P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2024.266

Effects of Two Local Live Dried *Saccharomyces cerevisiae* Yeasts on Ruminal Fermentation and Digestion in Ongole Crossed (*Bos Indicus*) Cattle

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Article History: 24-657 Received: 05-Oct-24 Revised: 02-Dec-24 Accepted: 03-Dec-24 Online First: 10-Dec-24

ABSTRACT

The purpose of this study was to determine the effect of two different strains of *Saccharomyces cerevisiae* yeast on rumen fermentation metabolites and feed digestibility in Ongole crossed (OC) cattle with diets comprising different forage-to-concentrate ratios. Twenty-one OC steers were randomly divided into three treatments, P1: (control) non-yeast, P2: +yeast 1 (YST1), and P3: +yeast 2 (YST2). The cattle were reared for 21 days of observation with a 7-day adaptation period and 14 days of feeding treatment. The cows were fed concentrate and forage at ratios of 60:40 (low concentrate) and 70:30 (high concentrate) in different periods. Yeast was supplemented by 1g (1 x 10^8 CFU/head/day). The rumen fluid fermentation metabolite products measured were VFA, lactic acid, ammonia, and pH. The results showed that concentrate consumption was higher at the 70:30 ratio compared to the 60:40 ratio. The results of this study show that the performance profile of yeast YST2 at a feed composition of 60:40 concentrate to forage can regulate rumen pH, reduce lactic acid levels, and raise the proportion of propionate while increasing feed digestibility, thereby demonstrating that this probiotic offers greater potential to improve the production performance of Ongole cross cattle.

Key words: Feed digestibility, Rumen metabolites, Ongole crossed, Yeast

INTRODUCTION

Beef is an animal product that plays an important role in improving food safety and is an important source of protein for humans. In Indonesia, while the requirement for beef as a nutritious food continues to increase, the Ongole Crossed (OC) beef cattle is a type of livestock with the potential for development. However, due to its low productivity, it is necessary to increase animal production through feeding management, which includes high-concentrate diets. However, a high-grain diet can have negative effects, including a reduction in ruminal pH that increases the incidence of ruminal acidosis, which has a negative impact on health (Monteiro and Faciola, 2020). Nevertheless, there are some practical means of overcoming the effects within the field, notably supplementation with feed supplements (antibiotics) that can suppress the negative effects. However, antibiotic use leads to resistance and residue in the product, and they have been banned for use in European Union countries since 2006 (Ahiwe et al. 2021). For this reason, as an alternative, direct fed microbials (DFMs) are used. These are safer to use as a feed supplement than antibiotics and are thought to have the potential to both reduce stress (Dunière et al. 2021) and increase livestock productivity (Pang et al. 2022).

Yeast is among the microorganisms with the potential to increase productivity and is one of the microbial additives in ruminant animal feed (Peng et al. 2020; Ribeiro et al. 2022) with the ability to modify rumen fermentation and improve livestock performance. The effects of yeast in the rumen include being able to modulate rumen microbes and reduce the inflammatory response (Baker et al. 2022). Yeast can make the rumen environment more anaerobic, enabling the rumen microbes to function more effectively. Yeast plays a role in supplying nutrients containing B vitamins, organic minerals, and peptides that can be used for the development of lactate-utilizing bacteria (Pantaya et al. 2022) and increasing microbial colonization in the rumen (Elghandour et al. 2020). Suntara et al. (2020) reported that the addition of yeast led to increased growth

Cite This Article as: Pantaya D, Pamungkas D, Hudhia KN, Wulandari S and Utami MMD, 2024. Effects of two local live dried *Saccharomyces cerevisiae* yeasts on ruminal fermentation and digestion in Ongole crossed (*Bos Indicus*) cattle. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2024.266

of the bacterium *Sellonomonas ruminantium*, *Megaspaera elsdenii* and changed the volatile fatty acid (VFA) profile, even supplying unknown growth factor ingredients. Other studies have reported an increase in pH accompanied by a decrease in acidosis time and inhibition of lactate production (Li et al. 2023; Glago et al. 2024). Pantaya et al. (2016) showed that yeast can inhibit a decrease in rumen pH and reduce acid pressure in non-lactating dairy cows fed high proportions of concentrate feed. This increase in rumen pH is highly conducive to the development of cellulolytic bacteria, which plays a role in the digestion of crude fiber and reduces production in the rumen (Peng et al. 2020; Coniglio et al. 2023).

There are many commercial products available, with wide variations in the strain of *S. cerevisiae* used and the number and viability of yeast cells present. The effect of supplemented *S. cerevisiae* on livestock varies depending on the strain. Animal and diet interactions may also alter the efficacy of some products. Several studies have been conducted on the use of yeast in *Bos taurus* (dairy) cattle and have shown positive results. To date, however, there has been a lack of information on the effect of yeast on the productivity of Ongole crossed beef cattle. This study therefore selected *S. cerevisiae* from several local products for its ability to stabilize pH and feed digestibility. This study aimed to determine whether the addition of two different local *S. cerevisiae* yeast strains had an effect on rumen fermentation products and the digestibility of beef cattle feed concentrate-based feed. We also determined the variable effects of these yeast strains on ruminal fermentation.

MATERIALS AND METHODS

Ethical approval

All experimental activities complied with standard operating procedures and were approved by the Ethics Committee with registration number: Balitbangtan/Lolitsapi/Rm/18/2021.

Yeast preparation

The *Saccharomyces cerevisiae* yeast used in this research was obtained from the Feed Technology Laboratory collection, Jember State Polytechnic and originated from local soil in Jember and peat soil from South Kalimantan, Indonesia. The yeast media for production contained peptone, sucrose, dextrose, and mixed minerals, as reported by Pantaya et al. (2022) and was ground using a 1-mm screen to form a powder.

Livestock and experimental procedure

The experiment was conducted at the Cattle Breeding Facility, Beef Cattle Research Center (Lolit Sapi Potong), Grati Pasuruan, East Java Province, Indonesia. Twenty-one male Ongole Crossed cattle (aged 1.5 years, initial body weight 329±11.8kg) were randomly assigned to one of three experimental treatments. The cattle were housed in individual tie stalls made of iron with dimensions of 1.12x2.5m and free access to feed and water. The experiment was divided into two periods, with a trial length of 21 days per period. Each period was divided into 7 days of adaptation time and 14 days of collection time. The experiment was conducted in a completely randomized design, with seven replications in each treatment, with one cow per replication.

The experimental treatments consisted of (1) a control diet (no yeast supplementation), (2) plus yeast 1, 1g live yeast/head/day (YST1), and (3) plus yeast 2, up to 1g live yeast/head/day (YST2), where each yeast contained (1×10^8CFU/g). The live yeast dose used in this study was based on the results of studies by Pantaya et al. (2016) and (Lettat et al. 2010). During the first 21-day period the cattle were fed a concentrate-to-forage ratio of 60:40. In the second period, they were fed a concentrate-to-forage ratio of 70:30 with an adaptation period of two weeks. The composition of the feed is presented in Table 1. Sampling was conducted every three days at the end of each period. The grain diet was fed at 08:00, and the forage was fed twice daily, at 08:00 (60%) and 14:00 (40%). The yeast was given to the cows via insertion into an alginate capsule before the morning feed. The cattle feed and drinking water were available *ad libitum*; any remaining feed was cleaned daily from the container, weighed and recorded to determine the dry matter intake (DMI). Feed composition concentrate is as in Table 1.

Table 1: Feed composition concentrate

Item	%
Ingredient (% of diet DM)	
Palm oil meal	22.5
Cassava meal	25.0
Coffee hulls	10.0
Ketchup waste	8.5
Rice bran meal	23.0
Pollard	10.0
Mineral vitamin mix	1.0
Nutrient composition (% of DM)	
Dry Matter	13.2
Crude Protein	13.5
ADF	41.5
NDF	24.5
Starch	30.5

Minerals (%), P (0.25), Ca (2.0), Mg (0.45), Na (0.35); trace elements (mg/kg): Cu (15); vitamins (IU/kg): vitamin A (6,000), vitamin D3 (1,250), and vitamin E (10mg/kg)
ADF : Acid detergent fiber, NDF : Neutral detergent fiber

Sampling and data analysis

Sample collection of rumen fluid was carried out during the final three days of each observation period according to the Petrovski method (Petrovski, 2017). It was conducted orally using a tube with a diameter of 5 cm and equipped with a further small tube to reach the rumen (ventral ruminal sac). The rumen fluid was then aspirated with a manual pump and collected in a 100mL polypropylene (PP) tube. The rumen liquor was filtered using polyester monofilament and the filtrate was analysed. Samples for pH, lactic acid, VFA, and NH3-N (ammonia) were collected at 0, 4 and 8 hours after the morning feeding. The digestibility test was conducted by collecting the total feces produced for three days at the end of each period according to the method used by (Welch et al. 2021). For VFA analysis, 0.8mL of rumen filtrate was transferred into a tube containing 0.5mL of crotonic acid (4g/L). For lactic acid, 2mL of the filtrate was transferred to the tube and stored at -20°C. An amount of 1mL of the filtrate was added to 0.1mL of ortho phosphoric. Ruminal pH was measured using a Mettler Toledo pH meter. VFAs (acetate, propionate, butyrate) were analyzed by gas chromatography (GC) (Shimadzu Corporation, Kyoto,

Japan) as previously described (Morgavi et al. 2013). Lactic acid was analyzed using the enzymatic method (Fernando et al. 2010) and ammonia was analyzed using the Berthelot reaction (Park et al. 2009). Blood samples were taken on day 14 during treatment at sampling times 0 and 4 hours after feeding. Blood was taken at the coccygeal venipuncture section and collected in a sodium heparin vacutainer tube containing an anticoagulant. The blood samples were centrifuged at 2500 x g at 4°C for 20min and blood plasma was stored at -20°C until the glucose were analyzed.

Statistical Analysis

Data on the feed consumption, rumen pH, lactic acid, ammonia, VFA concentration and the dry matter digestibility of feed were analysed using MIXED SAS version 9.1 (SAS Institute Inc., Cary, NC). If there was a significant difference, Duncan's test was used with the following mathematical model: $Yij=\mu+Pj+Aij+eij$. All statements of statistical significance are based on a probability of $P<0.05$. Trends are discussed at a statistical significance of $P<0.10$.

RESULTS

Experiment 1. feed concentrate: forage ratio 60:40

DMI and digestibility are shown in Table 2. Based on the statistical analysis, there was no difference in the average DMI between treatments ($P>0.05$). The average DMI in this study was 11.35kg/head/day. Supplementing live yeast had no effect on DMI. We observed a significant difference in terms of higher dry matter digestibility in response to YST2 supplementation compared to other treatments. The feed digestibility calculation showed a significantly higher result for the yeast (YST2) compared to the control feed and supplementation with YST1 ($P<0.05$). The digestibility of feed with the addition of YST2 yeast (69.42%) was higher than that of both the YST1 yeast treatment (64.70%) and control feed (65.85%). The result suggests that improved fiber fraction digestibility through yeast addition may support the function of cellulolytic bacteria to digest fiber and indicated better microbial fermentation. Similar results were seen for the pH value. Fig. 1 illustrates the dynamic change of ruminal pH. While there was no significant effect on pH value before the morning feed, a significant effect was visible between treatments ($P<0.05$) four hours after the morning feed. The YST2 (pH 7.0) and YST1 (pH 7.0) yeast treatments were significantly higher than the control (pH 6.6). Ruminal pH was higher 8h after feeding the YST2 yeast compared to the YST1 yeast (pH 6.7) and control (pH 7.0). The lactic acid concentration for YST1 was higher than for YST2 and the control before feeding, whereas 4h after feeding, YST1 and YST2 both had lower ruminal lactic acid concentrations than the control ($P<0.05$). At 8 hours after feeding, the YST2 yeast, YST1 yeast, and the control diet were different ($P>0.05$). The lactic acid present within the physiological range (4–8mM) in the concentration was lower with the addition of YST2 compared to YST1 and the control 4h after feeding. The results suggest that lower lactic concentration with yeast addition may support the population of lactate-utilizing bacteria, which will reduce lactic acid and increase pH.

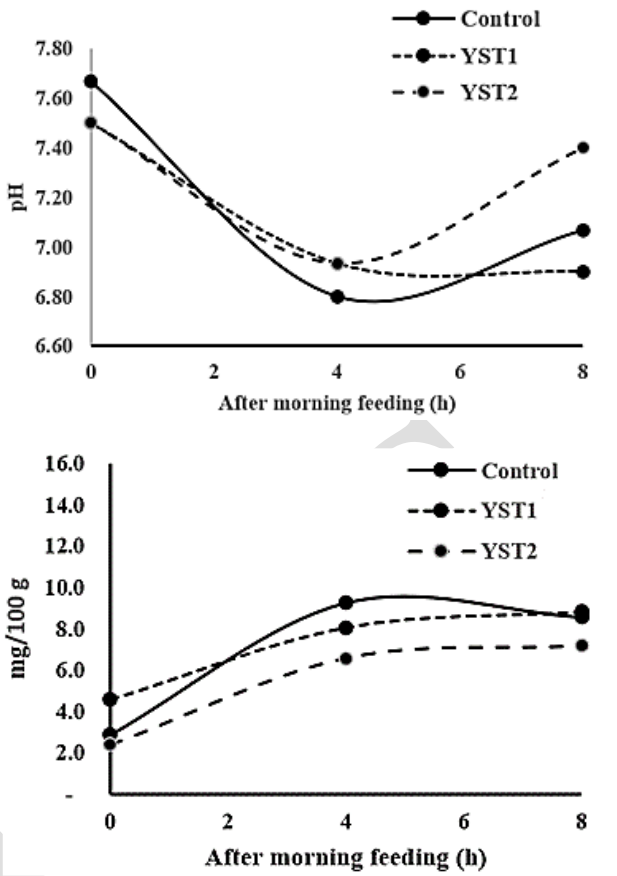

Fig. 1: Effect of yeast on ruminal pH and lactic acid feed concentrate diets (60:40).

The rumen metabolite product data are presented in Table 3. The acetate to propionate (A/P) ratio was significantly higher at 0h and then 4h after morning feeding ($P<0.05$), while no significant difference was found 8h after feeding. The A/P ratios for YST2 and YST1 were significantly higher than for the control treatment ($P<0.05$) (before feeding (0h)). In contrast, the propionate proportion was significantly lower for YST2 and YST1 yeast supplementation than for the control treatment. This lower A/P ratio was due to a lower proportion of acetate and a higher proportion of propionate. It is suggested that yeast supplementation stimulates the population of lactate-utilizing bacteria and enhances ruminal propionate. The butyric proportion before morning feeding and 8h after feeding showed no significant effect, while a significant effect between treatments ($P<0.05$) was observed 4h after morning feeding. The proportion of butyrate was significantly higher with yeast YST2 and YST1 supplementation compared to the control. This result suggests that yeast supplementation may stimulate the butyrate proportion and the population of lactate-utilizing bacteria, thus increasing butyrate.

With yeast supplementation, blood glucose was higher at 4h after feeding than at 0h before feeding. The blood glucose concentration of cattle consuming dietary YST2 tended to increase by 7mg/dL (from 13.7mg/dL at 0h before feeding to 20.9mg/dL 4h after feeding), compared to an increase of 6mg/dL (11.4 to 18.7mg/dL) for the control treatment and 3mg/dL (15.6 to 18.0mg/dL) for YST1. The increase in glucose levels in YST2 may be due to the increased transformation of propionic acid into blood

Table 2: Effect of yeast on dry matter intake and digestibility (n=7)

Item	Treatment			SEM[2]	*P value*
	Control (- yeast)	Yeast YST1	Yeast YST2		
Concentrate : Forage (60:40)					
● DMI, kg/d	11.18	11.35	11.57	1.09	0.881
● Dry matter digestibility (%)	65.85[a]	64.70[a]	69.42[b]	2.92	0.021
Concentrate : Forage (70:30)					
● DMI, kg/d	10.17	10.55	10.50	0.88	0.787
● Dry matter digestibility (%)	66.43	66.86	66.35	4.97	0.986

Different superscripts in the same row mean significant (P<0.05); Yeast Supplementation = 1 x 10^8/g/head/day; SEM[2]= Standard error mean; YST1 = Yeast *Saccharomyces cerevisiae* 1, YST2: Yeast *Saccharomyces cerevisiae* 2

Table 3: Effect of yeast on metabolite product of ruminant fed low-concentrate diets (concentrate: forage) (60:40)

Item	Treatment				*P value*
	Control (- Y)	YST1	YST2	SEM	
Before feeding (0 h)					
Total VFA[1] (mM)	44.18±17.31	64.60±30.3	77.81±19.71	23.13	0.173
Acetate (%)	68.10±2.75	70.81±2.85	72.44±1.33	2.87	0.084
Propionate (%)	19.93±1.58[a]	17.38±0.57[b]	16.02±0.85[b]	1..96	0.002
Butyrate (%)	7.65±1.24	6.94±2.77	7.42±0.69	1.66	0.851
Isobutirate (%)	2.48±0.51	3.17±0.99	2.11±0.26	0.76	0.128
Isovalerate(%)	1.85±0.36	1.70±0.24	2.01±1.16	0.66	0.829
Isoacid (%)	4.32±0.53	4.87±1.17	4.12±1.09	0.94	0.557
A/P ratio	3.44±0.38[a]	4.07±0.11[b]	4.53±0.28[b]	0.53	0.001
NH_3-N, mM	1.89±0.28	1.78±0.49	3.23±0.63	0.49	0.004
After feeding (4 h)					
Total VFA[1] (mM)	57.3±26	39.21±11.22	36.33±11.00	20.54	0.263
Acetate (%)	71.47±3.60	67.08±1.28	65.12±1.83	3.55	0.014
Propionate (%)	17.59±1.31[a]	18.84±0.86[a]	22.21±1.70[b]	2.37	0.002
Butyrate (%)	9.07±0.l97[a]	11.62±0.61[b]	10.68±1.14[b]	1.39	0.012
Isobutirate (%)	1.02±0.76	1.33±0.70	1.42±0.56	0.64	0.702
Isovalerate(%)	0.85±0.65	1.13±0.16	0.57±0.40	0.47	0.267
Isoacid (%)	1.87±1.41	2.46±0.82	1.99±0.86	1.00	0.721
A/P ratio	4.09±.52[a]	3.57±0.22[a]	2.95±0.28[b]	0.59	0.005
NH_3-N, mM	3.83±1.16	3.48±0.88	3.91±0.195	1.40	0.899
After feeding (8 h)					
Total VFA[1] (mM)	64.7±38	79.3±71	53.1±23	44.17	0.749
Acetate (%)	65.12±1.21	61.30±3.61	61.84±2.97	2.66	0.617
Propionate (%)	22.21±0.67	22.06±0.73	20.48±0.47	0.89	0.020
Butyrate (%)	10.68±1.54	12.89±0.70	13.90±1.84	1.38	0.600
Isobutirate (%)	1.42±0.26	2.56±1.76	2.62±1.10	1.19	0.451
Isovalerate (%)	0.57±0.53	1.19±0.80	1.15±0.76	0.65	0.852
Isoacid (%)	1.99±0.28	3.75±2.55	3.78±1.71	1.72	0.556
A/P ratio	2.78±0.05	3.03±0.27	3.00±0.12	0.19	0.143
NH_3-N, Mm	2.65±0.56	2.88±0.45	2.98±1.05	0.73	0.813

Different superscripts in the same row mean significant (P<0.05); SEM[2]= Standard error mean, CFU (Colony forming unit); A/P : acetate/propionate ratio; YST1 = Yeast *Saccharomyces cerevisiae* 1, YST2: Yeast *Saccharomyces cerevisiae* 2

glucose 4h after feeding through the process of gluconeogenesis. Propionic acid increases due to the action of yeast, which increases the development of lactate-utilizing bacteria; these are then converted to propionate.

Experiment 2, feed concentrate: forage ratio 70:30

Concentrate diets Table 2 shows the average dry matter consumption from the experiments with a high level of concentrates. Based on the statistical analysis, there was no difference in the average DMI between treatments (P>0.05). The average dry matter consumption was 10.5kg/head/day. The addition of yeast had no effect on dry matter and nutrient intake. Yeast supplementation had no significant effect on feed digestibility (P>0.05) in the feed containing a high proportion of concentrates. The same results were seen for the pH value, whereby a pH of around 7.4 was found for all treatments before feeding. Fig. 2 illustrates the dynamic change of ruminal pH. The pH value before morning feeding showed no significant effect (7.4), whereas a significant effect was observed between treatments (P<0.05) 4h after the morning feed. Treatment YST1 (pH 6.4) was significantly lower than YST2 (pH 6.6) and the control treatment (pH 6.6) (P<0.05), while 8h after feeding, YST1 (pH 6.4) was lower than both YST2 (pH 6.6) and the control (pH 6.6). The lactic acid concentration results before morning feeding were around 3mM for all treatments. This compared to a lower ruminal lactic acid concentration for YST1 (11 mM) compared to YST2 (13mM) and the control (13mM) (P<0.05) 4h after feeding, while at 8h after feeding, YST2 (12mM) was significantly higher than YST1 and the control (8mM). These results suggest that higher lactic acid concentration (11–13mM) was caused by an increase in the activity of lactate-producing bacteria, which led to a decrease in ruminal pH. The fact that the yeast was unable to stabilize rumen pH suggests an imbalanced capacity of lactate - consuming bacteria due to the increased production of organic acids in the rumen by increasing starch intake.

Table 4: Effect of yeast on metabolite product of ruminant product fed high-concentrate diets (C:F) (70:30)

Item	Treatment				
	Control (-yeast)	YST1	(YST2)	SEM[2]	P value
Before feeding (0 h)					
Total VFA[1] (mM)	147.8±34.9	124.9±18.2	212.5±37.6	34.15	0.045
Acetate (%)	73.53± 3.98	78.69±0.42	78.26±3.78	4.05	0,181
Propionate (%)	16.09±1.62	13.77±0.76	12.02±1.32	2.52	0,622
Butyrate (%)	7.27±1.24	5.26±0.95	6.56±0.73	1.18	0,136
Isobutirate (%)	1.24±0.83	0.95±0.86	0.79±0.16	0.64	0,705
Isovalerate (%)	1.11±0.41	0.91±0.51	0.70±0.25	0.38	0,337
Isoacid (%)	2.35± 1.23	1.86±1.37	2.71±0.65	1.05	0,410
A/P ratio	4.57±1.10	5.72±0.19	6.51±0.22	0.85	0,476
NH_3-N, mM	3.31±1.65	2.24±0.18	1.98±0.85	1.07	0.232
After feeding (4 h)					
Total VFA[1] (mM)	299.2± 39.1	326.5± 12.1	266.13 ±81	52.47	0.312
Acetate (%)	71.73±1.11	72.45±1.43	73.52±1.13	1.36	0.177
Propionate (%)	19.28±1.36	19.23±1.81	19.00±1.29	1.37	0.961
Butyrate (%)	7.58±0.56	7.20±0.28	6.51±0.89	0.73	0.100
Isobutirat (%)	0.26±0.04	0.22±0.09	0.25±0.09	0.07	0.754
Isovalerat (%)	0.45±0.07	0.39±0.18	0.33±0.18	0.12	0.370
Isoacid (%)	0.71±0.11	0.61±0.27	0.58±0.18	0.19	0.637
A/P ratio	3.72±0.33	3.77±0.47	3.87±0.30	0.32	0.858
NH_3-N, mM	2.68±2.61	2.01±0.63	8.2±13.46	7.90	0.506
After feeding (8 h)					
Total VFA[1] (mM)	327.89±38.7	377.22 ±64.2	220.81 ±67	78.3	0.027
Acetate (%)	75.91 ±1.28	75.29±3.88	73.78±1.46	2.50	0,578
Propionate (%)	15.73 ±1.43	13.15 0.78	17.74±0.59	1.87	0,018
Butyrate (%)	7.21±0.58	7.97±0.84	7.30±0.74	0.75	0,323
Isobutirat (%)	0.24±0.06	2.71±0.04	0.24±0.12	0.07	0,886
Isovalerat (%)	0.44±0.06	0.44±0.09	0.36±0.13	0.09	0,511
Isoacid (%)	0.68±0.11	3.15±0.09	0.60±0.25	0.15	0,474
A/P ratio	4.83±0.49	5.73±0.19	4.16±0.22	0.60	0,026
NH_3-N, mM	1.56±0.93	2.40±1.40	9.63±11.39	6.64	0.223

Different superscripts in the same row mean significant (P<0.05); SEM[2]= Standard error mean, CFU (*Colony forming unit*); A/P : acetate/propionate ratio; YST1 = Yeast *Saccharomyces cerevisiae* 1, YST2 : Yeast *Saccharomyces cerevisiae* 2

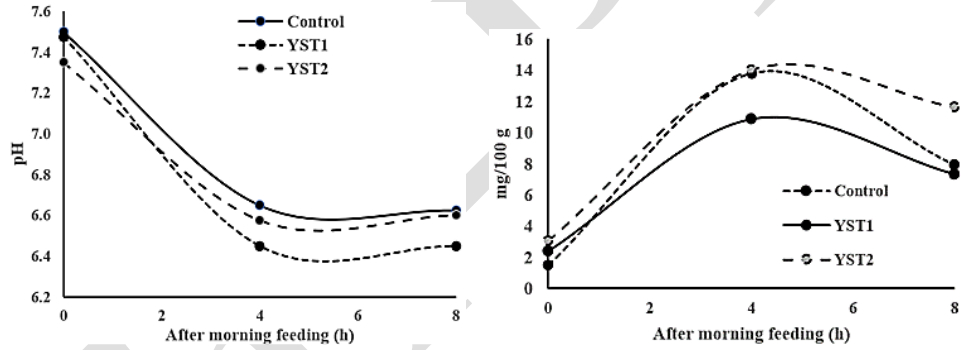

Fig. 2: Effect of yeast on ruminal pH and lactic acid feed concentrate diets (70:30).

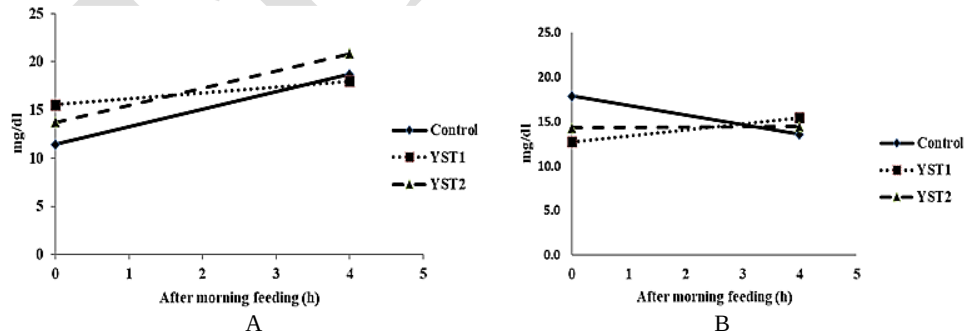

Fig. 3: Glucose blood concentration before and 4h after feeding A (low grain) and B (High grain).

At the sampling times of 0, 4 and 8 hours, statistical analysis of the acetic, propionic, and butyric acid content showed no significant difference (P>0.05) (Table 4). The acetate content ranged from 71 to 73%, while the acetic and butyric acid values were around 19% and 7.08%, respectively. The results also revealed no significant difference (P>0.05) for the A/P ratio at sampling times 0, 4, and 8 hours after eating, with a value range of 4–5.

Overall, ammonia concentration did not show a significant difference, with values in the range of 0.9–3mg/dL. The blood glucose content is shown in Fig. 3, where it can be seen that the highest increase in glucose levels in the control feed occurred at 0 hours by 18.7 to 12mg/dL at 4 hours after eating. This compared to an increase of 3mg/dL for the Yeast YST 1 and YST2 treatments.

DISCUSSION

The DMI of feed at low concentrate (11.5kg/head/day) was greater than at high concentrate (10.5kg/head/day), whereas the proportion of DMI was greater in the high concentrate (7.3kg/head/day) than low concentrate (6.6kg/head/day). This led to a higher starch intake with the high-concentrate feed compared to the low-concentrate feed. The concentrate is rich in starch, which was readily fermentable into carbohydrate in the rumen and converted to an organic acid for use as an energy source. A high-starch diet will produce a decrease in rumen pH, which has the potential to interfere with feed digestibility. The supplementation of starch at 3.88 kg has been found to reduce rumen pH to 5.56 in steers (Golder and Lean 2024), Orton et al. (2020) and Plaizier et al. (2022) reported that the addition of concentrate can lower it to pH 5.1. A low fiber content in feed will reduce saliva production and buffer capacity, which will lower pH. The simulation of adding concentrate is expected to lower the pH and determine whether yeast can regulate rumen pH when concentrate is added at different ratios (Phesatcha et al. 2021). The higher pH value compared to Gozo's opinion was due to the sampling in the reticulum, where the pH was 0.24–0.7 different than in the rumen (Elmhadi et al. 2022).

To evaluate the capacity of yeast to regulate feed starch, we compared the treatment of cattle feed diets with differing starch levels. It was shown that yeast YST2 can inhibit the decrease in pH 4 hours after feeding in low-concentrate diets, while in high-concentrate diets, yeasts YST1 and YST2 were unable to regulate pH up or down. Since the same effect was seen with the control, we suspected that the capacity of yeast to regulate pH was limited and dependent on the starch content. The fact that the yeast was unable to stabilize rumen pH suggests an imbalanced capacity of lactate-utilizing bacteria due to the increased production of organic acids in the rumen by increasing lactic acid. The difference in results was caused by the composition of the feed. It was proven that the yeast pH regulator function was not effective in the high concentrates, which was probably due to differences in the rumen environment and nutritional differences that limited the power of the yeast as a regulator. At this level, it is suspected that the microbes do not adapt well to the composition of the feed. This aligns with the opinion of, Cavallini et al. (2022) who reported that the fermentation rate of sugars and end products in the rumen can vary depending on the adaptation of feed treatment.

The total VFA in the high-concentrate feed was greater than in the low-concentrate feed. In the high concentrate, the VFA concentration ranged from 124 to 212mM at the time before the morning feeding. The increase in total VFA indicates a condition approaching sub-acute ruminal acidosis (SARA), the key criteria of which are VFA concentration in the rumen of around 150mM (Monteiro and Faciola, 2020; Plaizier et al. 2022) and pH in the range of 5.0–5.8 for longer than 3 h/day (Orton et al. 2020). Gelsinger et al. (2020) reported that cattle fed concentrates experienced a decrease in rumen pH and increased total VFA. These conditions also affect the proportion of acetate and propionate. A rate of production out of balance with absorption will affect the content of organic acids in the rumen.

Supplementation with YST2 yeast in the low-concentrate feed increased the proportion of propionate (C3) while the proportion of acetate (C2) decreased compared to the control feed. (Wang-Li et al. 2020) asserted that this may be due to the role of lactate-utilizing bacteria, which can convert lactic acid to C3 where growth is stimulated by yeast supplementation. When compared, the addition of yeast can increase the molar proportion of C3 content, decrease the proportion of C2, and increase the A/P ratio. Based on the fermentation results, YST2 yeast supplementation tends to produce a glucogenic fermentative pattern compared to YST1. This can be seen in the linear increase in glucose production at 4h in the low concentrate diet. YST2 showed a good impact with an increase in digestibility of 4%. Yeast supplementation increased dry matter digestibility, thereby aligning with the opinions expressed by Phesatcha et al. (2021) and Mombach et al. (2021) that the addition of yeast can stimulate the development of fibrolytic bacteria and contribute to reducing digestive disorders and enhance livestock health.

SARA is also indicated by lactic acid concentration. The high concentrates showed a greater increase in lactic acid compared to the low concentrates. The concentration of lactic acid in the rumen is influenced by the consumption of starch in the feed. Starch containing glucose will be converted by rumen microbes *Lactobacili* and *Streptococcus bovis* into lactic acid (McLoughlin et al. 2023). Greater production of lactic acid will lower the pH in the rumen. Lactate-utilizing bacteria include *Megasphaera elsdeni* and *Selonomonas rumiantium*, the development of which can be stimulated by yeast supplementation and increase the pH in the rumen (Golder and Lean 2024).

The variable effect of yeast is influenced by dose, yeast type, livestock psychology, and feeding system. These results align with Sukmawati et al. (2021), who stated that the addition of yeast can increase the population of cellulolytic bacteria (*R. flavefaciens* and *F. succinogenes*) and suppress the growth of lactic acid bacteria (*Streptococcus bovis*), which can increase feed digestibility, consistency of rumen fermentation, and degradation and total cellulolytic bacteria in the rumen. Han et al. (2021)reported that the addition of yeast can increase the population of bacteria, fungi, protozoa, lactate-utilizing bacteria, and the rate of fiber decomposition. Yeast cell walls contain nutrients, organic and amino acids, and vitamins that can increase cellulolytic bacterial and fungal colonization in the rumen (Pantaya et al. 2022). Yeast can also stimulate microbial proliferation and lactate-utilizing bacteria and reduce acidosis (Monteiro and Faciola, 2020). *Saccharomyces cerevisiae* is a facultative anaerobic that uses oxygen on the surface of the feed consumed and reduces redox potential, removes oxygen, and increases the growth of cellulolytic bacteria that are strict anaerobes, increasing their adhesion to particles and cellulolytic processes and increasing hemicellulolytic bacteria activity (Zhang et al. 2022).

The results obtained in this study indicate that the nutrient digestibility profile of yeast YST2 was more effective in the feed composition of low-concentrate diets (concentrate-to-forage ratio of 60:40). In contrast, YST1 supplementation did not increase digestibility. Yeast YST2

can regulate rumen pH, reduce lactic acid levels, and increase the proportion of propionate more effectively than YST1. However, since the YST2 strain tends not to work at high concentrate levels, further research is necessary to evaluate this strain in cattle fed high-forage diets in terms of the use of an increased yeast supplementation. Nevertheless, the application of selected yeast continues to offer the potential to increase local cattle productivity (Pantaya et al. 2023).

Conclusion

Yeast YST2 was more effective in the feed composition of low-concentrate diets (concentrate-to-forage ratio of 60:40). In contrast, YST1 supplementation did not increase digestibility. Yeast YST2 can regulate rumen pH, reduce lactic acid levels, and increase the proportion of propionate more effectively than YST1. However, since the YST2 strain tends not to work at high concentrate levels, further research is necessary to evaluate this strain in cattle fed high-forage diets in terms of the use of an increased yeast supplementation. Nevertheless, the application of selected yeast continues to offer the potential to increase local cattle productivity.

Author's contribution: Conceptualization: DP and SW. Investigation: MDU. Writing original draft: DP and SW. Writing – review, and editing: DP, DiP, SW, KNH, and MMDU. Data curation and validation: MMDU. Project administration and supervision: DP.

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