



A NEW STRAIN OF *SACCHAROMYCES CEREVISIAE* IN DIETS OF LACTATING HOLSTEIN COWS IMPROVED FEED EFFICIENCY AND LACTATION PERFORMANCE

Hossam H. Azzaz¹, Ahmed E. Kholif^{1,2*}, Hussein A. Murad¹, Noha A. Hassaan³, Einar Vargas-Bello-Pérez^{4,5*}

¹Dairy Science Department, National Research Centre, 33 Bohouth St. Dokki, Giza, Egypt

²Department of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, NC 27411, USA

³Department of Animal Production, National Research Centre, 33 Bohouth Street, Dokki, Giza, Egypt

⁴School of Agriculture, Policy and Development New Agriculture Building, Earley Gate Whiteknights Road, PO Box 237, Reading RG6 6EU, Berkshire, UK

⁵Faculty of Animal Husbandry and Ecology, Autonomous University of Chihuahua, Periférico R. Aldama Km 1, 31031 Chihuahua, México

*Corresponding authors: ae_kholif@live.com, aekholif@ncat.edu; einar.vargasbelloperez@reading.ac.uk

Abstract

This study compared the effects of feeding a new strain of *Saccharomyces cerevisiae* HSA2020 with a commercial strain on *in vitro* rumen fermentation and production performance of dairy cows. Permeate was used as a substrate for the laboratory production of the new strain of *S. cerevisiae* after the hydrolysis by β -galactosidase (5000 μ mL at 37°C). Two experiments were conducted: in Experiment 1, the effects of three levels (1, 2 and 3 g/kg dry matter) of *S. cerevisiae* on *in vitro* ruminal fermentation kinetics were evaluated. In Experiment 2, for 60 days, sixty multiparous Holstein cows (639 $\pm$ 24.8 kg BW, 3 $\pm$ 1 parity, 7 $\pm$ 1 days in milk, with a previous milk production of 23 $\pm$ 2.0 kg/d) during the previous lactation, were randomly assigned to 3 treatments in a completely randomized design. Cows were fed without any additives (control treatment) or supplemented with 2 g/kg feed daily of laboratory produced (PY) or commercial (CY) *S. cerevisiae*. In Experiment 1, inclusion of PY and CY increased ($P<0.05$) gas production, propionate, and nutrient disappearance, while decreased ($P<0.05$) methane production and protozoal count. Moreover, in Experiment 2, PY followed by CY increased ($P<0.01$) nutrient digestibility, and serum concentrations of total protein, albumin, and glucose ($P<0.05$). Higher daily milk yield, and milk energy output were observed with PY and CY without affecting concentrations of milk components or milk fatty acid profile. Compared to control, increased feed efficiency was observed with PY and CY. Compared to PY, CY increased serum concentrations of urea-N and decreased triglycerides, while PY decreased serum aspartate transaminase and increased concentration of conjugated linoleic acids in milk. In early lactating cow diets, both strains of *S. cerevisiae* improved production performance at 2 g/kg, and minimal differences between strains were found.

Key words: yeast, probiotics, milk production, digestion, feed efficiency

For many years prebiotics and yeast, as direct fed microbials (DFM), have been administered to improve feed efficiency and animal's health (Elghandour et al., 2015; Hamdon et al., 2022). One of the common additives in dairy feeding is *Saccharomyces cerevisiae* that has been shown to improve balance of ruminal microorganisms, improve immunity, relieve stress, and improve productivity (Sun et al., 2021). The most common mode of action of *S. cerevisiae* is the creation of an anaerobic and stable ruminal environment improving activity of fibrolytic microorganisms (Martin and Nisbet, 1992), stabilizing ruminal pH (Chaucheyras-Durand et al., 2008), enhancing ruminal microbial enzyme activity, enhancing microbial attachment to feed particles, scavenging ruminal oxygen (Newbold et al., 1996). To improve feed digestion and milk production, finding new strains of yeast with low cost and higher ability to enhance ruminal environment is very important in dairy production. In this regard, inclusion of *S. cerevisiae* culture in dairy cow's feed has been shown to improve milk production, feed efficiency and antioxidant capacity (Sun et al., 2021). Feeding *S. cerevi-*

siae affects performance and cow's health by improving antioxidant capacity, immune capacity, body hormone levels and balance of ruminal microorganisms (Sivinski et al., 2022). Sun et al. (2021) fed *S. cerevisiae* culture to Holstein cows at 100 g/d and reported an increase in rumen pH, milk production, milk fat content, and ruminal acetate.

The use of commercial *S. cerevisiae* may increase total mixed rations costs, and finding alternatives produced on byproducts, at almost no cost, to lower costs, and keep similar efficiency to that from commercial products is of great interest for dairy production (Sivinski et al., 2022). Therefore, the objective of the present study was to compare the effects of in-feed supplementation of a commercially available (Pakmaya, PAK GIDA Uretim, Istanbul, Turkey; contained 1×10^9 CFU/g) or laboratory produced *S. cerevisiae* HSA2020 on: 1) *in vitro* ruminal fermentation; 2) feed efficiency, milk production, milk composition and milk fatty acid profile in early lactating Holstein cows. Since strains of yeast have different gene sequencing, metabolites and modes of action of a new

strain might produce different outcomes compared to other strains. Therefore, the hypothesis of this study was that regardless of its origin, *S. cerevisiae* would improve feed digestion resulting in improved milk production and feed efficiency in early lactating Holstein cows.

Material and methods

Laboratory production of *Saccharomyces cerevisiae* HSA2020

A new strain of *S. cerevisiae* HSA2020 was produced on permeate as a medium after the hydrolysis by β -galactosidase (5000 μ /mL at 37°C) as described by Murad et al. (2019). *S. cerevisiae* isolated from compressed bakery yeast were reactivated by overnight culture in a yeast extract-peptone-dextrose (YPD) liquid medium, containing 3 g/L yeast extract, 5 g/L peptone, and 20 g/L glucose. Cells were sampled and plated after serial dilutions on YPD solid medium. A single colony was selected arbitrarily and streaked consecutively at least four times to obtain pure colonies. Strain was maintained on YPD agar plates and was matched to *S. cerevisiae* using 18S rRNA analysis (El-Helow et al., 2015).

The *S. cerevisiae* HSA2020 is a new strain of yeast that was initially isolated from compressed bakery yeast. The purity of the isolated *S. cerevisiae* HSA2020 was confirmed by 18S rRNA analysis as described by El-Helow et al. (2015). The sequencing data has been submitted to the NCBI under the name *Saccharomyces* sp. isolate *cerevisiae* HSA2020 and received the accession number MT163713. For production of *S. cerevisiae* HSA2020 biomass, hydrolyzed permeate by β -galactosidase (5000 μ /mL at 37°C) was used as a basal growth medium as described by Murad et al. (2019). The inoculum of yeasts was prepared using 250 mL conical flasks that contained broth medium of YPD. After autoclaving at 121°C for 15 min, flasks were inoculated by a loop full of yeast culture and incubated in a rotary shaker (150 rpm) adjusted at 30°C for 24 h. The growing yeast was used to inoculate the experimental flasks at 1% (v/v). The biomass production of yeast was carried out in 250 mL conical flasks each containing 50 mL of enzymatically treated permeate containing 35 g/L of reducing sugars. The flasks were sterilized in an autoclave at 121°C for 15 minutes. After cooling to room temperature, the flasks were inoculated by the above prepared inoculum, and then incubated in a rotary shaker adjusted to 30°C and 150 rpm for 48 h. For the pilot scale production of yeast biomass, the enzymatically hydrolyzed permeate was concentrated by evaporation until obtaining a syrup containing 20% glucose. A fed batch fermentation system was employed on a 7.5 L capacity BioFlo 310 fermentor (Eppendorf, Hamburg, Germany) for 24 h for production of *S. cerevisiae* HSA2020 biomass. Temperature, agitation, and aeration were controlled at 30°C, 200 rpm and 1 vvm, respectively. The initial volume of growth medium was 3 L inoculated by 10% liquid inoculum. A feeding volume of 1.5 L

was added to the fermentor batchwise by input of 100 mL of the medium every 2 h. After getting the yeast biomass, the total viable plate count was used for measuring the yeast viability after its enumeration on PDA medium at 30°C for 48 h (Fadel and Foda, 2001).

Experiment 1

In vitro fermentation

Management of the donor sheep was performed according to the 3rd edition (2010) of the guide of Agricultural Research and Teaching of Federation of Animal Science Societies, Champaign, IL, USA. Using a stomach tube and a pump, rumen liquor was obtained from three adult Barki sheep (51 $\pm$ 2.6 kg of BW) at the Agriculture Experimental and Research Station, Faculty of Agriculture, Al-Azhar University (Egypt). Ewes were fed a fixed amount of concentrate (500 g) and *ad libitum* berseem hay daily. Animals were fed two meals daily at 08:00 and 16:00 h in two equal amounts. The rumen contents (liquid and solid contents 1:1 v/v) were collected before morning feeding, kept in pre-warmed thermo containers at 39°C under anaerobic conditions. About 500 mL of ruminal fluid was collected from all ewes. To avoid saliva contamination, the first 50 mL of rumen fluid samples were discarded. The rumen fluid was mixed for 10 sec, squeezed through four layers of cheese cloth, and maintained in a water bath at 39°C under continuous CO₂ flushing until inoculation (Theodorou et al., 1994). Three incubation runs were performed in three different weeks. Rumen contents obtained from the three sheep were combined for each run.

A total mixed ration composed of (per kg dry matter (DM)) berseem clover (forage) and concentrate at 30:70 w/w was formulated. The concentrate was composed of: 429 g corn silage, 214 g soybean meal, 343 g ground corn, 7 g sodium chloride, and 7 g minerals and vitamins mixture were used as a substrate. The nutrient contents of feed ingredients and basal diet are shown in Table 1.

Laboratory produced (*S. cerevisiae* HSA2020 at 1 $\times$ 10⁹ CFU/g product) and commercially produced (Pakmaya, PAK GIDA Uretim, Istanbul, Turkey; contained 1 $\times$ 10⁹ CFU/g) *S. cerevisiae* was evaluated at 0 (Control treatment), 1, 2 or 3 g/kg DM. The *in vitro* total gas production (TGP) assay was conducted as described in Azzaz et al. (2023). The experiment continued for 48 h of incubation. At the end of incubation after 48 h, exactly, 5 ml of headspace gas was taken from each bottle, at the end of incubation, and infused into a Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK) to measure the concentration of methane (CH₄) and carbon dioxide (CO₂). The control treatment without *S. cerevisiae*, and experimental treatments with different levels of *S. cerevisiae* were tested in three incubation runs in three consecutive weeks with 3 bottles containing inoculum and buffer but no feed (blanks). The average GP, CH₄ and CO₂ in the empty bottles (empty corrected GP) was subtracted to obtain the net GP, CH₄ and CO₂.

Table 1. Chemical composition (g/kg DM) of ingredients and control diet used in the *in vitro* and *in vivo* experiments (mean $\pm$ SD)

	Ground corn	Soybean meal	Corn silage	Berseem clover	Control diet
Dry matter, wet weight	896 $\pm$ 13.7	898 $\pm$ 16.5	891 $\pm$ 18.0	889 $\pm$ 17.8	893 $\pm$ 14.9
Organic matter	985 $\pm$ 16.9	930 $\pm$ 18.5	915 $\pm$ 18.5	870 $\pm$ 20.6	921 $\pm$ 18.6
Crude protein	83 $\pm$ 2.37	422 $\pm$ 9.2	77 $\pm$ 3.5	170 $\pm$ 3.5	158 $\pm$ 4.3
Ether extract	51 $\pm$ 0.9	48 $\pm$ 0.92	21 $\pm$ 0.7	32 $\pm$ 1.9	36 $\pm$ 0.9
Ash	15 $\pm$ 0.4	70 $\pm$ 2.37	85 $\pm$ 6.3	130 $\pm$ 7.8	79 $\pm$ 5.9
Nonstructural carbohydrates ¹	666 $\pm$ 9.4	313 $\pm$ 6.1	286 $\pm$ 5.4	258 $\pm$ 6.6	377 $\pm$ 9.8
Neutral detergent fiber	185 $\pm$ 3.9	147 $\pm$ 4.4	531 $\pm$ 15.6	410 $\pm$ 17.5	351 $\pm$ 8.1
Acid detergent fiber	37 $\pm$ 2.2	65 $\pm$ 3.1	320 $\pm$ 9.2	270 $\pm$ 4.4	196 $\pm$ 5.9

¹Nonstructural carbohydrates = 100 – (% neutral detergent fiber + % crude protein + % ether extract + % ash).

At 48 h (the end of incubation), fermentation was terminated by immersing the bottles in ice. For each *S. cerevisiae* level, 3 bottles were used to measure pH, ammonia-N (NH₃-N) (AOAC, 2005) and short chain fatty acids (SCFA) by steam distillation and titration, whereas the other 3 bottles were filtered in pre-weighed crucibles and washed with hot water then acetone, and the residual DM and ash were used to determine true DM, organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) disappearance (*d*DM, *d*OM, *d*NDF and *d*ADF, respectively) according to López et al. (2010). The blank bottles were used to correct for the net nutrient disappearance.

Concentration of SCFA and its individual molar proportions were determined according to Galyean method (Galyean and May, 2010) using a gas chromatograph (Thermo Fisher Scientific, Inc., TRACE1300, Rodano, Milan, Italy) fitted with an AS3800 autosampler and equipped with a capillary column HP-FFAP (19091F-112; 0.320 mm i.d., 0.50 μ m i.d., and 25 m length; J & W Agilent Technologies Inc., Palo Alto, CA, USA). A mixture of known concentrations of individual short chain fatty acids (acetate, propionate, and butyrate) was used as an external standard (Sigma Chemie GmbH, Steinheim, Germany) to calibrate the integrator.

For the analysis of bacterial and protozoal count as described by Galyean and May (2010), 4 mL of the medium was mixed with 1 mL of 10% formaldehyde solution, shaken slightly, and then placed in a refrigerator at 4°C until. The total bacterial number was counted using a count chamber bacterium Petroff-Hausser (Hausser Scientific®, 3900, Horsham, PA) and a phase contrast microscope at a magnification of 100 $\times$. Protozoal number was counted using a Neubauer chamber (BRAND, 7178-10, Wertheim, Germany) and a contrast microscope at 400 $\times$ magnification. The viable protozoa were counted in eight quadrants (4 in each grid), which maintained their morphological integrity.

Experiment 2

Animals

The experiment was conducted at a private farm “Sanad Farms for Meat and Dairy Production”, in the

Basateen area next to Damietta Port, Damietta Governorate (Egypt). The management of the cows was in accordance with the 3rd edition (2010) of the guide of Agricultural Research and Teaching of Federation of Animal Science Societies, Champaign, IL, USA. During the first week of lactation, sixty lactating Holstein cows (639 $\pm$ 24.8 kg body weight, 3 $\pm$ 1 parity, 7 $\pm$ 1 days in milk), with a previous milk production of 23 $\pm$ 2.0 kg/d based on 305 d total milk yield during the previous lactation. All animals enrolled in the experiment within 7 days. Before calving, cows were fed the same diets as during lactation. To allow for individual monitoring, cows were kept in individual pens (2 $\times$ 2 m), under shade, without any bedding and with free access to water. Each pen had feeding bunk and drinking trough. Cows were fed *ad libitum* a total mixed ration containing (per kg DM) 300 g berseem clover, 300 g corn silage, 150 g soybean meal and 250 g ground corn, to meet nutrient requirements for a 650 kg cow with 20 kg DM intake and 35 kg daily milk production according to the NRC (2001) recommendations. Animals were fed 10% more of the expected dry matter intake to ensure collection of orts. The diet fed to cows was the same for the *in vitro* experiment.

Cows were assigned randomly to one of three experimental treatments in a complete randomized design with 20 cows per treatment for 60 days to be fed their diets without any additives (control treatment) or supplemented with 2 g/kg DM feed daily of laboratory produced (containing 1×10^9 CFU/g product) (PY treatment) or commercial food grade *S. cerevisiae* (Pakmaya, PAK GIDA Uretim, Istanbul, Turkey; contained 1×10^9 CFU/g) (CY treatment). Feeding rate was set at 110% to not restrict intake and to target refusals between 5 to 10% of feed offered. To avoid problems with yeast survival, additives were mixed daily with all feedstuffs just before feeding using a feeding wagon. Before use, *S. cerevisiae* was kept at 4°C. Samples of diet ingredients were taken daily, composited weekly, dried at 60°C in a forced-air oven for 48 h (method 930.15) (AOAC, 2005) and stored for chemical analyses. The nutrient contents of the feed ingredients are shown in Table 1.

Nutrient intake and digestibility

Feed intake was recorded daily by weighing the total daily amount of feed offered to each cow and the total daily amounts of weighedorts using a digital scale. On weeks 4 and 8, nutrient digestibility trials were conducted for 7 successive days, in which acid insoluble ash was used as an internal indigestibility marker (Sales and Janssens, 2003). Acid-insoluble ash contents of feeds and feces were determined gravimetrically after drying, burning, and boiling ash in hydrochloric acid, and then filtering and washing the hot hydrolysate, and re-burning. Coefficients of digestion were calculated according to Ferret et al. (1999).

The dried feed, orts and fecal samples were ground to pass a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA) and analyzed for DM (method ID 930.15), ash (method ID 942.05), nitrogen (method ID 954.01) and ether extract (EE; method ID 920.39) according to the AOAC (2005) official methods. Neutral detergent fiber and lignin were determined by the procedure of Van Soest et al. (1991). Acid detergent fiber was analyzed according to the AOAC (2005). Organic matter (OM) was calculated as: $1000 - \text{ash}$. Nonstructural carbohydrates (NSC) were calculated as: $\text{NSC} = \text{OM} - \text{CP} - \text{EE} - \text{NDF}$.

Sampling and analysis of blood serum

On the last day of the 4th and 8th weeks of the experiment, individual ($n = 60$) blood samples (10 mL) were taken before morning feeding at 03:00 h from the jugular vein. Blood samples were centrifuged at $4,000 \times g$ for 20 min at 4°C . The serum was separated into 2 mL Eppendorf tubes and frozen at -20°C until analysis. Blood parameters were measured using specific kits (Spinreact, Ctra. Santa Coloma, Girona, Spain) with following the manufacturers' instructions.

Milk sampling, and milk composition

Cows were mechanically milked three times daily at 04:00, 12:00 and 20:00 h, and individual milk samples (30 g/kg of milk yield) were collected at each milking. A mixed sample of milk (proportional to amounts taken in each milking time) was taken daily every two weeks to determine milk composition. Milk samples were analyzed using infrared spectrophotometry (Ekomilk-M ultrasonic milk analyzer, EON Trading 2000, INC, Bulgaria).

Fatty acids in milk were chromatography determined using methyl esters prepared by base-catalyzed methanolysis of glycerides (potassium hydroxide in methanol) according to International Standards (International Standard ISO 15884-IDF 182:2002. Brussels, Belgium: International Dairy Federation).

The gross energy content was calculated according to Tyrrell and Reid (1965). The milk energy output (Mcal/d) was calculated as milk energy (Mcal/kg) $\times$ milk yield (kg/d). Fat corrected milk (4% FCM, kg/day) and energy-corrected milk (ECM, kg/day) were calculated according to Tyrrell and Reid (1965) as:

$$\text{ECM (kg/d)} = \text{milk (kg/day)} \times [38.3 \times \text{fat (g/kg)} + 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} + 20.7]/3140.$$

$$\text{FCM} = [0.4 \times \text{milk yield (kg/d)}] + [15.0 \times \text{fat yield (kg/d)}].$$

Feed efficiency was calculated and expressed as milk yield, FCM, and ECM per unit of DM intake.

Statistical analyses

The Shapiro–Wilk test was used to test the normal distribution of data. For the small number of variables that showed significance for the Shapiro–Wilk test, data transformation (e.g., natural log, inverse of the natural log, square root, or inverse of the square root) was applied before statistical analysis.

In vitro measurements data were analyzed using the GLM procedure (SAS Institute Inc., Cary, NC, USA) for a completely randomized design using the model:

$$Y_{ijkl} = \mu + Y_i + D_j + R_k + (Y \times D)_{ij} + \varepsilon_{ijkl}$$

where: Y_{ijkl} is the observation, μ is the population mean, Y_i is the *S. cerevisiae* type effect, D_j is the inclusion level effect, R_k is the run effect, $(Y \times D)_{ij}$ is the interaction between *S. cerevisiae* type and inclusion level, and ε_{ijkl} is the residual error. Data of each of the three runs of the same sample of the substrate were averaged prior to statistical analysis. Mean values of each individual run were used as the experimental unit. Linear and quadratic contrasts were used to examine dose responses within each *S. cerevisiae* type.

Data from *in vivo* measurements were analyzed as a completely randomized design with repeated measures using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA), considering sampling time as repeated measures and individual cow as the experimental unit. Data for variables measured daily for each week (period) were averaged before statistical analyses. The statistical model included the treatment effect, week (period) effect and the treatment $\times$ week (period) interaction. Animal nested within treatment was considered the random effect, while treatment was the fixed effect. Two covariance structures were considered in the REPEATED statement in PROC MIXED: compound symmetry (cs) and auto-regressive (AR(1)). The error structure, with the lowest Akaike information criteria, that fits the statistics was selected for the model. When the *F*-test was significant at $P < 0.05$, means were compared by applying the probability of difference option of the least squares means statement. Significance was declared at $P < 0.05$.

Results

Experiment 1 (*in vitro* fermentation)

Compared to control, PY inclusion linearly and quadratically increased ($P < 0.05$) TGP by 14, 21 and 114%, dDM by 7, 10 and 6%, dOM by 8, 10 and 6%, dNDF by 10, 13 and 8% and dADF by 15, 26 and 13% and con-

concentrations of $\text{NH}_3\text{-N}$ by 20, 22 and 19% and total SCFA by 13, 20 and 12% (Table 2). However, PY inclusion decreased ($P<0.05$) CH_4 production per g DM by 19, 16 and 20%, g $d\text{DM}$ by 24, 24 and 25%, g $d\text{OM}$ by 25, 24 and 25%, g $d\text{NDF}$ by 26, 26 and 27% and g $d\text{ADF}$ by 29, 33 and 30% for the level 1, 2 and 3%, respectively.

Compared to control, the inclusion of CY for the level 1, 2 and 3% linearly and quadratically increased ($P<0.05$) TGP by 18, 19 and 17% and decreased ($P<0.05$) CH_4 production per g DM by 18, 16 and 20%, g $d\text{DM}$ by 21, 22 and 22%, g $d\text{OM}$ by 21 for all levels, g $d\text{NDF}$ by 23, 24 and 25% and g $d\text{ADF}$ by 28% for all levels. The highest (quadratic effect, $P<0.05$) $d\text{DM}$, $d\text{OM}$, $d\text{NDF}$, $d\text{ADF}$, SCFA and $\text{NH}_3\text{-N}$ were observed with an inclusion of 2 g/kg DM of CY and PY compared to control.

Compared to control, *S. cerevisiae* (both of CY and PY) inclusion increased ($P<0.05$) TGP, $d\text{DM}$, $d\text{OM}$, $d\text{NDF}$ and $d\text{ADF}$, $\text{NH}_3\text{-N}$, total SCFA, propionate proportion and total bacterial count, and decreased ($P<0.05$) CH_4 production per g DM, $d\text{DM}$, $d\text{OM}$, $d\text{NDF}$ and $d\text{ADF}$, acetate proportion and total protozoal count.

Experiment 2 (animal performance)

Milk production, milk composition, and milk fatty acids

The treatment $\times$ week interactions were not significant for all measured parameters. Compared to control,

increased daily production of milk, ECM, FCM, total solids, solids non-fat, protein, fat, lactose (PY > CY) and milk energy output were observed with PY and CY treatments (Table 3). PY and CY did not affect concentrations of total solids, solids non-fat, protein, fat, lactose, or milk energy content compared to control treatment. Higher feed efficiency calculated as milk: DM intake, ECM: DM intake and FCM: DM intake was observed in PY and CY compared to control, with no differences between CY and PY.

Treatments did not affect the profile of fatty acids in milk (Table 4). The PY increased the concentration of the conjugated linoleic acid (C18:2 *trans*-10 *cis*-12) and α -linolenic acid (C18:3 *trans*-9 *cis* 12,15) compared to the CY and control treatments. C18:3 *trans*-9 *cis* 12,15 was decreased by CY.

Feed intake, nutrient digestibility, and blood measurements

The treatment $\times$ week interactions were not significant for all measured parameters. *S. cerevisiae* inclusion (PY and CY) did not affect feed intake (Table 5). Higher ($P<0.01$) digestibility of DM (PY > CY), OM, CP EE, NSC (PY > CY), NDF (PY > CY) and ADF (PY > CY) were observed with *S. cerevisiae* inclusion compared to control treatment.

Table 2. *In vitro* fermentation parameters of the control diet supplemented with laboratory produced or commercial *Saccharomyces cerevisiae* at 1, 2 or 3 g/kg DM after 48 h of incubation

	Control	PY ¹			CY ²			Pooled SEM	PY		CY	
		1	2	3	1	2	3		linear	quadratic	linear	quadratic
TGP, mL/g DM	104	119	126	119	123	124	122	3.2	0.016	0.004	0.003	0.016
CH_4 , mL/g DM	21.5	17.5	18.1	17.1	17.7	18.0	17.3	0.49	0.015	0.005	0.001	0.001
CH_4 /g $d\text{DM}$	42.7	32.4	32.3	31.9	33.8	33.2	33.3	1.12	0.047	0.001	<0.001	0.005
CH_4 /g $d\text{OM}$	38.2	28.7	29.2	28.7	30.0	30.0	30.2	0.90	0.030	<0.001	<0.001	0.001
CH_4 /g $d\text{NDF}$	61.0	45.0	45.1	44.5	46.9	46.4	46.0	2.05	0.045	0.008	<0.001	0.002
CH_4 /g $d\text{ADF}$	86.4	61.0	58.1	60.3	62.1	62.1	62.5	4.10	0.009	0.002	0.004	0.007
$d\text{DM}$, g/kg	506	540	559	536	525	544	520	7.4	0.019	0.004	0.008	0.728
$d\text{OM}$, g/kg	564	609	619	597	591	601	575	6.4	0.041	<0.001	<0.001	0.916
$d\text{NDF}$, g/kg	356	390	403	385	378	389	377	9.8	0.036	0.012	0.026	0.607
$d\text{ADF}$, g/kg	252	291	317	286	289	296	278	13.4	0.036	0.014	0.025	0.487
pH	6.52	6.85	6.83	6.87	6.87	6.85	6.88	0.030	0.222	0.821	0.536	0.060
SCFA, mmol/L	121	137	145	135	141	145	135	3.7	0.032	0.001	<0.001	0.188
Acetate, %	64.4	62.6	60.8	60.1	63.5	63.6	62.4	3.55	<0.001	0.029	0.031	0.003
Propionate, %	22.4	23.3	24.8	26.1	23.7	24.2	25.2	1.93	<0.001	0.032	0.042	0.011
Butyrate, %	10.5	10.2	10.8	10.5	10.4	10.9	10.4	1.84	0.111	0.056	0.200	0.306
$\text{NH}_3\text{-N}$, mg/dL	10.8	13.0	13.2	12.8	13.0	13.2	12.9	0.12	0.018	0.019	0.015	0.804
Total bacteria $\times 10^8$	10.6	11.9	11.2	11.6	11.3	11.2	11.3	0.25	0.010	0.033	0.021	0.048
Total protozoa $\times 10^5$	5.06	4.16	4.39	4.32	4.39	4.19	4.41	0.072	0.014	0.015	0.039	0.076

$d\text{ADF}$, acid detergent fiber disappearance; CH_4 , methane; $d\text{DM}$, dry matter disappearance; $d\text{NDF}$, neutral detergent fiber disappearance; $\text{NH}_3\text{-N}$, Ammonia-N (mg/dL); $d\text{OM}$, organic matter disappearance (g/kg); SCFA, short chain fatty acids (mmol/L); TGP, total gas production (mL/g DM).

¹The produced product contained *Saccharomyces cerevisiae* (1×10^9 CFU/g product) evaluated at 1, 2 or 3 g/kg DM.

²The commercial product (Pakmaya, PAK GIDA Uretim, Istanbul, Turkey) contained 2×10^9 CFU/g evaluated at 1, 2 or 3 g/kg DM.

Table 3. Milk production and composition of Holstein cows fed diets supplemented with laboratory produced or commercial *Saccharomyces cerevisiae* at 2 g/kg DM

	Treatment ¹			SEM	P value		
	control	PY	CY		treatment	period	treatment × period
Yield (kg/d)							
milk	30.0 b	33.5 a	33.2 a	0.18	<0.001	<0.001	0.964
energy corrected milk (ECM)	29.2 b	33.0 a	32.3 a	0.27	<0.001	0.279	1.000
fat corrected milk (4% FCM)	28.4 b	31.9 a	31.4 a	0.22	<0.001	0.231	0.999
total solids	3.80 b	4.32 a	4.25 a	0.027	<0.001	0.242	0.999
solids non-fat	2.71 b	3.09 a	3.04 a	0.020	<0.001	0.369	0.997
protein	1.00 b	1.15 a	1.13 a	0.010	<0.001	0.321	1.000
fat	1.10 b	1.23 a	1.21 a	0.089	<0.001	0.083	1.000
lactose	1.49 c	1.70 a	1.65 b	0.099	<0.001	0.021	0.990
milk energy output (Mcal/d)	90.9 b	102 a	100 a	0.9	<0.001	0.202	1.000
Composition (g/kg)							
total solids	127	129	128	0.8	0.179	<0.001	0.999
solids non-fat	90.1	92.1	91.7	0.62	0.055	<0.001	0.999
protein	33.4	34.2	34.0	0.29	0.152	0.957	1.000
fat	36.5	36.7	36.3	0.27	0.540	<0.001	0.996
lactose	49.7	50.8	49.8	0.34	0.059	<0.001	0.995
milk energy content (Mcal/kg)	3.03	3.07	3.03	0.02	0.236	<0.001	0.999
Feed efficiency							
milk: DM intake	1.55 b	1.67 a	1.69 a	0.065	0.037	0.053	0.988
ECM: DM intake	1.51 b	1.64 a	1.64 a	0.069	0.022	0.068	0.986
FCM: DM intake	1.46 b	1.59 a	1.59 a	0.069	0.035	0.069	0.989

Means in the same row with different letters differ (P<0.05).

SEM, standard error of the mean.

¹Treatment: Control diet contained 30% berseem clover + 30% corn silage + 15% soybean meal + 24% yellow corn + 5 g sodium chloride + 5 g minerals and vitamins mixture, without additives (Control), or supplemented with laboratory produced (PY) or commercial (CY) *Saccharomyces cerevisiae* at 2 g/kg DM.

Table 4. Milk fatty acid profile (g/100 g fatty acids) of lactating Holstein cows fed diets supplemented with laboratory produced or commercial *Saccharomyces cerevisiae* at 2 g/kg DM

	Treatment ¹			SEM	P value		
	control	PY	CY		treatment	period	treatment × period
1	2	3	4	5	6	7	8
C4:0	0.81	0.81	0.75	0.017	0.138	0.429	0.531
C6:0	0.97	0.95	0.98	0.016	0.391	0.159	0.602
C8:0	0.93	0.90	0.93	0.017	0.400	0.892	0.623
C10:0	2.68	2.74	2.75	0.128	0.907	0.611	0.245
C11:0	0.20	0.20	0.19	0.010	0.866	0.929	0.133
C12:0	3.33	3.38	3.35	0.193	0.983	0.477	0.051
C13:0	0.46	0.46	0.47	0.015	0.895	0.018	0.431
C14:0	12.3	12.2	12.3	0.18	0.829	0.007	0.646
C15:0	1.07	1.10	1.08	0.015	0.524	0.660	0.334
C16:0	34.4	34.2	33.9	0.24	0.502	0.006	0.305
C17:0	0.66	0.63	0.65	0.078	0.974	0.200	0.162
C18:0	9.36	9.20	9.21	0.199	0.817	0.671	0.808
C20:0	0.12	0.12	0.12	0.010	0.928	0.802	0.212
C22:0	0.05	0.05	0.05	0.002	0.936	0.007	0.111
C23:0	0.04	0.03	0.03	0.002	0.755	0.862	0.769
C24:0	0.04	0.04	0.04	0.001	0.494	0.004	0.084
Σ Saturated fatty acids (SFA)	67.4	66.9	66.8	0.48	0.698	0.000	0.204

Table 4 – contd.

1	2	3	4	5	6	7	8
C14:1 <i>cis</i> -9	0.28	0.28	0.27	0.009	0.740	0.022	0.145
C14:1 <i>trans</i> -9	0.99	0.98	0.99	0.004	0.650	0.150	0.443
C16:1 <i>cis</i> -9	0.34	0.32	0.34	0.026	0.859	0.694	0.372
C16:1 <i>trans</i> -9	1.86	1.83	1.81	0.037	0.668	0.280	0.768
C18:1 <i>cis</i> -9	19.9	20.8	21.1	0.57	0.412	0.998	0.838
C18:1 <i>trans</i> -9	0.24	0.26	0.26	0.017	0.671	0.994	0.900
C18:1 <i>trans</i> 11	0.97	1.04	1.00	0.039	0.525	0.316	0.154
Σ Monounsaturated fatty acids (MUFA)	24.5	25.5	25.7	0.58	0.411	0.271	0.790
C18:2 <i>trans</i> -9,12	1.76	1.76	1.72	0.084	0.911	0.005	0.470
C18:2 <i>cis</i> -9-12	0.17	0.19	0.19	0.011	0.650	0.003	0.810
C18:2 <i>cis</i> -9 <i>trans</i> -11	0.40	0.41	0.40	0.024	0.986	0.211	0.231
C18:2 <i>trans</i> -10 <i>cis</i> -12	0.01 b	0.02 a	0.01 b	0.001	0.019	0.002	0.298
C18:3 <i>trans</i> -9 <i>cis</i> 12,15	0.11 a	0.10 a	0.09 b	0.002	0.041	0.839	0.053
C18: 3 <i>cis</i> -9,12,15	0.40	0.39	0.40	0.026	0.954	0.003	0.293
C20:3 <i>cis</i> - 8,11,14	0.09	0.10	0.09	0.002	0.364	0.004	0.562
C20:4 <i>cis</i> -5,8,11,14	0.12	0.13	0.12	0.009	0.898	0.015	0.304
C20:5 <i>cis</i> - 5,8,11,14,17	0.03	0.03	0.03	0.001	0.604	0.017	0.958
C22:5, <i>cis</i> -7,10,13,16,19	0.17	0.16	0.16	0.004	0.354	0.382	0.541
Σ Polyunsaturated fatty acids (PUFA)	3.25	3.26	3.20	0.075	0.818	0.020	0.083
Σ Unsaturated fatty acids (UFA)	27.8	28.8	28.9	0.63	0.478	0.003	0.730
Σ Conjugated linoleic acid ²	0.41	0.42	0.41	0.024	0.969	0.167	0.602
UFA: SFA	0.41	0.43	0.43	0.012	0.493	0.559	0.183

Means in the same row with different letters differ ($P < 0.05$).

SEM, standard error of the mean.

¹Treatment: Control diet contained 30% berseem clover + 30% corn silage + 15% soybean meal + 24% yellow corn + 5 g sodium chloride + 5 g minerals and vitamins mixture, without additives (Control), or supplemented with laboratory produced (PY) or commercial (CY) *Saccharomyces cerevisiae* at 2 g/kg DM.

²C18:2 *cis*-9 *trans*-11 + C18:2 *trans*-10 *cis*

Table 5. Intake and nutrient digestibility of lactating Holstein cows supplemented (2 g/kg DM) with laboratory produced or commercial *Saccharomyces cerevisiae* at 2 g/kg DM

	Control	Treatment ¹		SEM	P value		
		PY	CY		treatment	period	treatment × period
Intake (kg/cow/d)	19.4	20.1	19.7	0.34	0.269	0.592	0.629
Digestibility (g absorbed/kg ingested)							
Dry matter	656 c	731 a	717 b	3.3	<0.001	0.334	0.641
Organic matter	656 b	723 a	702 a	3.9	<0.001	0.311	0.190
Crude protein	681 b	741 a	721 a	7.4	<0.001	0.476	0.422
Ether extract	702 b	761 a	753 a	10.6	0.001	0.451	0.395
Non-structural carbohydrates	683 c	743 a	725 b	3.8	<0.001	0.465	0.133
Neutral detergent fiber	572 c	666 a	637 b	7.0	<0.001	0.089	0.991
Acid detergent fiber	522 c	615 a	587 b	7.0	<0.001	0.089	0.991

Means in the same row with different letters differ ($P < 0.05$).

SEM, standard error of the mean.

¹Treatment: Control diet contained 30% berseem clover + 30% corn silage + 15% soybean meal + 24% yellow corn + 5 g sodium chloride + 5 g minerals and vitamins mixture, without additives (Control), or supplemented with laboratory produced (PY) or commercial (CY) *Saccharomyces cerevisiae* at 2 g/kg DM.

Except for urea-N ($P = 0.041$), the treatment × week interactions were not significant for all measured parameters. *S. cerevisiae* did not affect concentrations of serum globulin, albumin: globulin ratio, cholesterol, triglycerides, alanine transaminase (ALT), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) (Table 6).

Increased ($P < 0.05$) concentrations of serum total protein, albumin and glucose were observed with *S. cerevisiae* (PY and CY) inclusion. Higher ($P < 0.05$) concentrations of serum urea-N and lower concentration of aspartate transaminase (AST) were observed with CY compared to PY.

Table 6. Blood parameters of lactating Holstein cows fed diets supplemented with laboratory produced or commercial *Saccharomyces cerevisiae* at 2 g/kg DM

	Treatment ¹			SEM	P value		
	control	PY	CY		treatment	period	treatment × period
Total protein (mg/dL)	6.87 b	7.49 a	7.51 a	0.120	0.011	0.290	0.166
Albumin (mg/dL)	3.87 b	4.41 a	4.37 a	0.139	0.019	0.033	0.076
Globulin (mg/dL)	3.00	3.08	3.14	0.130	0.648	0.156	0.143
Albumin: globulin ratio	1.33	1.45	1.44	0.100	0.604	0.077	0.065
Urea-N (mg/dL)	20.2 b	19.71 b	21.8 a	0.46	0.007	0.400	0.041
Glucose (mg/dL)	77.0 b	84.5 a	83.3 a	1.39	0.002	0.971	0.650
Cholesterol (mg/dL)	169	148	141	10.2	0.062	0.392	0.317
Triglycerides (mg/dL)	102	99.1	94.9	5.33	0.066	0.082	0.448
AST (units/L)	40.3 a	33.0 b	37.0 a	1.51	0.008	0.051	0.194
ALT (units/L)	28.5	27.3	25.6	0.82	0.058	0.124	0.695
NEFA (mmol/L)	0.85	0.87	0.87	0.044	0.934	0.016	0.739
BHBA (mmol/L)	0.90	0.90	0.90	0.042	0.995	0.037	0.270

AST, aspartate aminotransferase; ALT, alanine aminotransferase, BHBA, β -hydroxybutyrate, NEFA, non-esterified fatty acids.

Means in the same row with different letters differ ($P < 0.05$).

SEM, standard error of the mean.

¹Treatment: Control diet contained 30% berseem clover + 30% corn silage + 15% soybean meal + 24% yellow corn + 5 g sodium chloride + 5 g minerals and vitamins mixture, without additives (Control), or supplemented with produced (PY) or commercial (CY) *Saccharomyces cerevisiae* at 2 g/kg DM.

Discussion

One of the main inconsistencies between experiments on *S. cerevisiae* is due to the strain of *S. cerevisiae* and if such yeast contained dead or live cells. Therefore, not all *S. cerevisiae* culture preparations are effective. Different strains of *S. cerevisiae* contain different concentrations of soluble growth factors such as organic acids, B vitamins, and amino acids (Callaway and Martin, 1997). Different concentrations of soluble growth factors stimulate cellulolytic bacteria growth and feed digestion, and modify microbial activity, and fermentation kinetics (Beev et al., 2007). Such differences may be responsible for the observed variations between the evaluated strains resulting in various abilities to shift rumen fermentation patterns. Therefore, more studies on gene sequencing, metabolites, and modes of action of yeasts are recommended.

Experiment 1 (*in vitro* fermentation)

In the present study, yeast inclusion increased the total bacterial number. It is well documented that *S. cerevisiae* supplementation stimulates the microbial cellulolytic activity resulting in improved nutrient digestion and TGP (Elghandour et al., 2016). Moreover, the PY at all levels and medium level of CY increased *d*DM, *d*OM, *d*NDF and *d*ADF indicating an improved ruminal condition with *S. cerevisiae* supplementation (Ogunade and McCoun, 2021). In an *in vitro* experiment, Xue et al. (2022) observed that yeast administration at 0.3, 0.6 and 1.20% did not affect ruminal pH and increased the concentrations of $\text{NH}_3\text{-N}$, SCFA, acetic acid, propionic acid and digestibility of DM, NDF, and ADF at 0.6% of yeast inclusion. In another *in vitro* experiment, Dai et al.

(2023) reported for *S. cerevisiae* culture that low-dose SC (0.10%), and high-dose SC supplementation (0.30%) increased DM, CP and digestibility. Recently, Benchaar et al. (2024) stated that dried *S. cerevisiae* at 600 and 1200 mg/L incubation medium increased total gas production and SCFA and acetate concentrations as well as CH_4 production without affecting propionate concentration.

Improved nutrient degradation could be explained by the increased amounts of total bacteria, fungi, and lactate-utilizing bacteria in the rumen, and the decreased amount of starch-degrading and lactate-producing bacteria (Ding et al., 2014; Dai et al., 2023). Yeast can produce or increase the activity of enzymes including hemicellulase, pectanase, protease, amylase, lipase and phytase, resulting in improved nutrient degradability (Michalak et al., 2021). Moreover, increasing the total number of ruminal bacteria cannot be ignored as a reason for improved nutrient disappearance. One striking feature from this study was that compared to the commercial strain, the isolated strain improved disappearance of DM and OM, which confirmed our assumption that the genome of both strains differed. The possible different production of metabolites may affect the composition of rumen microbiome, especially in those microorganisms involved in fiber digestion (Beev et al., 2007). Further studies should determine effects on rumen microbiome.

The inclusion of PY and CY decreased CH_4 production by 16.2 to 20.6% and 16.3 to 19.6%, respectively, which was paralleled with improved fiber disappearance and ruminal propionate. Reducing CH_4 production in the rumen may increase feed energy, which could positively affect livestock performance, in many studies. *S. cerevisiae* supplementation can stimulate acetogens

to compete for H_2 with methanogens, thereby reducing CH_4 emissions (Patra, 2022). *S. cerevisiae* can stimulate the development of ruminal microbes to digest feed so that fermentation of carbohydrates in the rumen results in high production of propionate, which requires H_2 bound to glucose (Zain et al., 2022). Another reason for such results in our study is that *S. cerevisiae* decreased ruminal protozoa. Phesatcha et al. (2021) observed that *S. cerevisiae* supplementation to Thai native beef cattle fed diets with different roughage concentrate ratios decreased protozoal population, which may explain the lowered CH_4 production because protozoa populations are closely related to rumen CH_4 production (Dai and Faciola, 2019).

The PY inclusion increased the concentrations of total SCFA by 11.7 to 20%, while the medium level of CY inclusion increased SCFA by 19.8%. Moreover, both PY and CY caused some changes in the proportions of individual SCFA resulting in increased proportions of propionate while decreasing acetate (Hassan et al., 2016). Increasing propionate may partially explain the observed improved milk production in Experiment 2; however, the lowered acetate production was not consistent with the results of unchanged fat concentrations in milk. Perdomo et al. (2020) observed increased concentrations of SCFA with *S. cerevisiae* feeding to cows. The improved nutrient disappearance with *S. cerevisiae* inclusion may be the main reason of the increased total SCFA and propionate (Elghandour et al., 2014). Lowering acetate proportion was not paralleled with fiber disappearance; however, concentrations of acetate and propionate were increased with yeast inclusion. At the same time, the PY and medium level of CY inclusion linearly and quadratically increased the concentrations of NH_3 -N by 18.6 to 22.3% and 22.2%, respectively. Increasing ruminal NH_3 -N and SCFA at the same time improves the synchronization between dietary energy and protein, which is expected to increase microbial-N production within the rumen.

One of the main purposes of feeding *S. cerevisiae* to animals is to maintain ruminal pH. In the present study, treatments did not affect fermentation pH; however, all values were above the minimal value (5.6) reported for the activity of cellulolytic microorganisms (Tajima et al., 2001). Bennett et al. (2021) observed unaffected fermentation pH with *S. cerevisiae* inclusion in a dual-flow continuous culture system.

Experiment 2 (animal performance)

Milk production, milk composition, and milk fatty acids

S. cerevisiae inclusion increased daily production of milk by 11.7 and 10.7%, respectively, ECM by 13.2 and 10.8%, respectively, and FCM by 12 and 10.2%, respectively. Similar results were reported by Perdomo et al. (2020) when *S. cerevisiae* supplementation did not affect DM intake in cows, and thus, increase in FCM and ECM cannot be attributed to increased nutrient intake. The increased total tract digestibility of nutrients and probable lowered CH_4 production (as observed in Experiment 1),

result in increased content and availability of digestible energy needed to support additional milk yields (Perdomo et al., 2020). Other factors that may explain our results could be an improved function of mammary epithelial cells and increased level of cortisol that ensured nutrient mobilization and availability in the body (Chaiyabutr, 2012). However, that study did not explain the mechanisms behind increases in cortisol. During lactation, cortisol increases glucocorticoids that provide energy for galactose production and increase the expression of milk protein genes (Chaiyabutr, 2012). Sun et al. (2021) reported an increased milk yield by 1.36 kg/d, 3.5% FCM by 2.63 kg/d and the concentrations of milk fat and lactose with cows fed with a diet supplemented with *S. cerevisiae* at 100 g/d without affecting milk protein content.

Almost no information is available about the effect of *S. cerevisiae* on milk fatty acid profile; therefore, more experiments are required to explore these effects. Half of fatty acids in milk are driven from plasma uptake of fatty acids while the rest amount is synthesized in the mammary gland (Bionaz et al., 2020; Kholif and Olafadehan, 2022). The minor effects of *S. cerevisiae* on milk fatty acids are similar to that reported by Aoki et al. (Aoki et al., 2021) who fed lactating cows with a diet supplemented with *S. cerevisiae*. However, in this study, PY increased the concentration of C18:2 *trans*-10 *cis*-12 compared to CY and control. This is a striking finding since this conjugated linoleic acid (CLA) isomer is related to milk fat depression (Rinaldi et al., 2022) but milk yield and milk fat were increased by PY. C18:2 *trans*-10 *cis*-12 is associated with down regulation of genes related to milk fat synthesis (Bauman et al., 2011) and desaturation such as ACACA, FADS2, FASN, and SCD (Vargas-Bello-Pérez et al., 2020).

Perhaps the amount of *S. cerevisiae* used in this study was enough to improve productive traits without affecting milk fatty acid profile. Another explanation is that *S. cerevisiae* did not cause disruptions in the rumen environment and therefore microorganisms related to rumen biohydrogenation (Cancino-Padilla et al., 2021) were not affected and that led to an undisturbed rumen microbiome. Milk fat depression occurs when there is a shift in bacteria populations due to changes in rumen pH (which was not observed in our *in vitro* data) or by dietary polyunsaturated fatty acids and that increases the rumen outflow of biohydrogenation intermediates (Dewanckele et al., 2020). Aside from the increased in milk C18:2 *trans*-10 *cis*-12, the lack of changes in milk fatty acid profile was expected; however, further studies should analyze rumen microbiome to fully understand these findings.

Feed intake, nutrient digestibility, and blood measurements

Both PY and CY did not affect feed intake. Perdomo et al. (2020) observed minimal effects on feed intake when feeding *S. cerevisiae* to lactating cows. Weak ef-

fects of *S. cerevisiae* on feed intake were observed in many experiments when beef calves (Maamouri and Salem, 2021) and Holstein cows (Perdomo et al., 2020) were fed rich concentrate feeds (high energy intake) and that was due to metabolic satiety. However, positive effects may be considered when animals are fed diets rich in fiber following the direct action of *S. cerevisiae* on the level of oxygen consumption by rumen microorganisms that degrade (Kholif et al., 2017; Sallam et al., 2020) and increase the fibrolytic activity by accelerating digestive transit and increasing DM intake (Chaucheyras-Durand and Durand, 2010).

As previously noted, treatments did not affect feed intake and, therefore, changes in digestibility are unlikely to be related to changes in intake and retention of feed particles in the rumen. However, we did not analyze rumen microbiome; others (Ding et al., 2014) have reported an increase in total bacteria, fungi, protozoa, and lactate-utilizing bacteria in steers fed *S. cerevisiae*.

The inclusion of PY and CY increased CP digestibility by 8.9 and 6%, respectively, which may be a result of increased rumen fiber and OM digestion which could favor N use and increase microbial protein yield. Additionally, the increase in fiber and OM digestion in the rumen could favor N recycling and increase microbial protein yield and would reduce the amount of OM reaching the large intestine and therefore reduce the amount of N recycled to the cecum and colon, which would be expected to reduce fecal N and improve CP digestion (Perdomo et al., 2020).

In this study, PY improved digestibility of DM, NSC, NDF and ADF than the CY indicating different concentrations of soluble growth factors that stimulate growth of cellulolytic bacteria and digestion of feeds in the rumen (Beev et al., 2007), which confirm our assumption that the genome of both strains differed in their mode of action and production of metabolites leading to a different architecture of the rumen microbiome with special regard to those microorganisms related to fiber disappearance (Beev et al., 2007).

All blood metabolites determined in the present study were within the reference range for healthy animals (Etim et al., 2013). *S. cerevisiae* (PY and CY) inclusion increased concentrations of serum total protein (by 9 and 9.3%, respectively) and albumin (by 13.8 and 12.7%, respectively) which are indicators for improved nutritional and physiological status of cows. In this study, the increased total tract CP digestibility and levels of total protein and albumin in blood were paralleled with the improved production performance.

S. cerevisiae inclusion increased the concentrations of serum glucose by 9.8 and 8.2%, respectively, indicating that the *S. cerevisiae* supplementation improved cow's energy metabolism. The high concentration of serum glucose may be a result of improved OM digestibility and could produce more rumen propionate production, as observed in the *in vitro* experiment. Ruminal propionate is main precursor for gluconeogenesis and favorably im-

pacts milk production (Kholif et al., 2017). Additionally, greater blood glucose enhances mammary glucose supply and thus stimulates milk lactose synthesis.

The PY decreased AST by 18.1% without affecting ALT concentration; however, both were within the normal physiological range for healthy dairy cows (Petersson et al., 2008), indicating normal liver function. It has been indicated that *S. cerevisiae* supplementation positively influences the metabolic status and might have a liver-protecting effect in high-yield cows (Sun et al., 2021). The AST is regarded as the most sensitive indicator of fat deposition processes in the liver of cattle, and its activity increases with increasing fat contents in the liver (Pechova et al., 2002). However, little has been published on the effect of live yeast dietary supplementation on metabolic profile parameters in transition cows so far and this issue requires to be studied to a greater detail.

Urea is the end-product of $\text{NH}_3\text{-N}$ and amino acid metabolism in ruminants. The CY increased concentrations of serum urea-N by 8% may be related to a probable increase in ruminal ammonia-N, as indicated in the *in vitro* experiment. Moreover, feeding *S. cerevisiae* may have increased vitamin B (Ogunade et al., 2019) which may be linked to a positive influence on liver metabolism (Khan et al., 2015). Moreover, increased urea-N in cow's serum suggests increased N wastage either at digestive or at metabolic levels (Patra and Aschenbach, 2018).

Conclusion

Daily supplementation of cows with the new produced strain or commercial strain of *S. cerevisiae* at 2 g/kg DM improved feed digestion, milk production and feed efficiency; however, the laboratory produced strain of *S. cerevisiae* had greater results compared to the commercial strain. The economic production of the new strain of *S. cerevisiae* recommends its use compared to the commercial strains. Minor effects were observed with *S. cerevisiae* supplementation on milk fatty acid profile. Our data could be useful for producers looking for low-cost *S. cerevisiae* additives that can improve feed efficiency and milk production.

Reference

- AOAC (2005). The official methods of analysis. Washington DC, AOAC International.
- Aoki N., Yanli Z., Kanda S., Kurokawa Y., Sultana H., Itabashi H. (2021). Effect of *Saccharomyces cerevisiae* fermentation product on ruminal fermentation, blood metabolites, and milk production in dairy cows. *Jap. Agr. Res. Q.*, 55: 265–271.
- Azzaz H.H., Kholif A.E., Abd El Tawab A.M., El-Sherbiny M., Murad H.A., Hassaan N.A., Vargas-Bello-Pérez E. (2023). Lactation performance and feed utilization of Rahmani ewes fed with either a newly produced bacteriocin-like substance or a commercial bacteriocin. *Transl. Anim. Sci.*, 7: txad010.
- Bauman D.E., Harvatine K.J., Lock A.L. (2011). Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. *Annu. Rev. Nutr.*, 31: 299–319.
- Beev G., Todorova P., Tchobanova S. (2007). Yeast cultures in ruminant nutrition. *Bulg. J. Agricu. Sci.*, 13: 357–374.
- Benchaar C., Hassanat F., Yang W.Z. (2024). Effects of active dried

- yeast (*Saccharomyces cerevisiae*), a non-ionic surfactant, or their combination on gas production, rumen microbial fermentation and methane production in vitro. *Anim. Feed Sci. Technol.*, 307: 115844.
- Bennett S.L., Arce-Cordero J.A., Brandao V.L.N., Vinyard J.R., Agostinho B.C., Monteiro H.F., Lobo R.R., Tomaz L., Faciola A.P. (2021). Effects of bacterial cultures, enzymes, and yeast-based feed additive combinations on ruminal fermentation in a dual-flow continuous culture system. *Transl. Anim. Sci.*, 5: txab026.
- Bionaz M., Vargas-Bello-Pérez E., Busato S. (2020). Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. *J. Anim. Sci. Biotechnol.*, 11: 110.
- Callaway E.S., Martin S.A. (1997). Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. *J. Dairy Sci.*, 80: 2035–2044.
- Cancino-Padilla N., Catalán N., Siu-Ting K., Creevey C.J., Huws S.A., Romero J., Vargas-Bello-Pérez E. (2021). Long-term effects of dietary supplementation with olive oil and hydrogenated vegetable oil on the rumen microbiome of dairy cows. *Microorganisms*, 9: 1121.
- Chaiyabutr N. (2012). Milk Production – An Up-to-Date Overview of Animal Nutrition, Management and Health. London, UK, InTech.
- Chaucheyras-Durand F., Durand H. (2010). Probiotics in animal nutrition and health. *Benef. Microbes*, 1: 3–9.
- Chaucheyras-Durand F., Walker N.D., Bach A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Anim. Feed Sci. Technol.*, 145: 5–26.
- Dai D., Liu Y., Kong F., Guo C., Dong C., Xu X., Li S., Wang W. (2023). *Saccharomyces cerevisiae* culture's dose–response effects on ruminal nutrient digestibility and microbial community: an in vitro study. *Fermentation*, 9: 411.
- Dai X., Faciola A.P. (2019). Evaluating strategies to reduce ruminal protozoa and their impacts on nutrient utilization and animal performance in ruminants – a meta-analysis. *Front. Microbiol.*, 10: 2648.
- Dewanckele L., Toral P.G., Vlaeminck B., Fievez V. (2020). Invited review: Role of rumen biohydrogenation intermediates and rumen microbes in diet-induced milk fat depression: An update. *J. Dairy Sci.*, 103: 7655–7681.
- Ding G., Chang Y., Zhao L., Zhou Z., Ren L., Meng Q. (2014). Effect of *Saccharomyces cerevisiae* on alfalfa nutrient degradation characteristics and rumen microbial populations of steers fed diets with different concentrate-to-forage ratios. *J. Anim. Sci. Biotechnol.*, 5: 24.
- Elghandour M.M.Y., Vázquez Chagoyán J.C., Salem A.Z.M., Kholif A.E., Martínez Castañeda J.S., Camacho L.M., Buendía G. (2014). *In vitro* fermentative capacity of equine fecal inocula of 9 fibrous forages in the presence of different doses of *Saccharomyces cerevisiae*. *J. Equine Vet. Sci.*, 34: 619–625.
- Elghandour M.M.Y., Salem A.Z.M., Castañeda J.S.M., Camacho L.M., Kholif A.E., Chagoyán J.C.V. (2015). Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants. *J. Integr. Agric.*, 14: 526–533.
- Elghandour M.M.Y., Kholif A.E., López S., Mendoza G.D., Odongo N.E., Salem A.Z.M. (2016). *In vitro* gas, methane, and carbon dioxide productions of high fibrous diet incubated with fecal inocula from horses in response to the supplementation with different live yeast additives. *J. Equine Vet. Sci.*, 38: 64–71.
- El-Helow E.R., Elbahloul Y., El-Sharouny E.E., Ali S.R., Ali A.A.-M. (2015). Economic production of baker's yeast using a new *Saccharomyces cerevisiae* isolate. *Biotechnol. Biotechnol. Equip.*, 29: 705–713.
- Etim N.N., Enyenihi G.E., Williams M.E., Udo M.D., Offiong E.E.A. (2013). Haematological parameters: indicators of the physiological status of farm animals. *Br. J. Sci.*, 10: 33–45.
- Fadel M., Foda M.S. (2001). A novel approach for production of highly active baker's yeast from fodder yeast, a byproduct from ethanol production industry. *J. Biol. Sci.*, 1: 614–620.
- Ferret A., Plaixats J., Caja G., Gasa J., Prió P. (1999). Using markers to estimate apparent dry matter digestibility, faecal output and dry matter intake in dairy ewes fed Italian ryegrass hay or alfalfa hay. *Small Rumin. Res.*, 33: 145–152.
- Galyean M., May T. (2010). Laboratory procedure in animal nutrition research. Lubbock, Texas, Department of Animal and Food Sciences, Texas Tech University.
- Hamdon H.A., Kassab A.Y., Vargas-Bello-Pérez E., Abdel Hafez G.A., Sayed T.A., Farghaly M.M., Kholif A.E. (2022). Using probiotics to improve the utilization of chopped dried date palm leaves as a feed in diets of growing Farafra lambs. *Front. Vet. Sci.*, 9: 1048409.
- Hassan A.A., Salem A.Z.M., Kholif A.E., Samir M., Yacout M.H., Hafsa S.H.A., Mendoza G.D., Elghandour M.M.Y., Ayala M., Lopez S. (2016). Performance of crossbred dairy Friesian calves fed two levels of *Saccharomyces cerevisiae*: Intake, digestion, ruminal fermentation, blood parameters and faecal pathogenic bacteria. *J. Agric. Sci.*, 154: 1488–1498.
- Khan N., Kewalramani N., Chaurasia M., Singh S., Haq Z. (2015). Effect of niacin supplementation on *in-vitro* rumen fermentation pattern in crossbred cattle. *J. Anim. Res.*, 5: 479.
- Kholif A.E., Olafadehan O.A. (2022). Dietary strategies to enrich milk with healthy fatty acids – A review. *Ann. Anim. Sci.*, 22: 523–536.
- Kholif A.E., Abdo M.M., Anele U.Y., El-Sayed M.M., Morsy T.A. (2017). *Saccharomyces cerevisiae* does not work synergistically with exogenous enzymes to enhance feed utilization, ruminal fermentation and lactational performance of Nubian goats. *Livest. Sci.*, 206: 17–23.
- López S., Makkar H.P.S., Soliva C.R. (2010). Screening plants and plant products for methane inhibitors. Springer Netherlands.
- Maamouri O., Salem M.B. (2021). Effect of yeast culture feed supply on growth, ruminal pH, and digestibility of fattening calves. *Food Sci. Nutr.*, 9: 2762–2767.
- Martin S.A., Nisbet D.J. (1992). Effect of direct-fed microbials on rumen microbial fermentation. *J. Dairy Sci.*, 75: 1736–1744.
- Michalak M., Wojnarowski K., Cholewińska P., Szeligowska N., Bawej M., Pacoń J. (2021). Selected alternative feed additives used to manipulate the rumen microbiome. *Animals*, 11: 1542.
- Murad H.A., Hosseiny E.N., Abd Elhamid S.M., Abu-El Khair A.G., Azzaz H.H., Zahran M.O. (2019). Utilization of hydrolyzed UF-permeate supplemented with different nitrogen sources and vitamins for production of baker's yeast. *Biotechnology*, 18: 55–63.
- Newbold C.J., Wallace R.J., McIntosh F.M. (1996). Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *Br. J. Nutr.*, 76: 249–261.
- NRC (2001). Nutrient Requirements of Dairy Cattle. Washington, D.C., USA, National Academies Press.
- Ogunade I.M., McCoun M. (2021). Effects of adding live *Saccharomyces cerevisiae* and *Aspergillus*-based enzyme extracts on ruminal fermentation, plasma polyamine concentrations, and fiber digestibility in beef steers fed a high-forage diet. *Appl. Anim. Sci.*, 37: 21–26.
- Ogunade I., Schweickart H., McCoun M., Cannon K., McManus C. (2019). Integrating 16S rRNA sequencing and LC–MS-based metabolomics to evaluate the effects of live yeast on rumen function in beef cattle. *Animals*, 9: 28.
- Patra A.K. (2022). Animal Feed Science and Nutrition – Production, Health and Environment. IntechOpen.
- Patra A.K., Aschenbach J.R. (2018). Ureases in the gastrointestinal tracts of ruminant and monogastric animals and their implication in urea-N/ammonia metabolism: A review. *J. Adv. Res.*, 13: 39–50.
- Pechova A., Illek J., Pavlata L. (2002). Metabolic profiles in dairy cows with fatty liver syndrome. *Wiener Tierärztliche Monatsschrift*, 89: 325–332.
- Perdomo M.C., Marsola R.S., Favoreto M.G., Adesogan A., Staples C.R., Santos J.E.P. (2020). Effects of feeding live yeast at 2 dosages on performance and feeding behavior of dairy cows under heat stress. *J. Dairy Sci.*, 103: 325–339.
- Pettersson J., Hindorf U., Persson P., Bengtsson T., Malmqvist U., Werkström V., Ekelund M. (2008). Muscular exercise can cause highly pathological liver function tests in healthy men. *Br. J. Clin. Pharmacol.*, 65: 253–259.
- Phesatcha K., Phesatcha B., Wanapat M., Cherdthong A. (2021). The effect of yeast and roughage concentrate ratio on ruminal pH and protozoal population in Thai native beef cattle. *Animals*, 12: 53.

- Rinaldi S., Contò M., Claps S., Marchitelli C., Renzi G., Crisà A., Faila S. (2022). Milk fat depression and trans-11 to trans-10 C18:1 shift in milk of two cattle farming systems. *Sustainability*, 14: 977.
- Sales J., Janssens G.P.J. (2003). Acid-insoluble ash as a marker in digestibility studies: A review. *J. Anim. Feed Sci.*, 12: 383–401.
- Sallam S.M.A., Abdelmalek M.L.R., Kholif A.E., Zahran S.M., Ahmed M.H., Zeweil H.S., Attia M.F.A., Matloup O.H., Olafadehan O.A. (2020). The effect of *Saccharomyces cerevisiae* live cells and *Aspergillus oryzae* fermentation extract on the lactational performance of dairy cows. *Anim. Biotechnol.*, 31: 491–497.
- Sivinski S.E., Meier K.E., Mamedova L.K., Saylor B.A., Shaffer J.E., Sauls-Hiesterman J.A., Yoon I., Bradford B.J. (2022). Effect of *Saccharomyces cerevisiae* fermentation product on oxidative status, inflammation, and immune response in transition dairy cattle. *J. Dairy Sci.*, 105: 8850–8865.
- Soest P.J., Van, Robertson J.B., Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583–3597.
- Sun X., Wang Y., Wang E., Zhang S., Wang Q., Zhang Y., Wang Y., Cao Z., Yang H., Wang W., Li S. (2021). Effects of *Saccharomyces cerevisiae* culture on ruminal fermentation, blood metabolism, and performance of high-yield dairy cows. *Animals*, 11: 2401.
- Tajima K., Aminov R.I., Nagamine T., Matsui H., Nakamura M., Benno Y. (2001). Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl. Environ. Microbiol.*, 67: 2766–2774.
- Theodorou M.K., Williams B.A., Dhanoa M.S., McAllan A.B., France J. (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.*, 48: 185–197.
- Tyrrell H.F., Reid J.T. (1965). Prediction of the energy value of cow's milk. *J. Dairy Sci.*, 48: 1215–1223.
- Vargas-Bello-Pérez E., Cancino-Padilla N., Geldsetzer-Mendoza C., Morales M.S., Leskinen H., Garnsworthy P.C., Looor J.J., Romero J. (2020). Effects of dietary polyunsaturated fatty acid sources on expression of lipid-related genes in bovine milk somatic cells. *Sci. Rep.*, 10: 14850.
- Xue L., Zhou S., Wang D., Zhang F., Li J., Cai L. (2022). The low dose of *Saccharomyces cerevisiae* is beneficial for rumen fermentation (both *in vivo* and *in vitro*) and the growth performance of heat-stressed goats. *Microorganisms*, 10: 1877.
- Zain M., Wijaya Setia Ningrat R., Suryani H., Jamarun N. (2022). Effect of various feed additives on the methane emissions from beef cattle based on an ammoniated palm frond feeds. In: *Animal Feed Science and Nutrition – Production, Health and Environment*, Patra A.K., Payan-Carreira R. (eds). London, UK, IntechOpen, pp. 1–18.

Received: 25 I 2024

Accepted: 12 IV 2024