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REVIEW ARTICLE

Dynamic role of single-celled fungi in ruminal microbial ecology and activities

M.M.Y. Elghandour[1], A. Khusro[2], M.J. Adegbeye[3], Z. Tan[4], S.H. Abu Hafsa[5], R. Greiner[6], E.A. Ugbogu[7], U.Y. Anele[8] and A.Z.M. Salem[1]

1 Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, México
2 Research Department of Plant Biology and Biotechnology, Loyola College, Nungambakkam, Chennai, Tamil Nadu, India
3 Department of Animal Science, College of Agriculture and Natural Sciences, Joseph Ayo Babalola University, Ikeji-Arakeji, Ilesha, Nigeria
4 Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Hunan, P.R. China
5 Department of Livestock Research, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El-Arab, Alexandria, Egypt
6 Department of Food Technology and Bioprocess Engineering, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany
7 Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria
8 North Carolina Agricultural and Technical State University, Greensboro, NC, USA

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Correspondence
Abdelfattah Z.M. Salem, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Estado de México, México.
E-mails: asalem70@yahoo.com and salem@uaemex.mx

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Summary

In ruminants, high fermentation capacity is necessary to develop more efficient ruminant production systems. Greater level of production depends on the ability of the microbial ecosystem to convert organic matter into precursors of milk and meat. This has led to increased interest by animal nutritionists, biochemists and microbiologists in evaluating different strategies to manipulate the rumen biota to improve animal performance, production efficiency and animal health. One of such strategies is the use of natural feed additives such as single-celled fungi yeast. The main objectives of using yeasts as natural additives in ruminant diets include; (i) to prevent rumen microflora disorders, (ii) to improve and sustain higher production of milk and meat, (iii) to reduce rumen acidosis and bloat which adversely affect animal health and performance, (iv) to decrease the risk of ruminant-associated human pathogens and (v) to reduce the excretion of nitrogenous-based compounds, carbon dioxide and methane. Yeast, a natural feed additive, has the potential to enhance feed degradation by increasing the concentration of volatile fatty acids during fermentation processes. In addition, microbial growth in the rumen is enhanced in the presence of yeast leading to the delivery of a greater amount of microbial protein to the duodenum and high nitrogen retention. Single-celled fungi yeast has demonstrated its ability to increase fibre digestibility and lower faecal output of organic matter due to improved digestion of organic matter, which subsequently improves animal productivity. Yeast also has the ability to alter the fermentation process in the rumen in a way that reduces methane formation. Furthermore, yeast inclusion in ruminant diets has been reported to decrease toxins absorption such as mycotoxins and promote epithelial cell integrity. This review article provides information on the impact of single-celled fungi yeast as a feed supplement on ruminal microbiota and its function to improve the health and productive longevity of ruminants.

Introduction

Optimal rumen health is a prerequisite for animal welfare and profitable production. A rumen is healthy when there are abundance of favourable/beneficial microbes, large rumen papillae for an efficient nutrient absorption, possesses good barrier function and support good fibre digestibility (Faniyi *et al.* 2019) and a well-regulated rumen pH. The pH is a direct homoeostatic result of the acid–base balance regulation efforts of the rumen and the host (Aschenbach *et al.* 2011). At the same time, the rumen pH is also a crucial parameter to ensure the normal functioning of the rumen and its microbiome. Therefore, it is the closest and most accurate indicator of rumen health or disorders such as subacute ruminal acidosis (SARA), because it provides direct information about the conditions within the rumen (Enemark 2008). Recently, Zhao *et al.* (2018) indicated that SARA produces a high concentration of ruminal lipopolysaccharides (LPS), which over-activates the inflammatory pathways and significantly increases the expression and synthesis of proinflammatory cytokines in the rumen epithelium, thus causing partial inflammation of the rumen. Low rumen pH ranging from 5–6 for prolonged periods can negatively affect feed intake, microbial metabolism, and nutrient degradation, erratic appetite, body weight loss, ruminal motility, stasis, hyperkeratosis, and leads to acidosis, inflammation, laminitis, diarrhoea and villi wear out and bloat (Rodríguez-Lecompte *et al.* 2014). One of the consequences of a low rumen pH is the reduction/elimination of protozoa, the most effective mycotoxin degraders among the rumen microbial community. This shift in the microbiome causes a decline in the Gram-negative *Bacteroidetes* and a drastic shift to Gram-positive lactic acid producers (*Streptococcus* and *Lactobacillus* sp.) in the foregut and hindgut of ruminants (Chiba 2014). Manipulating the rumen microbiome to enhance productivity and health of ruminants could be limited by the resilience of the microbial community in the rumen of mature animals (Yáñez-Ruiz *et al.* 2015). Although the effect of manipulating the rumen microbiome may not be permanent in the long term, it might have a positive effect on fattening/milk yield (Ogbuewu *et al.* 2018). Yeasts have given better results in ruminants (Seo *et al.* 2010). Broadway *et al.* (2015) reported that yeast supplements caused a range of effects in the rumen such as elevated ruminal pH and concentrations of volatile fatty acids (VFA), decreased methane production and increased the total number of micro-organisms and cellulolytic bacteria. Additionally, yeast supplements have been shown to improve the growth of lactic acid-utilizing micro-organisms and reduce lactic acid accumulation in the rumen (Marden *et al.* 2008), remove oxygen and increase the total rumen microflora (Chaucheyras-Durand and Durand 2010). Recently, live yeast supplements in the diet was reported to improve rumen fibre degradation in cattle grazing tropical pastures (Sousa *et al.* 2018). A positive effect of yeast in reducing the severity of SARA irrespective of its viability was observed by (Vyas *et al.* 2014) and this may also reduce the content of LPS in the rumen. Dietary supplements of both live yeast and yeast cell wall may enhance growth performance of beef cattle by reducing LPS concentrations in the rumen and plasma and improving other related aspects (Peng *et al.* 2019). Lei *et al.* (2013) showed that dietary supplementation of yeast cell wall at a dose of 2 g kg^{-1} DM could effectively bind LPS in the digestive tract, reduce the translocation of LPS from the digestive tract into circulation, and thereby improve growth performance in beef cattle. Yeast supplementation improved reproductive performance of dairy cows during heat stress due to the alteration of hormones and ovarian follicular dynamics (Nasiri *et al.* 2018). Habeeb (2017) reported that diet mixture containing yeast-improved appetite, resulting in increased feed intake, thus leading to increased daily weight gain. Kowalik *et al.* (2012) stated that supplementation of 10 g of yeast on heifer diet decreased blood protein, triacylglycerol and cholesterol content. Liu *et al.* (2018) noted that mannanoligosaccharide content is about 30% in the yeast cell wall and has a high antioxidant activity. Feed supplementation with yeast cell wall reduced the absorption of aflatoxin B1, increased the elimination of aflatoxin B1 and M1, and increased immune system in ewes (Firmin *et al.* 2011). This review therefore provides insight on the effect of single-celled fungi on the overall activities of rumen micro-organisms and the health of ruminants.

Effect of feeding single-cell fungi on ruminants

The rumen ecosystem

The rumen is a complex, dynamic ecosystem consisted of mostly anaerobic bacteria, anaerobic fungi, protozoa, methanogenic archaea and phages. These microbes interact closely with each other and have a symbiotic relationship—by supplying the host (ruminants) with microbial proteins, VFAs and vitamins (Mizrahi 2013) (Fig. 1). Studies on microbial communities and ruminal characteristics are important for understanding and manipulating ruminant performance and health (Huws *et al.* 2018). Recently, it was hypothesized that these microbes exhibit niche specialization in utilization of nutrients and they also engineer the rumen ecosystem in terms of subsequent microbial colonization and nutrient utilization (Pereira and Berry 2017; Shaani *et al.* 2018). In general, yeast supplementation manipulates rumen microbiome

population resulting in improved energy supply (enhanced VFAs production) and improved protein nutrition (greater microbial protein synthesis and more efficient conversion of dietary N to milk N) of lactating cows fed diets containing low-quality forages.

Rumen bacteria

Bacteria are the major abundant microbes in the foregut of ruminants, with approximately 10^{10}–10^{11} cells per ml and over 200 species (McSweeney and Mackie 2012). As a whole, they have many enzymatic activities (i.e. amylases, lipase, protease, cellulase and xylanase) that digest fibres, starch, proteins, lipids and plant cell walls to give useful compounds and elements necessary for the growth and productivity of the animals (Huws *et al.* 2018) (Table 1). In ruminant nutrition, yeast is commonly being used due to its efficient role in rumen stabilization and maintenance of microbial communities specifically fibrolytic bacteria. Supplementation of a *Saccharomyces cerevisiae* fermentation product stimulated the growth of cellulolytic bacterial population (*R. flavefaciens and F. succinogenes*) as well as reduced lactate-producing bacteria (*Streptococcus bovis*) thereby contributing to the stability of rumen fermentation (Zhu *et al.* 2017). The inclusion of *S. cerevisiae* at dose of 5 g per day per head in early

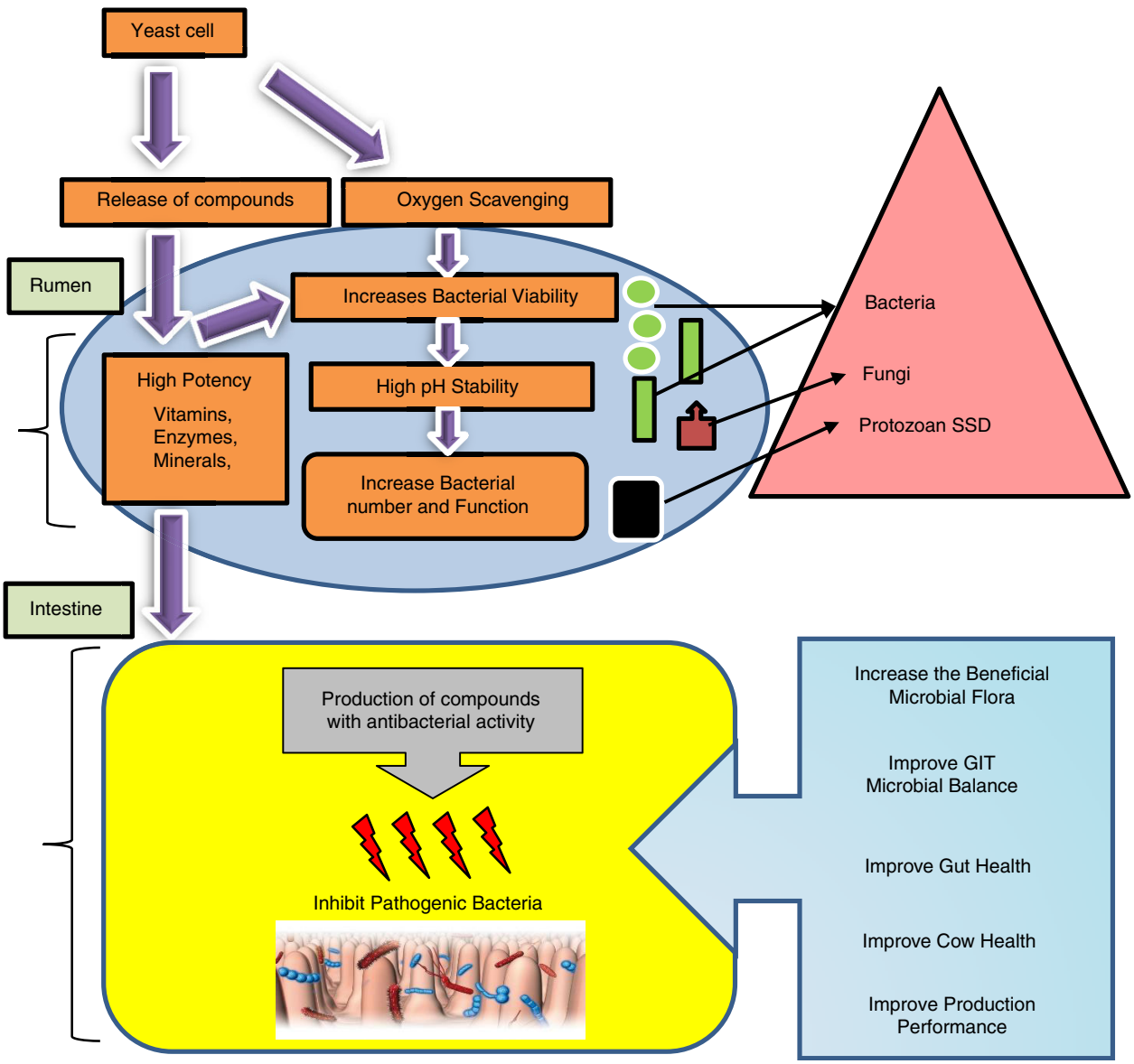

Figure 1 The effect of live yeast on the microbial flora of the gastrointestinal tract in ruminants (Figure adapted from Ghazanfar *et al.* (2017)). [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 Characteristics of principal ruminal bacteria

Micro-organisms	Gram stain	Morphology	Fermentation products	References
Cellulose-degrading bacteria:				
Fibrobacter succinogenes	Negative	Bacillus	Succinate, acetate, formate	Ivan *et al.* (2012)
Butyrivibrio fibrisolvens	Negative	Bacillus curve	Acetate, formate, lactate, butyrate, H_2, CO_2	Weimer (1996)
Ruminococci albus	Positive	Cocci	Acetate, formate, H_2, CO_2	Michalet-Doreau *et al.* (2001)
Clostridium lochheadii	Positive	Bacillus (espores)	Acetate, formate, butyrate, H_2, CO_2	Weimer (1996)
Amylolytic bacteria:				
Bacteriodes ruminicola	Negative	Bacillus	Formate, acetate, succinate	Cotta (1988)
Ruminobacter amylophilus	Negative	Bacillus	Formate, acetate, succinate	
Selenomonas ruminantium	Negative	Bacillus curve	Acetate, propionate, lactate	Cotta (1992)
Succinomonas amylolítica	Negative	Oval	Acetate, propionate, succinate	
Streptococci bovis	Positive	Cocci	Lactate	Cotta (1988), McAllister *et al.* (1990)
Lipolytic bacteria:				
Anaerovibrio lipolytica	Negative	Bacillus	Acetate, propionate, acetate	Fuentes *et al.* (2009)
Lactate-degrading bacteria:				
Selenomonas lactilytica	Negative	Bacillus curvado	Acetate, succinate	Brown *et al.* (2006)
Megasphaera elsdenii	Positive	Cocci	Acetate, propionate, butyrate, valerate, H_2, CO_2	
Pectin-degrading bacteria				
Lachnospira multiparus	Positive	Bacillus curve	Acetate, formate, lactate, H_2, CO_2	Duskova and Marounek (2001)
Ruminal archaea (methanogens)				
Methanobrevibacter ruminantium	Positive	Bacillus	CH4 (of H_2+CO_2 or formate)	Yanagita *et al.* (2000), Hook *et al.* (2010)
Methanomicrobium mobile	Negative	Bacillus	CH4 (of H_2+CO_2 or formate)	
Lactic acid-utilizing bacteria	Lactic acid-utilizing bacteria	Lactic acid-utilizing bacteria	Lactic acid-utilizing bacteria	Lactic acid-utilizing bacteria
Megasphaera elsdenii	*Megasphaera elsdenii*	*Megasphaera elsdenii*	*Megasphaera elsdenii*	*Megasphaera elsdenii*

lactating cows increased rumen pH, fibrolytic and lactate-utilizing bacteria (Pinloche *et al.* 2013). Zhu *et al.* (2017) reported that rumen microbial population was altered in response to *S. cerevisiae* supplementation. Supplementation with *S. cerevisiae* could provide various growth factors, provitamins, and/or micronutrients that help stimulate the growth of ruminal bacteria (Newbold *et al.* 1995; Fig. 1). Vallejo-Hernández *et al.* (2018) reported that high bacterial counts were observed with the inclusion of 4 mg of *S. cerevisiae* in goat and sheep inocula. Increases in bacterial numbers recovered from the rumen are the most reproducible effects of dietary yeast supplementation. Yeast cells in the rumen use available oxygen on the surfaces of freshly ingested feed to maintain metabolic activity and help remove oxygen in the rumen (Newbold *et al.* 1996). This creates better conditions for the growth of strict anaerobic cellulolytic bacteria, stimulates their attachment to forage particles and increases the initial rate of cellulolysis (Seo *et al.* 2010) (Fig. 2). Supplementation of yeast increases the diversity of microbes and stimulates the growth of fibrolytic bacteria (Ruminococcaceae) for colonization, leading to increased production of butyrate, and a reduction in the incidence of diarrhoea in large intestine (Xiao *et al.* 2016). Yeast supplements have been reported to enhance the production of organic acids and vitamins to activate the growth of the lactic acid bacteria (Campanile *et al.* 2008).

Rumen protozoa

Ciliate protozoa represent a large proportion of 10^4–10^6 cells per ml in rumen fluid and are responsible for 30–40% of overall fibre digestion (McSweeney and Mackie 2012) (Table 2). Protozoa play different roles in the rumen especially on the pH of the rumen and detoxification of mycotoxin. However, they are also well-known for their contribution to the production of greenhouse gases (GHGs) including methane. In addition to their ability to degrade fibres, protozoa have been closely associated with methanogenesis as defaunation reduces methane production by about 11% (Morgavi *et al.* 2010; Newbold *et al.* 2015). This is probably due to the fact

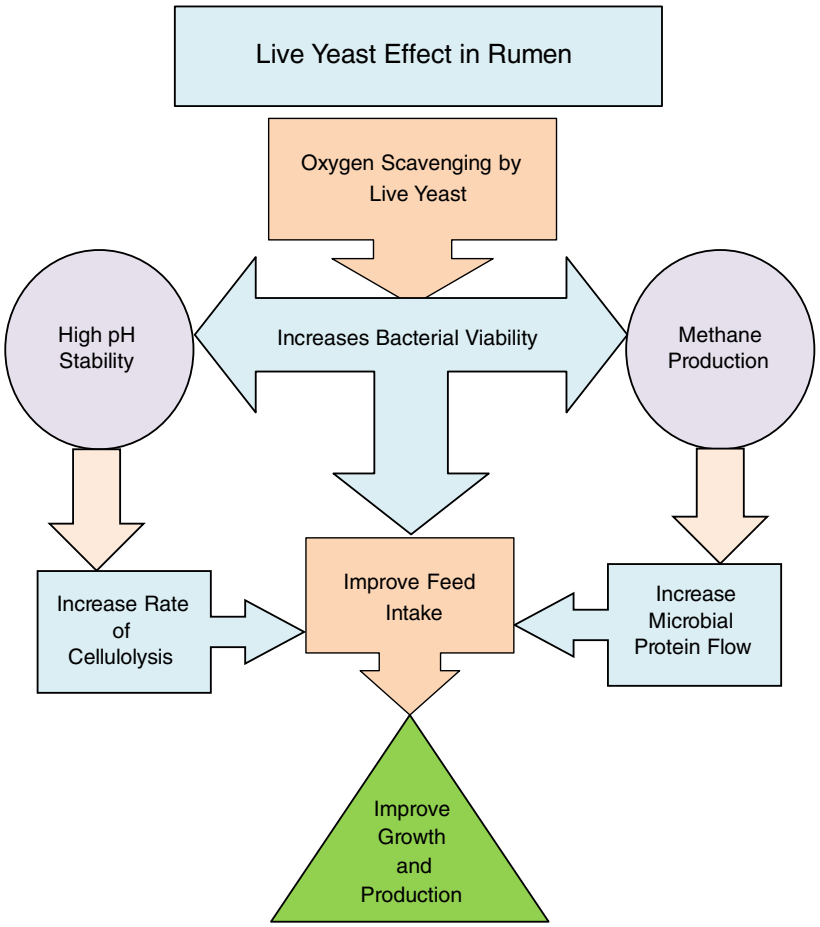

Figure 2 The mode of action of yeast culture (Figure adapted from Ghazanfar *et al*. (2017)). [Colour figure can be viewed at wileyonlinelibrary.com]

that rumen protozoal hydrogenosomes produce H_2, which then acts as a substrate for methanogens to lower CO_2 to methane via the hydrogenotrophic pathway (Belanche *et al.* 2014). This suggests that protozoa removal may be a strategy to lower methane production by ruminants. However, ruminal protozoa vary substantially in its contribution to plant degradation and methane production. The population of protozoa in the rumen fluctuates with changes in the diet consumed by the animal, a reduction in the number of protozoa in response to *S. cerevisiae* fermentation product may reduce bacteria engulfing with concomitant increase in microbial protein supply to the small intestine (Zhu *et al.* 2017). The contribution of protozoa in the fermentation of nutrients in the rumen remains controversial. Studies have shown that removal of protozoa (defaunation) from the rumen decrease the rate of organic matter degradation, especially of neutral and acid detergent fibres (Newbold *et al.* 2015). The *Entodinium* genus is the most dominant protozoan in high grain diets. This genus rapidly degrades starch, engulfing it and converting it to an iodophilic storage polymer (McSweeney and Mackie 2012), and generally holotrichs support methanogens and methanogenesis (Belanche *et al.* 2014). It was reported that total rumen protozoa decreased 3 h postfeeding without altering the population of *holotrich* and *entodiniomorph* protozoa when supplementing cannulated bulls diet with 5 g of *S. cerevisiae* (Ghasemi *et al.* 2012). Similarly, Kumar *et al.* (2013) observed that 0·5 g per animal per day of *S. cerevisiae* improved the average total protozoa growth in buffalo bulls. A 26% increase in the total population of protozoa and a 140% increase in the number of *Diplodinium* in yeast-fed animals was reported by Kowalik *et al.* (2011).

Rumen fungi

Rumen fungi (10^3–10^6 zoospores per ml) are anaerobic, and also represent approximately 10–20% of the rumen microbiome (Elekwachi *et al.* 2017), belongs to the class

Table 2 Main ruminal protozoa

Protozoa	Fermentation products	Reference
Cellullolytic protozoa *Enoploplastron triloricatum* *Eudiplodinium maggii* *Diploplastron affine* *Epidinium ecaudatum* *Diplodinium monacanthum* *Diplodinium pentacanthum*	Reducing sugars	Coleman *et al.* (1976)
Proteolytic protozoa *Entodinium caudatum* *Eudiplodinium medium*	Amonium, VFA	Ivan *et al.* (2000); Forsberg *et al.* (1984)

Table adapted from Castillo-Gonzáleza *et al.* (2014).

Neocallimastigomycetes, consisting of six previously recognized genera (Anaeromyces, Caecomyces, Cyllamyces, Neocallimastix, Orpinomyces and Piromyces; Table 3). Rumen fungi have amylolytic (Gordon and Phillips 1998) and proteolytic activities (Gruninger *et al.* 2014). The activity of anaerobic fungi is enhanced by methanogenic archaea (Cheng *et al.* 2009), which are known to be intrinsically attached to anaerobic fungal biomass. About 25 new species exist in the guts of herbivores (Paul *et al.* 2018), which are still uncharacterized. Anaerobic fungi are among the most active organisms in fibre digestion in the known biological world, mainly due to their efficient and extensive types of enzymes for the degradation of structural polymers of plants (Solomon *et al.* 2016). Supplementation of *S. cerevisiae* fermentation product increased the rumen fungi population (Zhu *et al.* 2017), and similar results were reported in a previous *in vitro* study (Mao *et al.* 2013).

Rumen methanogenic archaea

Methanogenic archaea have a wide range of unusual and distinctive metabolism, enabling them to survive in a variety of environments. Rumen archaea are strictly anaerobic and are the only known micro-organisms in the rumen capable of producing methane (Hook *et al.* 2010). Morgavi *et al.* (2012) reported that archaea exist in the rumen in the range 10^6–10^8 cells per ml, representing <4% of the microbial community. Archaea are found at the bottom of the trophic chain due to their need to use the end products of fermentation as substrates. Most methanogens remove H_2 gas by reducing CO_2 with hydrogen gas to form methane. In contrast, *Methanosphaera stadtmanae* only produces methane through reducing methanol with H_2, having one of the strictest energy metabolism of all methanogenic archaea. Methane production keeps hydrogen concentrations in the rumen low, allowing methanogens to promote the growth of other species, and enabling a more efficient fermentation (Ishler *et al.* 1996). However, methane produced in the rumen is eructated, leading to atmospheric pollution. Supplementation of active dried yeast in dairy cattle rations could alter the population composition and sizes of faecal methanogenic archaea in dairy cattle faeces, the reduction in methanobrevibacter occurred with a commensurate increase in the genera *Methanocorpusculum* and *Thermoplasma* (Jin *et al.* 2017).

Table 3 Main ruminal fungi

Fungi	Fermentation products	References
Cellulolytic fungi: *Neocallimastix frontalis* *Piromyces communis* *Orpinomyces joyonii*	Lactate, formate, acetate, succinate, ethanol, celobiose, celooligosacarides glucose	Moniello *et al.* (1996), Dashtban *et al.* (2009) Hodrova *et al.* (1995)

Table adapted from Castillo-Gonzáleza *et al.* (2014).

Impact of feeding single-cell fungi on neonates

The first microbial inoculums are vertically transmitted from the dam to the offspring, major colonization begins at birth and is complemented during lactation (Power *et al.* 2017) and later in life. The microbial environment of the pregnant mother affects the offspring. The dam's exposure to a diverse microbiota appears to be beneficial (Torow and Hornef 2017). Recently, microbe-derived compounds have been elegantly introduced to enhance the differentiation of gut-specific innate lymphoid cells in the murine foetus (Gomez de Aguero *et al.* 2016). However, it remains unclear whether intact microbe or only their components or secreted products reach the foetus. The identity and composition of the primary rumen bacterial populations acquired shortly after birth, and the changes occurring in these populations at different growth stages of the animal remain largely unknown, despite their importance for understanding the forces governing this microbial ecosystem and its similarities to others (Elie *et al.* 2013). Microbes in neonate's gut are acquired from the dam at birth form saliva or through faecal contact. However, the transition from liquid feed to solid feed via creep feed causes an imbalance in rumen microbes that may cause poor growth, diarrhoea and sometimes death. Diarrhoea accounts for 56·5% of deaths in preweaned calves (Gott 2018) and is caused by the release of enterotoxins into the lumen of the small intestine produced by pathogenic enterotoxigenic *E. coli* and *Salmonella* (Alugongo *et al.* 2017). Dobicki *et al.* (2006) reported that yeast could help enhance early microbial

colonization of the rumen in the neonates of small and large ruminants. Galvão *et al.* (2005) noted that the duration of diarrhoea was decreased in neonates receiving active dry yeast with feed. Similarly, Hassan *et al.* (2016) reported that 2·5 and 5 g of *S. cerevisiae* improved intake of starter diet, berseem hay and total solids in crossbred Friesian calves resulting in an increase in fermented by-products such as VFAs as well as a decrease in the concentration/production of rumen ammonia nitrogen.

Impact of feeding single-cell fungi on rumen fermentation activity and metabolites

Rumen pH, acidosis and its implications

Rumen pH affects all aspects of rumen functions. Dynamic rumen pH is an important factor affecting microbial populations and activities beside the diet (Jenkins 2018). The inclusion of *S. cerevisiae* at a dose of 5 g per day per head in early lactating cows increased rumen pH (Pinloche *et al.* 2013). Ruminal pH is also a crucial parameter to ensure the normal functioning of the rumen and its microbiome. Therefore, it is the closest and most accurate indicator of rumen health or disorders such as SARA, because it provides direct information about the conditions within the rumen (Enemark 2008). Rumen pH <6 for a prolonged period for several hours a day can adversely leads to acidosis, (Rodríguez-Lecompte *et al.* 2014). Recently, Zhao *et al.* (2018) indicated that SARA produces a high concentration of ruminal LPS, which over activates the inflammatory pathways and significantly increases the expression and synthesis of pro-inflammatory cytokines in the rumen epithelium, thus causing partial inflammation of the rumen. Therefore, it is expedient to stabilize rumen pH and prevent it from reaching the acidosis state. Acidosis occurs in the rumen when VFA production exceeds the absorption rate, and this is the case when ruminants consume high grains or rapidly fermentable diets. Ruminal acidosis is associated with inflammations of various organs resulting in negative impacts on animal health and profitability (Zebeli and Ametaj 2009; Kleen *et al.* 2013). Ruminal acidosis (subacute or acute) causes severe changes in the rumen wall (Steele *et al.* 2011), resulting in increased absorption of LPS released from Gram-negative bacteria in rumen and the lower gut (Plaizier *et al.* 2012). In addition, acidosis forces more fluid into the rumen leading to watery faeces and increased absorption of LPS through the epithelial tight junction (Jenkins 2018). Increased concentration of LPS in the blood is associated with metabolic disorders such as increased blood glucose and nonesterified fatty acid that decrease feed intake and affect patterns of hydroxybutyric acid, cholesterol and minerals, such as Ca, Z and Fe (Zebeli *et al.* 2010). Several studies have dealt with various strategies to reduce the incidence of ruminal acidosis. A study by (González *et al.* 2012) showed that the addition of feed additives in cow diets, such as yeasts, could decrease the prevalence of ruminal acidosis. Limiting lactic acid production/accumulation would help to reduce or prevent the extent of acidosis in the rumen. A positive effect on lactic acid concentration as well as rumen pH was also observed under *in vivo* conditions (Mohammed *et al.* 2017). Pinloche *et al.* (2013) observed a decrease in the concentration of D- and L-lactate by 58% when diet of cattle was supplemented with 5 g day^{-1} of yeast. Malekkhahia *et al.* (2016) observed an improvement of rumen pH in primiparous cows induced with subacute rumen acidosis (rumen pH 5·57–6·16 *vs* control: 5·40–6·05) in the presence of 10 g active dry yeast.

Impact on volatile fatty acid production

Volatile fatty acids (acetate, propionate and butyrate) are the main by-product of microbial fermentation in the rumen and can be absorbed across the gut wall to serve as an energy source for ruminants (Matthews *et al.* 2019). Numerous literatures mentioned the effect of yeast culture on proportion of VFA. Variable response in VFA production with the addition of yeast culture is a consequence of yeast culture's effect on the growth of different species of rumen microbes. Vallejo-Hernández *et al.* (2018) observed that metabolizable energy and VFA concentrations were greater when 4 mg of *S. cerevisiae* were supplemented to goat and sheep inocula. Supplementation of 5 g of yeast per day in dairy cows improved VFA production by 9·65% after 30 days and 2% after 60 days (Doleźal *et al.* 2012). Sousa *et al.* (2018) reported that yeast supplementation increased total VFA, decreased acetate proportion and increase propionate proportion. There was a tendency for higher valerate proportion when steers were fed live yeast. No significant effect on butyrate, and branched-chain fatty acids was observed. Muhammed and He (2018) reported that the addition of *S. cerevisiae* improved rumen environment across treatments with greater total VFA, propionic and butyric acids. Live yeast used as a dietary feed additive permits a better utilization of diet in dairy cows (Julien *et al.* 2015) and moreover, increased ruminal total VFA. *Saccharomyces cerevisiae* fermentation product supplementation manipulated rumen microbial population and resulted in improved energy supply (enhanced VFA production) and improved protein nutrition (higher microbial protein synthesis and more efficient conversion of dietary N to milk N) of lactating cows fed diets containing low-quality forages (Zhu *et al.* 2017). Increased VFA production

could be attributed to an increase in rumen fungi and fibre-digesting bacteria population. *In vivo* and *in vitro* studies have documented positive effects of *S. cerevisiae* fermentation products on rumen fermentation (Mao *et al.* 2013). Increases in rumen propionic acid concentration was observed when *S. cerevisiae* fermentation products was fed (Zhu *et al.* 2017) that would lead to increased glucogenic potential of the diet and milk production. Monnerat *et al.* (2013) observed that 1 g of *S. cerevisiae* increased total VFA in cattle fed high concentrate diet of two starch levels. In Murrah bulls, *S. cerevisiae* increased the proportion of VFA (Kiran and Kumar 2013). Ghasemi *et al.* (2012) fed four experimental diets containing *S. cerevisiae* to mature cannulated bulls and observed an increase in the concentration of propionate, but acetate and butyrate concentrations as well as the ratio of acetate to propionate were not affected. In an *in vitro* study, the addition of 0·50 and 0·75% of yeast to ammoniated rice straw increased total VFA production by 18·9 and 16·7%, respectively (Zain *et al.* 2011). However, Yoon *et al.* (2016) reported that addition of 14 g of yeast in subacute rumen acidosis induced animals did not have an effect on the total VFA, individual VFA, mol per 100 mol of rumen fluid, caecal digesta and faeces. The increase in total VFA concentration in the rumen in the presence of *S. cerevisiae* was suggested to be due to their effects on rumen pH and rumen lactic acid concentration. Moreover, Pinloche *et al.* (2013) reported that the inclusion of *S. cerevisiae* at 5 g per day per head in early lactating cows increased VFA concentrations.

Impact on methane emission

Livestock production contributes to global climate change by emitting GHGs from enteric fermentation and the major GHGs from the livestock sector are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) (Ugbogu *et al.* 2019).. Methane is the most important GHGs from the animal production system and has very high global warming potentials. Methane emission is also associated with loss of dietary energy; hence, reduce feed efficiency as well as the negative environmental impacts (Haque 2018). As a GHG, CH_4 is 23 times more potent than CO_2 (IPCC 2014). A significant portion of the ingested feed energy is lost as CH_4, ranging from 1·5 to 12% of the gross energy intake in cattle (Franz *et al.* 2010). Greenhouse gas emissions, excessive excretion, inefficient or poorly digested feeds from livestock production cause nutrient wastage, resulting in the loss of feed energy during ruminant production (Hristov *et al.* 2015). During anaerobic fermentation of organic matter, H_2 is produced and some microbes, such as methanogens, use the H_2 to produce CH_4 from CO_2. Thus, reducing CH_4 production would have a positive impact on feed efficiency. Methane is produced by methanogens, but other members of the microbiota can determine or profoundly influence the rate and yield of methanogenesis (Kittelmann *et al.* 2014; Danielsson 2016). By 2050, the total CH_4 emission from ruminants is expected to increase significantly due to the increasing demand of meat and milk for a rapidly growing world population (Gerber *et al.* 2013). Therefore, it is important to mitigate CH4 emission from the livestock industry. There are several strategies for CH_4 mitigation from ruminants that have been reviewed (Martin *et al.* 2010). Live-cell yeast and yeast products have recently been proposed to mitigate CH4 and it has been shown to reduce methane production in the rumen by stimulating acetogens to consume more hydrogen for acetate production (Darabighane *et al.* 2018). In a previous study, Wang *et al.* (2016) reported that red yeast rice (*Monascus purpureus*) reduced the production of methane in goats. Lu *et al.* (2016) reported that the addition of 6 and 12 g day^{-1} of yeast reduced enteric methane emissions without altering the numbers and diversity of methanogens. Most of the studies that evaluated the population of methanogens and diversity revealed that majority of methanogens population are relatively constant and did not really change when methane production declined. Yeast also is able to alter the fermentation process in the rumen in a way that decreases methane formation (CH_4) (Chung *et al.* 2011). Therefore, it is concluded that yeast as a dietary supplement may lead to better nutrient digestibility (Fig. 2). A reduction in the concentration of NH_3-N was observed when a yeast culture was used in *in vitro* fermentation of a high fibre diet (Lattimer *et al.* 2007) and on dairy cows (Moallem *et al.* 2009). Methane production was reduced in the presence of 4 mg of *S. cerevisiae* and can be used in an environmentally friendly and sustainable way to reduce biogas emissions from livestock; thereby improving environmental conditions (Vallejo-Hernández *et al.* 2018).

Impact on microbial protein synthesis

Constant supply of nutrients is required for optimization of microbial growth and production. There is a link between protein of microbial origin present in the rumen and amino acid absorption by the ruminant when their diet is supplemented with *S. cerevisiae* (Erasmus *et al.* 1992). Microbial protein synthesis was increased by 9·3% when 56 g per head per day of *S. cerevisiae* were added to dairy cows diet (Hristov *et al.* 2010), also inclusion of *S. cerevisiae* fermentation products at 120 and 180 g per head per day have been reported to increase microbial protein synthesis in cows by 12·8 and 9·7%, respectively. Moya *et al.* (2007) reported that addition of live yeast

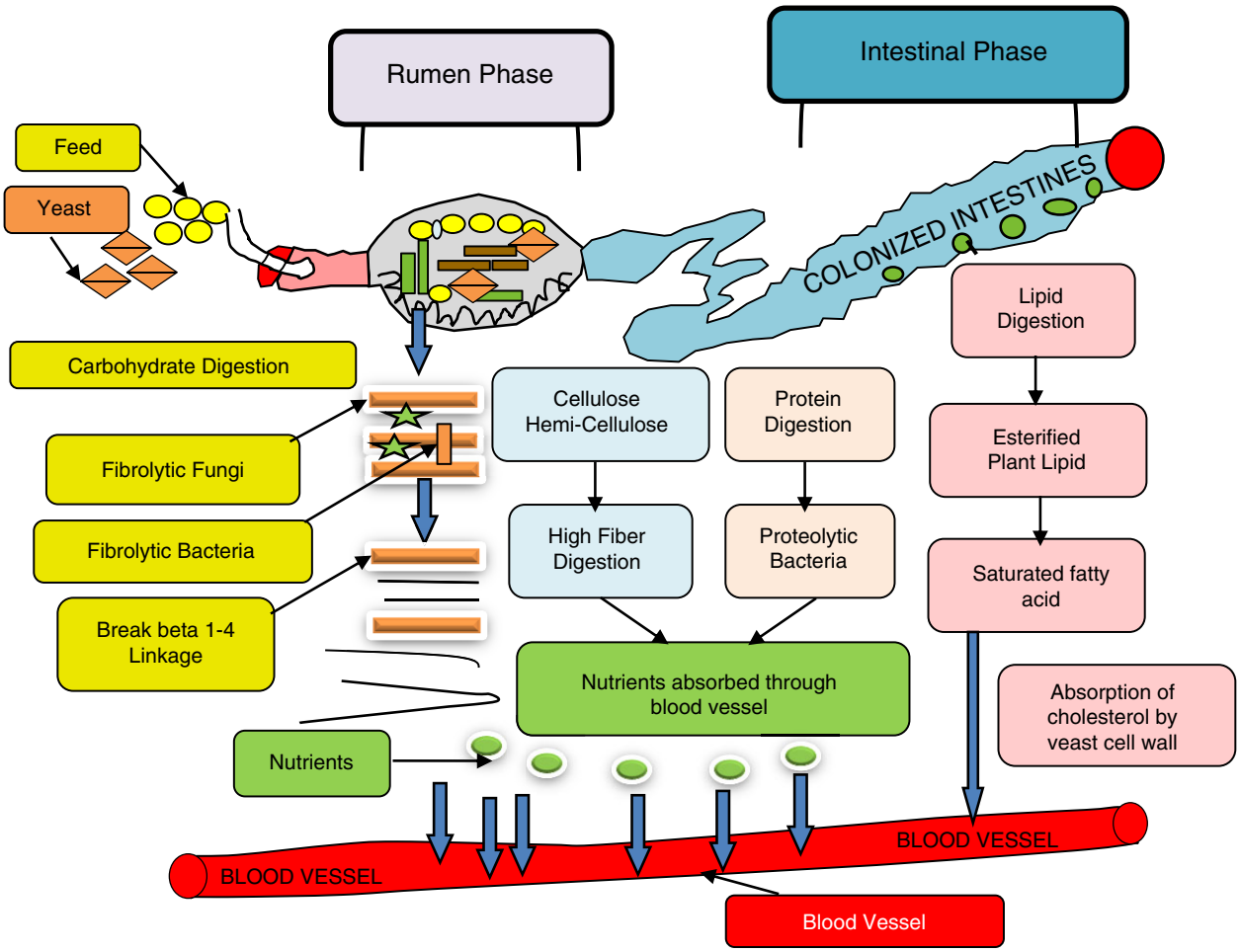

Figure 3 Mode of action of yeast in rumen and post-ruminal GIT (Figure adapted from Ghazanfar *et al.* (2017)). [Colour figure can be viewed at wileyonlinelibrary.com]

increased efficiency of microbial protein synthesis in a continuous culture system. The decrease in number of protozoa in response to *S. cerevisiae* fermentation product supplementation may decrease bacterial preying and allows more microbial protein to reach the small intestine (Zhu *et al.* 2017). Such effects have been reported in some other studies (Mao *et al.* 2013). *Saccharomyces cerevisiae* supplementation was associated with an increased flow of microbial protein leaving the rumen and enhanced supply of amino acids entering the small intestine (Biricik and Yavuz 2001) (Fig. 2).

Impact on fibre digestibility

The importance of dietary fibre fractions in animal feeding is due to its effect on the rate of passage, mucosal functionality and its role as substrate for gut microbiota associated with performance and digestive health. The complexity of the physical structure and chemical composition of polysaccharides in plant cell walls explains the wide and different physiological effects of this large range of fibre fractions (Gidenne 2015). Therefore, fibre degradation is an essential process in feeding ruminants because of significant amounts of cellulose, hemicelluloses, pectin, etc. embedded in plant cell walls (Chaucheyras-Durand *et al.* 2012). Therefore, it was reported that yeast supplementation enhanced fibre digestion in the rumen (Chaucheyras-Durand *et al.* 2012). *Saccharomyces cerevisiae* fermentation products improved the rumen fermentation of both low-quality forages and their mixed diets by stimulating the number of fibre-digesting rumen microbes, especially fungi populations in vitro (Mao *et al.* 2013). Ruminants depend on a close symbiosis with their ruminal microbiota for proficient conversion of plant biomass to microbial cell protein and VFA (McCann *et al.* 2014). By breaking down the plant cell walls of lignocellulosic feedstuffs, the micro-organisms fulfil their host's nutritional demands while thriving in a

suitable environment where they are provided a constant influx of energy and relative environmental stability (Naas and Pope 2019) (Fig. 3). Since ruminants receive most of their energy from their symbiotic microbiota, the efficiency of feed conversion and the quality of the end product of meat and milk in bovines is tightly related to the dynamics of the function of the rumen microbiome (Naas and Pope 2019). Yeasts have been shown to increase dry matter intake when supplemented in early lactation, and an increase in rate and extent of NDF degradation may stimulate intake and productivity in dairy cows (Poppy *et al.* 2012). Live yeast supplementation in dairy cows increased the relative abundance of cellulolytic, amylolytic and lactate-utilizing micro-organisms, whereas dead yeast supplementation increased amylolytic populations and lactate-utilizing micro-organisms (Jiang *et al.* 2017). Changes in rumen microbial population are expected to stimulate digestion of carbohydrates in the rumen, which likely explains the increased rumen NDF digestion (Diaz *et al.* 2018). Recently, supplementing live yeast in the diet of cattle grazing tropical pastures can benefit fibre-degrading bacteria and increase fibre digestibility (Sousa *et al.* 2018). Hassan *et al.* (2016) reported that supplementation with 2·5 and 5 g of yeast/calf increased digestion of ADF and NDF. In addition, the inclusion of 4 mg of *S. cerevisiae* resulted in increased dry matter degradability in sheep and steer inocula and increased degradation of organic matter in goat and sheep inocula (Vallejo-Hernández *et al.* 2018). Vyas *et al.* (2014) reported that an increase in fibre digestibility is normally associated with an increase in fibre degrading bacteria population. This is further supported by the findings from Peng *et al.* (2019) that the fibre degrading bacteria population (e.g. *F. succinogenes S85, R. albus 7 and R. flavefaciens FD-1*) in the rumen, was ~2 times higher in beef cattle supplemented with live yeast at 2 g per cattle per day compared with the control diet and therefore, increased apparent digestibility of NDF and ADF. A significant increase in digestion of NDF, ADF and hemicellulose was observed with *S. cerevisiae* NCDC42 culture at 1 ml kg^{-1} body weight (Tripathi and Karim 2011). Furthermore, Chaucheyras-Durand *et al.* (2016) reported that *Saccharomyces* may indirectly promote microbial fibre degradation by stabilizing ruminal pH and increasing dry matter intake. Lascano *et al.* (2012) reported that supplementation of *S. cerevisiae* at $3{\cdot}13 \times 10^7$ CFU per gram in lactating cows increased fibre digestibility, improved milk and fat contents and improved gastrointestinal tract microbial balance. They also reported that supplementation of *S. cerevisiae* at $2{\cdot}5 \times 10^9$ CFU per gram in dairy Holstein heifers improved feed efficiency and increased dry matter digestibility. In contrast, the use of red yeast rice (*M. purpureus*) in goat had no effect on ADF and NDF digestibility (Wang *et al.* 2016).

Impact on rumen physiology and lipopolysaccharide concentration

The intestinal epithelium functions as a barrier, preventing and controlling the penetration of food and bacterial pathogens into the tissues. At the same time, it has to be permeable to allow the translocation of nutrients, electrolytes and water. This intestinal permeability allows the exchange of solutes and fluids between the intestinal lumen and tissue (Odenwald and Turner 2013). A loss of the barrier function would negatively affect the health of ruminants by increased absorption of endotoxins such as mycotoxins. Endotoxin could trigger inflammation through the pattern recognition receptors (Emmanuel *et al.* 2008), but there is paucity of information on the influence of yeast as alternative to synthetic feed additive on rumen histology. The prolong depression in rumen pH to the point of acidosis causes lesion to the gastrointestinal barrier (Zebeli and Metzler-Zebeli 2012; Chang *et al.* 2015). Interestingly, Peng *et al.* (2019) reported that dietary supplementation of both live yeast and yeast cell wall might promote growth performance of beef cattle through reducing LPS production in the rumen and LPS absorption into plasma and decrease inflammatory parameters and improving other related aspects (Fig. 3). Vyas *et al.* (2014) also reported positive effects of both live and killed dried yeast in reducing the severity of SARA irrespective of its viability and this may also reduce the LPS content in the rumen. Recently, Liu *et al.* (2018) indicated that mannanoligosaccharide has a high antioxidant activity and its content is about 30% yeast cell wall. Dietary supplements of yeast cell wall with a dose of 2 g kg^{-1} DM can effectively bind LPS in the digestive tract, reduce the translocation of LPS from the digestive tract into circulation, and thus improve growth performance in beef cattle (Lei *et al.* 2013). Supplementation of a grain-based diet for sheep with 0, 2 g kg^{-1} DM of live *S. cerevisae* and 2 g kg^{-1} DM of the yeast +2 g kg^{-1} DM of mannan-oligosaccharide resulted in reduced plasma level of LPS by 51·0 and 95·7% for yeast and yeast + mannan-oligosaccharides respectively (Diaz *et al.* 2018). However, no effect on ruminal and duodenal LPS concentrations was observed. Live yeast improved the rumen papillae total width, improved epithelial thickness and lowered stratum corneum thickness. Stratum corneum is usually increased as a result of rumen epithelial response to high levels of nonfibrous carbohydrates in the diet (Steele *et al.* 2011) indicating that the yeast protects rumen epithelium against acidosis damage (Diaz *et al.* 2018). The low

permeability of the rumen epithelium to LPS in the presence of yeast may be due to increased epithelial thickness. However, (Zhang *et al.* (2013) reported that inclusion of *S. cerevisiae* lowered faecal LPS and plasma LPS which could indicate endotoxin absorption from/or production in the rumen was low. In beef cows, Lee *et al.* (2013) reported that dietary yeast wall supplements can effectively decrease the concentration of free LPS in plasma, digesta and faeces. The absorption of endotoxin puts enormous pressure on the liver for detoxification, and in extremes cases, they instigate the uptake of mycotoxin; such as trichothecenes and fumonisins that elicit inflammatory and immunosuppressive responses (Jenkins 2018). Feed supplementation with yeast cell wall reduced the absorption of aflatoxin B1 and increased the elimination of aflatoxin B1 and M1 in ewes (Firmin *et al.* 2011).

In summary, inclusion of live yeast cells (single-celled fungi) in the diets of ruminants stabilizes rumen microbiota and stimulates the growth of rumen microbes, which classifies yeast as a probiotic and a prebiotic. Yeast is also able to maintain rumen health by increasing rumen pH, improving barrier function, improving microbial protein synthesis in the rumen, enhancing fibre digestibility and animal health.

Conflict of Interest

The authors declare no conflict of interest.

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