

Chapter 18

Use of *Saccharomyces cerevisiae* as Probiotic in Ruminants Feed

Amna Tabassum^{1*}, Javeria Nousheen², Muhammad Mobashar³, Özlem KARADAĞOĞLU⁴, Shahid Hussain Farooqi⁵, Muhammad Arfan Zaman⁶, Shamreza Aziz¹, Tayyaba Akhtar², Ghulam Murtaza⁷ and Hizqeel Ahmed Muzaffar¹

¹KBCMA College of Veterinary and Animal Sciences, Narowal, Sub-campus UVAS-Lahore, Pakistan

²Department of Epidemiology and Public Health University of Veterinary and Animal Sciences Lahore, Pakistan

³Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, University of Agriculture, Peshawar, Pakistan

⁴Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

⁵Department of Clinical Sciences, KBCMA College of Veterinary and Animal Sciences, Narowal, Sub-campus UVAS-Lahore, Pakistan

⁶Department of Pathobiology, College of Veterinary and Animal Sciences Jhang, Sub-campus UVAS-Lahore, Pakistan

⁷National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad, Pakistan

*Corresponding author: syedaamnatabassum@gmail.com

ABSTRACT

Probiotics are live bacteria that give the host health benefits when given in sufficient doses. Yeast products have a variety of impacts, such as improving the operation of the intestinal barrier, changing the levels of bacteria in the gastrointestinal (GI) tract, and giving host bacteria useful substrates. By promoting the growth of the bacteria engaged in the process, yeast can affect the rumen's bio-hydrogenation pathway. Any alteration in WBC concentrations could indicate a better or more severe state of the illness. Cytokine production is necessary for immune system activation, which includes WBC recruitment and the induction of illness behaviour. Cachectin, B-cell stimulatory factor 2 (BSF-2), and type II interferon are examples of cytokines associated with inflammation that induce fever, illness behaviour, and the synthesis of APP. In contrast to untreated cows, nursing milking cows treated using *Saccharomyces cerevisiae* cultures or living yeast had higher serum glucose and lower nitrogen in their blood.

KEYWORDS

Probiotic; *Saccharomyces cerevisiae*; Yeast products; Bio-hydrogenation; Immune system activation

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INTRODUCTION

Probiotics are live bacteria that give the host health benefits when given in sufficient doses (Czerucka et al., 2007; Kumprechtová et al., 2019). Aside from the well-researched and well-known 2 Hydroxypropanoic acid bacteria, yeasts may also function as live microorganisms for improving growth (Altmann, 2017; Fomenky et al., 2018). The interest in this topic has grown over the past few years; over 31,000 articles using the term probiotic are indexed by PubMed, and over 15,000 of those have been printed in the past five years (Fig. 1). However, probiotic *S. cerevisiae* study makes up a small portion of this, with lesser than 850 articles published by PubMed in the past five years. The printing goal is to analyze the most recent data regarding probiotic and possibly probiotic yeasts and their use in different types of health supplemented food.

Etiology

S. cerevisiae from many families have been extracted from food sources and natural environments. By producing a variety of fermented foods, these potentially probiotic yeasts can improve their nutritional and sensory qualities. On the other hand, *Saccharomyces* is the only yeast genus that is productive in double-masked studies (Czerucka et al., 2007). Probiotics are capable of developing at 37°C, bear the harsh conditions of the human gastrointestinal tract (such as acidic pancreatico-biliary secretion, bile acids, and an acidic pH), and improve the health of an individual's health by modulating the microbes that reside there and carrying out biological activities (Table 1). Some probiotics can even stick to the secretions of gut epithelial cells (Sanchez et al., 2014). It is one of the most extensively used commercial treatments for diarrhoea in the world, having been used since the 1950s (McFarland, 1996; Altmann, 2017).

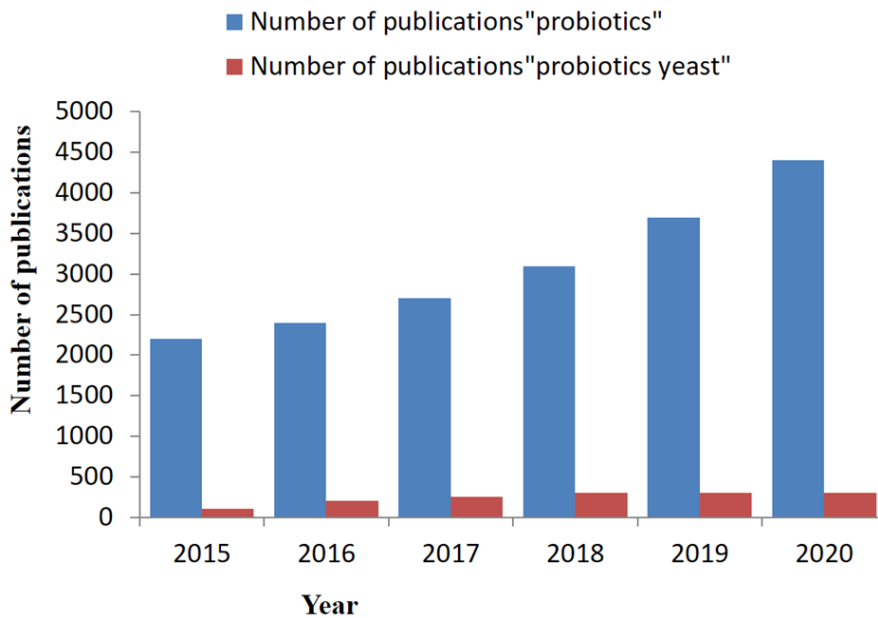


Fig. 1: Analysis of publications by PubMed in the past five years.

Table 1: Distinctive features of *Saccharomyces cerevisiae* var. *boulardii* and *Saccharomyces cerevisiae* (Altmann, 2017).

No.	Features	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces cerevisiae</i> var. <i>boulardii</i>
1.	Capability to grow at 37 °C	negative	positive
2.	Milk sugar used as a source of carbon	positive	Negative
3.	Ascospores generating capacity	positive	Negative
4.	Capability to withstand pH 2.5	Negative	positive
5.	Extra versions of chromosome 9	Negative	positive
6.	Increased capacity for pseudohyphal transitioning	Negative	positive
7.	No. of chromosome sets	bipartite or monoploid	double

Furthermore, compared to *S. cerevisiae*, *S. cerevisiae* var. *boulardii* has more copies of the genes involved in response to stressed conditions and translation. These genes might be involved in pseudo-hyphal transitioning, rapid development rates, and enhanced resilience to raise pH (Czerucka et al., 2007). Heat shock proteins, elongation factors, ribosomal proteins, enzymes activating inactivated proteins, carriers, and fluoride efflux are usually encoded by duplicated and triplicated genes, and these proteins may be useful in adapting to stressful environments. Additionally, it has been found that *S. cerevisiae* var. *boulardii* possesses many genes related to pseudo-hyphal development, each with varying copies (Altmann, 2017).

Impact of *S. cerevisiae*

According to research, *S. cerevisiae* has no discernible impact on ammonia content, methane generation, or overall VFA concentrations (Durand-Chaucheyras et al., 1997). The type of diet being evaluated appears to have a significant impact on how yeast supplementation affects pH stabilization. Yeast augmentation has often been associated with greater rumen pH, while the regulated pH tended to be lower than 6. According to Fiems et al. (Fiems et al., 1999), sheep cater for a concentrate diet consisting of maize silage and cereals with a high dextrose and glycogen content showed a greater influence of yeast on the ruminal pH compared to sheep fed grass hay and sugar beet gloop. Yeast products have a variety of impacts, such as improving the operation of the intestinal barrier, changing the levels of bacteria in the gastrointestinal (GI) tract, and giving host bacteria useful substrates. Controlling agitation in the GI tract can have a significant positive effect on the host by improving nutrition uptake and minimizing the migration of pathogenic microorganisms and the production of toxins systemically. This frequently happens as a result of changes in the GI tract's crypt and villi properties, tight connections between epithelial cells, and mucin formation. For instance, it was observed that pigs given yeast supplements had thinner intestinal mucus than control pigs, which may enhance nutrient absorption (Bontempo et al., 2006; Di Giancamillo et al., 2007). Furthermore, newborn pigs treated with yeast showed increases in crypt depth and villus height, two indicators of good intestinal health (Di Giancamillo et al., 2007). Dairy calves' GI tract development was enhanced by YCW supplementation (Ma et al., 2020). High-grain diets are frequently fed to cattle to boost effectiveness, but doing so comes at the cost of a rise in acidification prevalence, which is linked to reductions in rumen pH and dry matter intake (DMI), as well as increases in inflammation and disarray of the gastrointestinal barrier's function (Bradford et al., 2015). Before and after the calving in cows, yeast was discovered to alter the transcription of genes associated with the immune system in the rumen. This finding suggests that yeast is controlling inflammation and gut barrier integrity during this transitional phase (Bach et al., 2018). Moreover, it has been discovered that yeast not only boosts the number of cellulolytic microorganisms in the rumen that crash incomprehensible fibre but also scavenges oxygen, which may

contribute to pH stabilization and enhanced absorption of fibre-based feedstuffs (Chaucheyras-Durand et al., 2008; Pinloche et al., 2013; Habeeb, 2017). Reduced lactic acid levels in the rumen are typically linked to pH stabilization. Yeast-induced declines in 2-hydroxypropanoic acid concentrations and the required ruminal pH regulation may be explained by the enhancement of lactic acid-utilizing bacteria. It has been demonstrated that lactic acid-consuming bacteria such as *S. ruminantium*, which uses mannitol, are promoted in the laboratory using *Saccharomyces cerevisiae* in a blend of stomach fluid cultures (Newbold et al., 1998).

Additionally, *S. cerevisiae* can contend with *Streptococcus gallolyticus*, the primary rumen 2-Hydroxypropanoic acid generator, for the uptake of soluble carbohydrates (Chaucheyras et al., 1995). Girard (Girard, 1996) demonstrated that several yeast cell fractions contained both heat-stable (short-chain peptides) and heat-labile (likely lipidic) stimulating components. It has been demonstrated that yeast supplies vitamins, particularly vitamin B1, to promote the growth of rumen fungus (Chaucheyras et al., 1995). It was also proposed to remove oxygen, which would prevent the rumen's exclusively anaerobic bacteria from growing. Although the liquids that come out of the rumen are mainly oxygen-deprived, during the daily feeding cycle, a decreased amount of dissolved oxygen can be observed. While the animal is feeding, oxygen is taken up by the rumen from both the feed and the saliva. The availability of oxygen in the rumen during consumption of food, grinding, and water consumption is mostly responsible for the increase in redox potential seen in sheep following meals (Mathieu et al., 1996). Linoleic acid undergoes ruminal biohydrogenation, which turns it into rumenic acid. It then hydrogenates this acid to create trans-11 (vaccenic acid), which is then converted into octadecanoic acid (Harfoot, 1981; Kim et al., 2000). The hydrogenation by biosynthesis of cis-9,12-Octadecadienoic and cis-9,12-Octadecadienoic acid in the rumen is facilitated by several bacterial species. Groups A and B can be created from these bacteria based on the products that they produce during fermentation. While group B is specialized in producing stearic acid (18:0), group A's bacteria are in charge of converting linoleic and linolenic fatty acids into trans-11 (vaccenic acid). Some strains of *Butyrivibrio fibrisolvens* are among the rumen bacteria in group A, while two strains of *Fusocillus* are found in group B (Harfoot, 1981). By promoting the growth of the bacteria engaged in the process, yeast can affect the rumen's biohydrogenation pathway. Since the enzyme Δ^9 -desaturase converts trans-11 to cis-9, trans-11 CLA in the tissue, an increase in the ruminal manufacturing of trans-11 C18:1 indicates that beef and dairy products will contain more cis-9, trans-11 CLA (Figure 2) (Bauman et al., 1999; Bauman et al., 2020).

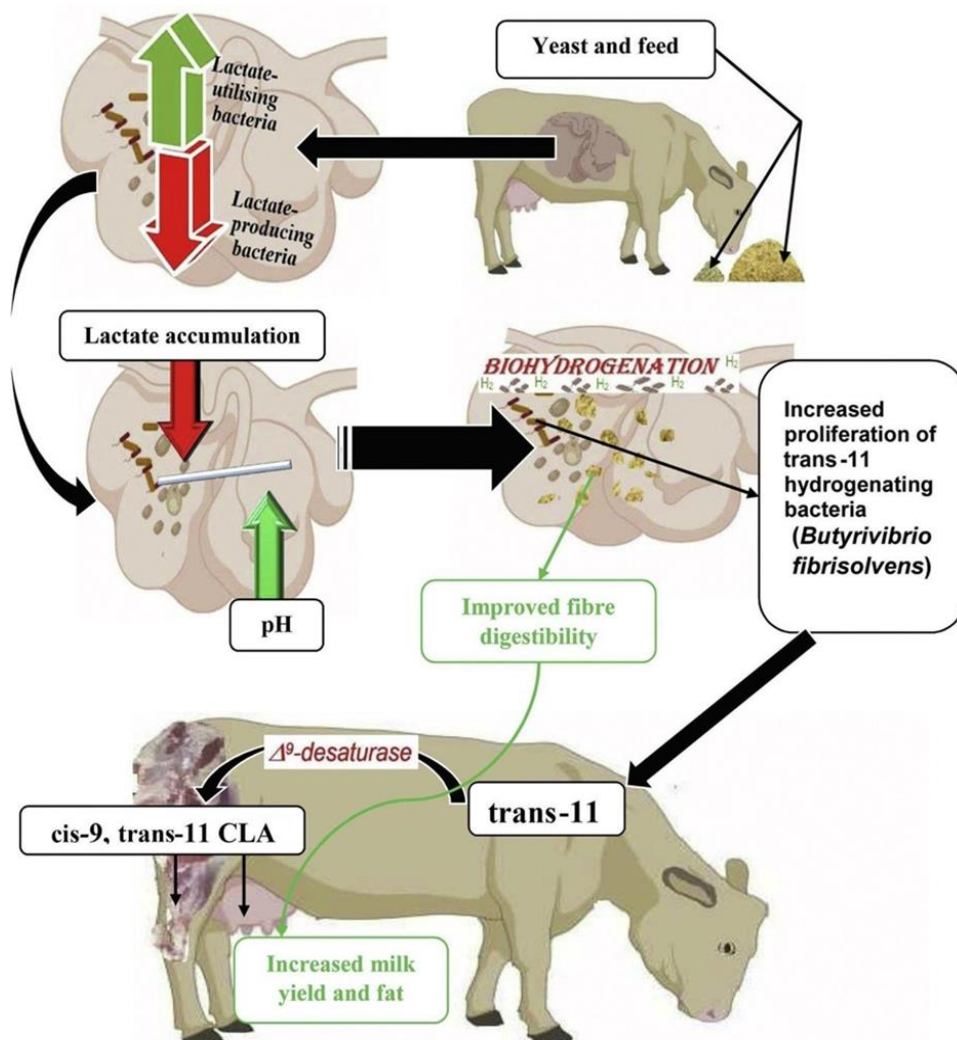


Fig. 2: The benefits of adding yeast culture to feeds with varying carbohydrate contents on cow milk production efficiency and eating behaviour.

Anti-adhesive Properties of Yeast

It is widely acknowledged that the initial stage of bacterial infection of mucous membranes is epithelial adherence. Similar to the link between antigens and antibodies, bacteria have attaching molecules on their surfaces that can interact stereospecifically with host-cell membranes. There is proof that some strains of salmonella or *E. Coli* have a fibroblast adherence that attaches to mannose residues on the membranes of epithelial cells (Ofek et al., 1977). According to Korhonen (1979), these bacteria or their separated fimbriae will also agglutinate yeast that has mannan in the outer layer of their cell wall. D- mannose solutions prevent this agglutination (Korhonen, 1979).

Pathogens that bind to the cell wall of yeast have an antimicrobial effect because the combined structure mix of *S. cerevisiae* and pathogen is quickly removed from the gastrointestinal tract (Gedek, 1989). Yeast's helpful activity may be explained by pathogens and yeast competing for intestinal cell adhesion, which is essential for the manifestation of the cytopathogenic effect (Oyofe et al., 1989; Line et al., 1998). Both mannose and yeast therapy dramatically decreased the frequency of *Salmonella typhimurium* colonization in broilers; however, the addition of yeast had little effect on *Campylobacter* colonization. It has also been demonstrated that *S. cerevisiae* inhibits the adherence of *Entamoeba histolytica* trophozoites and *Staphylococcus aureus* to human cells (Rigothier et al., 1990; Kvidera et al., 2017).

Yeast and Immunity Stimulation in Ruminants

Studies showing that when engaged, the defence systems of pigs and cattle utilize about 1 kg of glucose in 12 hours provide evidence for the energy need (Bontempo et al., 2006; Kvidera et al., 2016). When yeast is added to cattle and pigs, the immune system responds in different ways (Table 2). The non-specific response, which includes the first, is generally innate to an infective agent, and the natural immune response, which is an infection-specific response, comprises the two components of the immune system. Supplementing with yeast has been shown to influence both of these immunological responses.

WBC fluctuations may be a sign of an infection. Therefore, any alteration in WBC concentrations could indicate a better or more severe state of the illness. The most common WBC subtype in pigs and cattle is T cells, which are accompanied by monocytes. Steers' circulating neutrophils and lymphocytes significantly decreased in response to a vaccination challenge; however, this response was not observed in steers given a disintegrated yeast supplement (Kim et al., 2011).

Table 2: The outcome of *saccharomyces cerevisiae* and cerevisiae-based products ingestion on the immunological reaction in bovine and pigs.

No.	Classification	<i>Saccharomyces cerevisiae</i>	Effect on Immunological Response	Reference
1.	Beef Cattle	decomposed <i>Saccharomyces cerevisiae</i>	restrained decline in leukocyte	1 (Kim et al., 2011)
2.	Beef Cattle	YCW3	Diminished IL-6 concentrations	(Sanchez et al., 2013)
3.	Beef Cattle	YCW	Diminished acute phase proteins	(Lei et al., 2013)
4.	Dairy Calves	Yeast	Raised neutrophil ability	(Ryman et al., 2013; Fomenky et al., 2018)
5.	Sows	SCFP	Decreased leukocyte amounts	(Shen et al., 2009)
6.	Weaned pigs	YCW	Decreased leukocyte amounts	(Burdick Sanchez et al., 2020)
7.	Weaned pigs	<i>Saccharomyces cerevisiae</i>	Raised leukocyte; diminished inflammation causing lymphokines levels	(Collier et al., 2011)
8.	Weaned pigs	<i>Saccharomyces cerevisiae</i> culture	Reduced type II interferon level and TH cells	(Shen et al., 2009)
9.	Weaned pigs	β -glucan	Raised CD4+ T cells	(Hahn et al., 2006)
10.	Weaned pigs	SCFP	Raised amount of inflammation causing lymphokines	(Sanchez et al., 2018)
11.	Weaned pigs	MOS-4	Raised WBC; decreased cytokine concentrations	(Che et al., 2011)
12.	Weaned pigs	β -glucan	Raised cytokine amounts	(Li et al., 2006)
13.	Weaned pigs	β -glucan	Diminished cachetin and BSF2; Increased IL-10	(Li et al., 2005)
14.	Weaned pigs	Yeast	Unchanged lymphocyte amount	(Lessard et al., 2009)

SCFP: *S. cerevisiae* fermentation product; 2 YCW: Yeast cell wall; 3 MOS: mono oligosaccharide 4 B-cell stimulatory factor 2.

On the other hand, it was shown that beef cows treated with a Yeast fermented product (SCFP) before being challenged with LPS had greater quantities of thrombocyte and Leukocyte populations, but that after the immunological obstacle, their levels of cytokines that promote inflammation were lower (Burdick Sanchez et al., 2020). According to the authors, the higher WBC numbers before the LPS challenge primed the immune system, getting it ready for the obstacle and eventually leading to the lower mediator amounts seen after the test. However, supplementing heifers with a mixture of live yeast and YCW products did not alter the level of WBCs before or after an outbreak of breathing disorders caused by bacteria and viruses (Word et al., 2019).

Hence, WBC populations appear to respond differently to yeast supplementation in pigs, much like they do in cattle. Alterations in immune cell subtypes have also been examined in research. For instance, weaned pigs were given yeast culture supplementation and exhibited a decrease in CD4+ cells or T- helper cells (Shen et al., 2009). Nonetheless, it was discovered that weaned pigs given nutritional β -glucan derived from yeast had a higher number of CD4+ cells (Hahn et al., 2006). These results show that variations in particular leukocyte communities are also impacted by the difficult setting and saccharomyces food additive employed. Cytokine production is necessary for immune system activation, which includes WBC recruitment and the induction of illness behaviour. Cachectin, B-cell stimulatory factor 2 (BSF-2), and type II interferon are examples of cytokines associated with inflammation that induce fever, illness behaviour, and the synthesis of APP. They also promote the creation of other cytokines that aid in the immunological response. On the other hand, anti-inflammatory cytokines like cytokine synthesis inhibitory factor (CSIF) and Pitracinrabort stimulate the adaptive immune system and lessen inflammation. As a result, mediator ratios may be a useful marker of stimulation of the immune system and systemic inflammation. When beef heifers are fed with two distinct YCW products, their BSF2 values in blood samples after an LPS defiance were lower than those of non-supplemented heifers in terms of both amplitude and durability (Sanchez et al., 2013). Decreased temperature of the bowel was also noted in the study, which may indicate a general decrease in the inflammatory response in addition to this TNF response. On the other hand, inflammation-causing lymphokines cachexin and BSF2 were shown to be enhanced in the serum for a comparable amount of time in weaned pigs supplemented with SCFP as opposed to non-supplemented pigs (Sanchez et al., 2018). Before an LPS challenge, supplementing young pigs with β -glucan led to decreased levels of BSF-2 and cachexin, but raised IL-10 during the acute (3–6 hours) period that followed LPS injection (Li et al., 2006). The authors hypothesized that this cytokine response could explain the better pig performance seen in the study by indicating a reduction in an allergic reaction that promotes swelling and an elevation in the inhibition of inflammation. In a similar vein, it was discovered that weaned pigs given MOS supplements and confronted by the virus that causes pig fertility and lung infections had lower TNF- α but higher IL-10 concentrations after infection, as well as higher WBC concentrations in the early stages (Che et al., 2011). Thus, depending on the product and challenge model, there is a variance in serum lymphokine amount, which is comparable to changes in WBC populations. These variations are probably responsible for variations in cytokine duration as well as amplitude. The APP binds pathogens, initiates complements, and binds cellular debris, among other actions, that assist the immune response. Furthermore, while cytokines that cause inflammation have significance for the invulnerable reaction excess stimulation of these inflammatory mediators for a longer duration can lead to an inflammatory hypercondition that could be harmful to the animal's well-being and healing (Gruys et al., 2005).

Impact of Yeast on Metabolic Activity and Expected Mechanisms of Action

When yeast products are added to cow and swine feed, significant variations in the immunological response have been noted; however, the metabolic process seems to be more stable (Table 3). Weaned beef cows supplemented with yeast cell walls had different metabolic responses to LPS challenge; higher glucose and insulin responses were seen, while non-esterified fatty acid (NEFA) concentrations were decreased (Sanchez et al., 2014). Steers supplemented with SCFP after an LPS exposure similarly showed increased glucose responses (Burdick Sanchez et al., 2020). According to these two studies, adding yeast products to an immunological challenge may increase its energy availability, which could help hasten its resolution. Likewise, higher sucrose amounts were noted in Holstein calves that did not exhibit passive transfer after being supplemented with yeast (Galvão et al., 2005). Furthermore, contrasted to untreated cows, nursing milking cows treated using *S. cerevisiae* cultures or living yeast had higher serum glucose and lower nitrogen in their blood (Doležal et al., 2011; Dehghan-Banadaky et al., 2013).

Table 3: Impact of supplementing with saccharomyces and saccharomyces-based commodities on pigs and bovine metabolic metrics. YCW: yeast cell wall; NEFA: non-esterified fatty acid; SCFP: *Saccharomyces cerevisiae* fermentation product.

No.	Family	<i>Saccharomyces cerevisiae</i> Derivatives	Digestion Domain	Reference
1.	Beef calves	YCW 1	Raised glucose and insulin decreased NEFA 2	(Sanchez et al., 2014)
2.	Beef calves	SCFP 3	Accentuated glucose	(Burdick Sanchez et al., 2020)
3.	Feeder calves	Live yeast and YCW	Kindred glucose; decreased urea nitrogen	(Word et al., 2019)
4.	Dairy calves	Yeast	Raised glucose	(Galvão et al., 2005)
5.	Milking cows	Live yeast or Yeast culture	Raised glucose; diminished urea nitrogen	(Galvão et al., 2005; Doležal et al., 2011)
6.	Milking cows	Live yeast	No effect on glucose	(Dehghan-Banadaky et al., 2013)
7.	Weaned pigs	YCW	Diminished NEFA levels	(Burdick Sanchez et al., 2020)
8.	Sows	SCFP	Ability for decreased urea nitrogen	(Shen et al., 2009)

Living yeast-fed cows showed increased numbers of fibrolytic and lactate-utilizing bacteria (Pinloche et al., 2013). As previously mentioned, this is one of the possible ways that yeast works and could lead to a more stable pH. Elevations in

both fibrolytic and cellulolytic bacteria facilitate the breakdown of fibre-based livestock feed, hence stabilizing the pH of the rumen and increasing the amount of swallowed tubular animal feed that is utilized. Furthermore, it has been shown that yeast products, like SCFP, raise rumen propionate concentrations, which may contribute to an increase in cattle's serum glucose levels (Zhu et al., 2017). Dairy cows treated with live yeast showed higher levels of total VFA and acetate, indicating increased substrate availability for microbial glucose synthesis (Kumprechtová et al., 2019). Dairy cows treated with live yeast showed higher levels of total VFA and acetate, indicating increased substrate availability for microbial glucose synthesis (Kumprechtová et al., 2019). Furthermore, compared to non-supplemented cows, glucose was higher and NEFA was less at the height of lactation, indicating a potential role for yeast in reducing the stress associated with an imbalance in energy. All the same, it seems that yeast contributes to higher blood sugar levels which might offer more energy for the immune system to use and possibly hasten the healing process following an infection.

Conclusion

The field of probiotic research has experienced rapid growth in the last several years, with a growing interest in the use of probiotic yeasts, which has been used sparingly until now. When yeast is added to cattle and pigs, the immune system responds in different ways WBC fluctuations may be a sign of an infection. Therefore, any alteration in WBC concentrations could indicate a better or more severe state of the illness. Compared to *S. cerevisiae*, *S. cerevisiae var. boulardii* has more copies of the genes involved in response to stressed conditions and translation. These genes might be involved in pseudo-hyphal transitioning, rapid development rates, and enhanced resilience to raise pH. It might be feasible in future that probiotics may be recommended as drugs either in addition to or as substitute of antibiotics for certain medical conditions.

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