bacteria population*	7.6 ^a	0.08	7.25 ^a	0.07	8.1 ^b	0.1	7.5 ^a	0.2	0.03	0.02	0.26

Table 4. Effect of YA supplementation on rumen fermentations parameters and bacterial population (Log₁₀ copies/μL DNA) in the rumen of camels and sheep. Bacteria population = Log₁₀ of 16S rDNA copy number / μL DNA. ^{a,b,c}: Means in the same row with different superscripts differ significantly ($P < 0.05$).

nificantly ($P < 0.05$). For example, the molar proportions of propionic was increased in supplemented sheep (TR) and decreased in the supplemented camel (TC). The molar proportion of isobutyric was increased in camels and sheep by YA supplementation. Additionally, the bacterial population was affected by YA supplementation and animal species ($P < 0.05$) as it was increased in supplemented camels (TC).

Blood parameters. The effect of YA supplementation and animal species on serum biochemical parameters and serum immunoglobulins IgA, IgG, and IgM are presented in Table 5. Animal species and YA supplementation affected the concentration of most of the serum biochemical parameters and serum immunoglobulins ($P < 0.05$). Higher IgA was observed in camel group CC; and camel group TC showed higher glucose, triglycerides, IgG, and IgM. Moreover, sheep group TR showed higher total protein.

Bacterial diversity and composition. The sequencing of the V4 region on the 16S rDNA gene in 12 rumen samples resulted in 393 690 high-quality sequence reads with an average of 32 807 sequences per sample. Table 6 presents the effect of animal species and YA supplementation on the alpha diversity metrics. The results showed that feeding treatment and animal species affected the microbial diversity indices significantly ($P < 0.05$). Chao1 index was increased in camel group TC compared to CC and was declined in sheep group TR compared to CR. Shannon and InvSimpson indices were increased by YA supplementation. Principle Coordinate Analysis (PCoA) based on Bray Curtis dissimilarity metrics showed that bacterial communities of CC, CR, TC, and TR groups were separated distinctly by feeding treatment and animal species (Fig. 1).

	Control				Treatment				P values		
	Camel (CC)		Sheep (CR)		Camel (TC)		Sheep (TR)		A	T	A×T
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Glucose, mg/dl	72 ^b	2	45.33 ^a	0.88	92.33 ^c	11.33	45 ^a	3.38	0.002	0.001	0.18
Total Protein, g/dl	6.1 ^a	0.23	6.7 ^a	0.15	6.3 ^a	0.05	7.12 ^b	0.23	0.002	0.02	0.66
Albumin, g/dl	4.1 ^b	0.05	3.06 ^a	0.20	3.8 ^b	0.28	3.4 ^a	0.2	0.042	0.83	0.12
Urea, mg/dl	26.7 ^a	1.2	36 ^b	3.8	22.3 ^a	1.85	29 ^{ab}	2	0.024	0.09	0.06
Triglycerides, mg/dl	42.3 ^c	1.8	14.7 ^a	0.33	43.3 ^c	3.3	27.7 ^b	2.02	<0.0001	0.048	0.07
Total lipids, mg/dl	148.8	5.5	141.63	9.24	156.51	17.41	147.50	3.06	0.55	0.41	0.90
IgG, mg/dl	228 ^c	6.24	102.8 ^a	3	235.75 ^c	2.8	139.3 ^b	7.13	0.0001	0.001	0.007
IgM, mg/dl	42.7 ^b	2.02	19.5 ^a	3.4	52.96 ^c	1.21	35.84 ^b	1.7	0.0001	0.007	0.31
IgA, mg/dl	30 ^c	0.57	18.25 ^a	0.63	25.6 ^b	0.81	26.13 ^b	1.23	0.007	0.023	0.0001

Table 5. Effect of YA supplementation on the blood metabolic profile and immunological response (mg/dl) in camels and sheep. *IgG* Immunoglobulin G, *IgM* Immunoglobulin M, *IgA* Immunoglobulin A. ^{a,b,c}: Means in the same row with different superscripts differ significantly ($P < 0.05$).

	Control				Treatment				P value		
	Camel (CC)		Sheep (CR)		Camel (TC)		Sheep (TR)		A	T	A × T
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Chao1	453.85 ^a	5.3	1408.4 ^c	46.7	720.6 ^b	60.15	602.43 ^{ab}	112.3	0.002	0.025	0.002
Shannon	5.9 ^a	0.05	6.34 ^b	0.14	6.35 ^b	0.11	6.6 ^b	0.16	0.003	0.023	0.56
InvSimpson	283.9 ^a	0.9	250.06 ^a	33.04	447.75 ^b	39	541.13 ^b	32.75	0.327	0.002	0.131

Table 6. Averages of ASVs number, Chao1, Shannon, and Inverse Simpson indices of microbial communities in the rumen of camel and sheep supplemented and non-supplemented with YA mixture. NS Non-significance at $P < 0.05$. ^{a,b,c}: Means in the same row with different superscripts differ significantly ($P < 0.05$).

The taxonomic analysis showed that the bacterial community was assigned to 17 bacterial phyla. The bacterial community was predominated by phylum Bacteroidetes and Firmicutes. Other bacterial phyla that represented more than 1% were Planctomycetes, Proteobacteria, Spirochaetes, and Tenericutes. Furthermore, bacterial phyla that were found in less than 1% were Cyanobacteria, Fibrobacteres, and Kiritimatiellaeota (Table 7). Some phyla were observed exclusively in a specific group, including Actinobacteria and Synergistia, which were found in CR and TC groups. Phylum Chloroflexi was found only in CR and TR. Phylum Verrucomicrobia was observed in CC and TC whereas, phylum Elusimicrobia was observed in TC and TR groups. Phylum Lentisphaerae was not detected in TR, and phylum Patascibacteria was found only in the TC group. The results showed that the inclusion of YA mixture in animals' diets affected the relative abundance of some bacterial phyla significantly ($P < 0.05$) (Table 7; Fig. 2a).

Phylum Bacteroidetes was not affected by feeding treatment and animal species. The members of this phylum were assigned to six families, BS11_gut_group, RF16_group, F082, Muribaculaceae, Prevotellaceae, and Rikenellaceae (Table 8). The members of the family Prevotellaceae and Rikenellaceae represented the majority of Bacteroidetes. Family Prevotellaceae was dominated by genus *Prevotella* that was affected significantly ($P < 0.05$) by YA supplementation, whenever it was declined in camel group TC compared to CC and was increased in sheep group TR compared to CR. Family Rikenellaceae was dominated by genus *RC9_gut_group* (Table 8 and Fig. 2).

Phylum Firmicutes was not affected by dietary supplementation and animal species. This phylum was further classified into 13 families dominated by Christensenellaceae, Lachnospiraceae, Family_XIII, Ruminococcaceae, and Acidaminococcaceae (Table 8 and Fig. 2). Family Ruminococcaceae was assigned mainly to Unclassified Ruminococcaceae, *Papillibacter*, Ruminococcaceae_NK4A214_group, Ruminococcaceae_UCG-005, Ruminococcaceae_UCG-014, *Ruminococcus*, *Saccharofermentans*. Family Christensenellaceae was assigned mainly to R-7_group. Lachnospiraceae was dominated by *Butyrivibrio*, *Lachnoclostridium*, and AC2044_group. Genus *Anaerovibrio* was found only in the CC group and disappeared from the camel by YA supplementation. Family_XIII was dominated by *Anaerovorax*. Genus *Succiniclaticum* dominated family Acidaminococcaceae, decreased in camel and increased in sheep by YA supplementation (Table 8 and Fig. 2).

Phylum Proteobacteria was affected by animal species. This phylum was dominated by the family Desulfotribionaceae, which was assigned mainly to the genus *Desulfotribion*. Phylum Spirochaetes was increased in camels and was dominated by genus *Sphaerochaeta* and *Treponema*. The relative abundances of phylum Cyanobacteria, Planctomycetes, and Tenericutes were increased by YA supplementation. While phylum Kiritimatiellaeota was declined by YA supplementation. Moreover, phylum Fibrobacteres was increased in sheep and declined in camels by YA supplementation; and the opposite trend was found in phylum Lentisphaerae (Table 8 and Fig. 2).

- Group-CC
- ▲ Group-CR
- Group-TC
- ◆ Group-TR

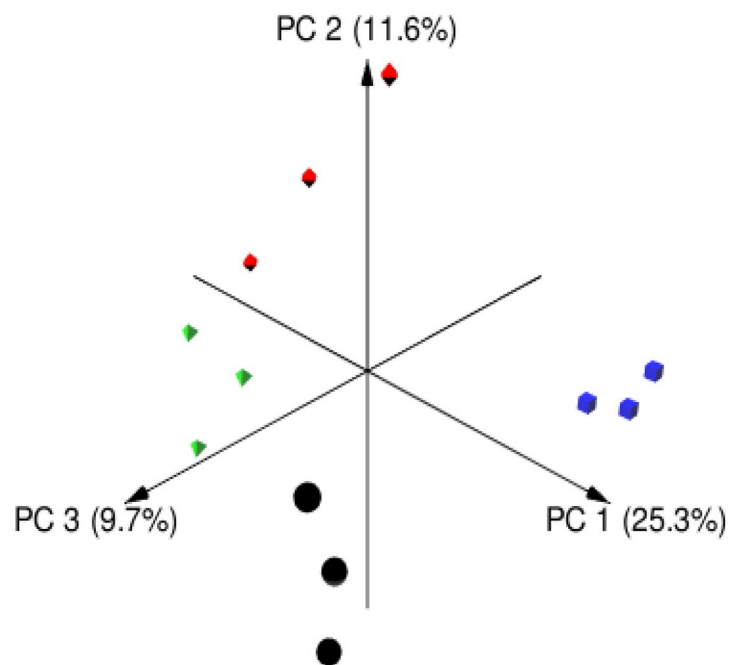


Figure 1. Principal coordinates analysis of bacterial communities in the rumen of supplemented and non-supplemented camels and sheep based on Bray–Curtis dissimilarity. The analysis was conducted between four experimental groups: black circles for non-supplemented camels, green triangles for non-supplemented sheep, blue squares for supplemented camels, and red triangles for supplemented sheep.

Pearson correlation analysis. Pearson correlation analysis (Fig. 3) was conducted between the relative abundances of dominant bacteria and other parameters. The results showed several positive and negative correlations. For example, NDFI was positively correlated with blood glucose and immunoglobulins, total VFA, NDFD, *Butyrivibrio*, *Unclassified_Ruminococcaceae*, *Saccharofermentans*, and *Fibrobacteres*. In addition, NDFI had a negative correlation with blood protein, rumen ammonia, and *R-7_group*.

Discussion

Using a combination of prebiotic and probiotic is a possible way to stimulate the rumen microbiota to improve rumen fermentation and enhance animal efficiency^{31,44}. This study examined the effect of the inclusion a combination of live yeast and algae into the sheep and camels' diets on feed intake, rumen fermentation, barley straw degradation, blood biochemical and immunology parameters, and rumen microbiota. Animals in this study offered free barley straw that is abundant lignocellulosic residue in desert regions and characterized by high-fiber and low-protein contents^{2,5}. The chemical composition of barley straw (Table 1) in the current study was in the line with previous studies^{45,46}. Higher NDF content in lignocellulosic biomass represents the main constrain against its digestibility in the rumen⁶. Thus, it is necessary to apply different strategies to enhance the functions of rumen microflora and stimulate fiber fermentation in the rumen¹⁸. Live yeast and algae were used previously separately in animal feeding, and to the best of our knowledge, there is no information available on the impact of combining these types of algae and yeast on feed utilization and rumen fermentation in ruminant animals. The chemical compositions of microalgae vary according to species, production system, growing condition, and harvesting method⁴⁷. Therefore, different algae species could provide different nutrients and bioactive compounds for animal feeding⁴⁸.

The feed intake of camels and sheep in this study was improved by YA supplementation and was in the range obtained by previous studies on camels and sheep^{4,15,49} (Table 2). The improvement in the feed intake was accompanied by improvement in the rumen disappearance of DM, CP, and NDF of barley straw at 48 h, which

Phylum	Control (C)				Treatment (T)				P value		
	Camel(CC)		Sheep (CR)		Camel (TC)		Sheep (TR)		A	T	A×T
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
Actinobacteria			0.6		0.06				ND	ND	ND
Bacteroidetes	53.4	3.04	62.2	8.7	45.5	1.32	63.9	3.77	0.055	0.56	0.39
Chloroflexi			0.047				0.12		ND	ND	ND
Cyanobacteria	0.12 ^a	0.05	0.06 ^a	0.02	1.5 ^b	0.8	0.10 ^a	0.02	0.013	0.034	0.051
Elusimicrobia					0.48		0.06		ND	ND	ND
Fibrobacteres	0.7	0.2	0.09	0.005	0.35	0.17	0.3	0.09	0.051	0.66	0.16
Firmicutes	38.8	3.5	31.6	7.7	41.7	0.8	29.9	3.37	0.12	0.90	0.62
Kiritimatiellaeota	0.22 ^a	0.06	0.27 ^a	0.081	0.68 ^b	0.14	0.28 ^a	0.11	0.228	0.048	0.051
Lentisphaerae	0.12	0.04	0.02	0.01	0.2	0.025	0	0	ND	ND	ND
NA	0.11 ^{ab}	0.05	0.02 ^a	0.008	0.23 ^b	0.05	0.13 ^{ab}	0.03	0.167	0.0013	0.896
Patescibacteria					0.13				ND	ND	ND
Planctomycetes	0.24 ^a	0.06	1.8 ^b	0.55	0.28 ^a	0.03	1.8 ^b	0.28	0.015	0.917	0.955
Proteobacteria	2.5 ^c	0.44	0.7 ^a	0.12	1.89 ^{bc}	0.29	0.97 ^{ab}	0.15	0.0018	0.552	0.198
Spirochaetes	1.38 ^{ab}	0.45	0.85 ^a	0.10	2.07 ^b	0.4	0.45 ^a	0.17	0.043	0.63	0.11
Synergistia			0.02		0.12				ND	ND	ND
Tenericutes	2.18 ^a	0.16	1.55 ^a	0.29	4.5 ^b	1.07	1.9 ^a	0.4	0.053	0.03	0.181
Verrucomicrobia	0.07				0.57				ND	ND	ND

Table 7. Effect of YA supplementation on the average of relative abundance (%) of bacterial phyla in the rumen of camels and sheep under investigation. ND Non-determined, NA Nonclassified phylum. ^{a,b,c}: Means in the same row with different superscripts differ significantly ($P < 0.05$).

is similar to other studies on live yeast and algae^{23,50–54}. The improvement in feed intake could be attributed to the increase in the digestibility and higher passage rate due to lipid content in *Spirulina* and *Chlorella*^{51,53}, which is supported by the positive correlation between NDFI and NDFD. Furthermore, higher protein disappearance in the rumen of sheep resulted in higher rumen ammonia, which agrees with previous studies^{23,55–57} and that was supported with a positive correlation between CPI and rumen ammonia.

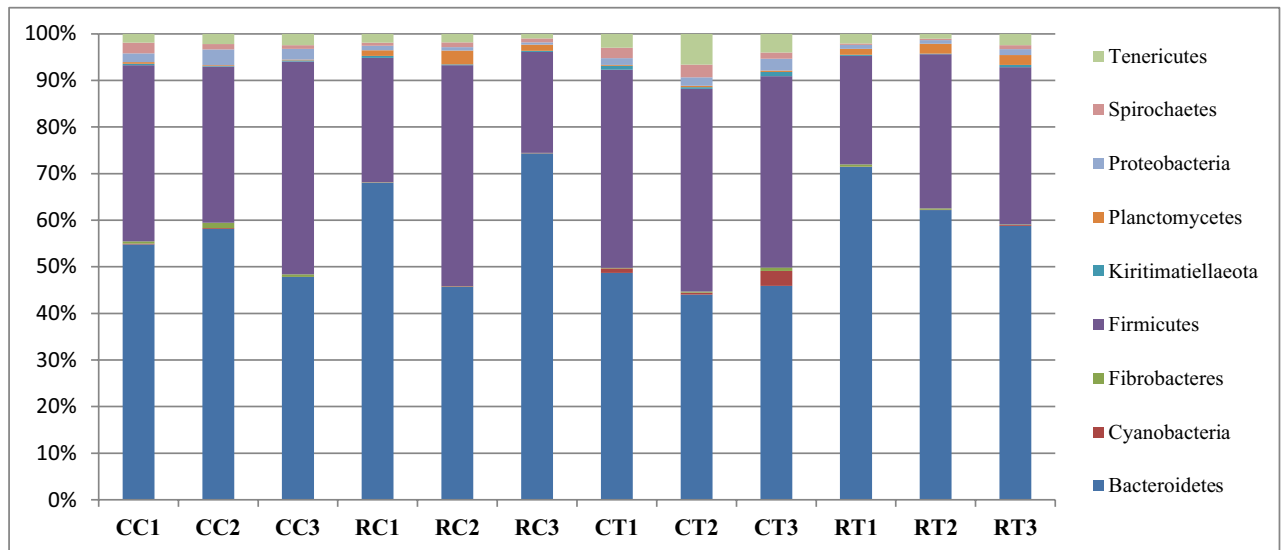
Blood glucose was improved in supplemented camels (TC) and total protein were enhanced in supplemented sheep (TR) (Table 5) by supplementation, which is similar to the previous studies that used live yeast or microalgae^{51,58,59}. The enhancement in blood glucose could be explained by the increase in the disappearance of DMD and NDF⁵¹; this speculation is supported by the positive correlation between blood glucose and NDFD (Fig. 3). The decline of blood urea could be a result of the decrease in protein catabolism and normal kidney function due to YA supplementation^{51,60}. The concentrations of blood immunoglobulins, IgA, IgG, and IgM, were increased by YA supplementation, which agrees with other findings on yeast and algae^{28,58}. Previous studies indicated that microalgae contain active compounds such as n-3 fatty acids, α -glucan, lutein, and other growth factors that work as immunostimulants^{21,51,54}. Also, *Saccharomyces* contain compounds such as β -glucans and oligosaccharides that work as immunomodulators⁶¹.

It was observed that discrepancies in total VFA production and VFA profile between camel and sheep, which might be attributed to discrepancies in microbial diversity and the relative abundance of bacterial groups^{21,62}. The concentration of acetic was declined and propionate was increased in sheep, which was also indicated in previous studies^{48,51,53}. Song et al.⁶³ included the yeast culture in the diet of growing lambs and observed that rumen pH and total VFA were not affected; rumen ammonia and butyric acid were increased, and acetic acid was decreased. Previous studies^{54,57} revealed that fiber degradation stimulates acetic acid production, which could demonstrate higher acetic acid in supplemented camel group (TC). The increase in the propionic acid production and the decline in the acetic acid could be a positive point for the YA supplementation to reduce the methane production in the rumen as the propionic acid is an alternative hydrogen sink that depresses the methanogenesis⁶⁴. The higher bacterial population in supplemented camels (TC) could be attributed to the availability of growth substrates in animal diets^{7,56}.

The reduction in total VFA production in supplemented camels (TC) was previously reported by studies that evaluated the microalgae supplementation^{48,65} and yeast/ microalgae combination supplementation (YA) in the horse²¹. Grimm et al.²¹ attributed the reduction in total VFA production after the YA supplementation to the changes in the taxonomy of hemicellulolytic and pectinolytic bacteria such as genus *Prevotella*. This speculation is supported by the decline in the relative abundance of *Prevotella* in supplemented camels (TC). However, investigating the bacterial functions and enzymes is recommended to get a better understanding of the supplementation impact²¹.

The chemical composition of the animal diet and animal species are the main determiner of rumen microbial community structure⁸. YA supplementation and animal species affected the diversity and relative abundances of bacterial communities in the rumen of camels and sheep (Tables 6, 7, 8; Figs. 1, 2). Similar findings were obtained by Song et al.⁶³ who investigated the effect of yeast supplementation in the diet of lambs on rumen microbiota. Limited information is available about the effect of the microalgae *Spirulina* and *Chlorella* on rumen

a



b

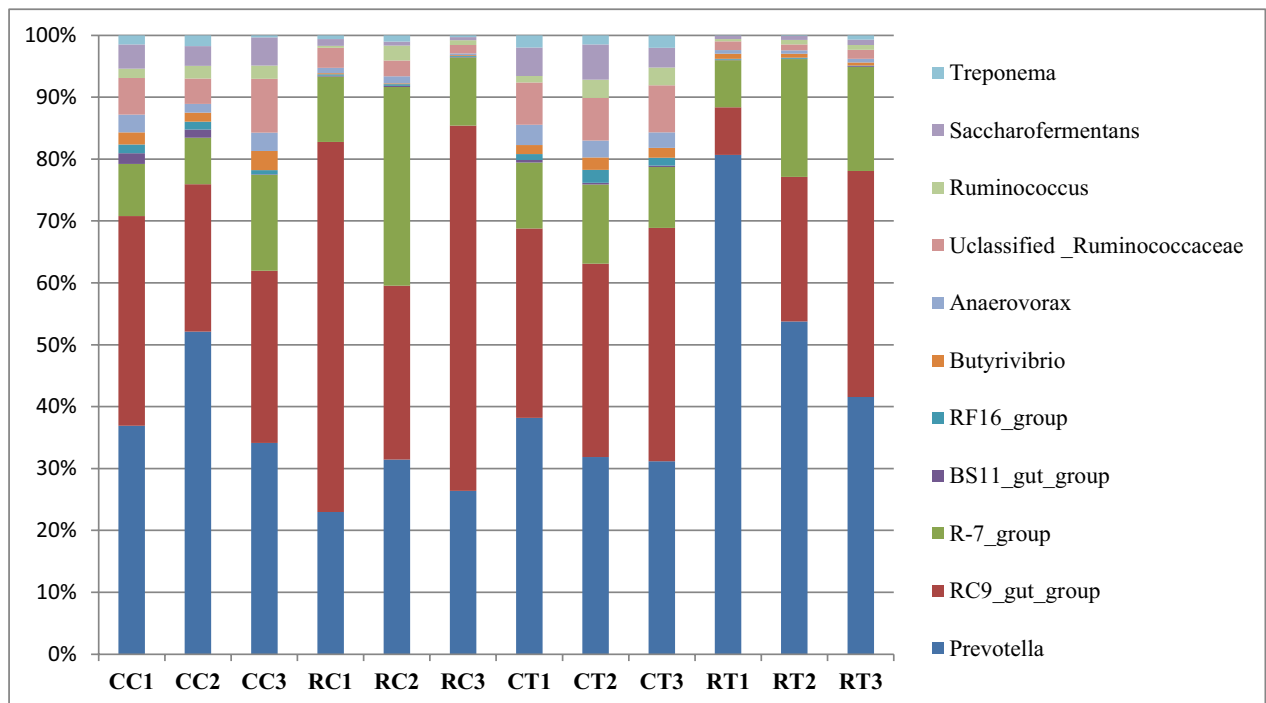


Figure 2. Relative abundance of bacterial families and genera. The relative abundances of dominant bacterial phyla (a) and bacterial genera (b) in the rumen of non-supplemented camels (CC1–CC3), non-supplemented sheep (CR1–CR3), supplemented camels (TC1–TC3), and supplemented sheep (TR1–TR3).

microbiota, and few studies quantified some of rumen bacterial genera using qPCR and concluded that algae supplementation affected the copy number of those genera, which supports our results^{28,66,67}. The majority of phylum Bacteroidetes was assigned to genus *Prevotella* and RC9_gut_group, reflecting the importance of these genera in rumen fermentation⁶³. Genus *Prevotella* is involved in the degradation of different substrates in the rumen, including protein, xylan, pectin, and starch, and can produce propionate^{6,7}, which might demonstrate the positive correlation between genus *Prevotella*, propionic acid, and CPD (Fig. 3). Also, the higher relative abundance of this genus and higher propionic in the TR group is explained. Genus RC9_gut_group, within phylum Bacteroidetes, is highly specialized in lignocellulose degradation⁷.

Members of phylum Firmicutes have an important role in fiber degradation in the rumen⁷. This phylum was dominated by Christensenellaceae_R-7_group, *Butyrivibrio*, *Ruminococcus*, *Saccharofermentans*, *Anaerovorax*, *Succiniclasticum* (Table 8; Fig. 2), which agree with previous studies^{7,63,68–71}. *Christensenellaceae_R-7_group*

	Control (C)				Treatment (T)				P values		
	Camel (CC)		Sheep (RC)		Camel (CT)		Sheep (RT)		A	T	A × T
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
P: Bacteroidetes											
F: BS11_gut_group	0.58	0.28	0.11	0.007	0.15	0.03	0.07	0.01	0.14	0.12	0.2
F: RF16_group	0.6 ^b	0.14	0.15 ^a	0.017	0.7 ^b	0.16	0.11 ^a	0.016	0.01	0.78	0.49
F: F082	6.8 ^c	1.01	1.18 ^a	0.49	3.62 ^b	0.15	1.17 ^a	0.28	0.0011	0.080	0.08
F: Muribaculaceae	1.27	0.07	0.57	0.30	1.07	0.12	1.17	0.37	0.43	0.17	0.028
F: Prevotellaceae	26.9 ^a	3.50	21.5 ^a	1.01	21.5 ^{a6}	1.59	44.1 ^{b6}	9.33	0.12	0.04	0.06
G: Prevotella	22.3 ^a	3.99	20.3 ^{a7}	1.39	16.9 ^a	1.60	43.5 ^b	9.3	0.05	0.02	0.07
G: RC9_gut_group	15.3 ^a	1.80	38.3 ^b	9.8	16.5 ^a	0.63	16.3 ^a	5.7	0.11	0.14	0.11
P: Firmicutes											
G: R-7_group	5.5 ^a	0.99	13 ^{ab}	4.27	5.5 ^a	0.52	11 ^b	2.54	0.04	0.03	0.55
G: Butyrivibrio	1.1 ^b	0.19	0.12 ^a	0.02	0.84 ^b	0.08	0.46 ^a	0.08	0.001	0.85	0.07
G: Lachnospirillum	0.65	0.23	0.17	0.01	0.38	0.08	0.00	0.00	ND	ND	ND
G: Anaerovorax	1.3 ^a	0.23	0.5 ^b	0.16	1.4 ^a	0.16	0.4 ^b	0.006	0.009	0.9	0.41
G: unclassified Ruminococcaceae	3.3 ^b	0.53	1.8 ^a	0.39	3.5 ^b	0.04	0.9 ^a	0.11	0.004	0.37	0.15
G: Papillibacter	3.5	0.09	0.22	0.02	2.44	0.66	0.00	0.00	ND	ND	ND
Ruminococcaceae_NK4A214	2.37	0.79	4.62	1.09	2.61	0.07	3.59	1.53	0.30	0.44	0.25
G: Ruminococcaceae_UCG-005	0.77 ^b	0.14	0.8 ^a	0.23	1.5 ^b	0.02	1.3 ^{ab}	0.19	0.71	0.01	0.46
G: Ruminococcaceae_UCG-014	1.40	0.43	1.42	0.68	3.72	1.30	2.24	0.17	0.4	0.1	0.37
G: Ruminococcus	1.01	0.10	0.83	0.41	1.13	0.30	0.46	0.09	0.24	0.54	0.28
G: Saccharofermentans	2.06 ^b	0.12	0.6 ^a	0.14	2.25 ^b	0.40	0.5 ^a	0.06	0.001	0.84	0.66
G: Succinilasticum	7.5 ^b	1.37	0.6 ^a	0.35 ^a	5.3 ^b	0.26	5.4 ^b	1.3	0.04	0.15	0.01
G: Anaerovibrio			0.27						ND	ND	ND
F: Desulfovibrionaceae	0.65	0.26	0.44	0.06	0.75	0.17	0.47	0.23	0.08	0.8	0.89
G: Desulfovibrio	0.63	0.26	0.32	0.07	0.50	0.14	0.40	0.22	0.16	0.9	0.67
G: Succinivibrio	0.062	0.02	0.00	0.00	0.077	0.02	0.035	0.01	ND	ND	ND
P: Spirochaetes, F: Spirochaetaceae											
G: Sphaerochaeta	0.64 ^{ab}	0.35	0.35 ^b	0.10	0.97 ^b	0.10	0.2 ^a	0.03	0.02	0.68	0.37
G: Treponema_2	0.66 ^{ab}	0.26	0.5 ^a	0.12	0.9 ^b	0.09	0.2 ^a	0.14	0.02	0.96	0.26

Table 8. Effect of YA supplementation on the average of relative abundance (%) of dominant bacterial families and genera in the rumen of camels and sheep under investigation. *ND* Non-determined, *P* phylum, *F* family, *G* genus. ^{a,b,c}: Means in the same row with different superscripts differ significantly ($P < 0.05$).

was also found in the rumen of goats and Yak and has a potential role in cellulose degradation^{69,72}. Genus *Anaerovorax* is involved in butyrate production and has a role in ruminal biohydrogenation^{68,73}. Additionally, *Butyrivibrio* has proteolytic and fibrolytic activities and is involved in the production of butyrate and ruminal biohydrogenation^{6,74}, which could explain the positive correlation between genus *Butyrivibrio* and butyric acid. Genus *Anaerovibrio* was not detected in the rumen of the camels after YA supplementation; also, the relative abundance of genus *Anaerovorax* and *Butyrivibrio* were affected by YA supplementation; these genera are involved in the ruminal biohydrogenation^{69,74,75}, which indicate that the biohydrogenation pathway could be regulated by yeast and algae supplementation.

Papillibacter genus was previously found in a high proportion in cattle- fed corn stover⁷⁶, indicating that this genus could be involved in fiber digestion⁷⁷. Moreover, genus *Succinilasticum* has a potential role in fiber degradation^{70,78} and converts succinate to propionate⁷⁹, which could illustrate the increase of this genus and propionic acid in the TR group. Genus *Saccharofermentans* is involved in polysaccharides degradation and produces acetate and propionate⁷¹, which could support the positive correlation between this genus and NDFD. Unclassified Ruminococcaceae represented a higher proportion of the bacterial community and some of which were increased by YA supplementation; these bacteria might have a role in fiber degradation^{6,8,76}. Phylum Spirochaetes was dominated by genus *Treponema* and *Sphaerochaeta*, which were increased by the supplementation. Previous studies^{7,80} indicated that *Treponema* is fiber-associated bacteria, with potential fibrolytic activities. *Sphaerochaeta* is involved in pectin degradation and produces acetate, lactate, and ethanol⁸¹.

Members of phylum Lentisphaerae are involved in cellobiose fermentation⁸². Phylum Verrucomicrobia was found only in camel and increased by YA supplementation, and has potential role the digestion of plant polysaccharides⁷. Planctomycetes were previously observed in the rumen of the dromedary camel⁸³ and have a potential role in cellulose and hemicellulose degradation⁸⁴. Phylum Cyanobacteria that was increased by supplementation are aerobic bacteria capable of scavenging oxygen in the rumen and fermenting carbohydrate during the deficiency of nitrogen⁸⁵. Therefore, this phylum ensures the anaerobic environment to facilitate cellulose degradation by anaerobic microorganisms⁸⁶. Overall, the results showed that the bacterial community

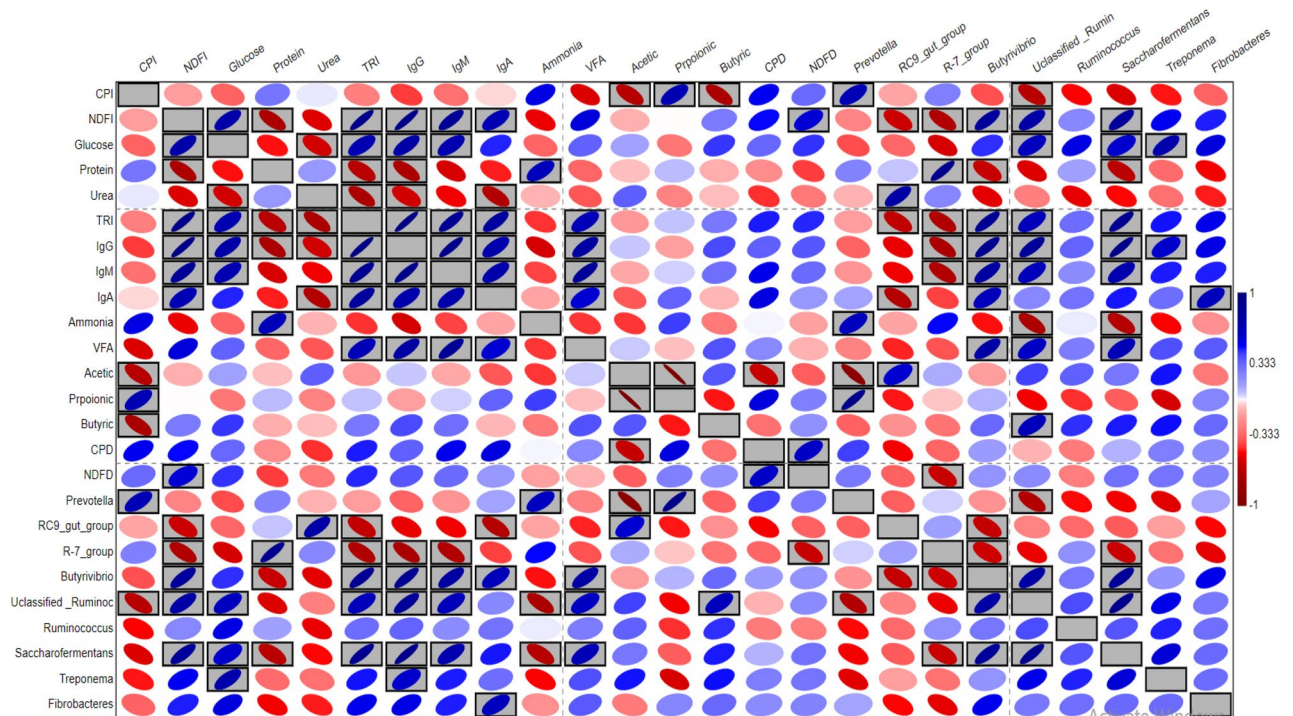


Figure 3. Heatmap based on Pearson correlation analysis. The heatmap shows the correlation relationships between feed intake (NDFI, CPI), blood biochemical and immunological parameters, rumen fermentation parameters, rumen disappearance of CP and NDF, and relative abundances of dominant bacterial genera in the rumen of camels and sheep under investigation. The black boxed ellipses refer to the significant correlations.

was dominated by polysaccharides degrading bacteria, such as, *Preotella*, *RC9_gut_group*, *Butyrivibrio*, *Ruminococcus*, *Saccharofermentans*, *Christensenellaceae_R-7_group*, and *Treponema*, *Fibrobacter*^{6,69,72,78}. These findings indicate the importance of these genera in the utilization of forage in the rumen. In addition, the relative abundances of some of these genera were affected by YA supplementation, which is in agreement with Ghazanfar et al., and Jiang et al.^{61,87}, who reported that yeast promotes the colonization of cellulolytic bacteria to dietary fiber as well as stabilize the rumen pH, which could explain the increase in NDFD at 48 h by YA supplementation.

Rumen microbiota plays a pivotal role in animal immunity⁶³. Previous studies^{59,61} mentioned that the improvement in animal immunity could be attributed to the increase in the activity of some cellulolytic bacteria that represent a barrier against pathogens. This trend was observed in the current study, wherever positive correlations between immunity parameters and some cellulolytic bacteria such as *Butyrivibrio*, *Unclassified_Ruminococcaceae*, *Saccharofermentans*, *Treponema*, and *Fibrobacteres* (Fig. 3). Estrada-Angulo, et al.⁸⁸ explained that combining the yeast with prebiotics such as carbohydrates stabilized the rumen pH, modulated the immune response, and improved fiber digestion; besides it inhibited pathogenic bacteria in the gut and alleviated the heat stress. Previous studies^{89,90} that linked animal efficiency with rumen microbiota, showed that efficient cows showed lower methane emission; higher feed intake, milk solid, higher relative abundance of *Preotella*, *Succin-clasticum*; *Treponema*, *Fibrobacter*, *Ruminococcus*; except for milk quality and methane emission that were not studied in this study, all the previous trends were observed in the supplemented animals. These findings highlight the yeast and algae combination as potential promising feed additives. Previous studies showed that live yeast and algae provide a diverse of chemical compounds that are required for the growth of rumen microbiota, such as carotenoids, phycobiliproteins, polysaccharides, lipids, essential fatty acids, n-3 fatty acids peptides, amino acids, and vitamins^{50,64}, which encourage the use of yeast and algae combination to supplement the animal diet.

Our results indicated that feed intake, the rumen disappearance of DM, CP, and NDF, and blood immunity were improved in both camels and sheep by YA supplementation. Furthermore, the response of rumen bacteria, rumen fermentation, some of the blood parameters to YA supplementation varied according to animal species. A similar pattern was observed by Lamminen et al.⁵² who noted an individual variation in animals in response to microalgae supplementation. Ghazanfar et al.⁶¹ concluded that the impact of live yeast on animal performance relies on animal breed, physiological stage, type of animal diet, feeding system, type of yeast, and supplementation dose. It is well known that the anatomy and motility of the camel rumen is different from other domestic ruminant animals^{12,14}, and the retention time of ingested feed is longer in the rumen of the camels than in other ruminants, which affects the response to dietary intervention, diet fermentation, the composition of rumen microbiota, metabolism, and blood biochemical parameters^{7,14,91,92}.

The study provides insights into the effect of YA supplementation on feed intake and the rumen ecosystem. The results suggest that YA supplementation modulated the rumen microbial community towards increasing degradation of dietary fiber and crude protein, which improved feed intake and affected rumen fermentation

parameters. Moreover, blood immunological parameters were improved. The response to YA supplementation varied according to animal species. Combining the microalgae with yeast might be beneficial to animals' performance.

Data availability

All the sequences were deposited to the sequence read archive (SRA) under the accession number: PRJNA767400
Via this link: <https://www.ncbi.nlm.nih.gov/sra/PRJNA767400>.

Received: 5 October 2021; Accepted: 19 July 2022

Published online: 29 July 2022

References

1. McSweeney, C. S., Odenyo, A. & Krause, D. O. Rumen microbial responses to antinutritive factors in fodder trees and shrub legumes. *J. Appl. Anim. Res.* **21**, 181–205. <https://doi.org/10.1080/09712119.2002.9706369> (2002).
2. Mutreja, R., Das, D., Goyal, D. & Goyal, A. Bioconversion of agricultural waste to ethanol by SSF using recombinant cellulase from *Clostridium thermocellum*. *Enzyme Res.* **1**, 1–6 (2011).
3. Moghazy, N. H. & Kaluarachchi, J. J. Sustainable agriculture development in the western desert of Egypt: A case study on crop production, profit, and uncertainty in the Siwa Region. *Sustainability* **12**, 6568 (2020).
4. Kewan, K. Z., El-Essawy, A. M., Shawket, S. M. & El-Shaer, H. M. Effect of energy sources supplement for camels fed berseem hay on: Feed intake, nutrients digestibility and concentration of some blood constituents. *J. Agric. Sci. Mansoura Univ.* **34**, 2803–2817 (2009).
5. Thabet, M. Drip irrigated barley (*Hordeum vulgare L.*) in arid regions of South Tunisia: Plant Growth and Yield Parameters. *Turk. J. Agric. Food Sci. Technol.* **4**, 470–475 (2016).
6. Liu, J., Zhang, M., Xue, C., Zhu, W. & Mao, S. Characterization and comparison of the temporal dynamics of ruminal bacterial microbiota colonizing rice straw and alfalfa hay within ruminants. *J. Dairy Sci.* **99**, 9668–9681. <https://doi.org/10.3168/jds.2016-11398> (2016).
7. Rabee, A. E., Forster, R., Elekwachi, C., Sabra, E. & Lamara, M. Comparative analysis of the metabolically active microbial communities in the rumen of dromedary camels under different feeding systems using total rRNA sequencing. *PeerJ* **8**, e10184. <https://doi.org/10.7717/peerj.10184> (2020).
8. Henderson, G. *et al.* Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci. Rep.* **5**, 14567. <https://doi.org/10.1038/srep14567> (2015).
9. Tajima, K. *et al.* Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl. Environ. Microbiol.* **67**, 2766–2774 (2001).
10. Gaughan, J. B., Sejian, V., Mader, T. L. & Dunshea, F. R. Adaptation strategies: Ruminants. *Anim. Front.* **9**, 47–53. <https://doi.org/10.1093/af/vfy029> (2019).
11. Clauss, M., Hume, I. D. & Hummel, J. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. *Animal* **4**, 979–992 (2010).
12. Kay, R. N. B. Maloiy MO (1989) Digestive secretions in camels. *Options Méditerranéennes Série Séminaires* **2**, 83–87 (1989).
13. Abousoliman, I. *et al.* Analysis of candidate genes for growth and milk performance traits in the Egyptian Barki sheep. *Animals* **10**, 197 (2020).
14. Wilson, R. T. The nutritional requirements of camel. *Option méditerranéennes-série séminaires-no 2*, 171 (1989).
15. Gihad, E. A., El-Gallad, T. T., Sooud, A. E., Farid, M. F. A. & Abou El-Nasr, H. M. Feed and water intake, digestibility and nitrogen utilization by camels compared to sheep and goats fed low protein desert by-products. *Options Méditerranéennes Série Séminaires* **2**, 75–81 (1989).
16. Rabee, A. E., Sayed Alahl, A. A., Lamara, M. & Ishaq, S. L. Fibrolytic rumen bacteria of camel and sheep and their applications in the bioconversion of barley straw to soluble sugars for biofuel production. *PLoS ONE* **17**(1), e0262304. <https://doi.org/10.1371/journal.pone.0262304> (2022).
17. Marcus, C. & Hummel, J. Physiological adaptations of ruminants and their potential relevance for production systems. *Revista Brasileira de Zootecnia*. **46**, 606–613. <https://doi.org/10.1590/S1806-92902017000700008> (2017).
18. Arowolo, M. A. & He, J. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. *Anim. Nutr.* **4**, 241–249. <https://doi.org/10.1016/j.aninu.2018.04.010> (2018).
19. Michalak, M. *et al.* Selected alternative feed additives used to manipulate the rumen microbiome. *Animals* **11**, 1542. <https://doi.org/10.3390/ani11061542> (2021).
20. Patra, A. K. & Saxena, J. Dietary phytochemicals as rumen modifiers: A review of the effects on microbial populations. *Antonie Van Leeuwenhoek* **96**, 363–375. <https://doi.org/10.1007/s10482-009-9364-1> (2009).
21. Grimm, P., Combes, S., Pascal, G., Cauquil, L. & Julliand, V. Dietary composition and yeast/microalgae combination supplementation modulate the microbial ecosystem in the caecum, colon and faeces of horses. *Br. J. Nutr.* **28**(123), 372–382. <https://doi.org/10.1017/S0007114519002824> (2020).
22. Belanche, A. *et al.* Editorial: Gut microbiome modulation in ruminants: Enhancing advantages and minimizing drawbacks. *Front. Microbiol.* **11**, 622002. <https://doi.org/10.3389/fmicb.2020.622002> (2021).
23. Mohamed, M. I., Maarek, Y. A., Abdel-Magid, S. S. & Awadalla, I. M. Feed intake, digestibility, rumen fermentation and growth performance of camels fed diets supplemented with a yeast culture or zinc bacitracin. *Anim. Feed Sci. Technol.* **149**, 341–345. <https://doi.org/10.1016/j.anifeedsci.2008.07.002> (2009).
24. Pinloche, E. *et al.* The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. *PLoS ONE* **8**, e67824. <https://doi.org/10.1371/journal.pone.0067824> (2013).
25. Abdel-Aziem, S. H., Abd El-Kader, H. A. M., Ibrahim, F. M., Sharaf, H. A. & El makawy, A. I. Evaluation of the alleviative role of *Chlorella vulgaris* and *Spirulina platensis* extract against ovarian dysfunctions induced by monosodium glutamate in mice. *Genet. Eng. Biotechnol. J.* **16**, 653–660 (2018).
26. Andrade, L. M., Andrade, C. J., Dias, M., Nascimento, C. A. O. & Mendes, M. A. *Chlorella* and *spirulina* microalgae as sources of functional foods, nutraceuticals, and food supplements; an overview. *MOJ Food Process Technol.* **6**, 45–58. <https://doi.org/10.15406/mojft.2018.06.00144> (2018).
27. Holman, B. W. B. & Malau-Aduli, A. E. O. *Spirulina* as a livestock supplement and animal feed. *J. Anim. Physiol. Anim. Nutr.* **97**, 615–623. <https://doi.org/10.1111/j.1439-0396.2012.01328.x> (2013).
28. Tsiplakou, E. *et al.* The effect of dietary *Chlorella vulgaris* inclusion on goat's milk chemical composition, fatty acids profile and enzymes activities related to oxidation. *J. Anim. Physiol. Anim. Nutr.* **102**(1), 142–151 (2018).
29. Gotteland, M. *et al.* The pros and cons of using algal polysaccharides as prebiotics. *Front. Nutr.* **7**, 163. <https://doi.org/10.3389/fnut.2020.00163> (2020).

30. Patel, A. K. *et al.* Emerging prospects of macro- and microalgae as prebiotic. *Microb. Cell Fact.* **20**(1), 112. <https://doi.org/10.1186/s12934-021-01601-7> (2021).
31. Markowiak, P. & Ślizewska, K. The role of probiotics, prebiotics and synbiotics in animal nutrition. *Gut Pathogens* **10**, 21. <https://doi.org/10.1186/s13099-018-0250-0> (2018).
32. El-Sayed, A.E.-K. & El-Sheekh, M. Outdoor cultivation of spirulina platensis for mass production. *Notulae Scientia Biologicae* **10**, 38–44. <https://doi.org/10.15835/nsb10110177> (2018).
33. El-Feky, A. M. *et al.* Phytoconstituents and in vitro antioxidant, antiviral, antihyperlipidemic and anticancer effects of chlorella vulgaris microalga in normal and stress conditions. *Der Pharma Chemica* **12**, 9–20 (2020).
34. Van Soest, P. J., Robertson, J. B. & Lewis, B. A. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* **74**, 3583–3597 (1991).
35. AOAC. Association of Official Analytical Chemists Official Methods of Analysis, 16th ed. AOAC, Arlington (1997).
36. Annon, E. F. Studies on the volatile fatty acids of sheep blood with special reference to formic acid. *Biochem. J.* **58**, 670–680 (1954).
37. Weimer, P. J., Shi, Y. & Odt, C. L. A segmented gas/liquid delivery system for continuous culture of microorganisms on solid substrates, and its use for growth of *Ruminococcus flavefaciens* on cellulose. *Appl. Microbiol. Biotechnol.* **36**, 178–183 (1991).
38. Walters, W. *et al.* Improved bacterial 16S rRNA gene (V4 and V4–5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* **1**, e00009-15. <https://doi.org/10.1128/mSystems.00009-15> (2015).
39. Comeau, A. M., Douglas, G. M. & Langille, M. G. I. Microbiome helper: A custom and streamlined workflow for microbiome research. *mSystems* **2**, e00127-e216. <https://doi.org/10.1128/mSystems.00127-16> (2017).
40. Denman, S. E. & McSweeney, C. S. Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. *FEMS Microbiol. Ecol.* **58**, 572–582. <https://doi.org/10.1111/j.1574-6941.2006.00190.x> (2006).
41. Callahan, B. *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583. <https://doi.org/10.1038/nmeth.3869> (2016).
42. SPSS. Statistical package for social science "Release 15, SPSS INC, Chicago, USA (1999).
43. Hammer, Ø., Harper, D. A. T. & Ryan, P. D. PAST: Paleontological statistics software package for education and data analysis. *Palaeo. Electr.* **4**, 9 (2001).
44. Pranckute, R., Kaunietis, A., Kuisiene, N. & Citavicius, D. J. Combining probiotics with probiotic bacteria can enhance bacterial growth and secretion of bacteriocins. *Int. J. Biol. Macromol.* **89**, 669–676. <https://doi.org/10.1016/j.ijbiomac.2016.05.041> (2016).
45. Viljoen, M., Brand, T. S. & Hoffman, L. C. Differences in the chemical composition and digestibility of cereal hay and straw produced in a Mediterranean rainfall area of South Africa. *S. Afr. J. Plant Soil.* **22**(2), 106–109. <https://doi.org/10.1080/02571862.2005.10634690> (2005).
46. Griffith, C. L., Ribeiro, G. O. Jr., Oba, M., McAllister, T. A. & Beauchemin, K. A. Fermentation of ammonia fiber expansion treated and untreated barley straw in a rumen simulation technique using rumen inoculum from cattle with slow versus fast rate of fiber disappearance. *Front. Microbiol.* **7**, 1839 (2016).
47. de Morais, M. G., Vaz, B., de Morais, E. G. & Costa, J. A. Biologically active metabolites synthesized by microalgae. *Biomed. Res. Int.* <https://doi.org/10.1155/2015/835761> (2015).
48. Meehan, D., Cabrita, A. R. J., Silva, J. L., Fonseca, A. J. M. & Maia, M. R. G. Effects of *Chlorella vulgaris*, *Nannochloropsis oceanica* and *Tetraselmis* sp. supplementation levels on in vitro rumen fermentation. *Algal Res.* <https://doi.org/10.1016/j.algal.2021.102284> (2021).
49. Laameche, F., Chehma, A. & Faye, B. Effect of diet composition on dry matter intake of dairy she-camels. *Trop Anim. Health Prod.* **51**, 2513–2519. <https://doi.org/10.1007/s11250-019-01960-9> (2019).
50. Rossow, H. A., Riordan, T. & Riordan, A. Effects of addition of a live yeast product on dairy cattle performance. *J. Appl. Anim. Res.* **46**(1), 159–163. <https://doi.org/10.1080/09712119.2017.1281810> (2018).
51. Kholif, A. *et al.* Dietary *Chlorella vulgaris* microalgae improves feed utilization, milk production and concentrations of conjugated linoleic acids in the milk of Damascus goats. *J. Agric. Sci.* **155**, 508–518. <https://doi.org/10.1017/S0021859616000824> (2017).
52. Lamminen, M., Halmemies-Beauchet-Filleau, A., Kokkonen, T., Jaakkola, S. & Vanhatalo, A. Different microalga species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets. *Anim. Feed Sci. Technol.* **247**, 112–126 (2019).
53. Panjaitan, T., Quigley, S. P., McLennan, S. R., Swain, A. J. & Poppi, D. P. Spirulina (*Spirulina platensis*) algae supplementation increases microbial protein production and feed intake and decreases retention time of digesta in the rumen of cattle. *Anim. Prod. Sci.* **55**, 535–543 (2014).
54. Kholif, A. E. *et al.* *Chlorella vulgaris* microalgae and/or copper supplementation enhanced feed intake, nutrient digestibility, ruminal fermentation, blood metabolites and lactational performance of Boer goat. *J. Anim. Physiol. Anim. Nutr.* **104**, 1595–1605. <https://doi.org/10.1111/jpn.13378> (2020).
55. Hamchara, P., Chanjula, P., Cherdthong, A. & Wanapat, M. Digestibility, ruminal fermentation, and nitrogen balance with various feeding levels of oil palm fronds treated with *Lentinus sajor-caju* in goats. *Asian-australas. J. Anim. Sci.* **31**, 1619–1626. <https://doi.org/10.5713/ajas.17.0926> (2018).
56. Khadem, A. A., Pahlavan, M., Afzalzadeh, A. & Rezaeian, M. Effects of live yeast *Saccharomyces cerevisiae* on fermentation parameters and microbial populations of rumen, total tract digestibility of diet nutrients and on the in situ degradability of alfalfa hay in Iranian Chall sheep. *Pak. J. Biol. Sci.* **15**(10), 590–597. <https://doi.org/10.3923/pjbs.2007.590.597> (2007).
57. Pallara, G. *et al.* Effect of stoned olive pomace on rumen microbial communities and polyunsaturated fatty acid biohydrogenation: An in vitro study. *BMC Vet. Res.* **10**, 271. <https://doi.org/10.1186/s12917-014-0271-y> (2014).
58. Mahmoud, M. M. *et al.* Influence of probiotic and yeast culture supplementation on selected biochemical and immunological parameters of growing lambs. *Pol. J. Vet. Sci.* **23**, 5–12. <https://doi.org/10.24425/pjvs.2019.131413> (2020).
59. El Shoukary, R. D. & Mahmoud, F. A. Effect of dietary yeast on male dromedary camels during the rut: Behavioral and biochemical insights. *Asian J. Anim. Vet. Ad.* **16**, 20–29. <https://doi.org/10.3923/ajava.2021.20.29> (2021).
60. Mašek, T. *et al.* Influence of live yeast culture (*Saccharomyces cerevisiae*) on milk production and composition, and blood biochemistry of grazing dairy ewes during the milking period. *Acta Vet. Brno* **77**, 547–554. <https://doi.org/10.2754/avb200877040547> (2008).
61. Ghazanfar, S., Khalid, N., Ahmed, I. & Imran, M. *Probiotic Yeast: Mode of Action and Its Effects on Ruminant Nutrition* (IntechOpen, Berlin, 2017). <https://doi.org/10.5772/intechopen.70778>.
62. Boguhn, J., Zuber, T. & Rodehutschord, M. Effect of donor animals and their diet on in vitro nutrient degradation and microbial protein synthesis using grass and corn silages. *J. Anim. Physiol. Anim. Nutr.* **97**, 547–557. <https://doi.org/10.1111/j.1439-0396.2012.01295.x> (2013).
63. Song, B. *et al.* Dietary supplementation of yeast culture into pelleted total mixed rations improves the growth performance of fattening lambs. *Front. Vet. Sci.* **8**, 657816. <https://doi.org/10.3389/fvets.2021.657816> (2021).
64. Johnson, K. A. & Johnson, D. E. Methane emissions from cattle. *J. Anim. Sci.* **73**, 2483–2492. <https://doi.org/10.2527/1995.73824.83x> (1995).
65. Sucu, E. Effects of microalgae species on in vitro rumen fermentation pattern and methane production. *Ann. Anim. Sci.* **20**, 207–218. <https://doi.org/10.2478/aoas-2019-0061> (2020).
66. Mavrommatis, A. *et al.* Alterations in the rumen particle-associated microbiota of goats in response to dietary supplementation levels of *Schizochytrium* spp. *Sustainability*. **13**, 607. <https://doi.org/10.3390/su13020607> (2021).

67. Toral, P. G. *et al.* Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. *J. Dairy Sci.* **95**, 794–806. <https://doi.org/10.3168/jds.2011-4561> (2012).
68. Huws, S. A. *et al.* As yet uncultured bacteria phylogenetically classified as Prevotella, Lachnospiraceae incertae sedis and unclassified Bacteroidales, Clostridiales and Ruminococcaceae may play a predominant role in ruminal biohydrogenation. *Environ. Microbiol.* **13**, 1500–1512. <https://doi.org/10.1111/j.1462-2920.2011.02452.x> (2011).
69. Liu, C. *et al.* Dynamic alterations in yak rumen bacteria community and metabolome characteristics in response to feed type. *Front. Microbiol.* **10**, 1116. <https://doi.org/10.3389/fmicb.2019.01116> (2019).
70. Qian, W., Ao, W., Jia, C. & Li, Z. Bacterial colonisation of reeds and cottonseed hulls in the rumen of Tarim red deer (*Cervus elaphus yarkandensis*). *Antonie Van Leeuwenhoek* **112**, 1283–1296 (2019).
71. Xue, Y., Lin, L., Hu, F., Zhu, W. & Mao, S. Disruption of ruminal homeostasis by malnutrition involved in systemic ruminal microbiota-host interactions in a pregnant sheep model. *Microbiome*. **8**, 138. <https://doi.org/10.1186/s40168-020-00916-8> (2020).
72. Wang, Y., Shen, Q., Zhong, S., Chen, Y. & Yang, Y. Comparison of rumen microbiota and serum biochemical indices in white cashmere goats fed ensiled or sun-dried mulberry leaves. *Microorganisms*. **8**, 981. <https://doi.org/10.3390/microorganisms8070981> (2020).
73. Lau, S. K., Teng, J. L. & Chiu, T. H. Differential microbial communities of omnivorous and herbivorous cattle in Southern China. *Comput. Struct. Biotechnol. J.* **16**, 54–60 (2018).
74. Lourenço, M., Ramos-Morales, E. & Wallace, R. J. The role of microbes in rumen lipolysis and biohydrogenation and their manipulation. *Animal* **4**, 1008–1023. <https://doi.org/10.1017/S175173111000042X> (2010).
75. Prins, R. A., Lankhorst, A., van der Meer, P. & Van Nevel, C. J. Some characteristics of Anaerobivrio lipolytica a rumen lipolytic organism. *Antonie Van Leeuwenhoek* **41**, 1–11. <https://doi.org/10.1007/BF02565031> (1975).
76. Zhang, R., Zhu, W., Zhu, W., Liu, J. & Mao, S. Effect of dietary forage sources on rumen microbiota, rumen fermentation and biogenic amines in dairy cows. *J. Sci. Food Agric.* **94**, 1886–1895. <https://doi.org/10.1002/jsfa.6508> (2014).
77. Mao, S. Y., Zhang, R. Y., Wang, D. S. & Zhu, W. Y. Impact of subacute ruminal acidosis (SARA) adaptation on rumen microbiota in dairy cattle using pyrosequencing. *Anaerobe* **24**, 12–19. <https://doi.org/10.1016/j.anaerobe.2013.08.003> (2013).
78. Du, C., Nan, X., Wang, K., Zhao, Y. & Xiong, B. Evaluation of the digestibility of steam-exploded wheat straw by ruminal fermentation, sugar yield and microbial structure in vitro. *RSC Adv.* **9**, 41775–41782 (2019).
79. van Gylswyk, N. O. Succiniclasticum ruminis gen. nov., sp. Nov., a ruminal bacterium converting succinate to propionate as the sole energy-yielding mechanism. *Int. J. Syst. Bacteriol.* **45**, 297–300. <https://doi.org/10.1099/00207713-45-2-297> (1995).
80. Ishaq, S. L. & Wright, A. G. Insight into the bacterial gut microbiome of the North American moose (Alces alces). *BMC Microbiol.* **12**, 212. <https://doi.org/10.1186/1471-2180-12-212> (2012).
81. Kelly, W. J. *et al.* Occurrence and expression of genes encoding methyl-compound production in rumen bacteria. *Anim. Microbiome*. **1**, 15. <https://doi.org/10.1186/s42523-019-0016-0> (2019).
82. Zoetendal, E., Plugge, C. M., Akkermans, A. D. L. & Vos, W. M. Victivallisvadensis gen. nov., sp. Nov., a sugar-fermenting anaerobe from human faeces. *Int. J. Syst. Evol. Microbiol.* **53**, 211–215. <https://doi.org/10.1099/ijs.0.02362-0> (2003).
83. Samsudin, A. A., Evans, P. N., Wright, A. D. & Al Jassim, R. Molecular diversity of the foregut bacteria community in the dromedary camel (*Camelus dromedarius*). *Environ. Microbiol.* **13**, 3024–3035. <https://doi.org/10.1111/j.1462-2920.2011.02579.x> (2011).
84. Stewart, R. D. *et al.* Compendium of 4,941 rumen metagenome-assembled genomes for rumen microbiome biology and enzyme discovery. *Nat. Biotechnol.* **37**, 953–961. <https://doi.org/10.1038/s41587-019-0202-3> (2019).
85. Neves, A. L. A., Li, F., Ghoshal, B., McAllister, T. & Guan, L. L. Enhancing the resolution of rumen microbial classification from metatranscriptomic data using kraken and mothur. *Front. Microbiol.* **8**, 2445. <https://doi.org/10.3389/fmicb.2017.02445> (2017).
86. Weimer, P. J. Cellulose degradation by ruminal microorganisms. *Crit. Rev. Biotechnol.* **12**, 189–223. <https://doi.org/10.3109/07388559209069192> (1992).
87. Jiang, Y. *et al.* Effects of the dose and viability of Saccharomyces cerevisiae. 1. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. *J. Dairy Sci.* **100**, 325–342. <https://doi.org/10.3168/jds.2016-11263> (2017).
88. Estrada-Angulo, A. *et al.* The effects of single or combined supplementation of probiotics and prebiotics on growth performance, dietary energetics, carcass traits, and visceral mass in lambs finished under subtropical climate conditions. *Biology* **10**, 1137. <https://doi.org/10.3390/biology10111137> (2021).
89. Liu, K. *et al.* Ruminal bacterial community is associated with the variations of total milk solid content in Holstein lactating cows. *Anim. Nutr.* <https://doi.org/10.1016/j.aninu.2021.12.005> (2022).
90. Lyons, T., Bielak, A., Doyle, E. & Kuhla, B. Variations in methane yield and microbial community profiles in the rumen of dairy cows as they pass through stages of first lactation. *J. Dairy Sci.* **101**(6), 5102–5114. <https://doi.org/10.3168/jds.2017-14200> (2018).
91. Simon, O., Jadamus, A. & Vahjen, W. Probiotic feed additives: Effectiveness and expected modes of action. *J. Anim. Feed Sci.* **10**, 51–67. <https://doi.org/10.22358/jafs/70012/2001> (2001).
92. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), *et al.* Guidance on the assessment of the efficacy of feed additives. *EFSA J.* **7**(16), e05274. <https://doi.org/10.2903/j.efsa.2018.5274> (2018).

Author contributions

A.R., K.K., B.Y., E.S. and M.L. conceived the research. A.R., K.K. and B.Y. conducted the experiments. M.L., A.R. and E.S. analyzed the data. All authors wrote and reviewed the manuscript. All authors approved the final manuscript.

Funding

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.E.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2022