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Effects of Three Yeast Strains on In Vitro Rumen Fermentation of Corn Stover and a Total Mixed Ration

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Abstract: This study aimed to evaluate the effect of three yeast strains, *Pichia guilliermondii* (Levica 27), *Candida norvegensis* (Levazoot 15), and a commercial product (Levucell® SC 10) containing *Saccharomyces cerevisiae*, on *in vitro* rumen fermentation of two substrates: corn stover and a total mixed ration (TMR). Samples were taken 24 hours post-fermentation to measure pH, ammonia nitrogen (NH_3-N), gas production, and volatile fatty acid (VFA) concentrations. The addition of yeasts did not affect gas production at 24 hours for either diet. For pH, differences between yeast strains were noted when TMR was used. The lowest NH_3-N concentration (6.1 mM) was recorded with corn stover, showing similar results across strains. Conversely, NH_3-N levels with TMR ranged from 47 mM to 66 mM. The inclusion of all three yeast strains increased the molar concentrations of total VFA, as well as acetic (C_2), propionic (C_3), and butyric (C_4) acids in the rumen fluid. The C_2:C_3 ratio remained unchanged with corn stover but decreased with the inclusion of Levica 27 and Levazoot 15 in the TMR. With the commercial product Levucell® SC 10, this ratio was comparable to the control treatment. In conclusion, Levica 27 and Levazoot 15 demonstrated a stimulatory effect on *in vitro* rumen fermentation for the evaluated diets, evidenced by increased VFA concentrations.

Keywords: microbial feed additives; probiotic; *Candida norvegensis*; *Pichia guilliermondii*; *Saccharomyces cerevisiae*

1. Introduction

With the growth of the human population, the demand for agricultural products in developing countries is expected to double by 2030 [1]. This increased demand necessitates the intensification of livestock production, prompting the use of new strategies to enhance the production of animal-derived products [2]. In ruminants, this challenge has led nutritionists to manipulate rumen fermentation to improve animal performance and health [3]. One such strategy involves using yeast additives as probiotics to enhance the fermentation of fibrous foods and nutrient absorption [4]. These additives can stimulate the growth of beneficial microbes in the rumen, particularly cellulolytic bacteria and fungi [5,6], stabilize rumen pH, reduce methane production [7–9], improve fermentation patterns, reduce pathogen concentrations, and increase meat and milk production [10]. Despite extensive research on the efficacy of yeast additives in ruminant diets, there are still gaps and inconsistencies in the findings. These variations can be attributed to differences in animal species, yeast genus and species, dosage, and application methods, as some strains have specific actions while others are multifunctional [11–13].

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Institutions worldwide are conducting preliminary research on yeast strains other than *Saccharomyces spp*., such as *Pichia guilliermondii, Issatchenkia orientalis, Candida tropicalis*, and *Candida norvegensis*. *In vitro* results suggest these strains improve fermentation patterns [11,14,15]. In some cases, these benefits surpass those of commercial *Saccharomyces cerevisiae*, despite it being the only yeast currently authorized for commercial probiotic use in ruminants. Therefore, it is crucial to explore the advantages of different yeast genera for their potential use as probiotic feed additives in livestock systems. The primary objective of this study was to evaluate the effects of *Saccharomyces cerevisiae*, *Pichia guilliermondii*, and *Candida norvegensis* on rumen fermentation *in vitro* using corn stover and a total mixed ration as substrates.

2. Materials and Methods

2.1. Location of the Study

The study was conducted in Chihuahua, Chihuahua, Mexico, located at latitude 28°35'10.9" N, longitude 106°6'26.6" W, and an altitude of 1,440 meters above sea level. The part of the research that involved the use of animals was carried out in accordance with the regulations of the Institutional Bioethics Committee (case number: CFTZyE-ACTA-101/2015: AGREEMENT 4.2).

2.2. Experimental Design

A completely randomized design with a 4 x 2 factorial arrangement and four replications was used to study the effect of three yeast strains and a non-yeast control treatment on *in vitro* rumen fermentation of corn stover and a totally mixed ration (TMR) as substrates. The ingredients of the TMR are shown in Table 1.

Table 1. Ingredients of totally mixed ration.

Ingredients	Percentage
Corn stover	40
Cracked corn	35
Molasses	9
Cottonseed	9
Flour mill	4
Urea	1.5
Minerals	1.0
Ammonium sulfate	0.5

[1]Minerals: Micro FOS.

The yeast strains used in the experiment were: *Pichia guilliermondii* (Levica 27), *Candida norvengensis* (Levazoot 15), and *Saccharomyces cerevisiae* (Levucell® SC 10; Lallemand Animal Nutrition). The experimental treatments are presented in Table 2.

Table 2. Experimental treatments.

Lable	Treatment
Corn stover	
T1	Yeast-free control
T2	Levica 27[1]
T3	Levazoot 15
T4	Levucell® SC 10
Totally mixed ration	
T1	Yeast-free control
T2	Levica 27
T3	Levazoot 15

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T4	Levucell® SC 10

[1]Levica 27 at 5.23 x 10^9 cfu/mL; Levazoot 15 at 1.32 x 10^{10} cfu/mL; Levucell® SC 10 at 10^{10} cfu/g.

2.3. Experimental Procedure

The test was performed *in vitro* in 18-mL tubes, maintaining an effective volume of 15 mL. Corn stover and TMR used as substrates were pre-dried at room temperature (27°C) under the sun and ground through a 1.0-mm mesh prior to use. The chemical composition of the substrates was determined according to [16] and is shown in Table 3. Subsequently, 0.5 g of each substrate was weighed into the 18-mL test tubes for incubation.

Table 3. Chemical composition of substrates used in *in vitro* rumen fermentation.

Nutrients	**Dry matter (%)**	
	Corn stover	TMR[1]
Dry matter	91.44	86.75
Crude protein	5.90	15.83
NDF	67.20	42.68
ADF	37.75	25.20
Fat	3.10	4.60

[1]TMR: totally mixed ration; NDF: neutral detergent fiber; ADF: acid detergent fiber.

Rumen fluid was extracted from two fistulated Pelibuey male sheep (32 kg and approximately 12-month-old), which were kept in individual pens and fed for seven days a diet containing 40% corn stover and 60% concentrate, with free access to water. Rumen fluid extraction was performed on day eight and prior to the first feed offering (9:00 a.m.). The rumen fluid was filtered through muslin and was used to prepare the fermentation medium, which contained only filtered rumen fluid (LRF). The LRF (15 mL each) was distributed to each of four vials containing the substrate, and four tubes without substrate were used as blank controls. The tubes were prepared under a constant stream of nitrogen gas to maintain an anaerobic atmosphere then capped for incubation as indicated below.

Yeast cultures were added to the tubes just before the addition of the LRF. For the preparation of the Levica 27 and Levazoot 15 inocula, the strains were activated by two aerobic subcultures in malt extract broth (DIBICO®, Cuautitlán Izcallí, Mexico) at 110 rpm (Orbital Shaker incubator; New Brunswick Model Innova 4000, Nijmegen, Netherlands), 30°C, and 24 hours of incubation. From the activated cultures, 10% (v/v) was used as inocula for 50 mL of malt extract broth (DIBICO®) in 100-mL flasks then inoculated again at 30°C and 110 rpm (Orbital Shaker incubator) for 24 hours. From these cultures, 0.5 mL (equivalent to 10 g of yeast/adult animal daily) was added to the corresponding tubes for *in vitro* rumen fermentation. The cultures of Levica 27 and Levazoot 15 had final concentrations of 5.23 x 10^9 and 1.32 x 10^{10} cfu/mL, respectively, in each tube. In the case of Levucell® SC 10, 1 mg of the commercial product equivalent to 10 g recommended for an adult ruminant was added to respective tubes. Finally, the tubes were immediately capped and incubated at 39°C and 110 rpm (Orbital Shaker incubator). After 24 hours, the tubes were placed on ice to stop fermentation and prepared for the analysis of the samples.

2.4. Variables Evaluated

pH was measured using a Hanna Instruments Model HI 9017 (Arvore-Vila do Conde, Portugal) and NH_3-N concentration was measured by visible UV spectrophotometry (Varioskan Flash Thermo Scientific v4.00.53, Vantaa, Finland) following the method of Broderick and Kang [17]. Total gas production was measured using a FESTO® pressure transducer (Siemens, Munich, Germany). A 1-mL gas sample was taken from each tube to determine its composition by gas chromatography using a GOW-MAC Series 580 chromatograph equipped with a Carbosphere® 80/100 packed column (5682PC) (GOW-MAC Instrument Company). Carbon dioxide was used as the carrier gas at a flow

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rate of 20 mL/min to determine the individual production of hydrogen, methane, and carbon dioxide after 24 hours of sample incubation. The molar concentration of volatile fatty acids (VFA) was determined by gas chromatography with flame ionization detection (Claurus 400® gas chromatograph; Perkin Elmer, Shelton, CT, USA) with a Varian capillary CP-wax58 (FFAP) CB column (15 m x 0.53 mm, 0.5 µm). The injected sample volume was 0.6 µL, which was previously conditioned with meta-phosphoric acid according to [18].

2.5. Statistical Analyses

Analysis of variance was performed using the SAS GLM procedure (Statistical Analysis System, version 9.3; Cary, NC, USA). The model equation that was fitted is as follows (Equation (1)):

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} is the measured response variable, μ is the general mean, α_i is the fixed effect of the treatment (i = 1, 2, 3, 4), $\beta_j$is the fixed effect of diet (j = 1, 2), $\alpha\beta_{ij}$is the interaction effect between treatment and diet, and e_{ijk} is the random error term.

3. Results

Table 4 shows the effect of yeast on gas production and pH. There was no interaction between diet and yeast strain (P > 0.05) for total gas production (TGP) or its composition (H_2, CO_2, and CH_4). However, these variables were different (P < 0.05) for each type of diet.

Table 4. Effect of yeasts on gas production and pH at 24 hours of *in vitro* ruminal fermentation with corn stover and totally mixed ration.

Parameters	Substrate	T1	T2	T3	T4	EE	P-value		
							D	T	DxT
TGP[1] (mL)	Corn stover	47.8^{b}	49.6^{b}	49.0^{b}	50.2^{b}	2.19	<0.0001	0.74	0.08
	TMR	94.8^{a}	92.0^{a}	91.2^{a}	91.8^{a}				
H_2 (mM)	Corn stover	22.1^{a}	23.0^{a}	23.7^{a}	25.2^{a}	1.85	<0.0001	0.37	0.48
	TMR	0.0^{b}	0.0^{b}	0.0^{b}	0.2^{b}				
CH_4 (mM)	Corn stover	0.3^{b}	1.0^{b}	0.7^{b}	0.2^{b}	3.08	<0.0001	0.63	0.64
	TMR	126.8^{a}	126.9^{a}	123.5^{a}	125.8^{a}				
CO_2 (mM)	Corn stover	191.1^{b}	197.2^{b}	194.4^{b}	198.9^{b}	8.05	<0.0001	0.70	0.07
	TMR	296.1^{a}	283.6^{a}	283.5^{a}	283.6^{a}				
pH	Corn stover	6.4^{a}	6.4^{a}	6.3^{a}	6.4^{a}	0.06	<0.0001	0.006	0.22
	TMR	5.7^{bwy}	5.6^{bw}	5.6^{bw}	5.8^{bcy}				

^{abcd}Means (n = 32) in columns with different letters indicate significant difference at P < 0.05. ^{wy}Means (n = 32) in rows with different letters indicate significant difference at P < 0.05. [1]TGP: total gas production; TMR: totally mixed ration; T1: control without yeasts; T2: *Pichia guilliermondii* (Levica 27); T3: *Candida norvegensis* (Levazoot 15); T4: *Saccharomyces cerevisiae* (Levucell® SC 10); D: diet; T: treatment; DxT: Interaction between D and T; EE: standard error.

The molar concentration of hydrogen in the corn stover diet exceeded (P < 0.05) that obtained with the TMR. Conversely, the molar concentrations of CO_2 and CH_4 were higher (P < 0.05) with the TMR than with corn stover, demonstrating the advantage of using a balanced diet for the appropriate growth and activity of ruminal microorganisms.

There was no interaction (P > 0.05) between diets and yeasts for pH, but differences (P < 0.05) were obtained among the studied strains when the TMR was used. The yeast strain *S. cerevisiae* resulted in a higher pH value compared to the strains of the genera *Pichia* and *Candida*. However, no

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difference (P > 0.05) was found between any of these strains and the control treatment. With corn stover, no yeast effect (P > 0.05) was found.

Table 5 shows results for VFA and NH_3-N concentration, showing interaction (P < 0.05) between yeast strains and diets evaluated. The inclusion of the three yeast strains increased the molar concentration (P < 0.05) of total VFA and acetic, propionic, and butyric acids in ruminal fluid. The C_2:C_3 ratio remained unchanged (P > 0.05) when corn stover was used as the substrate and decreased (P < 0.05) with the inclusion of the Levica 27 and Levazoot 15 strains in the TMR treatment. In the case of the commercial product Levucell® SC 10, this ratio remained similar (P > 0.05) to the control treatment.

Table 5. Effect of yeasts on the production of volatile fatty acids (VFA) and NH_3-N concentration during *in vitro* ruminal fermentation with corn stover and totally mixed ration.

Parameters	Substrate	T1	T2	T3	T4	EE	P-value		
							D	T	DxT
C_2 (mM)[1]	Corn stover	16.97[a]	51.83[b]	63.54[b]	47.24[bd]	4.29	<0.0001	<0.0001	<0.001
	TMR	32.36[ad]	112.22[c]	114.35[c]	97.47[c]				
C_3 (mM)	Corn stover	9.51[a]	26.38[b]	36.60[bd]	25.99[b]	2.92	<0.0001	<0.0001	<0.001
	TMR	13.37[a]	62.38[c]	59.12[c]	46.37[d]				
C_4 (mM)	Corn stover	32.83[a]	77.23[c]	83.47[c]	74.18[c]	3.87	<0.0001	<0.0001	0.002
	TMR	6.69[b]	25.57[a]	26.75[a]	22.52[a]				
Total VFA	Corn stover	52.42[a]	155.46[c]	183.62[cd]	147.42[c]	7.75	0.0015	<0.0001	0.01
(mM)	TMR	60.00[b]	200.17[d]	200.22[d]	166.36[c]				
C_2:C_3	Corn stover	1.96[a]	1.78[a]	1.74[a]	1.82[a]	0.10	0.0017	0.027	0.004
	TMR	2.43[b]	1.80[a]	1.93[a]	2.10[ab]				
NH_3-N (mM)	Corn stover	6.1[a]	8.5[a]	9.5[a]	6.1[a]	1.16	<0.0001	<0.0001	<0.0001
	TMR	55.9[c]	67.2[d]	66.8[d]	47.3[b]				

[abc]Means (n = 32) with different letters for both rows and columns indicate a statistically significant difference at P < 0.05.[1]C_2: acetic acid; C_3: propionic acid; C_4: butyric acid; TMR: totally mixed ration; T1: control without yeasts; T2: *Pichia guilliermondii* (Levica 27); T3: *Candida norvegensis* (Levazoot 15); T4: *Saccharomyces cerevisiae* (Levucell® SC 10); D: diet; T: treatment; DxT: Interaction between D and T; EE: standard error.

NH_3-N levels (Table 5), there was an interaction between yeast strains and diets used in this study. The lowest (P < 0.05) molar concentration was obtained with corn stover (6.1 mM) and was similar among yeast strains with this substrate. However, with the TMR, NH_3-N concentration increased to values between 47 mM and 66 mM. Additionally, differences (P < 0.05) for NH_3-N were observed among yeast strains. With Levica 27 and Levazoot 15, NH_3-N concentration was higher than the control treatment, but with the commercial product Levucell® SC 10, this concentration was lower (P < 0.05) than the control treatment.

4. Discussion

4.1. Gas Production and Composition

Yeasts have the ability to alter the fermentation process in the rumen, resulting in variations in gas production and composition. These variables are commonly used to evaluate the efficiency of ruminal fermentation [19]. Generally, the use of yeasts in ruminant feeding is associated with improvements in dry matter (DM) and structural carbohydrate digestibility, which leads to higher gas production, an expected outcome in this study. However, no variation in gas production was found when yeasts were included. In agreement with our results, González et al. (2023) [15] found no effects on *in vitro* dry matter digestibility or *in vitro* neutral detergent fiber digestibility when

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adding a strain of yeast *P. guilliermondii* (Levica 27) to the *in vitro* fermentation of corn stover. The effectiveness of yeasts as additives for dry matter and structural carbohydrate digestibility has given variable results and can be influenced by several factors, including the characteristics of the diet used [20,21], the yeast strain employed [15], and the dosage used [22]. Results are also variable regarding the influence of diet on the effect of yeasts in rumen fermentation. Roa et al. (1997) [23] reported that the best results were obtained when yeasts were used with high-quality forages. In contrast to our results, previous studies by Marrero et al. (2014) [24] and Castillo et al. (2016) [14] reported significant increases ($P<0.05$) in gas production when using *Candida norvegensis* yeast strains Levica 25 and Levazoot 15, respectively, in the *in vitro* fermentation of fibrous substrates. Those authors attributed the results to yeasts in the rumen improving the degradation rates of structural carbohydrates and therefore gas production.

Total gas production was higher when TMR was used. This variable increased from 47 mL of gas with corn stover to 94.8 mL with TMR, likely due to the better nutritive value of the latter. Corn stover is a low-quality fibrous substrate that might not meet the requirements of ruminal microorganisms, generating low fermentative capacity. Supporting this statement, Ikhimioya (2008) [25] mentioned that for optimal microbial growth in the rumen and consequent high fermentative activity, a minimum of 80 g/kg of DM of crude protein is required, whereas the protein content in the stover used in this study was 59 g/kg of DM.

The inclusion of yeasts can not only increase total gas production but also cause changes in the composition of gases produced by improving rumen efficiency [26]. Studies evaluating the effect of yeasts on methane production vary significantly, with some reporting reductions [27,28], others increases [22,29], and still others no changes [30]. In the current study, yeast inclusion had no effect on methane production in either of the two diets used. The increase in CH_4 production with TMR compared to the corn stover diet was possibly due to greater substrate degradation, a result that aligns with results presented by Benchaar et al. (2024) [22].

4.2. pH

The pH in the stover diet with yeast inclusion showed values close to neutrality (6.4), consistent with the pH observed when low-quality fibrous substrates are supplied. These observations are in line with the results reported by Marrero et al. (2006) [31], working with *S. cerevisiae* and Levica 25, and Ruiz et al. (2016) [32], using *C. norvegensis*, when studying the effects of yeast on the *in vitro* ruminal fermentation of fibrous substrates. The lack of difference observed with this type of diet when yeasts were included may be due to the establishment of methanogenic bacteria populations in the rumen when high-fiber diets are used. These bacteria utilize H_2 and CO_2 for methane formation, and their utilization of these gases, especially H_2, contributes to ruminal pH stabilization [33]. Similar results, with no differences in pH when yeasts were added to fibrous substrate fermentation, have been reported by Díaz et al. (2017) [21] and Anjum et al. (2018) [34].

With TMR as substrate, pH values ranged from 5.6 to 5.8, with the highest value obtained with the *S. cerevisiae* strain. The effects of yeast cultures on ruminal pH reported in the literature are variable and mainly depend on experimental conditions [35], with the primary factors being the composition of the diets evaluated [8,21,34,36] and the yeast species used [32]. Authors like Chaucheyras-Durand and Fonty (2008) [37] demonstrated that yeasts, when high-concentrate diets are used, can mainly increase ruminal pH or reduce its variability due to a decrease in lactate concentration. These outcomes are attributed to the interactions between yeast cells and lactate-metabolizing bacteria [20].

4.3. Volatile Fatty Acids

Consistent with the results from the current study, González et al. (2023) [15] evaluated the *P. guilliermondii* strain Levica 27 with corn stover as a substrate and found increases in the molar concentrations of total VFA, acetic acid, and propionic acid in ruminal fluid and a decrease in the C_2:C_3 ratio within a range of 6 to 12 hours of incubation. Additionally, Ruiz et al. (2016) [32], studying

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the effect of a *C. norvegensis* on the *in vitro* ruminal fermentation of oat straw, reported increased molar concentrations of acetic, propionic, and butyric acids compared to the control.

However, Marrero et al. (2006) [31] did not report any effect on the molar concentration of total VFA when evaluating different yeast strains in the ruminal fermentation of cannulated Holsteins consuming fibrous diets. Similar to the latter study, Moya et al. (2009) [38] also did not observe differences with the inclusion of a *S. cerevisiae* in the molar concentration of VFA or the C_2:C_3 ratio in cannulated Holstein heifers consuming a transition diet.

In the present study, the three yeast strains increased the molar concentration of butyric acid. This acid is an important energy source for the enterocyte, stimulating its proliferation, health, and function, which leads to better feed utilization by the animal [39]. Increases in total and individual VFA concentrations are indicators of enhanced degradation of both the substrate and the yeast cultures added in each treatment. In the rumen environment, yeasts have a short lifespan and are believed to degrade or pass to the lower parts of the digestive tract a few hours after supplementation [40,41]. Once degraded, the basic components of yeasts can contribute to VFA production in the rumen [22]. In the current study, 1 mg of yeast was added to each tube. This additional substrate likely contributed to the increase in total and individual VFA concentrations, although this same contribution of the yeasts was not observed in the gas production variables.

The decrease in the C_2:C_3 ratio of the treatments with Levica 27 and Levazoot 15 compared to the control found in this study is explained by the higher molar concentration of propionate in the yeast treatments. Additionally, this increase resulted in changes in the molar proportion of VFA in the rumen. Similar results were reported by Lila et al. (2004) [30] and Ruiz et al. (2016) [32], who noted that one of the main effects of yeast cultures is to increase the concentration of propionate at the expense of acetate concentration. This outcome could be due to the peptide fractions of yeast cells that stimulate the growth of *Megasphaera elsdenii*, the main lactate-consuming microorganism in the rumen, which uses lactate to produce propionate through the acrylate pathway [36]. Additionally, yeast cultures provide vitamins (thiamine), glucans, mannanoproteins, and organic acids that stimulate the growth of fiber-digesting and lactic acid-consuming microorganisms [42].

4.4. NH_3-N Concentration

The NH_3-N concentration obtained with corn stover aligns with other studies, with values ranging from 5 to 12 mM when fibrous diets were used [32,43]. Levica 27 and Levazoot 15 increased NH_3-N levels in ruminal fluid, consistent with the results observed by Díaz et al. (2017) [21], who reported increases in NH_3-N concentration when adding a yeast hydrolysate to ruminal fluid using a 50:50 forage-to-concentrate ratio as substrate. In agreement with our results using Levica 27 and Levazoot 15 strains, Oeztuerk (2009) [44] observed that the increase in NH_3-N concentration in fermenters produced by a live yeast culture was greater than that caused by the same autoclaved cultures. He concluded that the difference was associated with the stimulation of the proteolytic activity of rumen bacteria by the live yeast culture. This author also found higher ammonia concentrations in the presence of different doses of live yeasts in two *in vitro* fermentation systems and attributed the result to microbial degradation of yeast cells due to their high protein content [44]. In contrast, Anjum et al. (2018) [34] stated that the addition of *S. cerevisiae* decreased the concentration of ammonia during buffalo ruminal fermentation. This result aligns with results reported in the current study with the *S. cerevisiae* strain Levucell® SC 10, where the molar concentration of NH_3-N decreased compared to the control. Some authors consider the effect might be due to increased microbial protein synthesis [42].

5. Conclusions

The results suggest that the yeast strains used in this study could improve the energy utilization of feed for production purposes due to the increased gluconeogenic potential of the diet as reflected by increased propionate production. It is well known that propionate is the only VFA that contributes to hepatic gluconeogenesis, and it is also the most energy-efficient because its production is indirectly related to methanogens through the use of metabolic hydrogen [43,45].

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Among the strains evaluated, *Pichia guilliermondii* and *Candida norvegensis* gave similar or slightly better values to *Saccharomyces cerevisiae* for VFA production for both diets studied. Therefore, the use of *Pichia guilliermondii* and *Candida norvegensis* strains in livestock systems should be further studied and considered to achieve better and optimal production results.

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References

1. Food and Agriculture Organization of the United Nations. FAO. World agriculture: towards 2015 / 2030: an FAO perspective. **2015.** London UK.
2. Lara, E.C.; Bragiato, U.C.; Rabelo, C.H.S.; Messana, J.D.; Reis, R.A. Inoculation of corn silage with *Lactobacillus plantarum* and *Bacillus subtilis* associated with amylolytic enzyme supply at feeding. 1. Feed intake, apparent digestibility, and microbial protein synthesis in wethers. *Anim. Feed Sci. Technol.* **2018**, *243*, 22–34. https://doi.org/10.1016/j.anifeedsci.2018.07.004
3. Arowolo, M.A.; He, J. Use of probiotics and botanical extracts to improve ruminant production in the tropics: A review. *Anim. Nutr.* **2018**, *4*, 241–249. https://doi.org/10.1016/j.aninu.2018.04.010
4. Pilajun, R.; Wanapat, M. Chemical composition and *in vitro* gas production of fermented cassava pulp with different types of supplements. *J. Appl. Anim. Res.* **2018**, *46*, 81–86. https://doi.org/10.1080/09712119.2016.1261029
5. Fomenky, B.E.; Chiquette, J.; Bissonnette, N.; Talbot, G.; Chouinard, P.Y.; Ibeagha-Awemu, E.M. Impact of *Saccharomyces cerevisiae boulardii* CNCMI-1079 and *Lactobacillus acidophilus* BT1386 on total lactobacilli population in the gastrointestinal tract and colon histomorphology of Holstein dairy calves. *Anim. Feed Sci. Technol.* **2017**, *234*, 151-161. https://doi.org/10.1016/j.anifeedsci.2017.08.019
6. Liu, S.; Shah, A.M.; Yuan, M.; Kang, K.; Wang, Z.; Wang, L.; Peng, Q. Effects of dry yeast supplementation on growth performance, rumen fermentation characteristics, slaughter performance and microbial communities in beef cattle. *Anim. Biotech.* **2022,** *33*, 1150-1160. https://doi.org/10.1080/10495398.2021.1878204
7. Elghandour, M.M.; Salem, A.Z.; Castañeda, J.S.M.; Camacho, L.M.; Kholif, A.E. Chagoyán, J.C.V. Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants. *J. Integr. Agri.* **2015**, *14*, 526-533. https://doi.org/10.1016/S2095-3119(14)60834-0
8. Vallejo-Hernández, L.H.; Elghandour, M.M.Y.; Greiner, R.; Uchenna, Y.; Rivas-Cáceres, R.R.; Barros-Rodríguez, M.; Abdelfattah, Z.M. Environmental impact of yeast and exogenous xylanase on mitigating carbon dioxide and enteric methane production in ruminants. *J. Clean. Prod.* **2018,** *189*, 40-46. https://doi.org/10.1016/j.jclepro.2018.03.310
9. Phesatcha, K.; Phesatcha, B.; Wanapat, M.; Cherdthong, A. Roughage to concentrate ratio and *Saccharomyces cerevisiae* inclusion could modulate feed digestion and *in vitro* ruminal fermentation. *Vet. Sci.* **2020,** *7*, 151. https://doi.org/10.3390/vetsci7040151
10. Leicester, H.C.W.; Robinson, P.H.; Erasmus, L.J. Effects of two yeast based direct fed microbials on performance of high producing dairy cows. *Anim. Feed Sci. Technol.* **2016,** *215*, 58–72. https://doi.org/10.1016/j.anifeedsci.2016.03.003
11. Marrero, Y.; Rodríguez, R.; Torres, V.; Jay, O.; Galindo, J. Effect of yeasts on the production of gas from *Cynodon nlemfuensis* in an *in vitro* rumen incubation. *Lives. Res. Rural Develop.* **2020**, *32*, 1–3. Available online: http://www.lrrd.org/lrrd32/1/ymarr32001.html (accessed on 10 June 2024).
12. Amin, A.B.; Mao, S. Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review. *Anim. Nutr.* **2021**, *7*, 31-41. https://doi.org/10.1016/j.aninu.2020.10.005

13. Baker, L.M.; Kraft, J.; Karnezos, T.P.; Greenwood, S.L. The effects of dietary yeast and yeast-derived extracts on rumen microbiota and their function. *Anim. Feed Sci. Technol.* **2022**, *294*,115476. https://doi.org/10.1016/j.anifeedsci.2022.115476
14. Castillo C.Y.; Ruiz B.O.; Burrola B.M.E. Isolation and characterization of yeasts from fermented apple bagasse as additives for ruminant feeding. *Brazilian J. Microbiol.* **2016**, *47*, 889-895. https://doi.org/10.1016/j.bjm.2016.07.020
15. González M.B.; Ruiz-Barrera, O.; Castillo R.F.; Castillo-Castillo, Y. Effect of live yeasts (*Pichia guilliermondii*) on *in vitro* fermentation of corn stover as a fibrous substrate. *Fermentation* **2023,** *9*, 17. https://doi.org/10.3390/fermentation9010017
16. AOAC, Association Official Analytical Chemists. Official methods of analysis of AOAC International. 18a ed. Maryland, E. U. A. **2005.** pp 486.
17. Broderick, G.A.; Kang, J.H. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Dairy Sci.* **1980**, *63*, 64–75. https://doi.org/10.3168/jds.S0022-0302(80)82888-8
18. Galyean, M.L. Analysis of volatile fatty acids in rumen fluid. En Laboratory procedures in animal nutrition research. Animal Nutrition Laboratory. Department of Animal and Food Science. Texas Tech. University. Lubbock. **1980,** pages 161-162.
19. Chung, Y.H.; Walker, N.D.; McGinn, S.M. Beauchemin, K.A. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. *J. Dairy Sci.* **2011**, *94*, 2431-2439. https://doi.org/10.3168/jds.2010-3277
20. Chaucheyras-Durand, F.; Walker, N.D.; Bach, A. Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Anim. Feed Sci. Technol.* **2008**, *145*, 5-26. https://doi.org/10.1016/j.anifeedsci.2007.04.019
21. Díaz, A.; Ranilla, M.J.; Saro, C.; Tejido, M.L.; Pérez-Quintana, M. Carro, M.D. Influence of increasing doses of a yeast hydrolyzate obtained from sugarcane processing on *in vitro* rumen fermentation of two different diets and bacterial diversity in batch cultures and Rusitec fermenters. *Anim. Feed Sci. Technol.* **2017**, *232*, 129-138. https://doi.org/10.1016/j.anifeedsci.2017.08.011
22. Benchaar, C.; Hassanat, F.; Yang, W.Z. 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.* **2024,** *307*, 115844. https://doi.org/10.1016/j.anifeedsci.2023.115844
23. Roa, J.B. Effect of fiber source and a yeast culture (*Saccharomyces cerevisiae* 1026) on digestion and the environment in the rumen of cattle. *Anim. Feed Sci. Technol.* **1997**, *64*, 327-336. https://doi.org/10.1016/S0377-8401(96)01015-2
24. Marrero, Y.; Ruiz, O.; Corrales, A.; Jay, O.; Galindo, J.; Castillo, Y. *In vitro* gas production of fibrous substrates with the inclusion of yeast. *Cuban J. Agri. Sci.* **2014**, *48*, 119-123. Available online: https://cjascience.com/index.php/CJAS/article/view/468 (accessed on 10 June 2024).
25. Ikhimioya, I. Acceptability of selected common shrubs/tree leaves in Nigeria by West African Dwarf goats. *Livest. Res. Rural Develop.* **2008,** *20*. http://www.lrrd.org/lrrd20/6/ikhi20090.htm
26. Adesogan, T.; Yang, W.; Lee, C.; Gerber, P.J.; Henderson, B.; Tricarico, J.M. Mitigation of methane and nitrous oxide emissions from animal—SPECIAL TOPICS. *J. Anim. Sci.* **2013**, *91*, 5045-5069. https://doi.org/10.2527/jas.2013-6583
27. Wang, Z.; He, Z.; Beauchemin, K.A.; Tang, S.; Zhou, C.; Han, X.; Tan, Z. Evaluation of different yeast species for improving *in vitro* fermentation of cereal straws. *Asian-Australasian J. Anim. Sci.* **2016**, *29*, 230-240. https://doi.org/10.5713/ajas.15.0188
28. Cagle, C.M.; Fonseca, M.A.; Callaway, T.R.; Runyan, C.A.; Cravey, M.D. Tedeschi, L.O. Evaluation of the effects of live yeast on rumen parameters and in situ digestibility of dry matter and neutral detergent fiber in beef cattle fed growing and finishing diets. *Appl. Anim. Sci.* **2020**, *36*, 36-47. https://doi.org/10.15232/aas.2019-01888
29. Mao, S.Y.; Huo, W.J.; Zhu, W.Y. Microbiome–metabolome analysis reveals unhealthy alterations in the composition and metabolism of ruminal microbiota with increasing dietary grain in a goat model. *Environ. Microbiol*. **2016**, *18*, 525-541. https://doi.org/10.1111/1462-2920.12724
30. Lila, Z.A.; Mohammed, N.; Yasui, T.; Kurokawa, Y.; Kanda, S.; Itabashi, H. Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation *in vitro*. J. Anim. Sci. **2004,** *82*, 1847-1854. https://doi.org/10.2527/2004.8261847x
31. Marrero, Y.; Galindo, J.; Elias, A.; Moreira, O.; Cueto, M. Efecto de preparados biológicos con levaduras viables en la población microbiana ruminal e indicadores fermentativos en vacas que consumen dietas fibrosas. *Cuban J. Agri. Sci.* **2006**, *40*, 339-348. Available online: https://www.redalyc.org/articulo.oa?id=193017723013 (accessed on 10 June 2024).
32. Ruiz, O.; Castillo, Y.; Arzola, C.; Burrola, E.; Salinas, J.; Corral, A.; Itza, M. Effects of *Candida norvegensis* live cells on *in vitro* oat straw rumen fermentation. *Asian-Australasian J. Anim Sci.* **2016**, *29*, 211-218. https://doi.org/10.5713%2Fajas.15.0166

33. Galindo, J.; Marrero, Y.; González, N.; Sosa, A.; Miranda, A.L.; Aldana, A.I.; Moreira, O.B.; Bocourt, R.; Delgado, D.; Torres, V.; Sarduy, L.; Noda, A. Efecto de preparados con levaduras *Saccharomyces cerevisiae* and Levica-25 viables en los metanógenos and metanogénesis ruminal *in vitro*. *Rev. Cubana Cienc. Agríc.* **2010**, *44*, 273-279. https://www.redalyc.org/pdf/1930/193015664010.pdf
34. Anjum, M.I.; Javaid, S.; Ansar M.S. Effects of yeast *(Saccharomyces cerevisiae)* supplementation on intake, digestibility, rumen fermentation and milk yield in Nili-Ravi buffaloes. Iranian. *J. Vet. Res.* **2018**, 19, 96–100. https://doi.org/10.22099/ijvr.2018.4852
35. Vohra, A.; Syal, P.; Madan, A. Probiotic yeasts in livestock sector. *Anim. Feed Sci. Technol.* **2016**, *219*, 31–47. https://doi.org/10.1016/j.anifeedsci.2016.05.019.
36. Cherdthong, A.; Prachumchai, R.; Supapong, C.; Khonkhaeng, B.; Wanapat, M.; Foiklang, S.; Milintawisamai, N.; Gunun, N.; Gunun, P.; Chanjula, P. Polyorach, S. Inclusion of yeast waste as a protein source to replace soybean meal in concentrate mixture on ruminal fermentation and gas kinetics using *in vitro* gas production technique. *Anim. Prod. Sci.* **2019**, *59*, 1682–1688. https://doi.org/10.1071/AN18491
37. Chaucheyras-Durand, F.; Fonty, G. Effects and modes of action of live yeasts in the rumen. *Biología (Bratislava)*. **2008**, *61*, 741-750. https://doi.org/10.2478/s11756-006-0151-4
38. Moya, D.; Calsamiglia, S.; Ferret, A.; Blanch, M.; Fandiño, J.I.; Castillejos, L.; Yoon, I. Effects of dietary changes and yeast culture (*Saccharomyces cerevisiae*) on rumen microbial fermentation of Holstein heifers. *J. Anim. Sci.* **2009**, *87*, 2874-2881. https://doi.org/10.2527/jas.2008-1446
39. Miguel, M.A.; Lee, S.S.; Mamuad, L.L.; Choi, Y.J.; Jeong, C.D.; Son, A.; Lee, S.S. Enhancing butyrate production, ruminal fermentation and microbial population through supplementation with *Clostridium saccharobutylicum*. *J. Microbiol. Biotech*. **2019,** *29*, 1083-1095. https://doi.org/10.4014/jmb.1905.05016
40. Kung, J.L.; Kreck, E.M.; Tung, R.S.; Hession, A.O.; Sheperd, A.C.; Cohen, M.A.; Swain, H.E.; Leedle, J.A.Z. Effects of a live yeast culture and enzymes on *in vitro* ruminal fermentation and milk production of dairy cows. *J. Dairy Sci.* **1997**, *80*, 2045–2051. https://doi.org/10.3168/jds.S0022-0302(97)76149-6.
41. Jouany, J.P. Optimizing rumen functions in the close-up transition period and early lactation to drive dry matter intake and energy balance in cows. *Anim. Reprod. Sci.* **2006**, *96*, 250-264. https://doi.org/10.1016/j.anireprosci.2006.08.005
42. Oeztuerk, H.; Schroeder, B.; Beyerbach, M.; Breves, G. Influence of living and autoclaved yeasts of *Saccharomyces boulardii* on *in vitro* ruminal microbial metabolism. *J. Dairy Sci.* **2005,** *88*, 2594–2600. https://doi.org/10.3168/jds.S0022-0302(05)72935-0
43. Guedes, C.M.; Gonçalves, D.; Rodrigues, M.A.M.; Dias-da-Silva, A. Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. *Anim. Feed Sci. Technol.* **2008**, *145*, 27-40. https://doi.org/10.1016/j.anifeedsci.2007.06.037
44. Oeztuerk, H. Effects of live and autoclaved yeast cultures on ruminal fermentation *in vitro*. *J. Anim. Feed Sci.* **2009**, *18*, 142-150. https://doi.org/10.22358/jafs/66378/2009
45. Miller-Webster, T.; Hoover, W.H.; Holt, M.; Nocek, J.E. Influence of yeast culture on ruminal microbial metabolism in continuous culture. *J. Dairy Sci*. **2002,** *85*, 2009-2014. https://doi.org/10.3168/jds.S0022-0302(02)74277-X