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Evaluation of the synergistic effect of yeast and Chicory-inulin on rumen fermentation parameters and estimation of methane emission

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SUMMARY

Probiotics and prebiotics are feed additives that have been extensively utilised in animal nutrition for a considerable duration. However, only some studies have investigated their synergistic effects on rumen fermentation parameters and their role in minimizing methane emission. Therefore, this study examined the impact of combining chicory-inulin with yeast (Saccharomyces cerevisiae) on rumen fermentation parameters and methane emissions in weaned dairy goats. The feeds were formulated into eight diets and offered to 24 Saanen×Toggenburg crossbred weaned female dairy goats weighing 14±0.5 Kg in a Completely Randomized Design with a (4x2) factorial arrangement. The diets were Rhodes grass hay and chicory supplementation at four levels: 0, 10, 20, 20, and 30% as the main effects and with (+) and without yeast (-) yeast as interaction levels. The findings indicated that the inclusion of yeast and Chicory had a significant effect ($p<0.05$) on rumen pH, ammonia nitrogen concentrations, production of volatile fatty acids), and estimated methane emission. The highest pH was recorded in T1- with 7.27. It was followed closely by T1+ with 6.73. T1+ recorded the highest methane (39.67 mmol/L), while T4- had the lowest (32.42 mmol/L). This study concludes that Chicory-inulin has prebiotic properties by maintaining pH levels and affecting amounts of ammonia nitrogen. The lack of a significant interaction effect between yeast and Chicory in methane emission implies that their combined influence may not significantly affect methane emissions in the current study's experimental settings.

Keywords: *Acetate, Prebiotics, Probiotics, pH, Ammonium-nitrogen, GHGs*

INTRODUCTION

The necessity to continually seek out and increase the pool of feeds and additives to increase ruminant production is crucial to meet the ever-escalating demand for animal products. However, increasing ruminant livestock production is faced with many constraints, among them, inadequate supply of feed resources that are necessary to supply adequate nutrients to the animals. This calls for the diversification of the available feed resources to meet the nutrient requirements of the animals and preserve the equilibrium between production and environmental sustainability.

Chicory has long been utilised as cattle feed in numerous parts of the world (Mahmoud, 2021). Due to its many beneficial medicinal, culinary, and nutritional properties, Chicory is rapidly gaining popularity, especially in Kenya. It is grown by farmers in most dairy farming areas. Also, chicory fodder has the same effect on animals as legumes and even more so than grass-based pastures. When added to basal pasture grasses during feeding, Chicory enhances milk production, as Mahmoud (2021) reported. According to Minneé (2017), the crude protein content of most chicory types is over 19% on a DM basis, which is 1.6–2.4 times greater than the value of most traditional grains like wheat, rice, corn, and barley; this indicates that Chicory is the best alternative source of protein. Lepczyński et al. (2021) found that chicory is abundant in inulin and other phytochemicals. Primarily found in temperate pastures and some plants like artichoke, chicory, and certain vegetables, inulin is frucstosan, a fructose polymer and an alternate storage carbohydrate to starch. Fresh chicory roots have many nutritional and physiological benefits, including a high concentration of inulin (68% DM base), a storage polysaccharide similar to starch, and potential richness in energy and protein.

According to Roodposhti and Dabiri (2012), probiotics are live, helpful microbes that support rumen functions and keep the gut microbiome well-balanced. For instance, the probiotic yeast *Saccharomyces cerevisiae* has a beneficial effect on the microorganisms in the rumen, specifically by raising the overall population of bacteria, anaerobic fungi, and protozoa and by enhancing the functionality of the rumen itself (Ding et al., 2014). Since *S. cerevisiae* is relatively inexpensive compared to other feed supplements and additives, it can increase animal output at no extra expense (Shurson, 2018). Yeast has been added to animal feed to improve the diets of domestic animals. *Saccharomyces cerevisiae* supplies growth-promoting substances, such as organic acids, B-complex vitamins, and amino acids, which support the growth of microorganisms in the rumen and, therefore, indirectly maintain a stable pH.

In contrast to probiotics, prebiotics function differently as a source of nourishment for specific microorganisms, resulting in advantageous outcomes for the host animal (Ding et al., 2014). Prebiotics exhibit resistance to gastric acidity, absorption, and hydrolysis by enzymes secreted in the gastrointestinal tract (Samal & Behura, 2015). The by-products of prebiotic fermentation by the microorganisms in the gut have the advantage of reducing the pH, thus inhibiting the growth of harmful bacteria in the gut (Gibson et al., 2010). Various prebiotics are utilised in cattle production, including fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan oligosaccharides (MOS), β-glucans, inulin, and lactulose (Patel & Goyal, 2012). Inulin is a complex

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carbohydrate composed of fructose molecules as the monomers linked together by a β 2, 1 glucosidic bond, with minor quantities of glucose present. Therefore, inulin is classified as a polysaccharide carbohydrate called fructosan (fructans). Chicory (*Cichorium intybus L.*) and Jerusalem artichoke (*Helianthus tuberosus L.*) are the primary sources of inulin (Masanetz et al., 2010). Inulin undergoes minimal digestion and absorption in the small intestine and, therefore, ends up in the lower part of the gastrointestinal tract (hindgut), where bacteria rapidly ferment it to volatile fatty acids and gases.

Lactobacillus bacteria, including Bifidobacterium, exert a favorable impact on gut metabolism. The consumption of inulin, therefore, provides nutrients to the cells in the intestines, reduces the acidity in the gut, and promotes the growth of the villi and micro-villi in the intestines, as well as an increase in the number of cells on each villus, which increases the absorptive area for nutrients (Xiao et al., 2016). Synbiotics are a combination of live microorganisms and specific substances consumed by the host microorganisms that positively affect the host's health. The concurrent use of a probiotic and a prebiotic can result in a mutually enhancing and advantageous outcome for the animal. The two feed supplements, when combined, can increase the gastrointestinal tract implantation rate and survival of a direct-fed microbial, as well as activate the metabolism of a small number of beneficial bacteria and encourage their growth.

Furthermore, a significant issue encountered in the rearing of ruminants is the release of methane, which is strongly linked to enteric fermentation in the gastrointestinal and is affected by various types of microbes, especially the cellulolytic flora (Zhou et al., 2018). Methane is released due to enteric microbial fermentation of feed both in the foregut and hindgut in herbivores, especially ruminant herbivores. Enteric fermentation is estimated to represent a loss of up to 12% of the feed's gross energy, which could have benefitted the host animal. The loss occurs because bacteria use organic acids, such as formic and acetic acids, to produce volatile fatty acids (Malmuthuge & Guan, 2016).

Additionally, methane is one of the major greenhouse gases that contribute to climate change. In recent years, there has been a strong global interest in decreasing methane gas emissions in animal production through the use of feed additives that impact the microbiome composition in the ruminant digestive system (Patel & Goyal, 2012; Uyeno et al., 2015). Probiotics and prebiotics are feed additives that have been extensively utilised in animal nutrition for a considerable duration. However, only some studies have investigated their synergistic effects on rumen fermentation parameters and their role in minimizing methane emissions. Therefore, the objective of this study was to determine the synergistic effect of yeast and chicory-inulin on rumen fermentation parameters and methane emission estimation.

MATERIALS AND METHODS

Study Site

The study was conducted at Egerton University's Tatton Agricultural Park (TAP), Njoro. The farm is situated in Kenya, the Njoro Sub County in Nakuru County in the Great Rift Valley of East Africa, on the eastern slopes of the Mau Escarpment. Its coordinates are 0º23'S latitude and 35º57'E longitude, and an elevation of 2,200 to 2,280 metres above sea level. The site is characterized by volcanic soils, which are well drained, and support the growth of herbs like Chicory. The region experiences a bimodal rainfall pattern with long rains from March to May, occasionally extending to June, and brief short rains from September to November.

Experimental Animals

The goats were confined in individual slatted-floor pens that were well ventilated. Fortnightly, each experimental goat was drenched with 10% Albendazole (anti-helminthic) to prevent internal parasites, while external parasites were prevented using acaricides. The feeds were formulated into eight diets replicated three times and offered to 24 Saanen×Toggenburg crossbred weaned female dairy goats weighing 14±0.5 Kg in a CRD with a (4x2) factorial arrangement. Each goat's initial weight was determined by averaging it over three days.

Experimental Diets

The basal diet comprised Rhodes grass (*Chloris gayana*) hay, with chicory leaves and yeast serving as supplements (*Table 1*). The hay was sourced within the Njoro area and shredded into 2 cm size using a tractor-driven forage shredding machine. The shredded hay was subsequently kept in plastic gunny bags while awaiting the feeding trial. Chicory was grown at the Egerton University Tatton farm and harvested, then dried. It was then chopped into 2 cm size using an electric power forage chopper and kept in plastic gunny bags.

Table 1. **Chemical composition of Rhodes grass hay and Chicory (g Kg^{-1} DM)**

Parameter	Chicory	Rhodes Grass Hay
DM	947.4	955.8
CP	220.6	99.0
Ash	132.9	88.8
EE	19.5	25.7
CF	118.6	324.0
NDF	291.0	732.0
ADF	247.3	473.0
ADL	102.9	34.9
TET	12.3	47.3

DM=Dry matter, CP=Crude protein, EE=Ether extracts, NDF=Neutral detergent fibre, ADF=Acid detergent fibre, ADL=Acid detergent lignin, CF=Crude Fiber, TET=Total extractable tannins.

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Two weeks before the start of the feeding trial, the feeds were transported to the experimental site in readiness for the feeding trial. The feeds were kept in a clean, dry, and temperature-controlled store with adequate ventilation throughout the experiment. The basal diet (T1), which was also the control, consisted of Rhodes grass hay, while the other experimental diets were as follows: T2, T3, and T4 with different levels of Chicory (10, 20, and 30%) with (+) and without yeast (-) at a rate of 10 g/goat—day as shown in *Table 2*. The higher DM intake level recorded for goats was used to compute dry matter intake, which was 5% of the live weight of each goat. The supplement diets were fed at 10, 20, and 30% of the expected daily DM intake.

In contrast, the basal Rhodes grass hay diet was provided *ad libitum* so that the unavailability of the basal forage did not restrict intake. The amount fed was adjusted at the start of each week to cater for changes in body weight. The supplement was provided to the animals as a priority before offering the basal diet *ad libitum* to guarantee that the goats eat the daily supplement.

Table 2. **The Dietary treatments for the study**

	Rhodes grass hay	Chicory	Yeast (Without)	With Yeast 10 g/goat/day
T1	100%	0	-	+
T2	90%	10%	-	+
T3	80%	20%	-	+
T4	70%	30%	-	+

Experimental Design

The study used a completely randomized design (CRD) in a (4x2) factorial arrangement replicated thrice. Eight dietary treatments were used in the study's experiment, which was based on the basal diet and different levels of Chicory (10, 20, 30%) and yeast supplements. The animals were confined in individual slatted-floor pens that were well-ventilated. Fortnightly, each experimental goat was drenched with 10% Albendazole (anti-helminthic) to prevent internal parasites, while external parasites were prevented using acaricides. Data collection was done for four weeks after a 14-day adaptation period. The eight dietary treatments were allocated to the animals in a randomized manner. The supplements were offered to the animals twice daily, in the morning at 08:00 hours and in the early afternoon at 14:00 hours. At the same time, the Rhodes grass hay basal diet was made available to the animals *ad libitum*.

Determination of the Rumen Fermentation Parameters

On the last data gathering day, rumen fluid samples were collected using a vacuum pump at 0 and 4 hours following feeding. Each time, a stomach tube connected to a vacuum pump was utilised to extract roughly 50 mL of rumen fluid from the lower region of the organ's core. The pH and temperature of the fluid in the rumen were quickly assessed using portable pH and temperature meters (specifically, the HANNA HI-8424 Portable pH/ORP Meter from Woonsocket, USA). The subsequent action involved passing the rumen fluid samples through four layers of cheesecloth in order to remove any particles. Right after filtering, 45 mL of rumen fluid was moved to a plastic container with 5 mL of sulfuric acid solution to eradicate lingering bacteria and stop the fermentation process. The mixture was centrifuged at a speed of 16,000 x g for 15 minutes using a Table Top Centrifuge PLC-02. The resulting supernatant was stored in a cold storage facility at -20 ºC for future analyses. These analyses involved measuring ammonia nitrogen (NH_3-N) with a Kjeltech Auto 1030 Analyzer and volatile fatty acids (VFAs) using a high-performance liquid chromatography (HPLC) method developed by Mathew and Kalyanasundaram in 2001. The HPLC setup included Controller Instruments, water model 600E, 484 UV detector, Novapak C18 column (4 mm 150 mm), and a mobile phase of 10 mM H2.

The equation utilised to predict ruminal methane (CH_4) production using proportions of volatile fatty acids (VFA) was based on the work of Moss et al. (2000). This calculation heavily influences the assessment and measurement of methane production from ruminant animals, which are among the largest emitters of greenhouse gases on earth. In all ruminant animals, the model assumes that there is homogeneity between portions of VFA and methane production. It is expressed as follows: CH_4 production=0.45 acetate + 0.275 propionate + 0.4 butyrate.

Statistical Analysis

The data collected on the various parameters was subjected to the analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system (SAS, 2002). Where significant differences were detected, means were separated using the least significance difference (LSD) at (p< 0.05).

RESULTS AND DISCUSSION

The results for the synergistic effects of yeast and inulin from Chicory are shown in *Table 3*. The highest pH was recorded in T1 (Rhodes grass with no yeast) at 7.27. It was followed closely by T1+ (Rhodes grass +yeast) with 6.73. The other treatments recorded had similar trends and no significant difference. There was a significant difference (p<0.05) in the basal diet with yeast (T1-) compared to T1+. The ammonia nitrogen values significantly (p<0.05) differed across the treatments, and the highest was T1- with 85.36 mg/L, while T4+ recorded the lowest with 56.07 mg/L. This study showed a decreasing trend with an increase in chicory level and yeast addition.

A similar trend was witnessed with acetate production, and T1- had the highest acetate concentration at 58.37 mmol/L, while T4+ had the lowest at 41.46 mmol/L. There was a significant difference (p<0.05) across diets on yeast addition and increased levels of Chicory. However, the synergistic effects of the levels and yeast addition were

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insignificant (p=0.4219) on acetate concentrations. Similar trends were witnessed in propionate (p=0.1849), butyrate (p=0.0851), and methane (p=0.4582) gas production. Yeast and chicory levels exerted their effects significantly (p<0.05) across the treatments and affected all the rumen parameters. Acetate to propionate ratio was only significant (p<0.05) on chicory levels. T1+ recoded the highest CH_4 (39.67 mmol/L) while T4- had the lowest (32.42 mmol/L).

Table 3. **The rumen fermentation parameters in the dairy goats fed on basal Rhodes grass hay supplemented with various levels of Chicory with and without yeast**

Parameter	Dietary composition								*p*-value		
	T1		T2		T3		T4		Diet	Yeast	Diet*Yeast
	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)			
pH	7.27^{a}	6.73^{b}	6.52^{c}	6.50^{c}	6.44^{c}	6.47^{c}	6.58^{bc}	6.50^{c}	<.0001	<.0001	<.0001
NH_3-N (mg/L)	85.36^{a}	82.62^{ab}	78.81^{bc}	77.50^{c}	75.24^{c}	65.83^{d}	62.98^{d}	56.07^{e}	<.0001	<.0001	0.0008
Acet (mmol/L)	58.37^{b}	53.83^{a}	47.67^{dc}	51.16^{bc}	43.66^{de}	46.78^{d}	35.66^{f}	41.46^{e}	<.0001	<.0001	0.4219
Prop (mmol/L)	53.83^{e}	58.37^{d}	51.18^{dc}	54.27^{c}	55.01^{c}	60.58^{b}	63.22^{ab}	65.92^{a}	<.0001	<.0001	0.1849
Buty (mmol/L)	38.60^{e}	44.72^{d}	49.51^{dc}	51.87^{bc}	55.99^{ab}	58.29^{a}	60.78^{a}	61.00^{a}	<.0001	0.0022	0.0851
Acet:Prop	1.32^{a}	1.23^{a}	0.93^{bc}	0.94^{b}	0.79^{bc}	0.77^{dc}	0.56^{e}	0.63^{dc}	<.0001	0.6654	0.1458
CH_4 (mmol/L)	36.0^{b}	39.67^{a}	35.79^{bc}	37.94^{ab}	36.07^{b}	37.39^{ab}	32.42^{c}	34.75^{bc}	0.0001	0.0003	0.4582

$^{a, b, c, d, e}$,f, means in the same row with different superscripts are significantly different at p<0.05) NH3-N= Ammonia nitrogen, Acet= Acetate, Prop=Propionate, Buty= Butyrate, Acet: Prop = Acetate: Propionate ratio, CH_4= Methane gas, with yeast (+), without yeast (-); T1-(Rhodes grass), T1+(Rhodes grass + yeast), T2-(10%Chicory), T2+(10%Chicory+Yeast), T3-(20%Chicory), T3+(20%Chicory+Yeast), T4-(30%Chicory), T4+(30%Chicory+Yeast)

The efficient breakdown and absorption of feed by a ruminant animal depend significantly on the rumen microbiome, which profoundly affects its nutritional well-being. The microorganisms in the rumen of ruminants play a crucial role in fermenting more than 70% of the energy substrates that the animal consumes and absorbs (Xu et al., 2021). Grass-fed ruminants typically maintain a pH range of 6 to 7, creating an ideal internal environment for anaerobic microbes to thrive and carry out fermentative processes (Zeitz et al., 2016). This research observed that the pH levels across all dietary treatments remained within the optimal range of 6–7, except for the basal diet, which had a slightly higher pH of 7.27. These results support the findings of Jonova et al. (2021), who demonstrated that yeast increases sugar availability by stimulating the growth of lactate-utilizing bacteria (*Megasphaera elsdenii*) while also competing with the lactate-producing bacteria (*Streptococcus bovis*) for the available sugar.

This study found that incorporating Chicory had a significant effect in stabilizing pH. These findings are consistent with the research of Liu et al. (2012), who observed that Chicory influences fermentation patterns and microbial populations, which may act together with yeast to control rumen pH. Prebiotics such as Chicory have improved rumen fermentation metabolism and gastrointestinal tract health. The addition of Chicory in this study significantly aided in stabilizing pH levels, which may be attributed to the prebiotic qualities of Chicory's inulin. These results align with previous findings that demonstrated an increase in Bifidobacterium count and acetic acid levels, as well as a decrease in intestinal pH, following the addition of prebiotic inulin to pig feed (Tzortzis et al., 2005). Similarly, another study reported a reduction in intestinal acidity in turkeys after being administered a 2% concentration of fructo-oligosaccharides (FOS) for eight weeks.

Based on research by Tripathi and Karim (2011), microbes can utilise ammonia nitrogen more effectively for growth in the presence of yeast. This is attributed to yeast's ability to enhance protein synthesis. Similarly, according to Mangwe (2020), Chicory's secondary metabolites, such as tannins, have the potential to impact rumen protein breakdown, thereby reducing ammonia levels positively. This combination can improve dietary nitrogen utilization and increase feed efficiency. The study revealed that supplementing animals' feed with yeast and increasing chicory levels significantly reduced NH_3-N levels.

Studies have indicated that Chicory can regulate the nitrogen metabolism in the rumen (Wang et al., 2021). Additionally, research conducted by Ganai et al. (2015) and Amin & Mao (2021) revealed that yeast can enhance protein utilization and reduce the accumulation of ammonia in the rumen. The combination of yeast and Chicory can have a collaborative effect in enhancing nitrogen utilization in the rumen, leading to a decrease in NH_3-N concentration. The supplementation of Chicory may have increased the availability of microbial protein to the dairy goats in the study by providing more fermentable organic matter (FOM), which is crucial for the growth of rumen microbes.

The variations in acetate, propionate, and butyrate levels across different diets illustrate the impact of yeast and Chicory on the fermentation processes in the rumen. The higher concentration of acetate in T1 suggests that including yeast may enhance the breakdown of fiber and increase acetate production (Guedes et al., 2008). The negative relationship between acetate levels and chicory levels is not unexpected, as the carbohydrate-rich supplement may

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have provided easily fermentable sugars, resulting in greater propionate production at the expense of acetate. Specifically, Chicory is known for its high fructosan (inulin) content, a storage polysaccharide that, much like starch, is readily fermentable in the rumen (Umucalilar et al., 2010). Moreover, a study by Liu et al. (2012) revealed that supplementing the diet of finishing beef steers with inulin improved their growth performance. This enhancement was likely due to changes in the fermentation pattern, particularly increased propionate production or the growth of beneficial bacteria in the rumen, which the dietary composition may influence.

The notable fluctuation in the acetate-to-propionate (A:P) ratio suggests that changes in the diet could be influencing alterations in the rumen fermentation pathways. The decrease in the A:P ratio seen with higher Chicory levels and yeast may be due to increased propionate levels at the acetate cost, indicating a potential shift towards more favorable fermentation and enhanced utilization of basic food.

The disparities in methane production among the different treatments illustrate the influence of yeast and Chicory on rumen methanogenesis. It has been observed that yeast can alter microbial populations and suppress methanogenesis, reducing methane emissions (Janssen, 2010; Gong et al., 2013). Despite this, the lack of significant interaction between Chicory and yeast suggests that their combined effect may not substantially impact methane synthesis under this study's specific experimental conditions. Though yeast and Chicory had noticeable effects on several rumen parameters, their combined influence appears to be additive rather than interactive. This finding underscores the importance of considering the effects of various ingredients when formulating diets for ruminant animals.

CONCLUSIONS

The results showed that yeast and Chicory levels significantly affected several parameters, including pH, ammonia nitrogen, acetate, propionate, butyrate, acetate to propionate ratio, and methane emissions. Yeast positively influenced pH, microbial protein production, and nitrogen utilization, while Chicory helped to stabilise pH and influenced the fermentation process. The research did not show a significant synergistic impact on methane emissions. However, adding yeast did reduce methane emissions. The combined effects of yeast and Chicory were additive rather than interactive, as anticipated, especially regarding methane emission. These findings underscore the importance of assessing dietary supplements' individual and combined influences on the rumen ecosystem and fermentation mechanisms. The collective impacts of yeast and Chicory on stabilizing pH, enhancing microbial protein synthesis, optimizing nitrogen utilization, and influencing fermentation patterns offer valuable insights for enhancing ruminant nutrition. This study recommends the inclusion of chicory and yeast in diets to improve animal health, productivity, and environmental sustainability.

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REFERENCES

Amin, A.B.; Mao, S. (2021): Influence of yeast on rumen fermentation, growth performance and quality of products in ruminants: A review. *Animal nutrition*, *7*(1), 31–41. https://doi.org/10.1016/j.aninu.2020.10.005

Armato, L.; Gianesella, M.; Morgante, M.; Fiore, E.; Rizzo, M.; Giudice, E.; Piccione, G. (2016): Rumen volatile fatty acids× dietary supplementation with live yeast and yeast cell wall in feedlot beef cattle. *Acta Agriculturae Scandinavica, Section A—Animal Science*, *66*(2), 119–124. https://doi.org/10.1080/09064702.2016.1272628

Chung, Y.H.; Walker, N.D.; McGinn, S.M.; Beauchemin, K.A. (2011): Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. *Journal of Dairy Science*, *94*(5), 2431–2439. https://doi.org/10.3168/jds.2010-3277

Dias, A.L.G.; Freitas, J.A.; Micai, B.; Azevedo, R.A.; Greco, L.F.; Santos, J.E.P. (2018): Effect of supplemental yeast culture and dietary starch content on rumen fermentation and digestion in dairy cows. *Journal of Dairy Science*, *101*(1), 201–221. https://doi.org/10.3168/jds.2017-13241

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. *Journal of Animal Science and Biotechnology*, *5*(1), 1–9.

Ganai, A.M.; Sharma, T.; Dhuria, R.K. (2015): Effect of yeast (Saccharomyces cerevisiae) supplementation on ruminal digestion of bajra (*Pennisetum glaucum*) straw and bajra straw-based complete feed in vitro. *Animal Nutrition and Feed Technology*, *15*(1), 145–153.

Gibson, G.R.; Scott, K.P.; Rastall, R.A.; Tuohy, K.M.; Hotchkiss, A.; Dubert-Ferrandon, A., ... & Buddington, R. (2010): Dietary prebiotics: current status and new definition. *Food Sci. Technol. Bull. Funct. Foods*, *7*(1), 1–19. https://doi.org/10.1616/1476-2137.15880

Gong, Y.L.; Liao, X.D.; Liang, J.B.; Jahromi, M.F.; Wang, H.; Cao, Z.; Wu, Y.B. (2013): *Saccharomyces cerevisiae* live cells decreased in vitro methane production in the intestinal content of pigs. *Asian-Australasian Journal of Animal Sciences*, *26*(6), 856.

DOI: 10.34101/ACTAAGRAR/2/13864

https://www.animbiosci.org/journal/view.php?doi=10.5713/ajas.2012.12663

Guedes, C.M.; Gonçalves, D.; Rodrigues, M.A.M.; Dias-da-Silva, A. (2008): Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. *Animal Feed Science and Technology*, *145*(1-4), 27–40. https://doi.org/10.1016/j.anifeedsci.2007.06.037

Janssen, P.H. (2010): Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal Feed Science and Technology*, *160*(1-2), 1–22. https://doi.org/10.1016/j.anifeedsci.2010.07.002

Jonova, S.; Ilgaza, A.; Zolovs, M. (2021): The impact of inulin and a novel synbiotic (yeast *Saccharomyces cerevisiae* strain 1026 and inulin) on the development and functional state of the gastrointestinal canal of calves. *Veterinary Medicine International*, *2021*. https://doi.org/10.1155/2021/8848441

Lepczyński, A.; Herosimczyk, A.; Barszcz, M.; Ożgo, M.; Michałek, K.; Grabowska, M.; Skomiał, J. (2021): Diet supplemented either with dried chicory root or Chicory inulin significantly influence kidney and liver mineral content and antioxidative capacity in growing pigs. *Animal*, *15*(2), 100129.

Liu, H.; Ivarsson, E.; Dicksved, J.; Lundh, T.; Lindberg, J.E. (2012): Inclusion of Chicory (*Cichorium intybus* L.) in pigs' diets affects the intestinal microenvironment and the gut microbiota. *Applied and environmental microbiology*, *78*(12), 4102–4109. https://doi.org/10.1128/AEM.07702-11

Mahmoud, Y.M. (2021): Effect of Supplementation of Dried Chicory (*Cichorium Intybus* L.) Leaves in Diets on Performance of Dairy Goats. *Egyptian Journal of Nutrition and Feeds*, *24*(1), 1–11.

Malmuthuge, N.; Guan, L.L. (2016): Gut microbiome and omics: a new definition to ruminant production and health. *Animal Frontiers*, *6*(2), 8–12. https://doi.org/10.2527/af.2016-0017

Mangwe, M.C. (2020): Strategic management of Chicory *(Cichorium intybus L.)* for improved milk quality and nitrogen utilization in dairy farm systems: A thesis submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy at Lincoln University (Doctoral dissertation, Lincoln University).

Masanetz, S.; Wimmer, N.; Plitzner, C.; Limbeck, E.; Preißinger, W.; Pfaffl, M.W. (2010): Effects of inulin and lactulose on the intestinal morphology of calves. *Animal*, *4*(5), 739–744. https://doi.org/10.1017/S1751731109991728

Mathew, N.; Kalyanasundaram, M. (2001): A high-performance liquid chromatographic method for the estimation of diethylcarbamazine content in medicated salt samples. *Acta tropica*, *80*(2), 97–102. https://doi.org/10.1016/S0001-706X(01)00163-2

Minneé, E.M.K.; Waghorn, G.C.; Lee, J.M.; Clark, C.E.F. (2017): Including Chicory or plantain in a perennial ryegrass/white clover-based diet of dairy cattle in late lactation: Feed intake, milk production, and rumen digestion. *Animal Feed Science and Technology*, *227*, 52–61.

Moss, A.; Jouany, J.P.; Newbold, J. (2000): Methane production by ruminants: its contribution to global warming. In *Annales de zootechnie. Vol. 49*, No. 3, pp. 231–253. https://doi.org/10.1051/animres:2000119

Patel, S.; Goyal, A. (2012): The current trends and future perspectives of prebiotics research: a review. *3 Biotech*, *pp. 2*, 115–125.

Roodposhti, P.M.; Dabiri, N. (2012): Effects of probiotic and prebiotic on average daily gain, fecal shedding of Escherichia coli, and immune system status in newborn female calves. *Asian-Australasian Journal of Animal Sciences*, *25*(9), 1255. https://doi.org/10.5713/ajas.2011.11312

Samal, L.; Behura, N.C. (2015): Prebiotics: an emerging nutritional approach for improving gut health of livestock and poultry. *Asian Journal of Animal and Veterinary Advances*, *10*(11), 724–739. DOI: 10.3923/ajava.2015.724.739

Samanta, A.K.; Jayapal, N.; Senani, S.; Kolte, A.P.; Sridhar, M. (2013): Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. *Brazilian Journal of Microbiology*, *44*, 1–14. https://doi.org/10.1590/S1517-83822013005000023

Tripathi, M.K.; Karim, S.A. (2011): Effect of yeast cultures supplementation on live weight change, rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and slaughtering performance of growing lamb. *Livestock Science*, *135*(1), 17–25. https://doi.org/10.1016/j.livsci.2010.06.007

Tzortzis, G.; Goulas, A.K.; Gee, J.M.; Gibson, G.R. (2005): A novel galactooligosaccharide mixture increases the bifidobacterial population numbers in a continuous in vitro fermentation system and in the proximal colonic contents of pigs in vivo. *The Journal of Nutrition*, *135*(7), 1726–1731.

Umucalilar, H.D.; Gulsen, N.; Hayirli, A.; Alatas, M.S. (2010): Potential role of inulin in rumen fermentation. *Revue Méd. Vét*, *161*(1), 3–9.

Uyeno, Y.; Shigemori, S.; Shimosato, T. (2015): Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environments*, *30*(2), 126–132. https://doi.org/10.1264/jsme2.ME14176

Wang, Y.; Nan, X.; Zhao, Y.; Jiang, L.; Wang, H.; Hua, D.; ... & Xiong, B. (2021): Dietary supplementation with inulin improves lactation performance and serum lipids by regulating dairy cows' rumen microbiome and metabolome. *Animal Nutrition*, *7*(4), 1189–1204. https://doi.org/10.1016/j.aninu.2021.09.007

Xiao, J.X.; Alugongo, G.M.; Chung, R.; Dong, S.Z.; Li, S.L.; Yoon, I; ... & Cao, Z.J. (2016): Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: Ruminal fermentation, gastrointestinal morphology, and microbial community. *Journal of Dairy Science*, *99*(7), 5401–5412. https://doi.org/10.3168/jds.2015-10563

Xu, Q.; Qiao, Q.; Gao, Y.; Hou, J.; Hu, M.; Du, Y.; ... & Li, X. (2021): Gut microbiota and their role in health and metabolic disease of dairy cow. *Frontiers in Nutrition*, *8*, 701511. https://doi.org/10.3389/fnut.2021.701511

Zeitz, J.O.; Ineichen, S.; Soliva, C.R.; Leiber, F.; Tschuor, A.; Braun, U.; ... & Clauss, M. (2016): Variability in microbial population and fermentation traits at various sites within the forestomach and along the digestive tract as assessed in goats fed either grass or browse. *Small Ruminant Research*, *136*, 7–17. https://doi.org/10.1016/j.smallrumres.2015.12.029

Zhou, M.; Hunerberg, M.; Chen, Y.; Reuter, T.; McAllister, T.A.; Evans, F.; ... & Guan, L.L. (2018); Air-Dried Brown Seaweed, &ITAscophyllum nodosum&IT, Alters the Rumen Microbiome in a Manner That Changes Rumen Fermentation Profiles and Lowers the Prevalence of Foodborne Pathogens. *MSPHERE*, *3*(1). https://doi.org/10.1128/msphere.00017-18

Zhu, W.; Wei, Z.; Xu, N.; Yang, F.; Yoon, I.; Chung, Y.; ... & Wang, J. (2017). Effects of *Saccharomyces cerevisiae* fermentation products on performance and rumen fermentation and microbiota in dairy cows fed a diet containing low quality forage. *Journal of Animal Science and Biotechnology*, *8*(1), 1–9.