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Effect of Yeast Supplementation During Various Stages of Beef Production

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

by

Elizabeth Palmer
Pennsylvania State University
Bachelor of Science in Animal Science, 2016

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Supplemental dietary yeast products are beneficial during times of stress. Calves experience increased levels of stress during birth, weaning, and the post-weaning receiving period. Therefore, 3 experiments were conducted to determine how yeast supplementation affects growth performance and health during this time. In experiment 1, 2 truckloads of highly stressed calves (n = 175; initial body weight [BW] = 226 ± 24.5 kg) were assigned randomly to 1 of 3 treatments; unsupplemented control, or supplementation with 2 commercial yeast products (Y1 and Y2). Average daily gain (ADG) was not different ($P = 0.99$) during the 28 d receiving period; nor was there any difference ($P = 0.29$) in the percentage of calves treated for bovine respiratory disease. In experiment 2, heifer calves (n = 95; initial BW = 165 ± 27 kg) were randomly assigned to pastures and pastures were randomly assigned to treatment: 1) no yeast (CON), or 2) addition of yeast product (YP). Heifers were supplied treatments for 35 d prior to weaning and through a 42-d backgrounding period. Average daily gain prior to weaning was not different ($P \geq 0.45$) between treatments. However, CON had increased ($P = 0.01$) ADG compared to YP from weaning to the end of the backgrounding period. Microbiome analysis found that supplemental yeast did not dramatically change α or β diversity nor was there a difference in community structure for rumen bacteria; fecal α or β diversity were different on d 34. In experiment 3, late gestation cows (n = 97) were supplemented YP approximately 45 d prior to parturition. At parturition colostrum and blood samples (n = 30) were collected to determine the effect on passive transfer. Supplementation ended 22 d after the last calf was born (d 85). Body weight on d 85 was greater ($P = 0.01$) for YP calves compared with CON. Cows that were supplemented YP had a lower ($P = 0.03$) neutrophil:lymphocyte at hour 0 and 48 after parturition. Similarly, calves on YP treatment had a lower ($P = 0.02$) neutrophil:lymphocyte at hour 48. Overall, effects of yeast supplementation have been variable between the 3 experiments.

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CHAPTER I

Introduction

Antibiotic use in animal agriculture has become a concern among the American population. Antibiotics are used in animals for the treatment and prevention of disease and as growth promoters. In 2017, the United States Food and Drug Administration (FDA) increased regulation regarding the use of antibiotics in animal agriculture that are classified as medically important to humans. Both consumer preference and government regulation has increased interest in a more natural livestock production. Therefore, greater importance has been placed on maintaining both animal health and production in order to reduce the amount of antibiotics administered.

Probiotics are naturally occurring additives that have gained popularity in the livestock industry to promote health and growth performance. Probiotics are defined as live microorganisms that provide health benefits to the host when consumed in adequate amounts and frequency (FAO-WHO, 2002). Probiotics can refer to microbial cultures, enzyme preparations, culture extracts, or any combination of the 3 (Yoon and Stern, 1995). In ruminants, the most common probiotic used is *Saccharomyces cerevisiae*, a live yeast to target the rumen, whereas the yeast, *Sacchromyces boulardii* and bacterial strains (*Lactobacillus* spp., *Enterococcus* spp., *Pendiococcus* spp., *Bacillus* spp.) are more popular in monogastrics to target the hindgut (Chaucheyras-Durand and Durand, 2010).

Prebiotics are an additional feed additive that can modify the microbial population in the gastrointestinal tract. Prebiotics are non-digestible feed ingredients that support the growth of gut microorganisms that are beneficial to the host (Gibson and Roberfroid, 1995). In order to be classified as a prebiotic, a feedstuff must meet certain criteria; 1) must be resistant to gastrointestinal absorption, 2) fermentable by microbes in the intestine, and 3) promote growth

of bacteria that are beneficial to the health of the individual (Gibson et al., 2004). Using pre- and probiotics in combination is known as synbiotics.

The FDA requires feed manufacturers to use the term direct-fed microbial (DFM), which is defined as a feed product that contains live microorganisms and is typically of fungal or bacterial origin (Miles and Bootwalla, 1991). Direct-fed microbials are added to the diet to modify the microbial population in the gut. In a survey done by New Mexico State University and Texas Tech University, feedlot consulting nutritionists reported that 62.5% of clients use DFM in receiving diets and 59.6% in finishing diets for beef cattle (Samuelson et al., 2016).

Yeast is a eukaryote, single-celled microorganism, and member of the fungi family with approximately 1,500 species identified. Yeast exhibits properties of both pro- and prebiotics. Yeast can survive in the rumen and remain metabolically active (Kung et al., 1997) therefore demonstrating a probiotic effect. Yeast products could also be classified as a prebiotic due to the growth factors and vitamins that they provide for the rumen microbes (Opsi et al., 2012). The utilization of yeast in animal nutrition is not a new concept and has been practiced for years, yet there is a lack of understanding of the mechanisms and benefits in beef production.

LITERATURE CITED

Chaucheyras-Durand, F., and H. Durand. 2010. Probiotics in animal nutrition and health. Beneficial Microbes. 1:3-9.

FAO-WHO. 2002. Guidelines for the evaluation of probiotics in food. Joint FAO/WHO Working Group on drafting guidelines for the evaluation of probiotics in food; London, Ontario, Canada.

Gibson, G. R., and H. M. Probert, J. V. Loo, R. A. Rastall, and M. B. Roberfroid. 2004. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr. Re. Rev. 17:259-275.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing concept of prebiotics. J. Nutr. 125:1401-1412.

Kung, L. E., Jr., M. Kreck, and R. S. Tung. 1997. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. J. Dairy. Sci. 80:2045-2051.

Miles, R. D., and S. M. Bootwalla. 1991. Direct-fed microbials in animal production. Pages 117–132 in Direct-fed Microbials in Animal Production. A Review. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Opsi, F., R. Fortina, S. Tassone, R. Bodas, and S. López. 2012. Effects of inactivated and live cells of *Saccharomyces cerevisiae* on in vitro ruminal fermentation of diets with different forage:concentrate ratio. J. Agric. Sci. 150:271-283.

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Löest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University Survey. J. Anim. Sci. 94:2648-2663.

Yoon, I. K., and M. D. Stern. 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. J. Anim. Sci. 8:533-555.

CHAPTER II

Review of literature

Overview of yeast products

The most common specie of yeast used in animal diets is *Saccharomyces cerevisiae* which is commonly referred to as baker's or brewer's yeast. *Saccharomyces cerevisiae* is most often used in the food and beverage industries due to its role in the fermentation process. *Saccharomyces cerevisiae* is found throughout nature and is also naturally occurring in the gastrointestinal tract. While *S. cerevisiae* is the most common, other species and strains of yeast are being utilized in livestock nutrition (Chaucheyras-Durand and Durand, 2010). The influence of yeast in the diet can change depending on yeast strain and the components of yeast fed.

There are many varieties of commercially available yeast products used in animal nutrition including but not limited to live yeast, yeast cell wall (**YCW**), brewer's yeast (spent yeast), yeast cultures, and yeast extracts. Live yeast is a dried product that contains a high concentration of viable cells (AAFCO, 2013). Inclusion rate of live yeast is often lower compared to other yeast products because it contains pure, metabolically active yeast. Yeast cell wall, also referred to as mannan oligosaccharides (**MOS**), is the outer layer of the yeast cell and is derived after lysis of the cell. The cell wall of yeast is composed primarily of polysaccharides such as glucans and mannans. Brewer's yeast is killed yeast that is a by-product from the beverage industry (AAFCO, 2013). Yeast cultures are products of yeast fermentation and include growth media that they are grown in (AAFCO, 2013). Yeast cultures are included in the diet at a higher rate because the number of live cells is not guaranteed. Yeast extract refers to the contents within the cell and excludes the yeast cell wall components (AAFCO, 2013).

It is also critical to consider the length of time that yeast products are fed. Live yeast can take up to 14 days to propagate in the rumen while YCW will start to take effect immediately after feeding. Commercial products can also be a blended mix of yeast products to capture the different benefits that each provide. Most of the research conducted with yeast products has been

highly variable which can be attributed to differences in management, diet types, and the health and production stage of the animal (Desnoyers et al., 2009). There is much yet to understand about the interaction of these factors.

Production in cattle

Feedlot receiving period

Calves new to the feedlot environment are stressed prior to arrival due to weaning, commingling, and transportation (Duff and Galyean, 2007). Stress can have a negative impact on feed intake (Cole, 1996) and reduces gain during the receiving period. Therefore, it is vital to properly manage receiving calves to minimize the decreased intake and gain. Use of yeast in receiving diets has been heavily studied intensively however the outcomes have been highly variable. The most consistent finding is the positive influence that yeast has on dry matter intake (**DMI**) during the receiving period (Finck et al., 2014; Ponce et al., 2012; Young et al., 2017). The improvement in DMI has been suggested to be the cause of increased average daily gain (ADG; Ponce et al., 2012). However, greater ADG is not always observed during this period (Finck et al., 2014; Vendramini and Arthington, 2007).

Finishing period

Yeast has been utilized in feedlots as a natural feed additive to improve both feed efficiency and rumen fermentation characteristics. As with the receiving period, performance results have been highly variable during finishing. Hinman et al. (1998) saw an advantage to feeding yeast culture in ADG and feed efficiency (**FE**). Contrasting results have found that yeast did not affect gain or FE (Swyers et al., 2009; Ovinge et al., 2018). Furthermore, carcass characteristics have been altered with inclusion of yeast in finishing diets. Yeast improved carcass quality by increasing the percentage of steers grading premium choice and choice along

with yield grades of 1 and 2 (Swyers et al., 2009). While carcass quality was improved, hot carcass weight (**HCW**) was less in steers fed yeast compared to control. In agreement, Ovinge et al. (2018) saw a linear increase in carcasses grading premium choice as the inclusion rate of yeast increased in the diet. Carcass quality was improved with yeast, however, cost of gain in the feedlot was greater for supplemented calves compared to control (Swyers et al., 2009). As the natural market grows in the United States producers could demand a greater price for their product which could offset the increased cost in the feed yard.

Milk production

Similar to finishing beef cattle, the effects of yeast products are variable in dairy cows. Yeast has been shown to improve milk yield (Moallem et al., 2009; Nocek et al., 2011; Jiang et al., 2017a) but these findings have not been consistent (Robinson and Garrett, 1999; Shwartz et al., 2009). Not only is there variability among studies, but Jiang et al. (2017a) found that a low dose of live yeast improved milk yield but a high dose of live yeast had no effect. Milk fat and protein yield increased with enzymatically hydrolyzed yeast (Nocek et al., 2011) although milk fat was the only component to improve as a percentage of milk composition. Similarly, Moallem et al. (2009) reported an increase in milk fat yield but percentage of milk fat and protein were not different. Similar conclusions have been made regarding DMI in primiparous and multiparous lactating cows (Robinson and Garrett, 1999).

Effect on digestibility

Yeast products exhibit beneficial effects on total tract digestibility of dry matter (**DM**) and dietary fiber components including neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and hemicellulose (Jiang et al., 2017a; Ovinge et al., 2018). Crude protein (**CP**) digestibility has been improved in steers (Ovinge et al., 2018) but the same affect has not been

found in lactating dairy cows (Jiang et al., 2017a; Moallem et al., 2009). Improvement in digestibility is likely due to the increase in the concentration of total bacteria present in the rumen (Newbold et al., 1995; Kumar et al., 1994). Along with a greater abundance of bacteria, the presence of specific bacterial species vital to rumen function also increase with yeast supplementation such as cellulolytic (Newbold et al., 1995; Kumar et al., 1994; Harrison et al., 1988) and fibrolytic bacteria (Chaucheyras-Durand and Fonty, 2006).

More recent studies have evaluated the influence of yeast on rumen microbial species by sequencing the V4 region of the 16S rRNA region. Jiang et al. (2017b) identified an increase in *Ruminococcus* and *Bifidobacterium* species which are associated with fiber and starch digestibility when yeast was fed. Additionally, Jiang et al. (2017b) reported a decrease in bacterial species *Coprococcus* and an unknown species in the *Lachnospiraceae* family. In a companion paper, Jiang et al. (2017a) found that an unknown species in the family *Lachnospiraceae* correlated negatively to DM and NDF digestibility. Not surprising, *Ruminococcus* correlated positively to ADF digestibility (Jiang et al., 2017a), indicating that yeast stimulated the growth of favorable bacteria and limited the production of undesirable bacteria. Yeast did not affect the inter- or intra-diversity of rumen samples nor did it change the microbial structure (AlZahal et al., 2017), or have noticeable differences in the abundance of certain observational taxonomy units (**OTU**) within the rumen microbiome. AlZahal et al. (2017) saw that active dry yeast increased the relative abundance of *F. succinogenes* and *SR1* when cows were switched from a high fiber to high grain diet which was accompanied by a reduction in *Provetella* in yeast supplemented cows compared to the control.

Yeast products influence the profile of volatile fatty acid (**VFA**) within the rumen. In buffalo (Bubalus bubalis), *Saccharomyces cerevisiae* increased total VFA, acetate, propionate,

and the acetate to propionate ratio (Kumar et al., 1994). Conflicting results described a reduced acetate:propionate ratio due to a reduction in acetate and an increase in propionate (Harrison et al., 1988). A lower acetate to propionate ratio is desirable energetically. As the acetate to propionate ratio decreases the amount of methane emitted into the environment is reduced (Russell, 1998). As acetate is produced, H_2 is produced and converted to methane (Ferry, 1992). In vitro studies suggest that yeast could be a potential tool to mitigate methane production by decreasing acetate production by acetogenic bacteria (Chaucheyras-Durand et al., 1997) and stimulating the growth of H_2-utilizing bacteria (Chaucheyras et al., 1995). Similar to other variables, the effect of yeast on methane emissions has been extremely variable. One in vitro study found that yeast reduced methane production by 20% (Lynch and Martin, 2002) while others saw an increase (Ando et al., 2004). The same mixed effects have been observed in vivo, some having found no reduction on methane emissions when providing supplemental yeast (McGinn et al., 2004) while others have reported that methane can be reduced but it is dependent on the strain of yeast used (Hristov et al., 2010).

Live yeast survive and remain metabolically active in the rumen and positively influence pH in high producing dairy cows and finishing steers. Rumen pH decreases as calves are switched from a diet consisting primarily of forage to one predominantly consisting of grain (Russell, 1998). A decrease in ruminal pH below 5.8 could lead to subacute ruminal acidosis (**SARA**; Beauchemin et al., 2003). Subacute ruminal acidosis has substantial consequences including a reduced fiber digestibility, lameness, and liver abscesses (Plaizier et al., 2001; Nocek, 1997). Active as well as killed dried yeast improve minimum pH and decrease the length of time that the rumen remains in an acidotic state (Vyas et al., 2014). Vyas et al. (2014) saw an increase in *Ruminococcus flavefaciens*, a fiber-digesting bacterium, when killed dried yeast was

included in the diet but there was no difference in the level of lactic acid producing bacteria. In high producing Holstein cows, the amount of lactic acid was reduced with yeast culture supplementation, which resulted in an increase in rumen pH (Willams et al., 1991). After evaluating the effect of yeast on rumen microbiota, supplemented cows have a lower abundance of lactate-utilzing bacteria such as *Megasphaera* and *Selenomas* accompanied by an increase in fibrolytic groups including *Fibrobacter* and *Ruminococcus* (Pinloche et al., 2013). Therefore, the change in microbial species is believed to influence the beneficial effects of yeast on rumen pH.

The rumen is anaerobic in nature and has a high reducing potential. Under normal conditions, the average redox potential (E_h) in the rumen of sheep is between -150 to -260 mV (Broberg, 1957; Barry et al., 1977) and -88 to -134 mV in lactating Holsteins (Marden et al., 2008). A lower E_h is indicative of favorable reducing conditions. Chaucheyras-Durand and Fonty (2002) found that live yeast reduced the E_h in lambs. A decrease in E_h has further been observed in dairy cows (Marden et al., 2008; Křížová et al., 2011; Pinloche et al., 2013) after consuming yeast products; providing evidence that yeast has the capability of improving the reducing potential in the rumen which could support the growth of bacterial communities. An improvement in the rumen environment would explain the increase in total, cellulolytic, and fibrolytic bacterial species found in previous research.

Gressley et al. (2016) evaluated the effect of *Saccharomyces cerevisiae* on hindgut fermentation in steers. *Saccharomyces cerevisiae* var. *boulardii* was infused into the abomasum for an 18-d period. On d 16, oligofructose was infused in the abomasum to stimulate a similar response to SARA. Prior to oligofructose infusion, NDF digestibility had increased with yeast infusion; however, digestibility was not affected after oligofructose infusion. Gressley et al. (2016) observed an improvement in fecal scores post infusion with yeast products. Further work

on the influence of yeast on hindgut fermentation and health has been performed in monogastric species. In post-weaning pigs fed yeast culture, there was an improvement on villus height in the jejunum which led to an increase in the villus to crypt depth ratio (Shen at al., 2009). In addition, intestinal mucosal macrophages increased in weaned piglets after consuming yeast supplement (Bontempo et al., 2006). Dogs supplemented YCW saw an improvement in ileal nutrient digestibility and a reduction in *E. coli* (Middelbos et al., 2007). These results indicate that yeast has the potential to improve intestinal gut integrity and function.

Livestock are natural reservoirs of bacterial pathogens, such as *Salmonella* and *E. coli,* which can negatively impact human health. Therefore, meat scientists are concerned about pathogen shedding and contamination in food production when hides are removed from animals during slaughter (Wheeler et al., 2014). Components of yeast and YCW products are believed to bind to pathogenic bacteria in the gastrointestinal tract to further improve gut health (White et al., 2002). In vitro studies demonstrate that *Saccharomyces cerevisiae* has the ability to bind to the cell wall of *Salmonella* spp. (Pérez-Sotelo et al., 2005). There is evidence supporting the use of probiotics to reduce pathogen shedding in feces (Gaggìa et al., 2010). The prevalence of fecal pathogens is not completely eliminated with the addition of yeast although yeast can decrease the rate of fecal shedding (Swyers et al., 2011). The ability of yeast to prevent pathogen colonization and fecal shedding will have greater application as the demand for the reduction of antibiotics continues.

Immune function

It has been well established in the literature that inclusion of yeast and yeast cell products in the diet can influence the health status of individuals. Yeast and yeast cell wall are composed of polysaccharides such as α- and β-glucans (Ruiz-Herrera, 1991) which can bind with receptors on leukocytes and stimulate an inflammatory response. Beta-glucans are composed of 1,3-linked

monomers of D-glucose with varying degree of 1,6 linkage side chains (Williams, 1997). The shape and ratio of 1,3 to 1,6 linkages differ among fungal species (Volman et al., 2008). These two factors are important in determining the function and response that β-glucans play in the body (Volman et al., 2008). Dectin-1 has been classified as a β-glucan receptor on the surface of macrophages, monocytes, and natural killer cells (Brown and Gordon, 2001; Brown, 2006) and promotes the production of TNF- α (Brown et al., 2003). Other potential receptors have been identified; however, the Dectin-1 receptor has shown the greatest biological response (Brown and Gordon, 2001). Dectin-1 can interact with toll-like receptor 2 (TLR-2) and promote stimulation of the cytokine IL-12 (Gantner et al., 2003). Additionally, B lymphocytes that have been activated by β-glucans have upregulated production of IL-6, IL-8, and TNF- α (Ali et al., 2015). Therefore, β-glucans can directly and indirectly stimulate the innate immune response.

While most research has been performed in vitro, there have been several studies that have investigated the effect that yeast components have on immune function and response. Receiving calves supplemented with live yeast, YCW, or a combination of the two had a lower neutrophil to lymphocyte ratio after a lipolysaccharide challenge (Finck et al., 2014). A smaller neutrophil to lymphocyte ratio is more desirable because it indicates a reduction in stress. Neutrophil function has been improved in dairy calves fed yeast culture from 2 to 70 d after birth (Magalhães et al., 2008). Contrasting results found that yeast treatment did not improve phagocytic activity or respiratory burst of neutrophil in pigs post-weaning (Sauerwein et al., 2007).

Finck et al. (2014) also reported an increase in TNF- α in control calves prior to a challenge. After the challenge, there was no difference based on treatment. In a similar study, heifer calves were fed different YCW products prior to and during a lipolysaccharide challenge

(Burdick Sanchez et al., 2013), and found that yeast treatment did not influence the levels of serum IFN-γ and TNF- α, but IL-6 was greater in control calves post-challenge. Yeast supplemented calves also had decreased cortisol concentrations and vaginal temperatures. These results differ from those found in weaned piglets where yeast culture increased IFN- γ gut concentration while plasma IFN- γ concentration was decreased (Shen et al., 2009). Increasing concentration of IFN-γ produced from T-lymphocytes have also been reported in swine (Xiao et al., 2004).

Yeast has shown potential to improve morbidity throughout various stages of production. In receiving calves, the percentage morbidity decreased with a commercially available yeast product compared to control (Ponce et al., 2012). Additionally, yeast has shown the potential to decrease the number of days treated with antibiotics in calves (Magalhães et al., 2008; Cole et al., 1992). However, other studies have reported no effect on morbidity, especially in receiving calves (Young et al., 2017; Finck et al., 2014). A more consistent finding is the improvement on DMI in calves after a vaccine challenge or antibiotic treatment. Cole et al. (1992) found that yeast supplemented calves had greater DMI after an infectious bovine rhinotracheitis virus (IBRV) challenge. Similarly, *Saccharomyces cerevisiae* subspecies *boulardii* stimulated intake following prophylactic treatment in calves after arrival to the feedlot (Keyser et al., 2007). Furthermore, Burdick Sanchez et al. (2014) reported a change in metabolic response after a lipopolysaccharide challenge in heifer calves consuming different varieties of YCW product.

Prenatal and calf nutrition

Nutritional status of the dam and the intake of colostrum at time of birth are two factors that can play a significant role in the health and performance of the calf later in life (Duff and Galyean, 2007). Immune function of the calf at birth is dependent on the passive transfer of

colostral immunoglobulins. Perino (1997) found that 10 to 25% of newborn calves do not receive acceptable transfer of immunoglobulins. Calves that have decreased plasma proteins 24 h after birth have increased risk for morbidity in the feedlot (Wittum and Perino, 1995). Additionally, a decrease in immunoglobulin concentrations has been observed in calves whose dams were restricted protein during late gestation (Burton et al., 1984; Blecha et al., 1981); suggesting that the nutritional status of the dam during late gestation can also impact the adequacy of passive transfer.

There has been very limited research done on the influence of yeast supplementation prepartum and the subsequent effect on beef calves. More research has been done on this area in the swine and dairy industry. Sows fed active live yeast during the last 3 wk of gestation had an increase in the number of total solids, crude protein, and gamma globulins in their milk (Jurgens et al., 1997). In the aforementioned study, there was no effect of dietary treatment on growth performance of pigs from birth to weaning; however, piglets who consumed yeast pre-weaning, and whose dams were fed supplemental yeast, had increased growth performance and feed efficiency post-weaning. Supplementing yeast during gestation and lactation to sows increased concentration of colostrum IgG which resulted in greater plasma IgG in their piglets (Jang et al., 2013). Furthermore, MOS have shown potential to increase passive immunity to dairy calves. Cows fed MOS had increased serum levels of rotovirus neutralization titers, which resulted in a greater serum titer concentration in calves (Franklin et al., 2005).

Yeast products are beneficial in the maturation of rumen microbial populations in young ruminants. In lambs fed *Saccharomyces cerevisiae*, there was improved development and stabilization of celluloytic bacteria present in the rumen (Chaucheyras-Durand and Fonty, 2001) and improved rumen morphology (Lesmeister et al., 2004; Xiao et al., 2016). As previously

mentioned, yeast can improve gut health by binding to pathogens. In dairy calves supplemented yeast pre-weaning, there was a reduction in the percentage of days with scours (Galvão et al., 2005; Seymour et al., 1995) which led to a decrease in the amount of antibiotics administered (Seymour et al., 1995). In agreement, Magalhães et al. (2008) reported a decrease in the incidence of diarrhea and fever. Supplementation of yeast to neonatal calves has shown health benefits above decreasing the severity of scours. Calves who consumed yeast through calf starter had increased IgA concentrations and elevated levels of serum haptoglobin after a vaccine challenge (Kim et al., 2011a).

Heat Stress

Heat stress can occur in cattle during elevated temperatures and high humidity. Annually the beef industry loses approximately $369 million dollars due to the negative effects that heat stress has on growth performance and reproduction along with increased mortality (St-Pierre et al., 2003). The temperature-humidity index (THI; Thom, 1959) is the most common index used to measure heat stress. According to the THI, temperatures above 24°C can indicate risk of heat stress (LCI, 1970). Feeding yeast products has been used as a method to mitigate the negative effects associated with heat stress.

In lactating dairy cows, addition of dietary yeast can improve milk production (Bruno et al., 2009; Moallem et al, 2009), increase DMI (Moallem et al., 2009), and enhance feed efficiency (Schingoethe et al., 2004) during extended exposure to high temperatures. However, results are variable, and in some instances, yeast did not alleviate the effects of heat stress on milk yield or intake (Shwartz et al., 2009). During heat stress, it is believed that the decrease in milk production is attributable to a decrease in energy intake (Beede and Collier, 1986). Shwartz et al. (2009) found that energy balance was not altered by yeast treatment during heat stress, nor

was there an impact on circulating glucose, non-esterified fatty acids (NEFA), and plasma urea nitrogen (PUN). Similar results on plasma glucose and NEFA concentrations have been reported (Bruno et al., 2009). Suggesting that yeast products do not influence blood metabolites when dairy cattle are experiencing elevated environmental temperatures. Supplemental yeast increased the concentration of plasma niacin in lactating dairy cows (Dias et al., 2018; Salvalti et al., 2015) which improves evaporative heat loss and aids in body temperature regulation (Zimbelman et al., 2010).

Heat stress is detrimental to the poultry industry and the use of yeast to ameliorate the negative response has been evaluated. Mannan oligosaccharide supplemented broilers had greater BW gains and reduced feed conversion ratios compared to un-supplemented birds during heat stress (Sohail et al., 2012). Similar results were identified by Haldar et al. (2011) where yeast supplemented broilers had improved performance when temperatures averaged above 32.4°C.

High ambient temperatures have a greater impact on finishing cattle compared with cattle grazing pasture. Average daily gain of finishing cattle was increased by feeding enzymatically hydrolyzed yeast during the last 90 d before slaughter when THI was above normal (Salinas-Chavira et al., 2015). Salinas-Chavira et al. (2015) suspected the improvement in ADG was related to the increase in DMI as net energy was not altered at this time. Broadway et al. (2016) also reported a decreased vaginal temperatures accompanied by an increase in water consumption and drinking bouts in finishing heifer calves during induced heat stress. Omnigen-AF (a yeast-based product) mitigated some of the negative effects of heat stress by decreasing white blood cells and lymphocytes, therefore, improving the leukocyte profile (Burdick Sanchez

et al., 2015). Research suggests that yeast could be a useful tool in mitigating negative effects of heat stress.

Objective

Yeast products appear to be beneficial during times of stress. Calves experience significant levels of stress at birth, weaning, transportation, and receiving. Management during these time points impacts later performance. After reviewing the literature, the influence of yeast at birth and pre-weaning has not been intensively studied. Therefore, the objective of the following experiments was to determine how yeast influences health and growth performance during 1) receiving, 2) pre-weaning, and 3) in late gestation cows and the subsequent effect on their calves.

LITERATURE CITED

Association of American Feed Control Officials Incorporated. 2013 Official Publication. Pages 450-451.

Ali, M. F., C. B. Driscoll, P. R. Walters, A. H. Limper, and E. M. Carmona. 2015. Beta-glucan activated human B-lymphocytes participate in innate immune responses by releasing pro-inflammatory cytokines and stimulating neutrophil chemotaxis. J. Immunol. 195:5318-5326.

AlZahal, O., F. Li, L. L. Guan, N. D. Walker, and B. W. McBride. 2017. Factors influencing ruminal bacterial community diversity and composition and microbial fibrolytic enzyme abundance in lactating dairy cows with a focus on the role of active dry yeast. J. Dairy Sci. 100:4377-4393.

Ando, S., R. I. Khan, J. Takahasi, Y. Gamo, R. Morikawa, Y. Nishiguchi, and K. Hayasaka. 2004. Manipulation of rumen fermentation by yeast: the effects of dried beer yeast on the in vitro degradability of forages and methane production. Asian-Aust. J. Anim. Sci. 17:68-72.

Barry, T. N., A. Thompson, and D. G. Armstrong. 1977. Rumen fermentation studies on two contrasting diets. 1. Some characteristics of the in vivo fermentation, with special reference to the composition of the gas phase, oxidation/reduction state, and volatile fatty acid proportions. J. Agric. Sci. Camb. 89:183-195.

Beauchemin, K. A., W. Z. Yang, and L. M. Rode. 2003. Effects of particle size of alfalfa based dairy cow diets on chewing activity, ruminal fermentation, and milk production. J. Anim. Sci. 86:630-643.

Beede, D. K., and R. J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62:543-554.

Blecha, F., R. C. Bull, D. P. Olson, R. H. Ross, and S. Curtis. 1981. Effects of prepartum protein restriction in the beef cow on immunoglobulin content in blood and colostral whey and subsequent immunoglobulin absorption by the neonatal calf. J. Anim. Sci. 53:1174-1180.

Bontempo, V., A. Di Giancamillo, G. Savoini, V. Dell'Orto, and C. Domeneghini. 2006. Live yeast dietary supplementation acts upon intestinal morpho-functional aspects and growth in weanling piglets. Anim. Feed Sci. Tech. 129:224-236.

Broadway, P. R., J. A. Carroll, N. C. Burdick-Sanchez, S. L. Roberts, K. P. Sharon, J. T. Richeson, and J. R. Corley. 2016. Yeast probiotic supplementation mitigates come of the negative effects of heat stress in feedlot heifers. J. Anim. Sci. 94 (E-supplemental 1):34.

Broberg, G. 1957. Measurements of the redox potential in rumen contents. I. In vitro measurements on healthy animals. Nord. Vet. Med. 9:918-931.

Brown, G. D. 2006. Dectin-1: a signaling non-TLR pattern-recognition receptor. Nat. Rev. Immunol. 6:33-43.

Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for beta-glucans. Nature 413:36-37.

Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. J. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of β-glucans. J. Exp. Med. 197:1119-1124.

Bruno, R. G. S., H. M. Rutigliano, R. L. Cerri, P. H. Robinson, and J. E. P. Santos. 2009. Effect of feeding *Saccharomyces Cerevisiae* on performance of dairy cows during summer heat stress. Anim. Sci. Feed Tech. 150:175-186.

Burton, J. H., A. A. Hosein, I. McMillan, D. G. Grieve, and B. N. Wilkie. 1984. Immunoglobulin absorption in calves as influenced by dietary protein intakes of their dams. Can. J. Anim. Sci. 64:185-186.

Burdick Sanchez, N. C., J. A. Carroll, M. L. Mcbride, X. A. Ortiz, J. L. Collier, J. D. Chapman, D. Mclean, and R. J. Collier. 2015. OmniGen-AF alters rectal temperature (RT) and leukocyte profiles in dairy cows exposed to heat stress (HS) following acute activation of the stress axis. J. Anim. Sci. Suppl. 93 (E-Supplement 3):323.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R. Corley, R. J. Rathmann, and B. J. Johnson. 2013. Yeast cell wall supplementation alters aspects of the physiological and acute phase responses of crossbred heifers to an endotoxin challenge. Innate Immunity 19:411-419.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R. Corley, R. J. Rathmann, and B. J. Johnson. 2014. Yeast cell wall supplementation alters the metabolic responses of crossbred heifers to an endotoxin challenge. Innate Immunity 20:104-112.

Chaucheyras, F., G. Fonty, G. Bertin, and P. Gouet. 1995. In vitro H_2 utilization by a ruminal acetogenic bacterium cultivated alone or in association with an archaea methanogen is stimulated by a probiotic strain of Saccharomyces cerevisiae. Appl. Environ. Microbiol. 61:3466-3467.

Chaucheyras-Durand, F. and G. Fonty. 2001. Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive *Saccharomyces cerevisiae* CNCM I-1077. Reprod. Nutr. Dev. 41:57-68.

Chaucheyras-Durand, F. and G. Fonty. 2006. Effects and modes of action of live yeasts in the rumen. Biologia (Bratislava). 61:741-750.

Chaucheyras-Durand, F., G. Fonty, and G. Bertin. 1997. Effects of a microbial additive, Levucell SC, on growth and metabolism of ruminal acetogenic bacterial strain in vivo. In: Proceedings of Rumen Function Conference, Chicago, USA, p. 33.

Chaucheyras-Durand, F., N. D. Walker, and A. Bach. 2008. Effects of active dry yeast on the rumen microbial ecosystem: Past, present, and future. Anim. Feed Sci. and Tech. 145:5-26.

Cole, N. A. 1996. Review of bovine respiratory disease: Nutrition and disease interactions. Pages 57-74 in Review of Bovine Respiratory Disease – Schering-Plough Animal Health. R. Smith, ed. Veterinary Learning Systems, Trenton, NJ.

Cole, N. A., C. W. Purdy, and D. P. Hutcheson. 1992. Influence of yeast culture on feeder calves and lambs. J. Anim. Sci. 70:1682-1690.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92:1620-1632.

Dias, J. D. L., R. B. Silva, T. Fernandes, E. F. Barbosa, L. E. C. Graças, R. C. Araujo, R. A. N. Pereira, and M. N. Pereira. 2018. Yeast culture increased plasma niacin concentration, evaporative heat loss, and feed efficiency of dairy cows in a hot environment. J. Dairy Sci. 101:1-13.

Duff, G. C., and M. L. Galyean. 2007. Board Invited Review: Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Ferry, J. G. 1992. Methane from acetate. J. Bacteriol. 174:5489-5495.

Finck, D. N., F. R. B. Ribeiro, N. C. Burdick, S. L. Parr, J. A. Carroll, T. R. Young, B. C. Bernhard, J. R. Corley, A. G. Estefan, R. J. Rathmann, and B. J. Johnson. 2014. Yeast supplementation alters the performance and health status of receiving cattle. Prof. Anim. Sci. 30:333-341.

Franklin, S. T., M. C. Newman, K. E. Newman, and K. I. Meek. 2005. Immune parameters of dry cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. J. Dairy Sci. 68:766-775.

Gaggía, F., P. Mattarelli, and B. Biavati. 2010. Probiotics and prebiotics in animal feeding for safe food production. Internat. J. Food Microbiol. 141:515-528.

Galvão, K. N., J. E. P. Santos, A. Coscioni, M. Villasenor, W. M. Sischo, and A. C. B. Berge. 2005. Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*. Reprod. Nutr. Dev. 45:427-440.

Gantner, B. N., R. M. Simmons, S. J. Canavera, S. Akira, and D. M. Underhill. 2003. Collaborative induction of inflammatory responses by dectin-1 and toll-like receptor 2. J. Exp. Med. 197:1107-1117.

Gressley, T. F., K. A. Davison, J. Macies, C. Leonardi, M. M. McCarthy, L. M. Nemec, and C. A. Rice. 2016. Effect of abomasal carbohydrates and live yeast on measures of postruminal fermentation. J. Anim. Sci. 94:284-296.

Haldar, S., T. K. Ghosh, Toshiwati, and M. R. Bedford. 2011. Effects of yeast (*Saccharomyces cerevisiae*) and yeast protein concentrate on production performance of broiler chickens exposed to heat stress and challenged with *Salmonella enteritidis*. Anim. Feed Sci. Tech. 168:61-71.

Harrison, G. A., R. W. Hemken, K. A. Dawson, and R. J. Harmon. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967-2975.

Hinman, D. D., S. J. Sorensen, and P. A. Momont. 1998. Effect of yeast culture on steer performance, apparent digestibility, and carcass measurements when used in a barley and potato finishing diet. Prof. Anim. Sci. 14:173-177.

Hristov, A. N., G. Varga, T. Cassidy, M. Long, K. Heyler, S. K. R. Karnati, B. Corl, C. J. Hovde, and I. Yoon. 2010. Effect of Saccharomyces cerevisae fermentation product on ruminal fermentation and nutrient utilization in dairy cows. J. Dairy Sci. 93:682-692.

Jang, Y. D., K. W. Kang, L. G. Piao, T. S. Jeong, E. Auclair, S. Jonvel, R. D'Inca, and Y. Y. Kim. 2013. Effects of live yeast supplementation to gestation and lactation diets on reproductive performance, immunological parameters, and milk composition in sows. Livestock Sci. 152:167-173.

Jiang, Y., I. M. Ogunade, K. G. Arriola, M. Qi, D. Vyas, C. R. Staples, and A. T. Adesogan. 2017a. Effects of the dose and viability of *Saccharomyces cerevisiae*. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. J. Dairy Sci. 100:8102-8118.

Jiang, Y., I. M. Ogunade, S. Qi, T. J. Hackmann, C. R. Staples, and A. T. Adesogan. 2017b. Effects of the dose and viability of *Saccharomyces cerevisiae*. 1. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. J. Dairy Sci. 100:325-342.

Jurgens, M. H., R. A. Rikabi, and D. R. Zimmerman. 1997. The effect of dietary active dry yeast supplement on performance of sows during gestation-lactation and their pigs. J. Anim. Sci. 75:593-597.

Keyser, S. A., J. P. McMeniman, D. R. Smith, J. C. MacDonald, and M. L. Galyean. 2007. Effects of *Saccharomyces cerevisiae* subspecies *boulardii* CNCM I-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J. Anim. Sci. 85:1264-1273.

Kim, M. H., J. K. Seo, C. H. Yun, S. J. Kang, J. Y. Ko, and J. K. Ha. 2011a. Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. Animal 5:953-960.

Křížová, L., M. Richter, J. Třináctý, J. Říha, and D. Kumprechtova. 2011. The effect of feeding live yeast cultures on ruminal pH and redox potential in dry cows as continuously measured by a new wireless device. Czech J. Anim. Sci. 56:37-45.

Kumar, U., V. K. Sareen, and S. Singh. 1994. Effect of *Saccharomyces cerevisiae* yeast culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet. J. Anim. Sci. 59:209-215.

LCI. 1970. Patterns of transit losses. Omaha, Neb. Livestock Conservation, Inc.

Lesmeister, K. E., A. Heinrichs, and M. T. Gabler. 2004. Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci. 87:1832-1839.

Lynch, H. A., and S. A. Martin. 2002. Effects of *Saccharomyces cerevisiae* culture and *Saccharomyces cerevisiae* live cells on in vitro mixed ruminal microorganism fermentation. J. Dairy Sci. 85:2603-2608.

Magalhães, V. J., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetance of dairy calves. J. Dairy. Sci. 91:1497-1509.

Marden, J. P., C. Julien, V. Monteils, E. Auclair, R. Moncoulon, and C. Bayourthe. 2008. How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? J. Dairy Sci. 91:3528-3535.

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82:3346-3356.

Middelbos, I. S., M. R. Godoy, N. D. Fastinger, and G. C. Fahey Jr. 2007. A dose-response evaluation of spray-dried yeast cell wall supplementation of diets fed to adult dogs: Effects on nutrient digestibility, immune indices, and fecal microbial populations. J. Anim. Sci. 85:3022-3032.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 92:343-351.

Newbold, C. J., R. J. Wallace, X. B. Chen, and F. M. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73:1811-1818.

Nocek, J. E. 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80:1005-1028.

Nocek, J. E., M. G. Holt, and J. Oppy. 2011. Effects of supplementation with yeast culture and enzymatically hydrolyzed yeast on performance of early lactation dairy cattle. J. Dairy Sci. 94:4046-4056.

Ovinge, L. A., J. O. Sarturi, M. L. Galyean, M. A. Ballou, S. J. Trojan, P. R. B. Campanili, A. A. Alrumaih, and L. A. Pellarin. 2018. Effects of a live yeast in natural-program finishing feedlot diets on growth performance, digestibility, carcass characteristics, and feeding behavior. J. Anim. Sci. 96:684-693.

Pérez-Sotelo, L. S., M. Talavera-Rojas, H. G. Monroy-Salazar, S. Lagunas-Bernabé, J. A. Cuarón-Ibargüengoytia, R. M. O. Jiménez, and J. C. Vázquez-Chagoyán. 2005. In vitro evaluation of the binding capacity of *Saccharomyces cerevisiae* Sc47 to adhere to the wall of *Salmonella spp*. Rev. Latinoam. Microbiol. 47:70-75.

Perino, L. J. 1997. A guide to colostrum management in beef cows and calves. Vet. Med. 92:75-82.

Pinloche, E., N. McEwan, J. Marden, C. Bayourthe, E. Auclair, and C. Newbold. 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS ONE 8:e67824.

Plaizier, J. C., J. E. Keunen, J-P. Walton, T. F. Duffield, and B. W. McBride. Short Communication: Effect of subacute ruminal acidosis on in situ digestion of mixed hay in lactating dairy cows. Can. J. Anim. Sci. 81:421-423.

Ponce, C. H., J. S. Schutz, C. C. Elrod, U. Y. Anele, and M. L. Galyean. 2012. Effects of dietary supplementation of a yeast product on performance and morbidity of newly received beef heifers. Prof. Anim. Sci. 28:618-622.

Robinson, P. H., and J. E. Garrett. 1999. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to postpartum diets and on lactational performance. J. Anim. Sci. 77:988-999.

Ruiz-Herrera, J. 1991. Biosynthesis of β-glucans in fungi. Antonie van Leeuwenhoek 60:73-81.

Russell, J. B. 1998. The importance of pH in the regulation or ruminal acetate to propionate ratio and methane production in vitro. J. Dairy Sci. 81:3222-3230.

Salinas-Chavira, J., C. Arzola, V. González-Vizcarra, O. M. Manríquez-Núñez, M. F. Montaño-Gómez, J. D. Navarrete-Reyes, C. Raymundo, and R. A. Zinn. 2015. Influence of feeding enzymatically hydrolyzed yeast cell wall on growth performance and digestive function of feedlot cattle during periods of elevated ambient temperature. Asian Australas. J. Anim. Sci. 28:1288-1295.

Salvati, G. G. S., N. N. Morais Júnior, A. C. S. Melo, R. R. Vilela, F. F. Cardoso, M. Aronovich, R. A. N. Pereira, and M. N. Pereira. 2015. Response of lactating cows to live yeast supplementation during summer. J. Dairy Sci. 98:4062-4073.

Sauerwein, H., S. Schmitz, and S. Hiss. 2007. Effects of a dietary application of a yeast cell wall extract on innate and acquired immunity, on oxidative status and growth performance in weanling piglets and on the ileal epithelium in fattened pigs. J. Anim. Physiology and Anim. Nutrition 91:369-380.

Schingoethe, D. J., K. N. Linke, K. F. Kalscheur, A. R. Hippen, D. R. Renich, and I. Yoon. 2004. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87:4178-4181.

Seymour, W. M., J. E. Nocek, and J. Siciliano-Jones. 1995. Effects of a colostrum substitute and of dietary brewer's yeast on the health and performance of dairy calves. J. Dairy Sci. 78:412-420.

Shen, Y. B., X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon, and Y. G. Zhen. 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87:2614-2624.

Shwartz, G., M. L. Rhoads, M. J. VanBaale, R. P. Rhoads, and L. H. Baumgard. 2009. Effects of supplemental yeast culture on heat-stressed lactating Holstein cows. J. Dairy Sci. 92:935-942.

Sohail, M. U., M. E. Hume, J. A. Byrd, D. J. Nisbet, A. Ijaz, A. Sohail, M. Z. Shabbir, and H. Rehman. 2012. Effect of supplementation of prebiotic mannan-oligosaacharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poultry Sci. 81:2235-2240.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. J. Dairy Sci. 86:E52-E77.

Swyers, K. L., B. A. Carlson, K. K. Nightingale, K. E. Belk, and S. L. Archibeque. 2011. Naturally colonized beef cattle populations fed combinations of yeast culture and an ionophore in finishing diets containing dried distiller's grains with solubles had similar fecal shedding of *Escherichia coli* O157:H7. J. Food Protection 74:912-918.

Swyers, K. L., J. J. Wagner, K. L. Dorton, and S. L. Archibeque. 2009. Evaluation of *Saccharomyces cerevisiae* fermentation products as a natural alternative to an ionophore on growth performance, cost of gain, and carcass characteristics of heavy-weight yearling beef steers. Proceedings from Western Section American Association of Animal Science. 60:377-381.

Thom, E. C. 1959. The discomfort index. Weatherwise 12:57-59.

Vendramini, J. M. B., and J. D. Arthington. 2007. Case Study: Effects of supplemental yeast fermentation products on performance of early-weaned calves on pasture and measures of stress and performance during a feedlot receiving period. Prof. Anim. Sci. 23:709-714.

Volman, J. J., J. D. Ramakers, and J. Plat. 2008. Dietary modulation of immune function by β-glucans. Physiol. Behav. 94:276-284.

Vyas, D., A. Uwizeye, R. Mohammed, W. Z. Yang, N. D. Walker, and K. A. Beauchemin. 2014. The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers. J. Anim. Sci. 92:724-732.

Wheeler, T. L., N. Kalchayanand, and J. M. Bosilevac. 2014. Pre- and post-harvest interventions to reduce pathogen contamination in the U.S. beef industry. Meat Sci. 98:372-382.

White, L. A., M. C. Newman, G. L. Cromwell, and M. D. Lindemann. 2002. Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs. J. Anim. Sci. 80:2619-2628.

Williams, P. E. V., C. A. G. Tait, G. M. Innes, and C. J. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3026.

Wittum, T. E., and L. J. Perino. 1995. Passive immune status at postpartum hour 24 and long-term health and performance of calves. Am. J. Vet. Res. 56:1149-1154.

Xiao, J. X., G. M. Alugongo, R. Chung, S. Z. Dong, S. L. Li, I. Yoon, Z. H. Wu, and Z. J. Cao. 2016. Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: Ruminal fermentation, gastrointestinal morphology, and microbial community. J. Dairy Sci. 99:5401-5412.

Xiao, Z., C. A. Trincado, and M. P. Murtaugh. 2004. B-glucan enhancement of t cell IFN-γ response in swine. Vet. Immunol. Immunopathol. 102:315-320.

Young, T. R., F. R. B. Ribeiro, N. C. Burdick Sanchez, J. A. Carroll, M. A. Jennings, J. T. Cribbs, R. J. Rathmann, J. R. Corley, and B. J. Johnson. 2017. Yeast cell wall supplementation alters the performance and health of beef heifers during the receiving period. Prof. Anim. Sci. 33:166-175.

Zimbelman, R. B., L. H. Baumgard, and R. J. Collier. 2010. Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. J. Dairy Sci. 93:2387-2394.

CHAPTER III

Influence of commercial yeast products in diets for beef cattle new to the feedlot environment

ABSTRACT

Bovine respiratory disease (BRD) is of great concern for stocker producers in the Southeastern US, thus this research was conducted to test the effects of yeast products on health and performance of high risk, newly received beef calves at the University of Arkansas Stocker Receiving Unit. Male beef calves (n = 175; initial BW = 226 ± 24.5 kg) were delivered in 2 arrival blocks (Arrival Block 1: n = 85 with 54 intact bulls and 31 steers; Arrival Block 2: n = 90 with 62 intact bulls and 28 steers). Within arrival block, calves were blocked by initial BW, stratified by castrate status, and assigned randomly to pen (5 or 6 calves/pen; 15 pens/truckload). Pens within each weight block were allocated to 1 of 3 treatments; an unsupplemented control, or supplementation with 2 commercial *Saccharomyces cerevisiae* yeast products (**Y1** or **Y2**) for 28 d. Each day, a mixture (0.14 kg/d for each calf) of 58% ground corn and 42% of the appropriate yeast product, or 100% ground corn (control) was added to the feedbunks immediately after basal diet delivery. Average daily gain from d 0 to 28 was not different ($P = 0.99$) between treatments. Providing yeast product did not affect DMI ($P \geq 0.92$) or G:F ($P \geq 0.91$). Percentage morbidity ($P = 0.36$), percentage of BRD relapses ($P = 0.32$), and days to first antibiotic treatment ($P = 0.17$) did not differ due to yeast supplementation treatment. Antibiotic treatments began on d 2 and by d 5 approximately 50% of calves had been administered their first antibiotic. In high risk calves with over 50% morbidity by d 5, providing yeast products did not have an effect on health and growth performance.

Key Words: health, performance, receiving calves, yeast

INTRODUCTION

The receiving period is a crucial time in finishing phase of production and can affect calf performance during finishing. Calves entering the feedlot are often stressed due to weaning, commingling, and transportation (Duff and Galyean, 2007). Stress leads to a reduction in DMI (Cole, 1996) in addition to adversely affecting the immune system (Blecha et al., 1984) which can increase susceptibility to disease. According to a USDA report in 2013, approximately 16.2% of calves exhibited signs of bovine respiratory disease (**BRD**) during their time in the feedlot. The incidence of BRD is a great source of economic loss to the beef industry. Economic losses are incurred through mortality, antibiotic treatment, and decreased production in calves. Schneider et al. (2009) reported that carcass values decreased by $23.23, $30.15, and $54.01 for calves treated once, twice, or thrice or more for BRD. There has been greater emphasize placed on the judicious use of antibiotics due to both public perception and recent government regulation. Therefore, in the beef industry feeding naturally occurring yeast products has been proposed to improve health and increase growth performance.

Yeast products are utilized in ruminant diets to target ruminal microbial populations. Different strains of *Saccharomyces cerevisiae* have been shown to increase total, cellulolytic (Newbold et al., 1995), and fibrolytic bacterial populations (Chaucheyras-Durand and Fonty, 2006) in ruminants. The most promising use of live yeast is seen in the stabilization of ruminal pH and decreasing the incidence of acidosis in cattle (Chaucheyras-Durand et al., 2008). Supplementing yeast products to high risk, lightweight calves during the receiving period demonstrated a potential to increase DMI (Finck et al., 2014; Ponce et al., 2012); however, more variable results were found in ADG. Therefore, the objective of this study was to determine the

effect of feeding 2 commercial yeast products on growth performance and morbidity in high risk calves during a 28-d receiving period.

MATERIALS AND METHODS

Animals

All experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (protocol # 16068). This experiment was performed in 2 arrival blocks. The first arrival block (Arrival Block 1) of male calves (n = 85; 54 intact bulls and 31 steers; BW = 244 ± 16.2 kg) was delivered to the University of Arkansas Stocker Cattle Receiving unit near Fayetteville, AR on May 13, 2016. The second arrival block of male calves (Arrival Block 2; n = 90; 62 intact bulls and 28 steers; BW = 208 ± 17.1 kg) arrived on September 21, 2016.

On d 0, calves from Arrival Block 1 were weighed, tagged with a unique individual identification number, and ear notched for detection of calves persistently infected with bovine viral diarrhea virus (**PI-BVDV**). Calves in Arrival Block 1 were allocated by BW and castrate status to treatment and processed later that afternoon. Arrival Block 2 was delivered on d -1 and calves were weighed, tagged with a unique individual identification number, and ear notched for detection of calves infected with PI-BVDV. Ear notches from both arrival blocks were sent to Cattle Stats (Oklahoma City, OK) and 2 calves (1 each on Y1 and Y2 treatment) from Arrival Block 2 were PI-BVDV positive and were pulled from the study pens on d 1. Calves from Arrival Block 2 were held overnight with access to hay and water. Processing and treatment allocation occurred at 0800 the following morning (d 0). At processing, calves from both arrival blocks were branded, dewormed (Valbazen®, Zoetis, Parsippany, NJ), castrated via banding (if needed), and administered a viral vaccine for respiratory pathogens (Bovi-Shield Gold, Zoetis)

and a clostridial vaccination (Covexin 8, Merck Animal Health, Madison, NJ). Weight at arrival and at processing were averaged together for an initial weight.

Treatment and Pen Assignment

Calves were grouped by arrival weight into 5 weight blocks and stratified by castration status (bull or steer) and assigned randomly to 1 of 3 pens (5 to 6 calves/pen) within each BW group. Pens within each weight group were assigned randomly to 1 of 3 treatments that were top-dressed over the basal diet: 1) ground corn (**CON**), 2) CON + commercial *Saccharomyces cerevisiae* yeast product 1 (**Y1;** brewer's yeast), and 3) CON + commercial *Saccharomyces cerevisiae* yeast product 2 (**Y2;** yeast culture). Treatments 2 and 3 were the appropriate yeast product (42% as fed basis) and ground corn (58% as fed basis). Prior to the start of each arrival block, top-dress was mixed at the University of Arkansas Animal Science feed mill in a paddle mixer.

DMI and BW

On d 0, calves were offered the basal diet (Table 1) at 1% of their BW formulated to meet their nutrient needs. Top dress was added to the basal diet daily at 0.14 kg/d for each calf and mixed in the bunk by hand. On d 0, 1, and 2, calves were offered 0.9 kg/d of long-stem hay to adapt to the basal diet. Calves were fed once a day in the morning using a slick bunk feeding method with the amount of feed adjusted accordingly, with a maximum daily feed increase of 0.91 kg/calf. Any orts exceeding approximately 10% of feed offered were collected and weighed.

On d 14, calves were weighed, revaccinated with a respiratory (Bovi-Shield Gold, Zoetis) and clostridial vaccine (Covexin 8, Merck Animal Health), and given a second dewormer (Dectomax®, Zoetis). Calves remained on treatment until d 28 of the study. Calves were weighed on d 27 and 28 and BW were averaged to determine final weight.

Feed Analysis

Basal diet and top-dress were sampled weekly and composited and a hay sample was collected once at the beginning of each arrival block. All samples were dried at 50°C in a forced-air oven until a stable weight was reached. Duplicates of the dried samples were ground through a Wiley Mill (Thomas Scientific, Swedesboro, NJ) using a 1 mm screen and analyzed for nutrient content (Table 2). Dried samples were analyzed for NDF and ADF (ANKOM Technology Corp., Fairport, NJ; Vogel et al., 1999) and CP by total combustion (Rapid Combustion Method, Elementar Americas, Inc., Mt. Laural, NJ). Mineral analysis was performed at the University of Arkansas Division of Agriculture Altheimer Laboratory (Fayetteville, AR) by inductively coupled plasma spectroscopy (Model 3560, Applied Research Laboratory, Sunland, CA) following wet ashing.

Calf Health

Calves were observed daily for BRD and assigned a clinical attitude score (**CAS**) of 0 to 4 (0 = normal, 1 = mild BRD, 2 = moderate BRD, 3 = severe BRD, 4 = moribund). If a calf received a CAS > 0 it was pulled from the pen. If a calf had a CAS of 1 or 2 and a rectal temperature ≥ 40°C, or the calf had a CAS of 3 or 4 regardless of rectal temperature were administered an antibiotic treatment based on a pre-determined antibiotic protocol. The antibiotic protocol consisted of florifenicol (Nuflor, Merck Animal Health) as the first antibiotic administered. Rectal temperatures were rechecked at the end of the post treatment interval (PTI; 2 d). If calves were pulled a second time and met the predetermined requirements for antibiotic treatment, they were administered ceftiofur crystalline free acid (Excede, Zoetis) as the second antibiotic treatment (PTI = 5 d). Lastly, if calves required a third antibiotic treatment, they were given tulathromycin (Draxxin, Zoetis). Calves were rechecked 7 d following administration;

however, no additional antibiotics were administered at this time. Antibiotics, BW, rectal temperatures, and day of treatment were recorded throughout the study.

Statistical Analysis

Body weight, ADG, G:F, DMI, and cost of antibiotic treatment were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Pen was set as the experimental unit for all dependent variables. In the initial statistical analysis, Weight Block was included in the model but was nonsignificant and thus was removed. Therefore, the model for quantitative variables included treatment as a fixed effect and Arrival Block as a random effect. The Kenward-Rogers option was used to adjust degrees of freedom to reduce prevalence of type 1 errors. Qualitative data were analyzed with the GLIMMIX procedure of SAS. Fixed effects included treatment while the random effect was Weight Block. Due to a lower number of observations, chronic and mortality data were analyzed using the GENMOD procedure with treatment being the only fixed effect. Least square means for growth performance, feed intake, and morbidity data were separated using orthogonal contrasts. Orthogonal contrasts were: 1) supplementation with yeast versus control and 2) the contrast between the 2 yeast products. The number of days until treatment was analyzed using the LIFETEST procedure of SAS. Treatment means were considered different at $P \leq 0.05$ and tendencies were observed at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

There were no differences ($P \geq 0.82$) in growth performance among the 3 treatment groups (Table 3). Final BW at the end of the 28-d period were not affected ($P = 0.99$) by supplemental yeast products. Furthermore, ADG did not differ ($P = 0.99$) among treatments during the receiving period. While it is difficult to detect differences in gain during a 28-d receiving period, previous research has found similar results in receiving cattle supplemented

yeast products for longer periods of time. Finck et al. (2014) reported that ADG was not affected by supplementation of live yeast, yeast cell wall (**YCW**), or a combination during a 56-d receiving period. Conversely, Ponce et al. (2012) reported a tendency for ADG to increase during a 35-d period when calves were fed an enzymatically hydrolyzed yeast product. Heifer calves supplemented YCW tended to have a greater ADG prior to and following a lipopolysaccharide challenge with differences observed in glucose, insulin, NEFA, and BUN concentrations post challenge (Burdick Sanchez et al., 2014). Indicating that supplementation with YCW could have the potential to alter energy metabolism in calves. Both yeast products in this study were strains of *Saccharomyces cerevisiae* and it is not well known how different varieties of yeast products effect growth performance. In a meta-analysis evaluating the impact that yeast has in ruminant species, primarily dairy, found that the form of diet, stage of production, and management could influence the beneficial effect of yeast (Desnoyers et al., 2009).

There was no effect of treatment on DMI ($P \geq 0.99$) or in G:F ($P \geq 0.97$) from d 0 to 28 (Table 3). Contrary to the current study, an increase in DMI was reported in receiving cattle when supplemented with yeast products (Finck et al., 2014; Ponce et al., 2012). In addition, there was a tendency for DMI to increase in feeder calves after induced stress when fed a yeast culture at 1 and 2% of the diet (Phillips and VonTungeln, 1985). Furthermore, Cole et al. (1992) observed an increase in DMI in calves fed yeast culture after exposure to IBRV challenge. Due to greater DMI in yeast fed calves, there was a tendency for increased weight gain compared to control. In regard to this study, a high occurrence of morbidity was observed across treatments with no change in performance due to treatment.

Percentages of calves treated for BRD once, twice, or thrice were not affected ($P \geq 0.36$; Table 4) by dietary treatment. The number of antibiotic treatments per calf was not different ($P = 0.74$) between CON, Y1, and Y2 treatments. Percentage of mortality ($P = 0.11$; Table 5) and percentage of calves classified as chronic ($P = 0.57$) were not different among dietary treatments. Two calves (1 each from Arrival Block 1 and 2) on the Y2 treatment died from BRD and six calves were classified as chronic. Additionally, a third calf from Arrival Block 2 was removed from the study on d 16 for reasons outside of respiratory illness and data from this calf were excluded. Cost of antibiotic treatment was not different ($P = 0.81$) between the three dietary treatments. As represented in Figure 1, calves started receiving antibiotic treatment for BRD on d 2. By d 5 approximately 54% of calves had been treated for respiratory disease, providing evidence that calves were previously exposed to respiratory pathogens prior to being delivered to the University Stocker Unit. Therefore, there was a reduced likelihood that nutritional intervention provided after arrival would have impacted BRD treatment rate.

Cole et al. (1992) proposed that supplemental yeast is more advantageous during periods of stress. Steers subjected to a lipopolysaccharide challenge had a lower neutrophil to lymphocyte ratio when supplemented with yeast product; indicating that steers supplemented with yeast experienced less stress from the immune challenge compared to the control (Finck et al., 2014). Furthermore, lightweight calves receiving *Saccharomyces cerevisiae* subspecies *boulardii* in the diet had greater DMI after administration of antibiotics upon arrival and fewer calves received a second round of antibiotic treatment (Keyser et al., 2007). Additionally, YCW supplemented in the diet resulted in decreased cortisol and IL-6 concentrations, and vaginal temperatures after exposure to an endotoxin (Burdick Sanchez et al., 2013), suggesting that yeast product may be more beneficial in recovery from respiratory disease in high risk, receiving

calves. An increase in DMI intake after exposure to a challenge (Cole et al., 1992; Phillips and VonTungeln, 1985; Keyser et al., 2007) provides further evidence that yeast may reduce the negative effects of an immune challenge. However, the current study did not observe a decrease ($P = 0.32$) in the percentage of morbid calves treated a second time for BRD. In high risk calves with over 50% morbidity by d 5, providing yeast did not improve health and growth performance during a 28-d receiving period.

Implications

Addition of yeast, *Saccharomyces cerevisiae*, did not affect growth performance during a 28-d receiving period in high risk calves. Furthermore, yeast supplementation did not impact the percentage of calves treated for BRD, nor did yeast decrease the administration or cost of antibiotic treatment in newly received stocker calves. Morbidity was high in the first 5 d after entry to the stocker facilities, indicating that calves were exposed to pathogens prior to arrival. Therefore, it could be advantageous to evaluate the effect of yeast prior to arrival at the feedlot and prior to exposure to BRD pathogens.

LITERATURE CITED

Blecha, F., S. L. Boyles, and J. G. Riley. 1984. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman × Angus feeder calves. J. Anim. Sci. 59:576-583.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R Corley, R. J. Rathmann, and B. J. Johnson. 2013. Yeast cell wall supplementation alters aspects of the physiological and acute phase responses of crossbred heifers to an endotoxin challenge. Innate Immunity 19:411-419.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R Corley, R. J. Rathmann, and B. J. Johnson. 2014. Yeast cell wall supplementation alters the metabolic responses of crossbred heifers to an endotoxin challenge. Innate Immunity 20:104-112.

Chaucheyras-Durand, F., and G. Fonty. 2006. Effects and modes of action of live yeasts in the rumen. Biologia 61:741-750.

Chaucheyras-Durand, F., N. D. Walker, and A. Bach. 2008. Effects of active dry yeast on the rumen microbial ecosystem: Past, present, and future. Anim. Feed Sci. and Tech. 145:5-26.

Cole, N. A., C. W. Purdy, and D. P. Hutcheson. 1992. Influence of yeast culture on feeder calves and lambs. J. Anim. Sci. 70:1682-1690.

Cole, N. A. 1996. Review of bovine respiratory disease: Nutrition and disease interactions. Pages 57-74 in Review of Bovine Respiratory Disease – Schering-Plough Animal Health. R. Smith, ed. Veterinary Learning Systems, Trenton, NJ.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92:1620-1632.

Duff, G. C., and M. L. Galyean. 2007. Board Invited Review: Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823-840.

Finck, D. N., F. R. B. Ribeiro, N. C. Burdick, S. L. Parr, J. A. Carroll, T. R. Young, B. C. Bernhard, J. R. Corley, A. G. Estefan, R. J. Rathmann, and B. J. Johnson. 2014. Yeast supplementation alters the performance and health status of receiving cattle. Prof. Anim. Sci. 30:333-341.

Keyser, S. A., J. P. McMeniman, D. R. Smith, J. C. MacDonald, and M. L. Galyean. 2007. Effects of *Saccharomyces cerevisiae* subspecies *boulardii* CNCM I-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J. Anim. Sci. 85:1264-1273.

Newbold, C. J., R. J. Wallace, X. B. Chen., and F. M. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73:1811-1818.

Phillips, W. A., and D. L. VonTungeln. 1985. The effect of yeast culture on the poststress performance of feeder calves. Nutr. Rep. Internat. 32:287-294.

Ponce, C. H., J. S. Schutz, C. C. Elrod, U. Y. Anele, and M. L. Galyean. 2012. Effects of dietary supplementation of a yeast product on performance and morbidity of newly received beef heifers. Prof. Anim. Sci. 28:618-622.

Schneider, M. J., R. G. Tait Jr., W. D. Busby, and J. M. Reecy. 2009. An evaluation of bovine respiratory disease complex in feedlot cattle: Impact on performance and carcass traits using treatment record and lung lesion scores. J. Anim. Sci. 87:1821-1827.

USDA. 2013. Types and costs of respiratory disease treatments in U.S. feedlots. In: USDA:APHIS:VS (ed.). National Animal Health Monitoring System, Fort Collins, CO.https://www.aphis.usda.gov/animal_health/nahms/feedlot/downloads/feedlot2011/Feed11_is_RespDis.pdf. Accessed 1 April 2018.

Vogel, K. P., J. F. Pederson, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276-279.

APPENDIX

Table 1. Ingredient list of basal diet fed during the 28-d receiving period

Item	% As-fed
Cracked corn	40.0
Cottonseed hulls	20.0
Dried distiller grains	18.3
Corn gluten meal	10.0
Soybean meal, hi protein	7.0
Limestone	1.5
Molasses	1.5
Yellow grease	1.0
Salt, white	0.4
Calcium monophosphate	0.1
Corn and Rumensin intermediate mix[3]	0.1
Vitamin A, D, E premix[1]	0.03
Vitamin E premix[2]	0.03
Trace mineral premix[4]	0.01

[1]Vitamin premix contained 8,800,000 IU Vitamin A, 1,760,000 IU Vitamin D, and 1,100 IU Vitamin E/kg
[2]Vitamin E premix contained 44,000 IU Vitamin E/kg
[3]Mix provides 22 g monensin/kg of intermediate mix
[4]Contains NB-8675 (Nutrablend, Neosho, Mo) 12% Zn, 8% Mn, 4% Cu, 1% Fe, 500 ppm Co, 2,000 ppm I, and 600 ppm Se

Table 2. Chemical composition of basal diet, hay, and the topdress for treatment delivery fed during the 28-d receiving period (DM basis)

		Treatment[1]			
	Basal diet	CON	Y1	Y2	Hay
Dry matter, %	90.1	88.3	90.4	89.7	89.3
Neutral detergent fiber, %	41.0	NA[2]	NA	NA	69.9
Acid detergent fiber, %	19.1	NA	NA	NA	39.3
Crude protein, %	24.0	8.5	25.2	12.6	10.2
Ash, %	3.2	0.7	1.8	2.1	5.3
Calcium, %	0.95	ND[3]	0.06	0.02	0.47
Phosphorus, %	0.57	0.26	0.53	0.66	0.46
Magnesium, %	0.18	0.09	0.18	0.25	0.30
Sodium, %	0.21	0.01	0.02	0.10	0.03
Sulfur, %	0.31	0.09	0.20	0.20	0.23
Potassium, %	0.92	0.33	0.90	0.98	2.43
Copper, mg/kg	19	5	15	6	6
Iron, mg/kg	151	25	95	71	85
Manganese, mg/kg	41	19	41	23	77
Zinc mg/kg	75	40	86	44	42

[1]CON = control; Y1 = commercial yeast product 1; Y2 = commercial yeast product 2
[2]Was not analyzed
[3]Nondetectable

Table 3. Effect of 2 commercial yeast products on growth performance during the 28-d receiving period

	Treatment[1]				*P*-value		
Item	CON	Y1	Y2	SEM[2]	*F*-test	CON vs. Y	Y1 vs. Y2
BW, kg							
Initial	226	227	226	8.0	0.99	0.96	0.99
Interim	231	231	232	8.0	0.99	0.97	0.99
Final	255	255	254	8.9	0.99	0.99	0.98
ADG, kg							
Day 0 to 14	0.4	0.3	0.4	0.10	0.98	0.98	0.86
Day 14 to 28	1.7	1.7	1.7	0.11	0.92	0.70	0.93
Day 0 to 28	1.0	1.0	1.0	0.09	0.99	0.89	0.98
ADFI, kg							
Day 0 to 14	3.4	3.4	3.4	0.22	0.98	0.94	0.87
Day 14 to 28	5.7	5.6	5.8	0.29	0.92	0.91	0.69
Day 0 to 28	4.5	4.5	4.5	0.27	0.99	0.93	0.89
G:F							
Day 0 to 14	0.10	0.10	0.10	0.028	0.99	0.92	0.98
Day 14 to 28	0.31	0.30	0.30	0.015	0.91	0.90	0.69
Day 0 to 28	0.23	0.23	0.22	0.012	0.97	0.97	0.82

[1]CON = control; Y1 = commercial yeast product 1; Y2 = commercial yeast product 2; Y = Y1 and Y2

[2]Pooled standard error of the mean

Table 4. Effect of commercial yeast products on morbidity during the 28-d receiving period

Item	Treatment[1]			SEM[2]	*P*-value		
						Contrast	
	CON	Y1	Y2		*F*-test	CON vs. Y	Y1 vs. Y2
Morbidity							
Treated once, %	67.2	77.2	64.9	6.38	0.36	0.60	0.18
Treated twice, %	27.6	31.6	35.1	6.12	0.69	0.45	0.69
Treated thrice, %	10.3	10.5	14.0	4.24	0.79	0.73	0.57
Number antibiotic treatments/calf	1.05	1.19	1.14	0.130	0.74	0.47	0.77
Relapse, %[3]	41.0	40.8	54.4	8.20	0.32	0.53	0.25
Treatment cost/head, $	20.38	22.97	22.02	2.868	0.81	0.55	0.82

[1]CON = control; Y1 = commercial yeast product 1; Y2 = commercial yeast product 2; Y = Y1 and Y2

[2]Pooled standard error of the mean

[3]Relapse is the percentage of morbid calves that received a second antibiotic

Table 5. Effect of commercial yeast products on days till treatment, percent chronic, and percent mortality for the 28-d receiving period.

	Treatment[1]				*P*-value
Item	CON	Y1	Y2	SEM[2]	Chi-Squared
Morbidity					
Days till antibiotic treatment 1	9.4	7.5	9.9	0.92	0.17
Days till antibiotic treatment 2	17.1	20.3	21.8	0.98	0.76
Days till antibiotic treatment 3	18.7	23.3	21.1	0.31	0.79
Chronic, %[3]	1.7	3.5	5.3	2.37	0.57
Mortality, %	0	0	3.5	2.44	0.11

[1]CON = control; Y1 = commercial yeast product 1; Y2 = commercial yeast product 2

[2]Pooled standard error of the mean

[3]Calves were considered chronic if they were treated 3 times with antibiotics for BRD and gained less than 0.23 kg

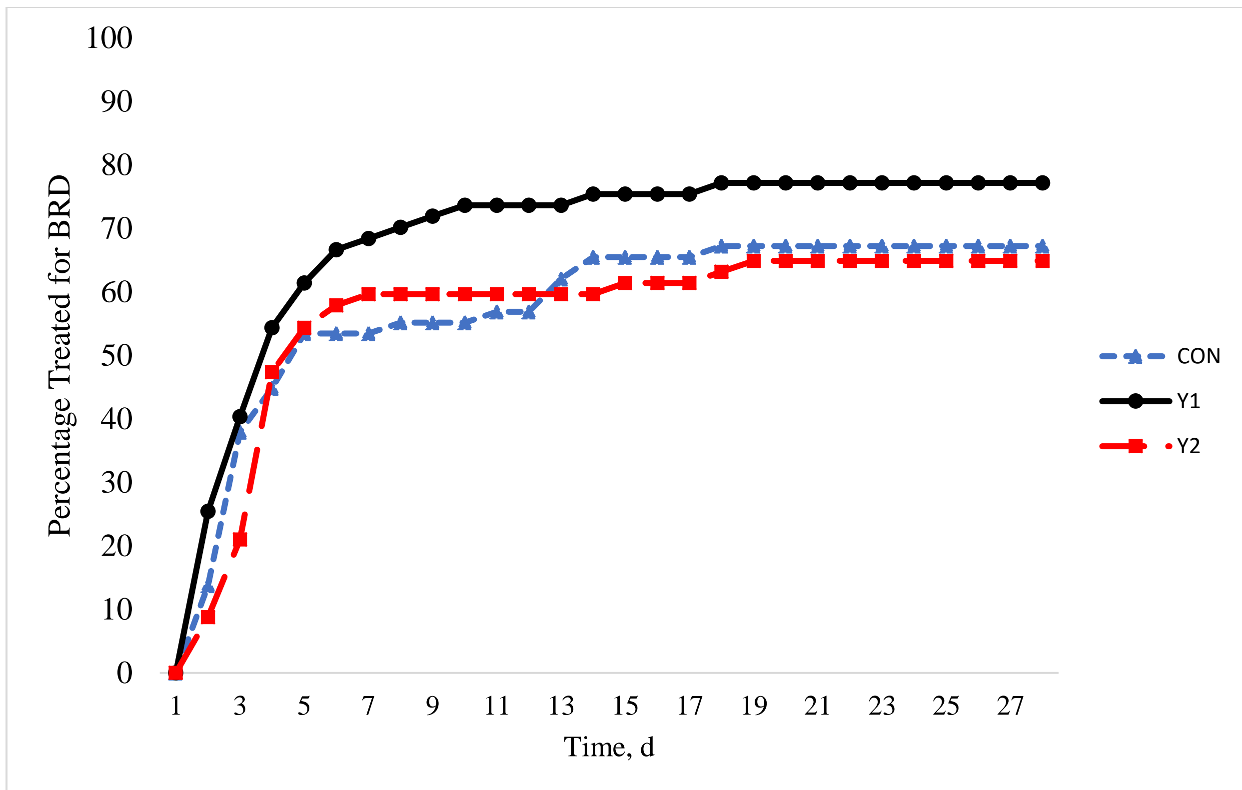


Figure 1. Days from arrival to the first antibiotic treatment was administered (*P* =0.29). Calves were administered their first antibiotic if they received a clinical attitude score of 1 or 2 and had a rectal temp higher than 40°C or if they score a 3 or 4 clinical attitude score rating regardless of rectal temperature. CON = control; Y1 = commercial yeast product 1; Y2 = commercial yeast product 2.

CHAPTER IV

Effect of a combination of live yeast and yeast cell wall products supplemented before and after weaning on heifer growth performance, immune function, and heat stress

ABSTRACT

Heifer calves (n = 95; initial BW = 165 ± 27 kg) were used to evaluate the effects of a combination of yeast products fed prior to weaning and through a backgrounding period. Heifer calves were stratified based on BW, birthdate, sire, and dam parity; and were assigned randomly to pasture (10 pastures, 9 or 10 cow-calf pairs/pasture). Pastures were assigned randomly to 1 of 2 treatments: 1) no yeast (CON), or 2) addition of yeast product (YP; Phileo Lesaffre Animal Care, Milwaukee, WI). Calves were offered creep-feed (limited using 5% salt) at 0.5% of BW for 35 d prior to weaning. The YP creep-feed was formulated to provide 4 g YP/d. After weaning, heifers remained in their pre-weaning groups and had ad libitum access to forage and were fed 1.8 kg/d of a grain supplement for 42 d; YP continued to provide 4 g of YP/d. Body weights were collected on d -1, 0, 21, 35 (weaning), 49, 63, 76, and 77. A subsample of calves (3 calves/pasture) were fitted with intra-vaginal temperature probes for 2-wk periods pre- and post-weaning. Daily water intake was recorded on 8 pens (4 pens/treatment) following weaning. Blood was collected for analysis of serum haptoglobin concentrations and complete blood cell counts on d -1, 35, 49, and 76. Blood collected on d 35 and 76 was analyzed by flow cytometry to determine phagocytic activity. Average daily gain prior to weaning was not different ($P \geq 0.45$) between treatments. However, CON had an increased ($P = 0.01$) ADG compared to YP from weaning to the end of the backgrounding period. Temperatures were not affected by treatment before weaning ($P = 0.31$) nor after weaning ($P = 0.37$). Dry matter intake was not different ($P = 0.41$) between CON and YP during the 35-d pre-weaning period. Water intake during the 42-d backgrounding period was not impacted ($P = 0.49$) by treatment. There was no difference ($P \geq 0.13$) observed for white blood cell concentrations or neutrophil to lymphocyte ratio. Serum haptoglobin concentrations were not affected ($P = 0.15$) by the addition of YP in the

diet; however, there was a day effect ($P < 0.01$). Furthermore, the percentages of cells that were positive for phagocytic activity were not different ($P = 0.97$). In summary, heifer calf growth performance, immune function, and heat tolerance were not affected by YP supplementation.

Keywords Heifer calf, immune function, yeast products

INTRODUCTION

Addition of yeast products is believed to be more advantageous when calves are exposed to stress (Cole et al., 1992). When calves are stressed, they have a reduction in DMI (Cole, 1996); however, yeast products can aid in offsetting this negative effect by stimulating DMI (Phillips and VonTungeln, 1985; Cole et al., 1992; Keyser et al., 2007). Furthermore, at receiving, calves supplemented with yeast have an advantage in ADG and improved morbidity rates (Ponce et al., 2012). However, the receiving period is not the only stressful time for a calf. Calves experience a great deal of stress during weaning indicated by an elevated neutrophil to lymphocyte ratio (Hickey et al., 2003), and when calves are stressed, they are more susceptible to disease (Blecha et al., 1984). This can negatively impact their performance in the feedlot; therefore, it is important to evaluate tools that could mitigate the negative effects of weaning.

Creep-feeding starts calves on a concentrate diet and increases calf weight at weaning (Martin et al., 1981). Preconditioning is another management strategy that producers can take advantage of prior to entry at the feedlot. Preconditioned calves have been weaned and acclimated to feed bunks and waterers for a certain amount of time, vaccinated, dehorned, castrated, and treated for internal parasites. Calves that have been preconditioned have lower incidence of bovine respiratory disease (BRD) in the feedlot compared to high-risk, auction barn calves (Roeber et al., 2001).

Yeast products supplemented prior to weaning have been shown to be beneficial in dairy calves. Yeast fed prior to weaning can reduce the degree of scours observed, ultimately decreasing the amount of antibiotics administered in young dairy calves (Seymour et al., 1995). Furthermore, providing yeast prior to weaning has improved DMI and ADG post-weaning (Terré et al., 2015). Nonetheless, dairy calves are weaned much earlier than beef calves and are

managed very differently. Limited research exists in this area in beef calves; therefore, the objective of the current study was to determine the effects of a combination of yeast products on growth performance and immune function when fed in creep-feed 35 d prior to weaning and during a 42-d backgrounding period in heifer calves.

MATERIALS AND METHODS

Animals and Treatments

Experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (protocol # 17060). Ninety-five cow-calf pairs with heifer calves (initial BW = 165 ± 27 kg) from the University of Arkansas Division of Agriculture Beef Unit near Fayetteville, AR were stratified based on initial BW, birthdate, sire, and cow parity. Calves were assigned randomly to pasture (10 pastures, 9 or 10 cow-calf pairs/pasture). Pastures were then assigned randomly to 1 of 2 treatments; 1) no yeast (**CON**), and 2) addition of yeast products (**YP**; combination of yeast and yeast cell wall). Cow-calf pairs were held in holding pens over night with access to bermudagrass hay and water. The following day they were moved to their assigned 2.4 ha pastures.

Calves were weaned from their dams 35 d after initiation of the study. At weaning calves were: 1) weighed, 2) branded, 3) vaccinated for respiratory pathogens (Virashield 6 VL 5, Elanco Animal Health, Greenfield, IN), 4) administered a clostridial vaccine (Covexin®8, Merck Animal Health, Madison, NJ), and 5) treated for internal parasites with an oral dewormer (Valbazen®, Zoetis, Parsippany, NJ). Cows were weighed and then removed from the study. Calves remained in their pre-weaning treatment groups and were moved to 0.4 ha pastures (10 pastures; 9 or 10 calves per pasture) with concrete bunks and automatic waterers for a 42-d backgrounding period. Calves were revaccinated 14 d after weaning with a respiratory vaccine

(Virashield 6 VL 5, Elanco Animal Health) and a clostridial (Covexin®8, Merck Animal Health). Calves remained on backgrounding diet for 42-d post weaning.

Diets and Nutrient Analysis

Calves were offered a 5% salt limited creep-feed (47.5% cracked corn and 47.5% dried distillers' grains) with or without YP at 0.5% of the average initial calf BW. Salt content was based on levels used by Lusby et al. (1985). Body weights were collected on d 21 and the amount of creep-feed offered was adjusted based on the average d 21 weight. Creep-feed with YP was formulated to provide a target intake of 4 g of YP/d (3 g of live yeast and 1 g of yeast cell wall product; Phileo Lesaffre Animal Care, Milwaukee, WI). Creep feeders were refilled twice a week; every Monday and Thursday afternoon which delivered creep supplement adequate for 3 or 4 d, respectively. Prior to refilling feeders, orts were collected, weighed, and subsampled. At weaning, calves were supplemented (Table 1) with 1.8 kg of grain daily. Grain diets that contained YP continued to provide 4 g of YP/d. Any orts remaining before feeding were collected at approximately 0800, weighed, and subsampled. Hay was offered as large round bales for ad libitum intake when pasture forage started to decline, a subjective decision by unit manager. All pens were offered hay beginning on the same day.

Samples were composited and dried in a forced air oven at 50°C until a stable weight was reached. Dried samples were ground through a Willey Mill (Thomas Scientific, Swedesboro, NJ) using a 1 mm screen. Samples were analyzed for NDF and ADF using the Van Soest method (ANKOM Technology Corp, Fairport, NJ; Vogel et al., 1999). Crude protein was determined by total combustion (Rapid Combustion Method, Elementar Americas, Inc., Mt. Laural, NJ). Mineral analysis was prepared by wet ashing and then analyzed at the University of Arkansas

Altheimer Laboratory (Fayetteville, AR) by inductively coupled plasma spectroscopy (Model 3560, Applied Research Laboratory, Sunland, CA). Nutrient analysis is presented in Table 2.

Morbidity

Calves were observed daily for morbidity and assigned a clinical attitude score (CAS) of 0 to 4 (0 = normal, 1 = mild BRD, 2 = moderately BRD, 3 = severe BRD, and 4 = moribund). If calves received a CAS of 1 or 2, and had a rectal temperature greater than 40°C, or if they received a score of 3 or 4 regardless of rectal temperature they were administered an antibiotic based on a pre-determined antibiotic protocol. The antibiotic protocol consisted of florifenicol (Nuflor, Merck Animal Health) as the first antibiotic administered. Rectal temperatures were rechecked at the end of the post treatment interval (PTI; 2 d). If calves were pulled a second time and met the predetermined requirements for antibiotic treatment, they were administered ceftiofur crystalline free acid (Excede, Zoetis) as the second antibiotic treatment (PTI = 5 d). Lastly, if calves required a third antibiotic treatment, they were given tulathromycin (Draxxin, Zoetis). Calves were rechecked 7 d following administration; however, no additional antibiotics were administered at this time. Antibiotics, BW, rectal temperatures, and day of treatment were recorded throughout the study.

BW and Blood Samples

Cow BW were recorded on d -1, 0, 34, and 35, while calf BW were collected on d -1, 0, 21, 34, 35, 49, 63, 76, and 77. Consecutive BW were recorded and averaged at the beginning, weaning, and end of study. Blood samples (n = 95) were collected on d -1, 34, 49, and 76 via jugular venipuncture. Whole blood was collected for complete blood cell counts in 6 mL vacuum blood tubes containing EDTA (BD Vacutainer®, Becton Dickson and Company, Franklin Lakes, NJ). Samples were stored over night at 4°C and processed on an automated analyzer (HemaVet

HV950; Drew Scientific, Miami Lakes, FL) the following day. To determine the effect of YP on the innate immune system, whole blood was used to analyze the percentage of cells positive for phagocytic activity. Whole blood (n = 20; 2 calves/pasture) was collected in a vacuum tube containing sodium heparin (6 mL) and immediately returned to the lab for analysis. Phagocytic activity was determined using pHrodo™ BioParticles® Phagocytosis Kit (Life Technologies, Carlsbad, CA) and samples were analyzed using flow cytometry (BD Accuri™ C6 Plus; BD Biosciences, San Jose, CA) and the gating strategy found in figure 1. Additional blood samples were collected in a plain vacuum tube (10 mL) and centrifuged at 3,000 g for 20 min. Samples were stored at -20°C until serum haptoglobin (Hp) analysis was performed. Serum Hp concentration was determined using a commercially available ELISA kit (Immunology Consultants Laboratory Inc, Portland, OR) with antibodies specific for bovine Hp.

Temperature Data and Water Intake

Three of the heaviest calves in each pasture (n = 30) based on initial BW were fitted with vaginal temperature probes 2 wk prior to weaning (d 21). Vaginal temperature probes consisted of a hormone-free controlled internal drug release devise (CIDR; Zoetis) that contained a digital temperature logger (iButton DS1922L thermosensors, Digi-key Thief River Falls, MN). Temperature loggers were programmed to record once every hour and were set at a resolution of 0.0625°C. The first set of temperature probes were removed on d 34 and a second subsample of 30 calves (3 second heaviest calves from each pen based on initial BW) were fitted with a temperature probe. Temperatures were collected for 14-d post-weaning, at time of revaccination probes were pulled from calves. Water intake was recorded daily during the 42-d backgrounding period. Water intake was measured by water meters (Recordall® Model 25; Badger Meter,

Milwaukee, WI) connected to 4 of the automatic waterers (2 pens on the same treatment shared a waterer).

Statistical Analysis

Data were analyzed using the MIXED procedure in SAS (SAS Inst., Inc., Cary, NC). Growth performance measurements for both cows and calves contained treatment as the only variable in the model. Water intake, DMI, temperature, and blood data were run as a repeated measure and analyzed with fixed effects of treatment, time, and treatment by time interaction. Pen was set as the experimental unit for all variables with the exception of water intake where 2 pens were designated as the experimental unit. Significance was declared at $P \leq 0.05$ and tendencies were assigned at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

During the 77 d period, only 2 calves (both on the YP treatment) were treated with antibiotics, but neither were BRD related (1 due to footrot and 1 from vaginitis while monitoring body temperature with the indwelling temperature probes). Supplementation of YP pre- and post-weaning did not influence ($P \geq 0.22$; Table 3) calf BW nor was there a difference in ADG from d 0 to 77 ($P = 0.22$). Prior to weaning ADG was not different ($P = 0.45$) between treatment groups, while post-weaning (d 35 to 77) ADG was greater ($P = 0.01$) in CON compared to YP calves. During the first 28 d after weaning, ADG was not impacted ($P \geq 0.11$) by dietary treatment; however, the last 14 d of backgrounding saw a decrease ($P = 0.05$) in ADG for YP calves. Disappearance of creep-feed prior to weaning was not impacted ($P = 0.41$) by the addition of yeast in the diet (data not shown). Consumption of creep-feed averaged 0.48 kg DM/d. Like creep-feed disappearance, there was no difference in supplement disappearance after weaning. On d 0, orts were collected from 6 of the 10 pens; however, in that first day there was

no difference (P = 0.67) in supplement intake between treatments and after d 0 calves consumed all supplement offered. Cow BW during the creep-feeding period was not affected (P = 0.87) by the calves consuming either YP or CON creep-feed. Furthermore, there was no difference (P = 0.34) in ADG in cows from the start of creep-feeding until weaning.

Addition of yeast products to the diet are promoted in the livestock industry to improve growth performance and feed efficiency. As reported growth performance was negatively affected by yeast product supplementation during the end of the backgrounding period. Reason for this decrease in ADG during the last 14 d is not well understood. In early weaned calves supplemented with 115 g/d of a yeast fermentation product from early January to mid-September, ADG was not impacted during the grazing or receiving period (Vendramini and Arthington, 2007). Finck et al. (2014) reported similar results during the receiving period when calves were fed live yeast at 5 g/d, yeast cell wall at 5 g/d, or a combination of the two (5 g/d of both live yeast and yeast cell wall) for 56 d. In contrast, Ponce et al. (2012) found that 1.8 g/d of an enzymatically hydrolyzed yeast cell wall and yeast cell metabolite product improved DMI in a 35-d receiving period which was complemented with greater ADG in yeast supplemented calves. Positive impacts on growth performance are attributed to the influence that yeast has on the microbial population in the rumen. Total (Newbold et al., 1995; Kumar et al., 1994), cellulolytic (Harrison et al., 1988), and fibrolytic (Chaucheyras-Durand and Fonty, 2006) bacteria are all augmented by the addition of yeast in the diet. Likewise, total tract dry matter and fiber digestibility was enhanced (Jiang et al., 2017; Ovinge et al., 2018).

Vaginal temperatures recorded throughout the day without interference from human handling make them an appropriate tool for research (Burdick et al., 2012). During the first 2-wk of temperature collection, data were successfully collected from 8 calves on CON and 5 calves

on YP due to complications with the iButtons recording and probes falling out. After weaning, data were successfully collected on 11 calves on CON and 14 calves on YP, with 5 probes falling out during the 2-wk period. After observing a high occurrence of probes falling out, ewe CIDR should be considered for future studies that are recording temperature data in young heifer calves.

Vaginal temperature was not affected by dietary treatment pre-weaning (P =0.31; Figure 2) or post-weaning (P = 0.37; Figure 3); while, a day effect (P < 0.01) was observed during both 2-wk periods. The spike in vaginal temperature during both periods coincided with an increase in ambient temperature. Additionally, water intake (Figure 4) was not different (P = 0.49) between treatments; indicating that supplementation of YP did not influence the calves' ability to tolerate heat stress. In the current study, the average high ambient temperature was 25.7°C with a low of 12.2°C, respectively. The thermal neutral zone (TNZ) for beef cattle falls between 22°C and 30°C. Therefore, these calves did not experience elevated levels of heat stress which may have influenced the observed lack of response due to YP.

In lactating dairy cows, supplementation of YP during heat stress increases milk yield (Moallem et al., 2009; Bruno et al., 2009) and feed efficiency (Schingoethe et al., 2004; Moallem et al., 2009). Similarly, in feedlot cattle, ADG and DMI have been shown to increase during high temperatures with the addition of a hydrolyzed yeast cell wall product (Salinas-Chavira et al., 2015). Yeast has also been shown to reduce vaginal temperatures and increase the amount of water consumed during heat stress (Broadway et al., 2016).

While yeast improves production parameters during heat stress, yeast also imparts physiological effects on the animal. Cattle experiencing an immune challenge had a contrasting effect on body temperature. After a lipopolysaccharide (LPS) challenge, heifers fed yeast cell

wall had higher vaginal temperatures but had greater ADG (Young et al., 2017). Young et al. (2017) hypothesize that heifers given yeast cell wall were more metabolically active during the immune challenge. This hypothesis is supported in a similar study that found increased blood urea nitrogen (BUN) and decreased non-esterified fatty acids (NEFA) in heifers supplemented yeast cell wall during an immune challenge (Burdick Sanchez et al., 2014).

The cell wall of yeast is composed of polysaccharides such as β-glucans (Ruiz-Herrara, 1991) which stimulate the innate immune response and bind to monocytes, macrophages, and natural killer cells (Brown and Gordon, 2001; Brown, 2006). An improvement in neutrophil function has been reported in dairy calves fed yeast culture from d 2 to 70 after birth (Magalhães et al., 2008). In the current study, stimulation of the innate immune system was examined by incubating cells with *E. coli* particles to determine degree of phagocytic activity. The number of live positive cells for phagocytic activity were divided by the frequency of total cells. Analysis showed that phagocytic activity was not affected ($P = 0.97$) by dietary treatment with the percentage of cells positive for phagocytosis being 23.5% for CON and 22.6% for YP at time of weaning and 16.6% for CON and 17.4% for YP at the end of the backgrounding period (Figures 5 and 6). Similarly in pigs, dietary yeast treatment did not improve phagocytic activity or enhance respiratory burst of neutrophil cells (Sauerwein et al., 2007).

Dietary treatment did not alter ($P = 0.32$) concentration of total white blood cells present. Concentration of lymphocytes tended to be greater in YP calves ($P = 0.06$; Table 4), but there was no difference in the concentration of neutrophils ($P = 0.38$; Table 4); ultimately leading to a similar ($P = 0.13$) neutrophil to lymphocyte ratio (Figure 7) between treatments. In contrast, Finck et al. (2014) reported a decrease in the neutrophil to lymphocyte ratio in steers supplemented with yeast product after exposure to a LPS challenge. The neutrophil to

lymphocyte ratio is a resource used to assess stress; a lower neutrophil to lymphocyte ratio indicates lower stress. As evident in Figure 7, a day effect was observed with the neutrophil to lymphocyte ratio being greatest on d 49, followed by d 35 and 77. This gradual increase in the ratio could be due to stress factors associated with weaning (Hickey et al., 2003). Furthermore, concentrations of monocytes, eosinophils, and basophils were not impacted ($P \geq 0.37$; Table 4) by yeast supplementation during this study.

Haptoglobin is an acute phase protein produced in the liver and released in the blood during periods of stress. Haptoglobin concentration is associated with inflammation and disease (Baumann and Gauldie, 1994) and is negatively correlated to ADG (Moriel and Arthington, 2013). Serum Hp levels were not different ($P = 0.15$; Figure 8) due to the main effect of treatment; however, a day affect ($P < 0.001$) was observed and Hp concentrations were greatest 14-d post-weaning. In agreement, yeast treatment did not affect Hp concentrations in calves upon entry to the feedlot (Vendramini and Arthington, 2007). Haptoglobin concentrations increase after weaning (Kim et al., 2011a) and administration of vaccine (Arthington et al., 2013). Kim et al. (2011b), found an increase in Hp concentrations after a vaccine challenge in young dairy calves supplemented yeast product. While not statistically different, Hp concentrations were numerically greater in YP calves 14 d after calves had been vaccinated. Production of haptoglobin is stimulated by pro-inflammatory cytokines IL-1, IL-6, and TNF-α. In vitro studies found that β-glucans bind to leukocytes and promote the production of TNF-α (Brown et al., 2003) and IL-6 (Ali et al., 2015). Furthermore, Burdick Sanchez et al. (2013) reported an increase in IL-6 in heifers supplemented yeast products after an endotoxin challenge compared with control.

Implications

Supplementing 4 g/d of a combination of live yeast and cell wall products prior to and after weaning did not improve performance. Consequently, it had a negative effect on performance from weaning to the end of the backgrounding period. Yeast products did not improve immune function of weaned calves based on the parameters measured in this study. Likewise, there was no effect of dietary treatment on water intake or vaginal temperatures. Therefore, providing YP to heifers in the thermal neutral zone and with low levels of stress may not be useful.

LITERATURE CITED

Ali, M. F., C. B. Driscoll, P. R. Walters, A. H. Limper, and E. M. Carmona. 2015. Beta-glucan activated human B-lymphocytes participate in innate immune responses by releasing pro-inflammatory cytokines and stimulating neutrophil chemotaxis. J. Immunol. 195:5318-5326.

Arthington, J. D., R. F. Cooke, T. D. Maddock, D. B. Araujo, P. Moriel, N. DiLorenzo, and G. C. Lamb. 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. J. Anim. Sci. 91:1831-1837.

Baumann, H., and J. Gauldie. 1994. The acute phase response. Immunol. Today 15:74-80.

Blecha, F., S. L. Boyles, and J. G. Riley. 1984. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman × Angus feeder calves. J. Anim. Sci. 59:576-583.

Broadway, P. R., J. A. Carroll, N. C. Burdick-Sanchez, S. L. Roberts, K. P. Sharon, J. T. Richeson, and J. R. Corley. 2016. Yeast probiotic supplementation mitigates come of the negative effects of heat stress in feedlot heifers. J. Anim. Sci. 94 (E-supplemental 1):34.

Brown, G. D. 2006. Dectin-1: a signaling non-TLR pattern-recognition receptor. Nat. Rev. Immunol. 6:33-43.

Brown, G. D., and S. Gordon. 2001. Immune recognition: a new receptor for beta-glucans. Nature. 413:36-37.

Brown, G. D., J. Herre, D. L. Williams, J. A. Willment, A. S. J. Marshall, and S. Gordon. 2003. Dectin-1 mediates the biological effects of β-glucans. J. Exp. Med. 197:1119-1124.

Bruno, R. G. S., H. M. Rutigliano, R. L. Cerri, P. H. Robinson, and J. E. P. Santos. 2009. Effect of feeding *Saccharomyces cerevisiae* on performance of dairy cows during summer heat stress. Anim. Sci. Feed Tech. 150:175-186.

Burdick, N. C., J. A. Carroll, J. W. Daily, R. D. Randel, S. M. Falkenberg, and T. B. Schmidt. 2012. Development of a self-contained, indwelling vaginal temperature probe for use in cattle research. J. Therm. Bio. 37:339-343.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R Corley, R. J. Rathmann, and B. J. Johnson. 2013. Yeast cell wall supplementation alters aspects of the physiological and acute phase responses of crossbred heifers to an endotoxin challenge. Innate Immunity 19:411-419.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R Corley, R. J. Rathmann, and B. J. Johnson. 2014. Yeast cell wall supplementation alters the metabolic responses of crossbred heifers to an endotoxin challenge. Innate Immunity 20:104-112.

Chaucheyras-Durand, F., and G. Fonty. 2006. Effects and modes of action of live yeasts in the rumen. Biologia 61:741-750.

Cole, N. A. 1996. Review of bovine respiratory disease: Nutrition and disease interactions. Pages 57-74 in Review of Bovine Respiratory Disease – Schering-Plough Animal Health. R. Smith, ed. Veterinary Learning Systems, Trenton, NJ.

Cole, N. A., C. W. Purdy, and D. P. Hutcheson. 1992. Influence of yeast culture on feeder calves and lambs. J. Anim. Sci. 70:1682-1690.

Finck, D. N., F. R. B. Ribeiro, N. C. Burdick, S. L. Parr, J. A. Carroll, T. R. Young, B. C. Bernhard, J. R. Corley, A. G. Estefan, R. J. Rathmann, and B. J. Johnson. 2014. Yeast supplementation alters the performance and health status of receiving cattle. Prof. Anim. Sci. 30:333-341.

Harrison, G. A., R. W. Hemken, K. A. Dawson, and R. J. Harmon. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967-2975.

Hickey, M. C., M. Drennan, and B. Earley. 2003. The effect of abrupt weaning of suckler calves on the plasma concentrations of cortisol, catecholamines, leukocytes, acute-phase proteins and in vitro interferon-gamma production. J. Anim. Sci. 81:2847-2855.

Jiang, Y., I. M. Ogunade, K. G. Arriola, M. Qi, D. Vyas, C. R. Staples, and A. T. Adesogan. 2017. Effects of the dose and viability of *Saccharomyces cerevisiae*. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. J. Dairy Sci. 100:8102-8118.

Keyser, S. A., J. P. McMeniman, D. R. Smith, J. C. MacDonald, and M. L. Galyean. 2007. Effects of *Saccharomyces cerevisiae* subspecies *boulardii* CNCM I-1079 on feed intake by healthy beef cattle treated with florfenicol and on health and performance of newly received beef heifers. J. Anim. Sci. 85:1264-1273.

Kim, M., J. Yang, S. D. Upadhaya, H. Lee, C. Yun, and J. K. Ha. 2011b. The stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in Holstein calves. J. Vet. Sci. 12:151-157.

Kim, M., J. K. Seo, C. Yun, S. J. Kang, J. Y. Ko, and J. K. Ha. 2011a. Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. Animal. 5:953-960.

Kumar, U., V. K. Sareen, and S. Singh. 1994. Effect of *Saccharomyces cerevisiae* yeast culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet. J. Anim. Sci. 59:209-215.

Lusby, K. S., K. C. Barnes, and J. W. Walker. 1985. Salt-limited creep feed for nursing calves. Oklahoma Agriculture Experiment Station. Anim. Sci. Resear. Report 249-251.

Magalhães, V. J., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetance of dairy calves. J. Dairy. Sci. 91:1497-1509.

Martin, T. G., R. P. Lemenager, G. Srinivasan, and R. Alenda. 1981. Creep feed as a factor influencing performance of cows and calves. J. Anim. Sci. 53:33-39.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 92:343-351.

Moriel, P., and J. D. Arthington. 2013. Metabolizable protein supply modulated the acute-phase response following vaccination of beef steers. J. Anim. Sci. 91:5838-5847.

Newbold, C. J., R. J. Wallace, X. B. Chen, and F. M. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. J. Anim. Sci. 73:1811-1818.

Ovinge, L. A., J. O. Sarturi, M. L. Galyean, M. A. Ballou, S. J. Trojan, P. R. B. Campanili, A. A. Alrumaih, and L. A. Pellarin. 2018. Effects of a live yeast in natural-program finishing feedlot diets on growth performance, digestibility, carcass characteristics, and feeding behavior. J. Anim. Sci. 96:684-693.

Phillips, W. A., and D. L. VonTungeln. 1985. The effect of yeast culture on the poststress performance of feeder calves. Nutr. Rep. Internat. 32:287-294.

Ponce, C. H., J. S. Schutz, C. C. Elrod, U. Y. Anele, and M. L. Galyean. 2012. Effects of dietary supplementation of a yeast product on performance and morbidity of newly received beef heifers. Prof. Anim. Sci. 28:618-622.

Roeber, D. L., N. C. Speer, J. G. Gentry, J. D. Tatum, C. D. Smith, J. C. Whittier, G. F. Jones, K. E. Belk, and G. C. Smith. 2001. Feeder cattle heath management: Effects on morbidity rates, feedlot performance, carcass characteristics, and beef palatability. Prof. Anim. Sci. 17:39-44.

Ruiz-Herrera, J. 1991. Biosynthesis of β-glucans in fungi. Antonie van Leeuwenhoek 60:73-81.

Sauerwein, H., S. Schmitz, and S. Hiss. 2007. Effects of a dietary application of a yeast cell wall extract on innate and acquired immunity, on oxidative status and growth performance in weanling piglets and on the ileal epithelium in fattened pigs. J. Anim. Physiology and Anim. Nutrition 91:369-380.

Salinas-Chavira, J., C. Arzola, V. González-Vizcarra, O. M. Manríquez-Núñez, M. F. Montaño-Gómez, J. D. Navarrete-Reyes, C. Raymundo, and R. A. Zinn. 2015. Influence of feeding enzymatically hydrolyzed yeast cell wall on growth performance and digestive function of feedlot cattle during periods of elevated ambient temperature. Asian Australas. J. Anim. Sci. 28:1288-1295.

Schingoethe, D. J., K. N. Linke, K. F. Kalscheur, A. R. Hippen, D. R. Renich, and I. Yoon. 2004. Feed efficiency of mid-lactation dairy cows fed yeast culture during summer. J. Dairy Sci. 87:4178-4181.

Seymour, W. M., J. E. Nocek, and J. Siciliano-Jones. 1995. Effects of a colostrum substitute and of dietary brewer's yeast on the health and performance of dairy calves. J. Dairy Sci. 78:412-420.

Terré, M., G. Maynou, A. Bach, and M. Gauthier. 2015. Effect of *Saccharomyces cerevisiae* CNCM I-1077 supplementation on performance and rumen microbiota of dairy calves. Prof. Anim. Sci. 31:153-158.

Vendramini, J. M. B., and J. D. Arthington. 2007. Case Study: Effects of supplemental yeast fermentation products on performance of early-weaned calves on pasture and measures of stress and performance during a feedlot receiving period. Prof. Anim. Sci. 23:709-714.

Vogel, K. P., J. F. Pederson, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276-279.

Young, T. R., F. R. B. Ribeiro, N. C. Burdick Sanchez, J. A. Carroll, M. A. Jennings, J. T. Cribbs, R. J. Rathmann, J. R. Corley, and B. J. Johnson. 2017. Yeast cell wall supplementation alters the performance and health of beef heifers during the receiving period. Prof. Anim. Sci. 33:166-175.

APPENDIX

Table 1. Composition of backgrounding diet fed for a 42-d period after weaning.

Item	Treatment[1]	
	CON	YP
Ingredient, %		
Cracked corn	68.4	68.3
Dried distillers' grains	26.0	26.0
Limestone	2.0	2.0
Molasses	2.0	2.0
Salt, white	1.0	1.0
Corn/Rumensin mix[4]	0.4	0.4
YP	NA	0.12
Vitamin A, D, E premix[2]	0.1	0.1
Ruminant trace mineral premix[5]	0.085	0.085
Vitamin E premix[3]	0.05	0.05

[1]CON = control, no addition of yeast product; YP = addition of yeast product.

[2]ADE premix contains 8,800,000 IU Vitamin A, 1,760,000 IU Vitamin D, and 1,100 IU/kg Vitamin E.

[3]Vitamin E contains 44,000 IU/kg.

[4]Corn/Rumensin mix provides 22 g monensin/kg.

[5]NB-8675 contains 12% Zn, 8% Mn, 4% Cu, 1% Fe, 500 mg/kg Co, 2,000 mg/kg I, and 600 mg/kg Se (Nutrablend, Neosho, MO).

Table 2. Nutrient analysis of creep-feed and backgrounding diets (DM basis).

	Creep-feed diet[1,2]		Backgrounding diet[1]		
	CON	YP	CON	YP	Hay[3]
DM, %	91.25	91.82	90.30	90.63	92.02
Ash, %	5.19	4.79	3.68	3.16	4.37
CP, %	17.95	17.30	16.17	13.92	12.32
P, %	0.702	0.681	0.619	0.527	0.37
K, %	0.961	0.925	0.836	0.712	1.63
Ca, %	0.047	0.024	1.109	0.715	0.66
Mg, %	0.231	0.221	0.204	0.177	0.25
S, %	0.358	0.345	0.306	0.237	0.21
Na, %	2.329	2.273	0.632	0.472	0.06
Fe, mg/kg	173.74	180.23	217.72	125.65	92.42
Mn, mg/kg	12.23	10.44	94.39	64.45	52.84
Zn, mg/kg	51.90	45.48	138.08	152.08	46.69
Cu, mg/kg	6.12	5.62	56.18	34.14	11.07

[1]CON = control, no addition of yeast product; YP = addition of yeast product.

[2]Creep-feed was fed for 35 d prior to weaning and backgrounding diet was fed for 42 d post-weaning.

[3]Hay offered ad libitum once forage began to decrease

Table 3. Effect of supplementing YP on heifer growth performance pre- and post-weaning.

	Treatment[1]			
Item	CON	YP	SEM	P-Value
Calf BW, kg				
D 0	165	165	0.3	0.85
D 21	193	194	0.9	0.48
D 35 (weaning)	199	201	1.2	0.44
D 49	215	213	1.5	0.40
D 63	232	232	1.3	0.67
D 77	244	240	1.9	0.22
Calf ADG, kg				
D 0 to 21	1.34	1.38	0.044	0.53
D 21 to 35	0.45	0.48	0.072	0.77
D 35 to 49	1.14	0.91	0.090	0.11
D 49 to 63	1.23	1.30	0.076	0.50
D 63 to 77	0.79	0.60	0.062	0.05
D 0 to 35 (wean)	0.98	1.02	0.032	0.45
D 35 to 77	1.05	0.93	0.026	0.01
D 0 to 77	1.02	0.97	0.025	0.22
Cow BW, kg				
D 0	445	452	7.1	0.51
D 35	469	471	9.1	0.87
Cow ADG d 0 to 35, kg	0.68	0.54	0.092	0.34

[1]CON = control, no addition of yeast product; YP = addition of yeast product.

[2]n = 95

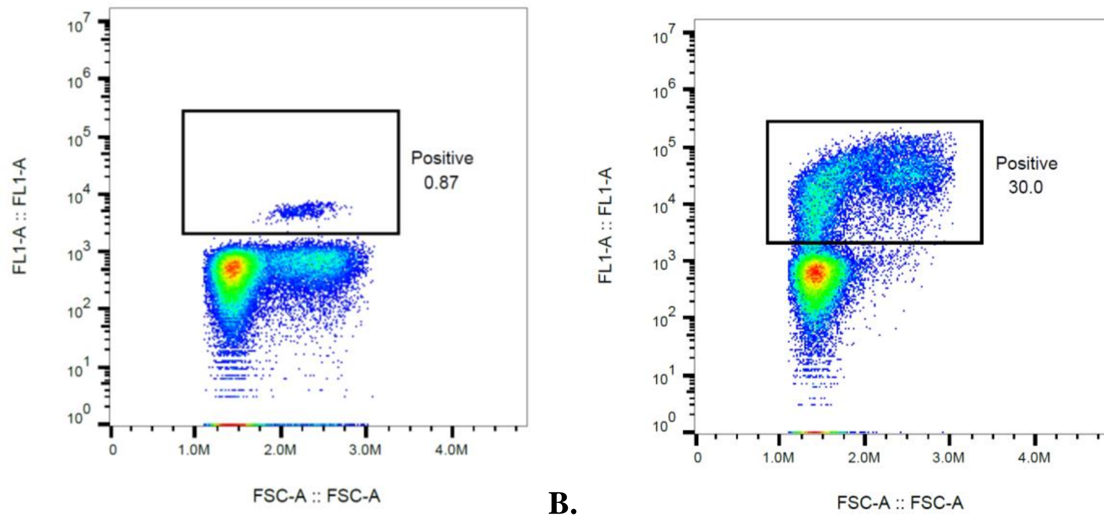
[3]Weaning occurred on d 35. Creep-feed fed from d 0 to 35 and the backgrounding diet fed d 35 to 77.

Table 4. Effect of YP on the concentration of white blood cells.

	Treatment[1]									*P*-Value		
	Control				YP							
	D 0	D34	D49	D76	D 0	D 34	D 49	D 76	SEM	Treatment	Time	Treatment x Time
Concentration, K/ µl												
White blood cells	8.54	11.54	9.54	9.29	8.87	11.08	10.73	9.93	0.59	0.32	<0.01	0.57
Neutrophil	2.88	4.12	4.08	3.41	2.95	4.12	4.49	3.43	0.20	0.38	<0.01	0.73
Lymphocyte	4.64	5.13	3.93	4.53	4.93	5.18	4.53	5.07	0.26	0.06	0.01	0.72
Monocyte	0.58	0.69	0.58	0.54	0.57	0.68	0.65	0.59	0.04	0.43	0.04	0.68
Eosinophil	0.40	1.22	0.87	0.75	0.38	0.96	0.98	0.75	0.12	0.63	<0.01	0.47
Basophil	0.03	0.38	0.08	0.07	0.04	0.15	0.08	0.08	0.09	0.37	0.04	0.45
Proportions, %												
Neutrophil	33.9	36.6	42.7	37.2	33.1	36.6	42.0	65.0	0.90	0.16	<0.01	0.66
Lymphocyte	54.2	46.0	42.7	49.0	55.6	47.3	42.5	51.1	1.22	0.18	<0.01	0.82
Monocyte	6.8	6.0	5.7	5.5	6.5	6.0	6.0	5.9	0.25	0.67	<0.01	0.53
Eosinophil	4.7	9.5	8.1	7.7	4.3	8.6	8.9	7.4	0.59	0.63	<0.01	0.57
Basophil	0.4	2.0	0.7	0.7	0.4	1.5	0.7	0.8	0.35	0.65	<0.01	0.84

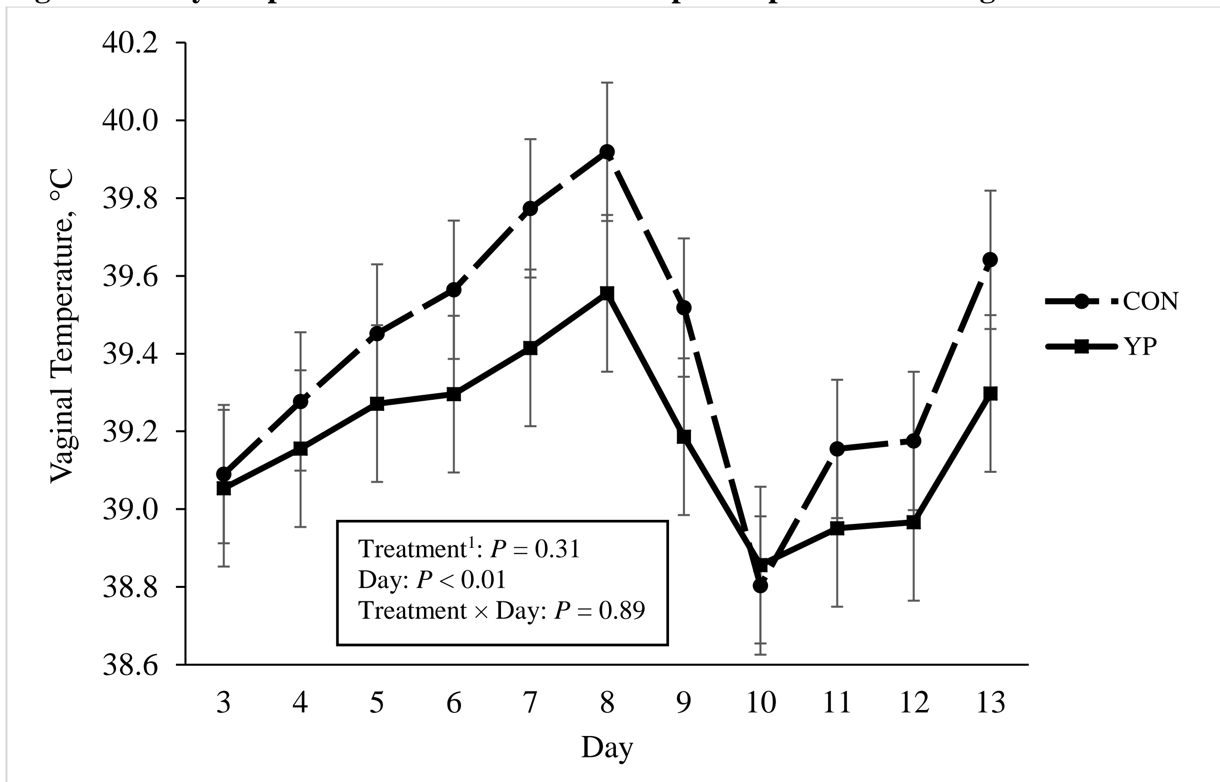
[1]CON = control, no addition of yeast product; YP = addition of yeast product

Figure 1. Gating strategy for negative and positive phagocytic controls.



A. Gating strategy for negative control incubated at 37°C to determine percent of cells positive for phagocytosis.
B. Gating strategy for positive control incubated at 37°C to determine percent of cells positive for phagocytosis. E. coli particles were added to positive controls to promote phagocytic activity in the blood.

Figure 2. Body temperature recorded for a 2 wk period prior to weaning.[2, 3]

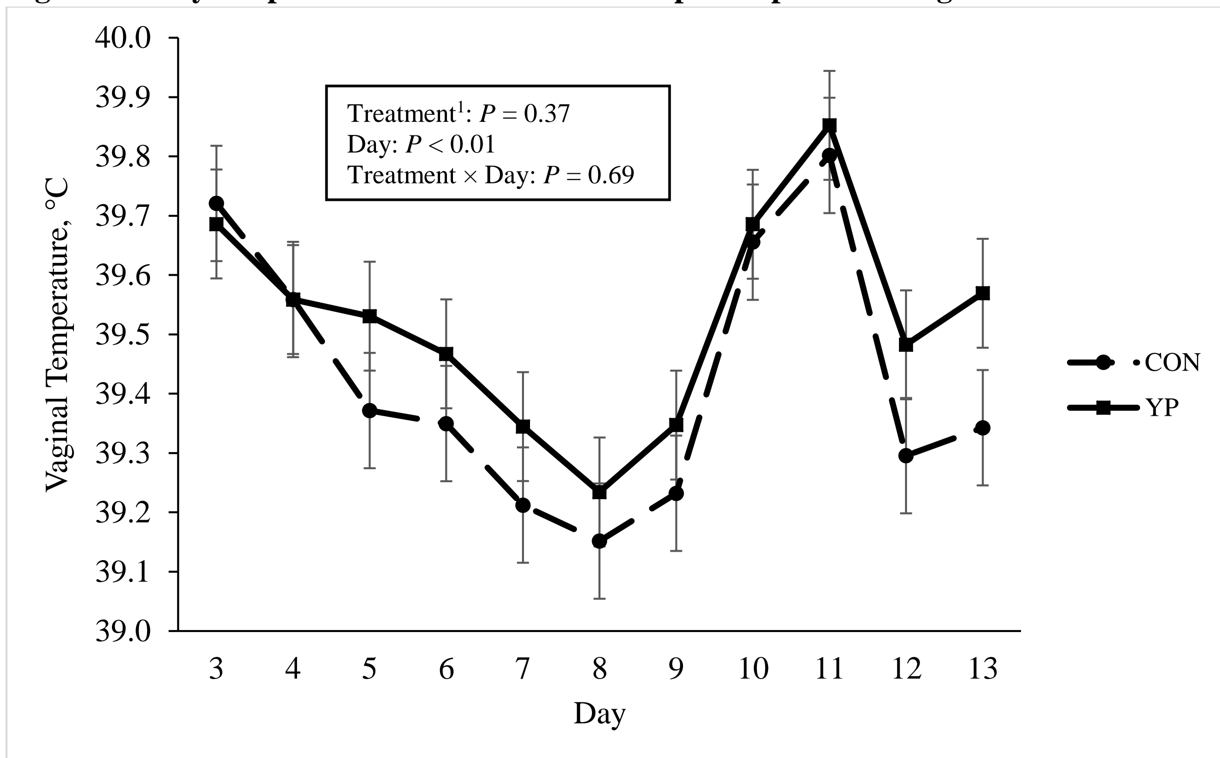


[1]CON = control, no addition of yeast product; YP = addition of yeast product.

[2]n = 30; data successfully obtained from 8 calves on CON and 5 on YP.

[3]Temperatures were collected using thermosensor iButtons embedded in hormone-free CIDR.

Figure 3. Body temperature recorded for a 2-wk period post-weaning.[2, 3]

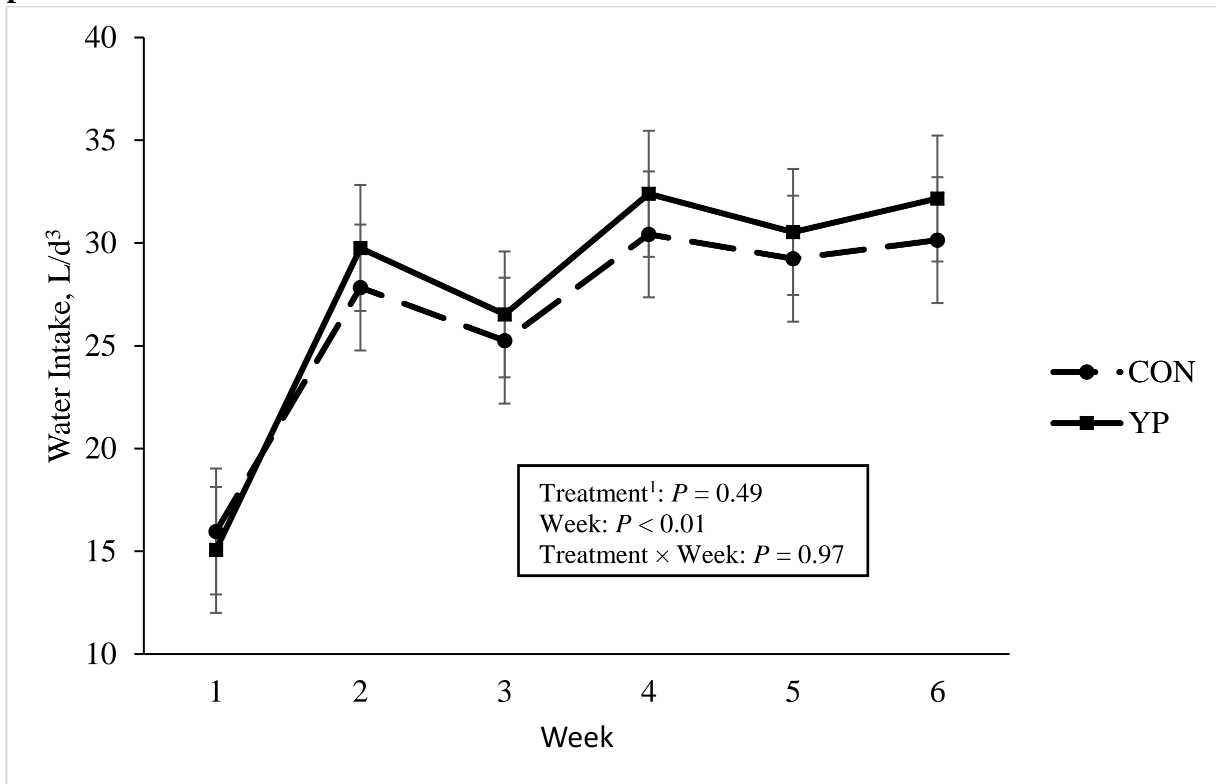


[1]CON = control, no addition of yeast product; YP = addition of yeast product.

[2]n = 30; successfully collected data from 11 calves on CON and 14 on YP.

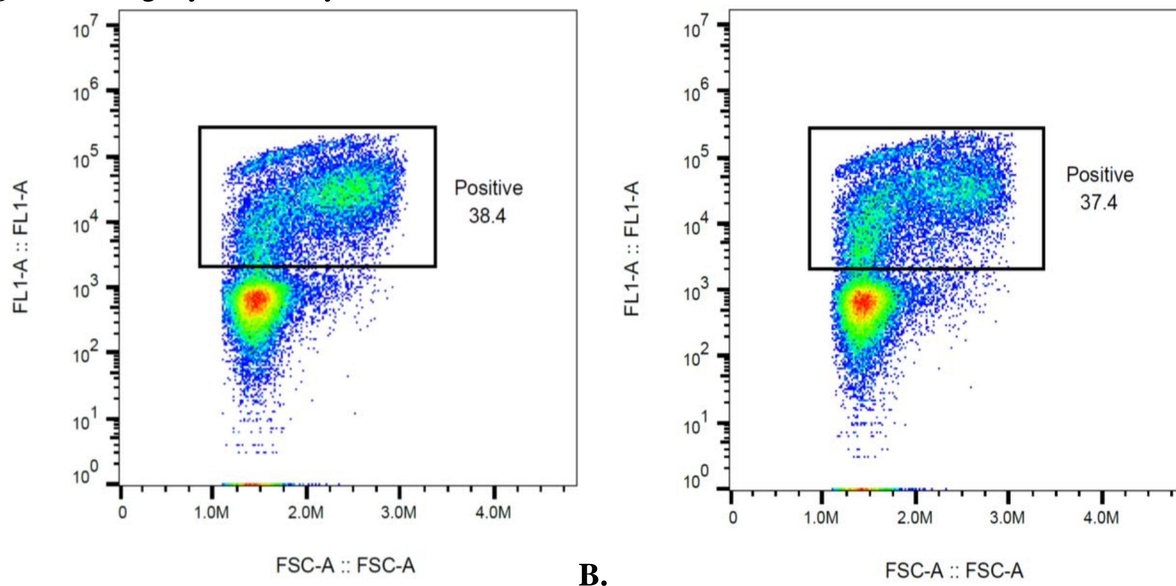
[3]Temperatures were collected using thermosensor iButtons embedded in hormone-free CIDR.

Figure 4. Effect of supplementing YP on water intake during the 42-d backgrounding period.[2, 3]



[1]CON = control, no addition of yeast product; YP = addition of yeast product.
[2]Water meters were installed on 4 automatic waterers which measured water intake for 8 pens (2 pens/waterer). The pens that shared a waterer were on the same dietary treatment.
[3]Water measurements were taken daily and the average daily water intake was calculated for each week.

Figure 5. Phagocytic activity on d 34 from a calf on CON and YP.[1]

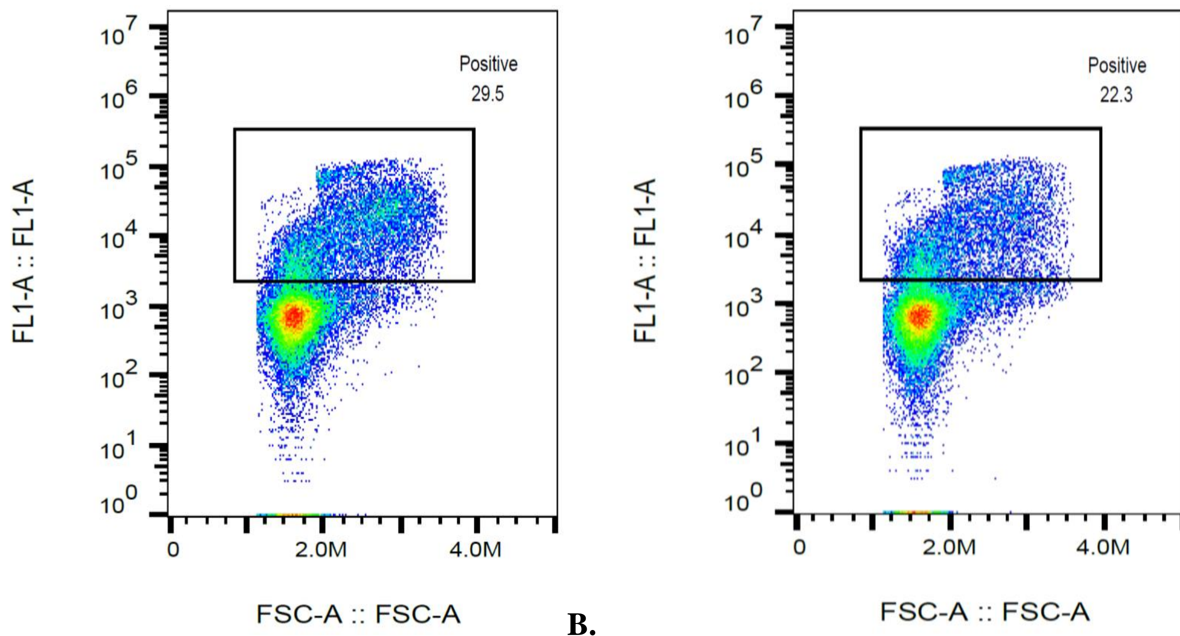


[1] CON = control, no addition of yeast product; YP = addition of yeast product.

A. Percentage of cells positive for phagocytic activity for a calf on CON diet.

B. Percentage of cells positive for phagocytic activity for a calf on YP diet.

Figure 6. Phagocytic activity on d 76 from a calf on CON and YP.[1]

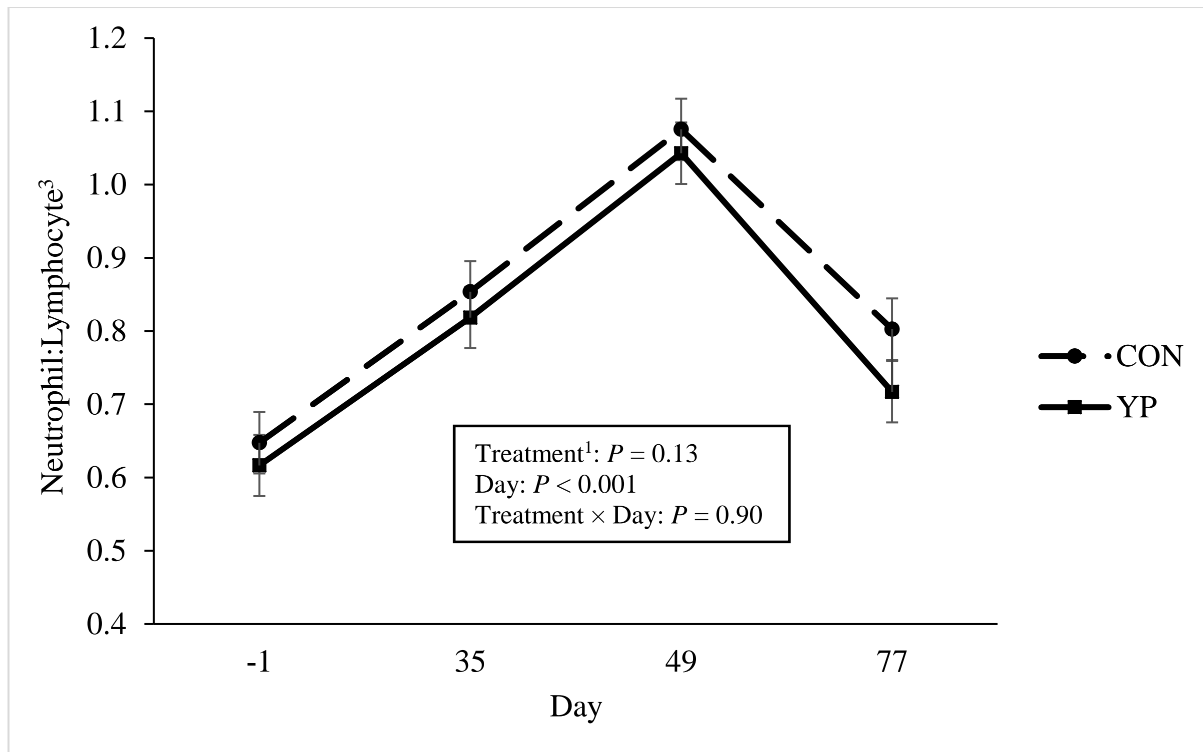


[1] CON = control, no addition of yeast product; YP = addition of yeast product.

A. Percentage of cells positive for phagocytic activity for a calf on CON diet.

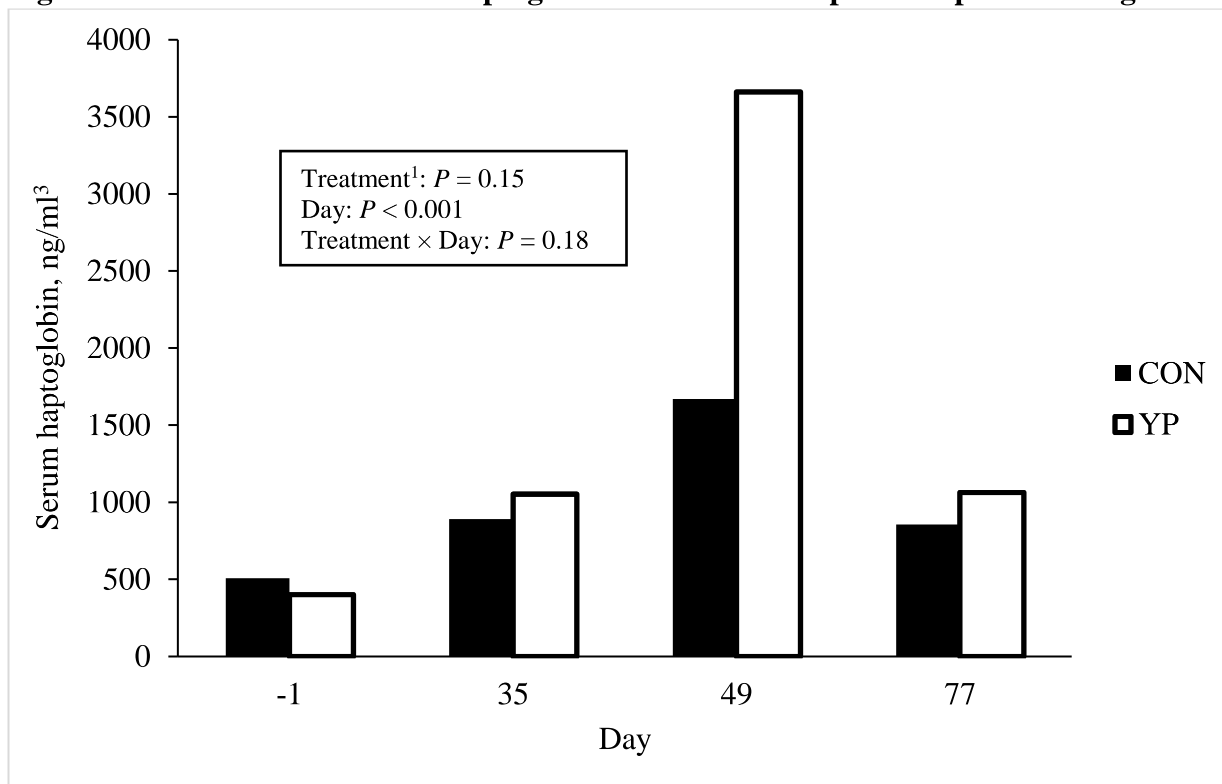
B. Percentage of cells positive for phagocytic activity for a calf on YP diet

Figure 7. Effect of YP on the neutrophil to lymphocyte ratio pre- and post-weaning.[2]



[1]CON = control, no addition of yeast product; YP = addition of yeast product.
[2]n = 95
[3]Neutrophil:Lymphocyte obtained from whole blood collected in a EDTA blood tube.

Figure 8. Effect of YP on serum haptoglobin concentrations pre- and post-weaning.[2]



[1]CON = control, no addition of yeast product; YP = addition of yeast product.

[2]n = 95

[3]Haptoglobin concentrations were log transformed to improve normality. Means represent back transformed data.

CHAPTER V

Characterization of rumen and fecal microbiota in heifer calves supplemented yeast products before and after weaning

ABSTRACT

Live yeast is utilized in ruminant diets to increase growth performance while improving immune function. Yeast is more beneficial in high producing animals and during times of stress. During weaning, calves suffer from elevated stress; therefore, heifer calves were used to determine the effects of supplementing yeast products 35 d prior to weaning and through a 42-d backgrounding period. Calves (n = 95; initial body weight [**BW**] = 165 ± 27 kg) were stratified based on BW, birthdate, sire, and dam parity; and were assigned randomly to pasture. Pastures were assigned randomly to 1 of 2 treatments: 1) supplement without yeast (C), or 2) supplement with yeast product (**YP**; 3 g of live yeast, and 1 g of yeast cell wall). Fecal samples were collected from a subsample of calves (n = 40; 4 calves/pasture) on d 0, 34 (weaning), and 76. Ruminal fluid samples were obtained from the same calves on d 34 and 76. Rumen and fecal microbiota were characterized by next generation sequencing the 16S V4 hypervariable region with the Illumina MiSeq platform. Addition of YP in the diet did not change the rumen microbial community structure or diversity. Fecal microbiome was impacted by supplementation on d 34. Calves receiving YP had a reduction in Shannon α-diversity ($P = 0.008$) and observed OTU ($P = 0.01$) compared to control. However, there was no effect of treatment observed on d 0 and 76 in feces. Among the top 50 most abundant operational taxonomic units (OTUs), that associated with *Paraprevotella* was greater in feces and that associated with *Prevotella* was greater in rumen fluid when calves consumed YP compared with control. Overall, YP did not change rumen and fecal diversity but affected relative abundance of certain OTU. Additionally, the fecal and rumen microbiomes were altered based on d of collection with microbial structure changing from time of weaning to the end of the backgrounding period.

Keywords: Microbiome, weaning, yeast products

INTRODUCTION

Probiotics are classified as live microorganisms that when consumed frequently and in adequate amounts can provide benefits to the host (FAO-WHO, 2002). Live yeast is a common probiotic used in livestock nutrition. It is able to maintain its integrity in the rumen and exert probiotic effects (Kung et al., 1997). When yeast is added to the diet it can increase diet digestibility (Jiang et al., 2017a; Ovinge et al., 2018) and promote the production of cellulolytic (Harrison et al., 1988) and fibrolytic (Chaucheyras-Durand and Fonty, 2006) rumen bacteria. Including yeast in the diet has influenced the relative abundance of various bacteria in the rumen of lactating dairy cows (Jiang et al., 2017b; AlZahal et al., 2016; Pinloche et al., 2013).

Using next generation sequencing has helped advance the understanding of the rumen microbiome by identifying bacteria down to the genus level (McCann et al., 2014). Diet is the greatest driver behind the variation in the gut microbiome between ruminants (Henderson et al., 2015). Age also influences the microbial populations within the rumen (Jami et al., 2013). Meale et al. (2016) found that weaning calves dramatically alters the gut microbiome. The aim of this study was to determine the influence that yeast has on the rumen and fecal microbiome of beef heifers during a 35-d creep-feeding period prior to weaning and through a 42-d backgrounding period.

MATERIALS AND METHODS

Animal Management

Experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (protocol # 17060). Heifer calves (n = 40; initial BW = 165 ± 27 kg) at the University of Arkansas Beef Unit were used to evaluate the effects that supplemental yeast has on the fecal and rumen microbiome. Heifer calves (n = 95) were stratified by BW, birthdate, dam

parity, and sire and assigned randomly to pasture (10 pastures). Pastures (2.4 h) were assigned randomly to 1 of 2 treatments: 1) grain supplement without yeast (C), or 2) grain supplement with yeast (YP).

Calves were offered creep-feed for 35 d prior to weaning at 0.5% of initial BW. Creep-feed consisted of 47.5% ground corn, 47.5% dried distiller's grains, and 5% white salt with or without the addition of YP. Creep-diets were formulated to provide 4 g of YP/d (3 g live yeast and 1 g yeast cell wall; Phileo Lesaffre Animal Care, Milwaukee, WI). Creep-feeders were refilled every 3 or 4 d. On d 21, calf BW were recorded and the amount of creep-feed offered was adjusted. Calves were weaned from their dams on d 35 at which time they remained in their pre-weaning treatment groups for a 42-d backgrounding period.

During the backgrounding period calves were housed on 0.4 h grass-traps (10 pastures; 9 or 10 calves/pasture) with access to concrete bunks and automatic waterers. Calves were offered a grain supplement (Table 1) with or without the addition of yeast supplement. Yeast supplement continued to provide 4 g of YP/d (3 g live yeast and 1 g yeast cell wall; Phileo Lesaffre Animal Care). When forage became limiting, calves had ad libitum access to bermudagrass hay. Calves remained on backgrounding diet for 42 d post-weaning.

Sample Collection

Fecal samples were collected on d 0, 34 (1 d prior to weaning), and 76 from a subsample of calves (n = 40; 4 calves/pasture). Rumen fluid was collected on the same 40 calves on d 34 and 76 via esophageal tubing. Due to the size of calves at the initiation of the study, rumen samples were not collected on d 0. Samples were put on ice immediately after collection and transported back to the lab, where they were stored at -20°C until time of DNA extraction.

DNA Extraction, library preparation, and sequencing

Both rumen and fecal DNA were isolated using DNeasy PowerSoils Kit (Qiagen Inc., Germantown, MD) which required 100 mg of feces or 100 µL of rumen fluid. Concentrations of isolated DNA were determined using Nanodrop One C (Fisher Scientific, Hanover Park, IL). Library preparation required samples to be diluted to 10 ng of DNA. Samples were amplified by PCR using dual index primers. Amplification occurred in the following steps: 1) 95°C for 2 min, 2) 30 cycles at 95°C for 20 s, then 55°C for 15 s, and 72°C for 5 min, and 3) 72°C for 10 min and then 4°C until the end (T100 Thermal Cycler; Bio-Rad Laboratories, Hercules, CA). Primers were selected to amplify the V4 region of the 16S rRNA gene in bacteria. Samples were checked for proper amplification by agarose gel electrophoresis. Amplified samples were then normalized using a SequalPrep™ Normalization Kit (Life Technologies, Grand Island, NY). After normalization, 5 µL aliquots from each sample were pooled together to create a library. Concentration of the pooled library was assessed using quantitative PCR (Eppendorf, Westbury, NY) and the Agilent 2100 Bioanalyzer System (Agilent, Santa Clara, CA). The pooled library was sequenced using the Illumina MiSeq® v2 (San Diego, CA) at the University of Arkansas Biomass Research Center (Fayetteville, AR).

Sequences produced were processed on the open-source mothur program (version 1.39.1) using the MiSeq SOP as described by the Schloss lab (Kozich et al., 2013). Sequences were screened, and ambiguous bases and sequences longer than 275 base pairs were removed (Schloss et al., 2009). Only unique sequences were kept to reduce the number of duplicated sequences present. Sample sequences were aligned with the SILVA release 128 (Quast et al., 2013) reference database. In order to remove excess noise, sequences were removed if they contained greater than 2 mismatches using the pre-cluster method (Huse et al., 2010). To further reduce sequencing error, chimeras were identified and removed from all samples (Edgar et al., 2011).

Operational taxonomic units (OTU) were classified at the genus level by way of the Bayesian method (Cole et al., 2009) using a cut-off of 97% similarity. Sequences were subsampled at 3,000 reads for both rumen and fecal samples, which were used to determine alpha and beta diversity measures.

Statistical Analysis

Two separate random forest analyzes were performed to determine the top rumen and fecal microbial predictors for treatment and ADG. Both regression and classification random forest were run using the randomForest package (Liaw and Wiener, 2002) in RStudios version 1.1.423 (RStudio Team, Boston, MA). Classification random forest was used to determine the best predictors based on dietary treatment at d 34 and 78 in both the rumen and fecal microbiome. All OTUs identified in both the rumen and fecal microbiome were used for classification random forest in order to reduce the out of bag error rate (OOB). Regression random forest evaluated the best predictors for ADG from d 34 to 76. First, the difference in the relative abundance of the top 1,000 OTUs on d 34 and 76 were calculated. Once the difference was determined this new number was used in the regression random forest.

Individual animal was set as the experimental unit for all data analysis. Alpha diversity measures including the Shannon Diversity Index (Shannon and Weaver, 1949) and the number of Observed OTUs, were analyzed using the Wilcoxon Rank Sum Test in RStudio. Beta diversity was measured using the Jaccard (Chao et al., 2005) and Bray-Curtis (Bray and Curtis, 1957) distance matrices. Statistical analysis for beta diversity measures were analyzed in mothur version 1.39.1 using the ANOSIM command (analysis of similarity). The relative abundance of the top 50 OTUs were analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC).

Treatment, time, and the treatment by time interaction were included in the model. Significant differences were deemed at P-value ≤ 0.05 and tendencies were set at $0.05 < P \leq 0.10$.

RESULTS AND DISSCUSSION

Calf Performance

Supplementation of YP pre- and post-weaning did not influence ($P \geq 0.22$) calf BW nor was there a difference in ADG from d 0 to 77 ($P = 0.22$). Prior to weaning ADG was not different ($P = 0.45$) between treatment groups, while post-weaning (d 35 to 77) ADG was greater ($P = 0.01$) in control compared to YP calves. Disappearance of creep-feed prior to weaning was not impacted ($P = 0.41$) by the addition of yeast in the diet with consumption of creep-feed, averaging 0.48 kg DM/d. Similar to creep-feed, there was no difference in the amount of supplement consumed during the backgrounding period. After the first day of feeding the new diet, calves consumed all supplement offered during the remainder of the backgrounding period.

Sequencing Analysis

Analysis of rumen and fecal samples produced a total of 2,362,426 and 1,790,860 reads, respectively. Rumen reads ranged from 6,664 to 246,428 with an average of 29,904 and fecal reads from 3,910 to 75,179 with an average of 14,924. Rumen and fecal samples were standardized and subsampled at 3,000 reads. One rumen sample was excluded from the dataset due to its low number of reads. This resulted in a total of 79 rumen samples and 120 fecal samples used for data analysis. The number of OTUs identified were 31,109 and 24,518 for rumen and fecal samples, respectively.

Alpha and Beta Diversity Measures

Within community (alpha) diversity was evaluated using the Shannon index (Shannon and Weaver, 1949) and the number of observed OTUs for both rumen and fecal samples. Dietary

treatment did not affect the community diversity within the rumen (Figure 1) as evident by both the Shannon index ($P \geq 0.41$) and the number of observed OTUs ($P \geq 0.51$). However, fecal (Figure 2) alpha diversity was impacted by supplemental yeast at time of weaning. At weaning (d 34), control calves had a greater ($P < 0.01$) Shannon index, indicating greater variety and distribution of species present. Similarly, the number of observed OTU were greater ($P = 0.01$) for control compared to calves supplemented yeast product. Nevertheless, both Shannon index ($P = 0.12$) and the number of observed OTUs ($P = 0.19$) were not different at the conclusion of the study. Others (Jiang et al., 2017b; AlZahal et al., 2017) have found no differences on alpha-diversity in the rumen when feeding yeast.

Rumen beta-diversity (Figure 3) was not different ($P \geq 0.24$) based on community membership and structure between calves offered control or supplemental yeast which has been supported (AlZahal et al., 2017). As with alpha-diversity, there was a difference (Jaccard: ANOSIM $P = 0.03$; Bray-Curtis: ANOSIM $P = 0.06$; Figure 4) observed in community structure and membership in the fecal microbiome on d 34 but once again, beta-diversity was not impacted by dietary treatment on d 76. This difference on d 34 for both alpha- and beta-diversity in fecal samples is interesting to note considering that there was no difference in creep-feed disappearance at this time. It would appear that at this time calves on YP had reduced diversity of bacterial populations in the fecal microbiome.

While dietary treatment did not have an influence in community structure and membership, there was an effect ($P < 0.01$) of time on rumen and fecal microbiota. As shown in Figures 3 and 4, clustering was present based on the day of sample collection. Prior to the initiation of the study (d 0) calves were on a forage based diet. Calves received a grain based creep supplement for 35 d prior to weaning and during the backgrounding period; although, these

two grain supplements differed. Having a change in the dietary treatment at both these points could be the cause of the clustering observed based on time of sample collection.

In young dairy calves, alpha-diversity in the rumen was greater in calves pre-weaning, this was different than alpha-diversity in the feces which showed greater richness and evenness post-weaning (Meale et al., 2016). Meale et al. (2016) further reported alterations in community structure in calves pre- and post-weaning. Furthermore, age of calves at weaning can affect the change in community structure. Meale et al. (2017) found that early weaned calves (5 wk of age) had a more abrupt shift in rumen community structure compared to calves weaned at 7 to 9 wk of age. However, these studies evaluating the microbiome at weaning were conducted in dairy calves which are managed and weaned very differently than beef calves.

OTU Distribution

The top 50 most abundant OTU in the rumen (Figure 5) accounted for, on average, 38% of the total sequences (Figure 5). The most abundant OTU in the rumen was OTU1 unclassified *Firmicutes* followed by OTU2 and OTU4, *Prevotella*. Based on the top 50 OTU, dietary treatment only impacted ($P = 0.02$) OTU32 *Prevotella* in the rumen which was increased with YP. Contrary to Pinloche et al. (2013) who found a decrease in *Prevotella* due to yeast supplementation. *Prevotella* has been well documented as one of the most abundant genus in the rumen (Mao et al., 2015; Jiang et al., 2017b).

Within the fecal microbiome, the 50 OTU with the greatest relative abundance accounted for approximately 50% of the population (Figure 6). The top 3 genus found in the fecal microbiome were: 1) OTU1 unclassified *Ruminococcacaea*, 2) OTU2 *Paraprevotella*, and 3) OTU3 *Clostridium* XIV. Out of the top 50 OTU found in feces, dietary treatment affected the relative abundance of OTU2 *Paraprevotella* ($P = 0.02$), OTU37 unclassified *Bacteroidales* ($P <$

0.01), and OTU47 unclassified *Ruminococcacaea* (*P* < 0.01). OTU2 *Paraprevotella* was increased by the addition of YP in the diet; whereas, OTU37 and OTU47 were greater in calves fed the control diet. *Paraprevotella* has been isolated in human feces previously and it is believed to produce succinic and acetic acids as fermentation end products (Morotomi et al., 2009). *Ruminococcacaea* is one of the most prominent families of bacteria in the gut and is generally related to gut health (Biddle et al., 2013).

To acquire a broader perspective of the effect that YP has on the microbial population in the gut, the top 50 bacterial families isolated from the rumen and feces were determined. In the rumen (Figure 7) the top 3 microbial families consisted of *Prevotellaceae*, unclassified *Bacteroidetes*, and *Lachnospiraceae*. *Prevotellaceae* has reportedly been the most dominant family of bacteria identified in the rumen and is an important property of enzymes involved in acetate and propionate formation (Deusch et al., 2017). Additionally, 2 of the top 50 most abundant microbial families in the rumen were influenced by adding YP; including an increase in unclassified SR1 (*P* = 0.05) accompanied by a decrease in *Victivallaceae* (*P* = 0.02). Similar to the current study, AlZahal et al. (2017) reported that adding active dried yeast to the diet increased the phylum *SR1* in the rumen. Bacteria from the SR1 phylum are anaerobic and have been isolated in the guts of humans and mice (Ley et al., 2008); while the specific function of this bacteria is not well understood, AlZahal et al. (2017) proposed that members of the phylum SR1 could potentially play a role in fiber digestion, as they found that SR1 was more abundant on a high fiber diet and decreased when cows were switched to a high concentration diet. As stated, a decrease in the family *Victivallaceae* was detected in the rumen of heifers fed YP, this microbe has been associated with increased average daily feed intake in heifers (Paz et al., 2018).

In the current study, the amount of supplement disappearance was not affected by treatment pre- or post-weaning.

The top 3 bacterial families identified in the fecal microbiome (Figure 8) were: 1) *Ruminococcaceae*, 2) *Lachnospiraceae*, and 3) unclassified family from the phylum *Bacteroidetes*. Interestingly, *Lachnospiraceae* and unclassified *Bacteroidetes* were in the top 3 most abundant bacterial families in both the rumen and feces. Families that were increased ($P \leq 0.10$) by YP included *Prevotellaceae*, *Veillonellaceae*, and *Acetobacteraceae*. In the rumen *Veillonellaceae* has been positively associated with gain (Myer et al., 2015) and is more abundant in the fecal microbiome when cows are fed a moderate grain diet (Kim et al., 2014). Whereas, *Ruminococcaceae*, *Spirochaetaceae*, *Fibrobacteraceae*, and *Victivallaceae* were greater ($P \leq 0.08$) in calves on the control diet.

Random Forest

Random forest was implemented on the genus level to determine the 50 most predictive OTUs on d 34 and 76 for both rumen and fecal samples based on the mean decrease accuracy (MDA; Figure 9). On d 34, the 2 best predictors for rumen fluid included OTU151 unclassified *Prevotellaceae* and OTU2 *Prevotella*. On d 76, the top 2 predictors included OTU13 *Fibrobacter* and OTU18 unclassified *Bacteroidetes*. The 2 best predictors for fecal samples on d 34 were OTU19 unclassified *Bacteroidetes* and OTU47 unclassified *Ruminococcaceae*; lastly, OTU141 unclassified *Bacteroidetes* and OTU233 *Clostridium_IV* were the top 2 predictors on d 76. While these were the top 2 predictors from each d and sample type, the MDA was relatively low for each. Likewise, the OOB for each sample was high even when using all OTU identified. Out of the bag error rate for rumen samples on d 34 and 76 were 59 and 50%, respectively. While OOB

error rate for fecal samples on d 34 and 76 were 42.5 and 52.5%, respectively. Thus, the microbial population may not be the best predictor for supplemental yeast treatment.

Average daily gain of calves supplemented yeast product was less than control from weaning to the end of the backgrounding. Therefore, there was interest in evaluating predictors on ADG based on the difference in the relative abundance of OTU on d 76 and 34. Within the rumen (Figure 10) the top 2 predictors for ADG were OTU492 unclassified *Porphyromonadaceae* and OTU640 unclassified *Lachnospiraceae* (Figure 10). Based on the top 1,000 OTU identified in the feces, the 2 best predictors for ADG were OTU798 unclassified *Ruminococcaceae* and OTU115 unclassified *Porphyromonadaceae* (Figure 11). Both rumen and fecal samples had an unclassified OTU from family *Porphyromonadaceae* which is reported to be greater in the rumen of cows on high starch diets (Zened et al., 2012). In rumen samples, *Porphyromonadaceae* was negatively correlated to ADG based on Pearson regression (slope = -0.52, $r^2 = 0.28$, $P < 0.01$). Others have reported that OTU from *Ruminococcaceae* and *Lachnospiraceae* correlate positively to ADG in steers, however, they were not associated with ADG in heifers (Paz et al., 2018). The unclassified OTU in family *Lachnospiraceae* was positively associated with ADG based on Pearson regression (slope = 0.53, $r^2 = 0.27$, $P < 0.01$).

Implications

In a previous paper, authors found that supplemental yeast did not affect overall growth performance, health, or heat stress. However, supplementing yeast product during the backgrounding period negatively affected ADG post-weaning. As with growth performance, yeast product did not impact the rumen or fecal microbiome of calves. There was no difference in diversity based on dietary treatment in the rumen; but there was an effect of day on community structure and membership. In regard to the fecal microbiome, there was a greater

community diversity in calves fed the control diet on d 34 compared to calves supplemented yeast product. Supplemental yeast had a greater impact on the relative abundance of OTU. There were several OTU identified that were increased with YP, including unclassified SR1 and *Paraprevotella*. Based on random forest analysis, rumen and fecal bacteria were not sufficient predictors to determine yeast supplementation. Overall, feeding live yeast in combination with yeast cell wall products did not improve phenotypic parameters or alter the microbial population in the rumen and feces.

LITERATURE CITED

AlZahal, O., F. Li, L. L. Guan, N. D. Walker, and B. W. McBride. 2017. Factors influencing ruminal bacterial community diversity and composition and microbial fibrolytic enzyme abundance in lactating dairy cows with a focus on the role of active dry yeast. J. Dairy Sci. 100:4377-4393.

Biddle, A., L. Stewart, J. Blanchard, and S. Leschine. 2013. Untangling the genetic basis of fibrolytic specialization by *Lachnospiraceae* and *Ruminococcaceae* in diverse gut communities. Diversity 5:627-640.

Bray, J. R. and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Mono. 27:325-349.

Chao, A., R. L. Chazdon, R. K. Colwell, and T. J. Shen. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecol. Let. 8:148-159.

Chaucheyras-Durand, F. and G. Fonty. 2006. Effects and modes of action of live yeasts in the rumen. Biologia (Bratislava). 61:741-750.

Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. S. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity, and J. M. Tiedje. 2009. The ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37:D141-D145

Deusch, S., A. Camarinha-Silva, J. Conrad, U. Beifuss, M. Rodehutscord, and J. Seifert. 2017. A structural and functional elucidation of the rumen microbiome influenced by various diets and microenvironments. Front. Microbiol. 8:1605.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200.

FAO-WHO. 2002. Guidelines for the evaluation of probiotics in food. Joint FAO/WHO Working Group on drafting guidelines for the evaluation of probiotics in food; London, Ontario, Canada.

Harrison, G. A., R. W. Hemken, K. A. Dawson, and R. J. Harmon. 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci. 71:2967-2975.

Henderson, G., F. Cox, S. Ganesh, A. Jonker, W. Young, Global Rumen Census Collaborators, and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep. 5:14567.

Huse, S. M., D. M. Welch, H. G. Morrison, and M. L. Sogin. 2010. Ironing out the wrinkles in the rare biosphere through improved OUT clustering. Environ. Microbiol. 12:1889-1898.

Jami, E., A. Israel, A. Kotser, and I. Mizrahi. 2013. Exploring the bovine rumen bacterial community from birth to adulthood. ISME J. 7:1069-1079.

Jiang, Y., I. M. Ogunade, K. G. Arriola, M. Qi, D. Vyas, C. R. Staples, and A. T. Adesogan. 2017a. Effects of the dose and viability of *Saccharomyces cerevisiae*. 2. Ruminal fermentation, performance of lactating dairy cows, and correlations between ruminal bacteria abundance and performance measures. J. Dairy Sci. 100:8102-8118.

Jiang, Y., I. M. Ogunade, S. Qi, T. J. Hackmann, C. R. Staples, and A. T. Adesogan. 2017b. Effects of the dose and viability of *Saccharomyces cerevisiae*. 1. Diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and quantitative PCR. J. Dairy Sci. 100:325-342.

Kim, M., J. Kim, L. A. Kuehn, J. L. Bono, E. D. Berry, N. Kalchayanand, H. C. Freetly, A. K. Benson, and J. E. Wells. 2014. Investigation of bacterial diversity in the feces of cattle fed different diets. J. Anim. Sci. 92:683-694.

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. K. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79:5112-5120.

Kung, L. E., Jr., M. Kreck, and R. S. Tung. 1997. Effects of a live yeast culture and enzymes on in vitro ruminal fermentation and milk production of dairy cows. J. Dairy. Sci. 80:2045-2051.

Ley, R. E., M. Hamady, C. Lozupone, P. J. Turnbaugh, R. R. Ramey, J. S. Bircher, M. L. Schlegel, T. A. Tucker, M. D. Schrenzel, R. Knight, and J. I. Gordon. 2008. Evolution of mammals and their gut microbes. Sci. 320:1647-1651.

Liaw, A. and M. Wiener. 2002. Classification and Regression by Random Forest. R News 2:18-22.

Mao, S., M. Zhang, J. Liu, and W. Zhu. 2015. Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: membership and potential function. Sci. Rep. 5:16116.

Meale, S. J., S. C. Li, P. Azevedo, H. Derakhshani, T. J. DeVries, J. C. Plaizier, M. A. Steele, and E. Khafipour. 2017. Weaning age influences the severity of gastrointestinal microbiome shifts in dairy calves. Sci. Rep. 7:198.

Meale, S. J., S. Li, P. Azevedo, H. Derakhshani, J. C. Plaizier, E. Khafipour, and M. A. Steele. 2016. Development of ruminal and fecal microbiome are affected by weaning but not weaning strategy in dairy calves. Front. Microbiol. 7:582.

McMann, J. C., T. A. Wickersham, and J. J. Loor. 2014. High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. Bioinformatics and Biology Insights 8:109-125.

Morotomi, M., F. Nagai, H. Sakon, and R. Tanaka. 2009. *Paraprevotella clara* gen. nov., sp. Nov. and *Paraprevotella xylaniphila* sp. nov., members of the family '*Prevotellaceae*' isolated from human faeces. Intern. J. System. And Evolut. Microbiol. 59:1895-1900.

Myer, P. R., T. P. L. Smith, J. E. Wells, L. A. Kuehn, H. C. Freetly. 2015. Rumen microbiome from steers differing feed efficiency. PLoS ONE 10;e012174.

Ovinge, L. A., J. O. Sarturi, M. L. Galyean, M. A. Ballou, S. J. Trojan, P. R. B. Campanili, A. A. Alrumaih, and L. A. Pellarin. 2018. Effects of a live yeast in natural-program finishing feedlot diets on growth performance, digestibility, carcass characteristics, and feeding behavior. J. Anim. Sci. 96:684-693.

Paz, H. A., K. E. Hales, J. E. Wells, L. A. Kuehn, H. C. Freetly, E. D. Berry, M. D. Flythe, M. L. Spangler, and S. C. Fernando. 2018. Rumen bacterial community structure impacts feed efficiency in beef cattle. J. Anim. Sci. 96:1045-1058.

Pinloche, E., N. McEwan, J. Marden, C. Bayourthe, E. Auclair, and C. Newbold. 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS ONE 8:e67824.

Quast, C., E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, and F. O. Glöckner. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41:D590-D596.

Schloss, P. D., S. L. Wescott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stress, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ, Microbiol. 75:7537-7541.

Shannon, C. E., and W. Weaver. 1949. The mathematical theory of communication. University of Illinois press, Urbana.

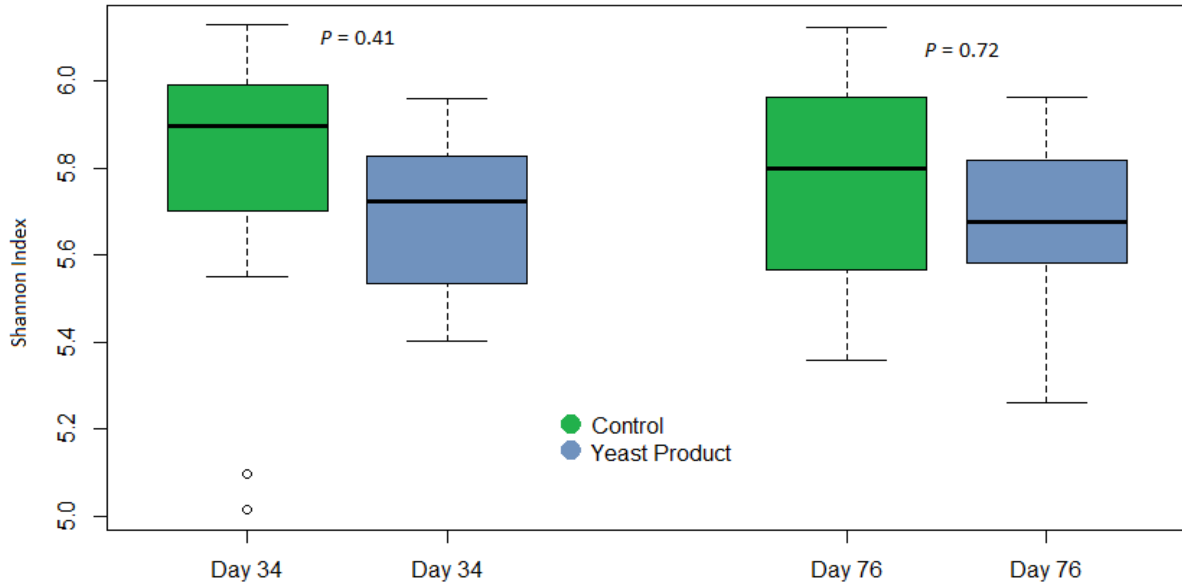
Zened, A., S. Combes, L. Cauquil, J. Mariette, C. Klopp, O. Bouchez, A. Troegeler-Meynadier, and F. Enjalbert. 2012. Microbial ecology of the rumen evaluated by 454 GS FLX pyrosequencing is affected by starch and oil supplementation of diets. FEMS Microbiol. Ecol. 83:504-514.

APPENDIX

Table 1. Ingredient list of basal diet fed during the 28-d receiving period

Item	% As-fed
Cracked corn	40
Cottonseed hulls	20
Dried distiller grains	18.33
Corn gluten meal	10
Soybean meal, high protein	7
Limestone	1.5
Molasses	1.5
Yellow grease	1
Salt, white	0.4
Corn and Rumensin intermediate mix[3]	0.1
Calcium monophosphate	0.1
Vitamin A, D, E premix[1]	0.03
Vitamin E premix[2]	0.03
Trace mineral premix[4]	0.01

[1]Vitamin premix contained 8,800,000 IU Vitamin A, 1,760,000 IU Vitamin D, and 1,100 IU Vitamin E/kg
[2]Vitamin E premix contained 44,000 IU Vitamin E/kg
[3]Mix provides 22 g monensin/kg of intermediate mix
[4]Contains NB-8675 (Nutrablend, Neosho, Mo) 12% Zn, 8% Mn, 4% Cu, 1% Fe, 500 ppm Co, 2,000 ppm I, and 600 ppm Se



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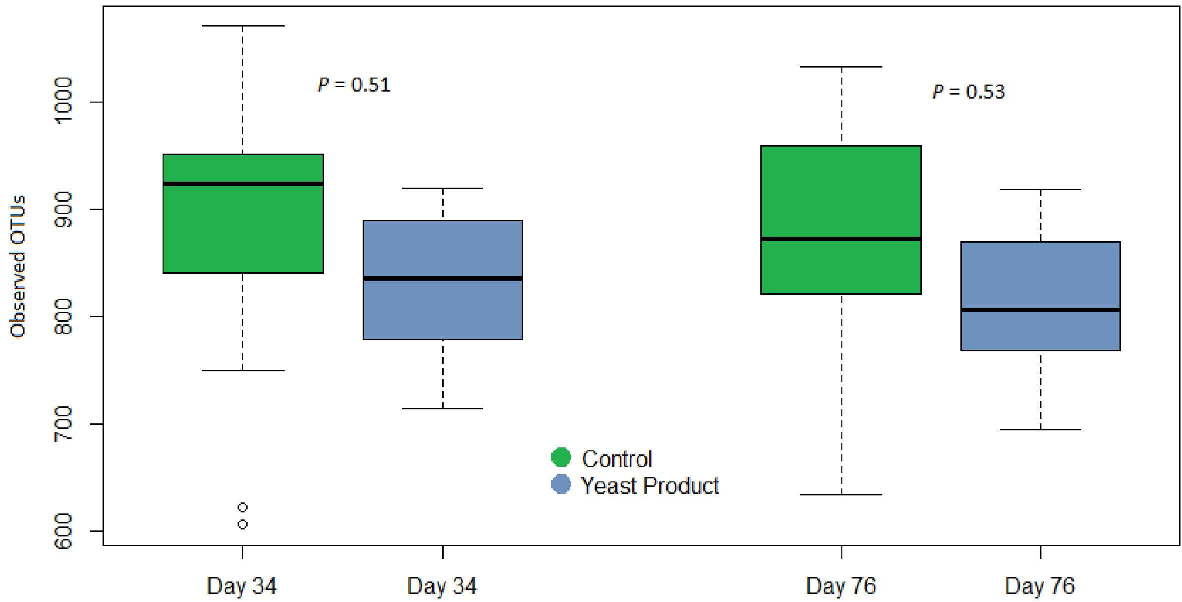


B.

Figure 1. Alpha diversity measures for rumen microbiome. Yeast product calves were supplemented with 3 g of live yeast and 1 g of yeast cell wall for 35 d prior to weaning and through a 42-d backgrounding period. A. Shannon Index B. number of Observed.

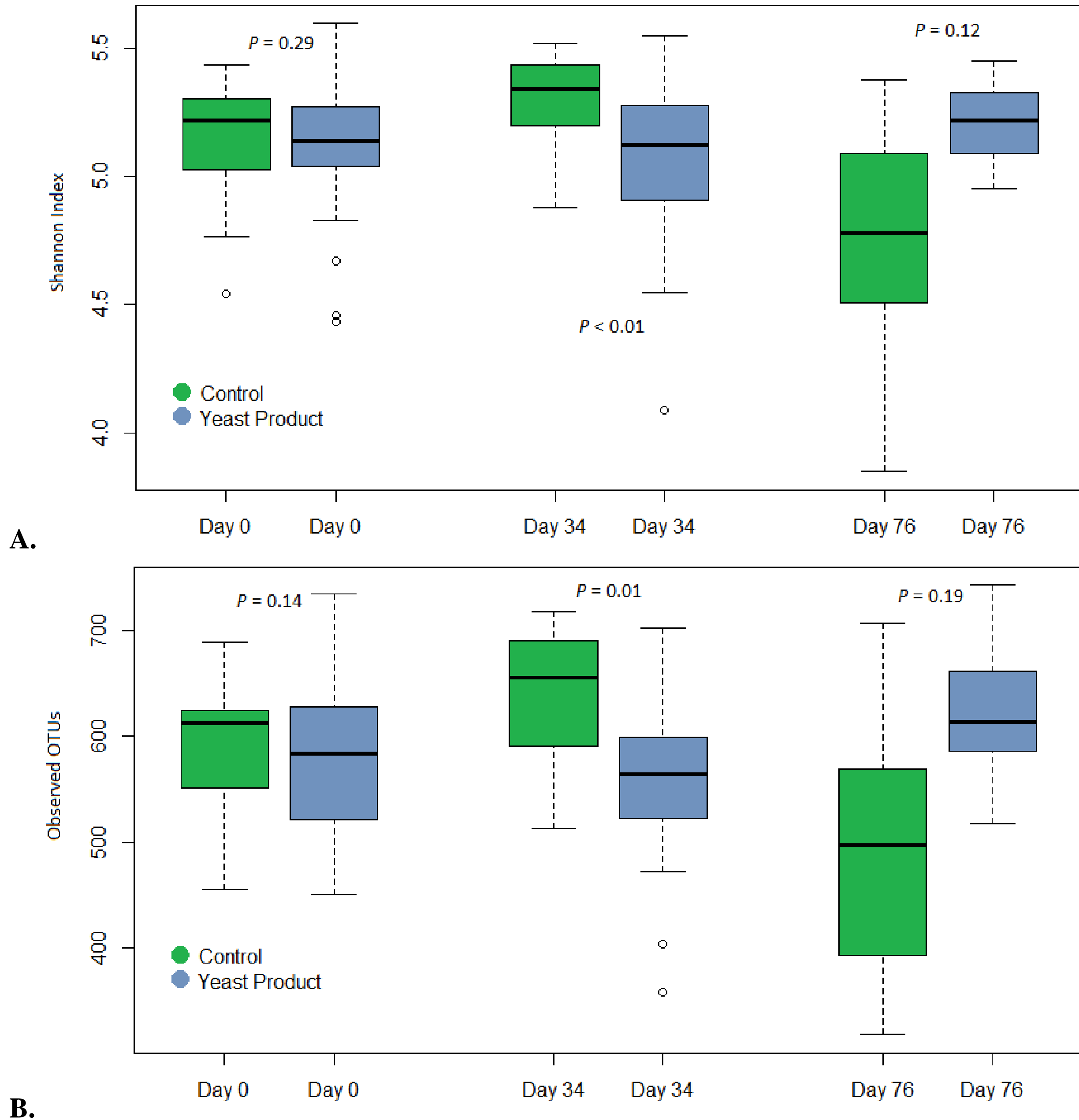
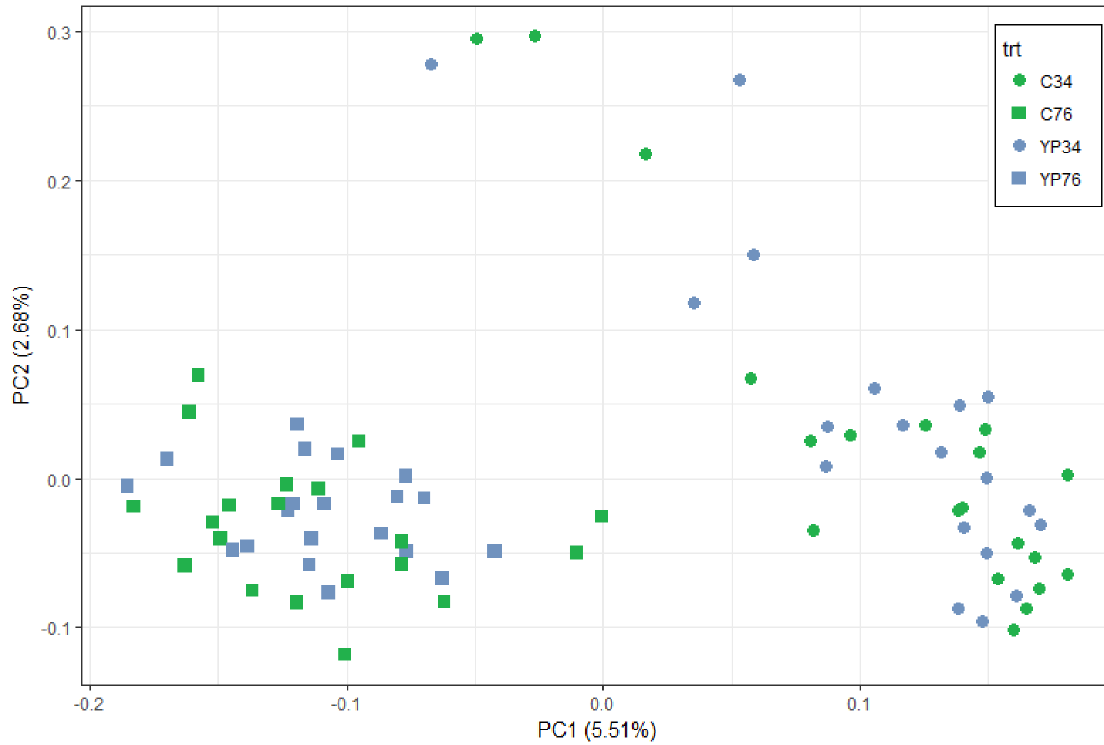


Figure 2. Alpha diversity measures for fecal microbiome. Yeast product calves were supplemented with 3 g of live yeast and 1 g of yeast cell wall for 35 d prior to weaning and through a 42-d backgrounding period. A. Shannon Index B. number of Observed OTUs.



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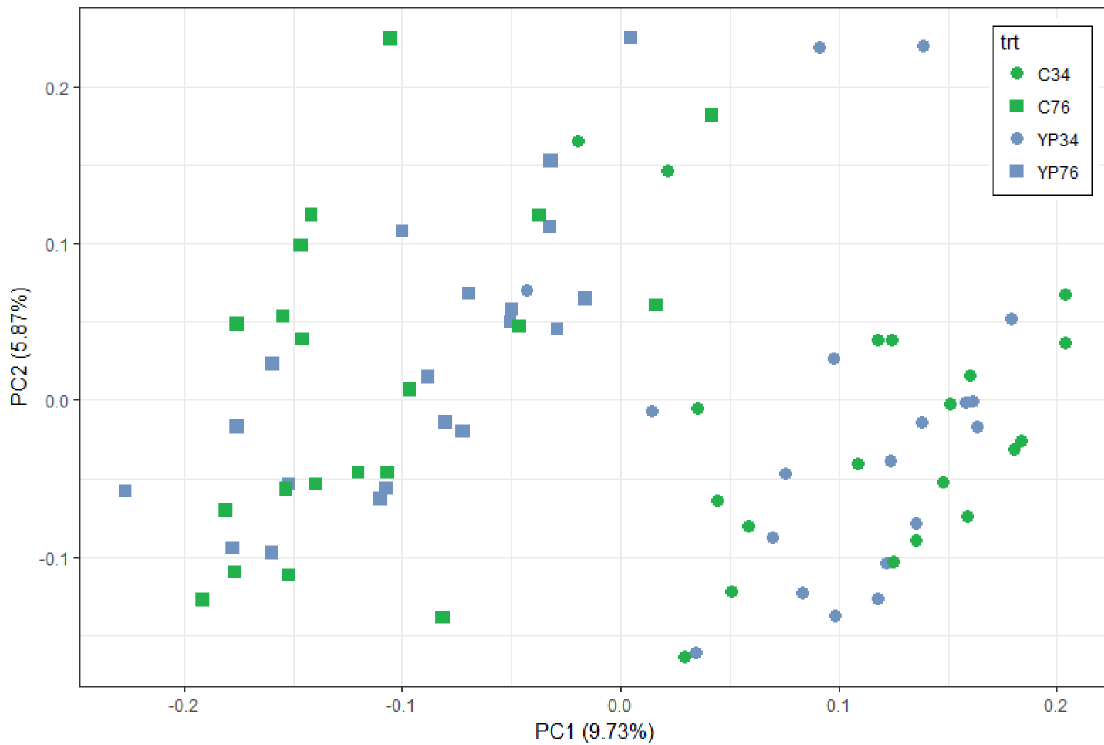
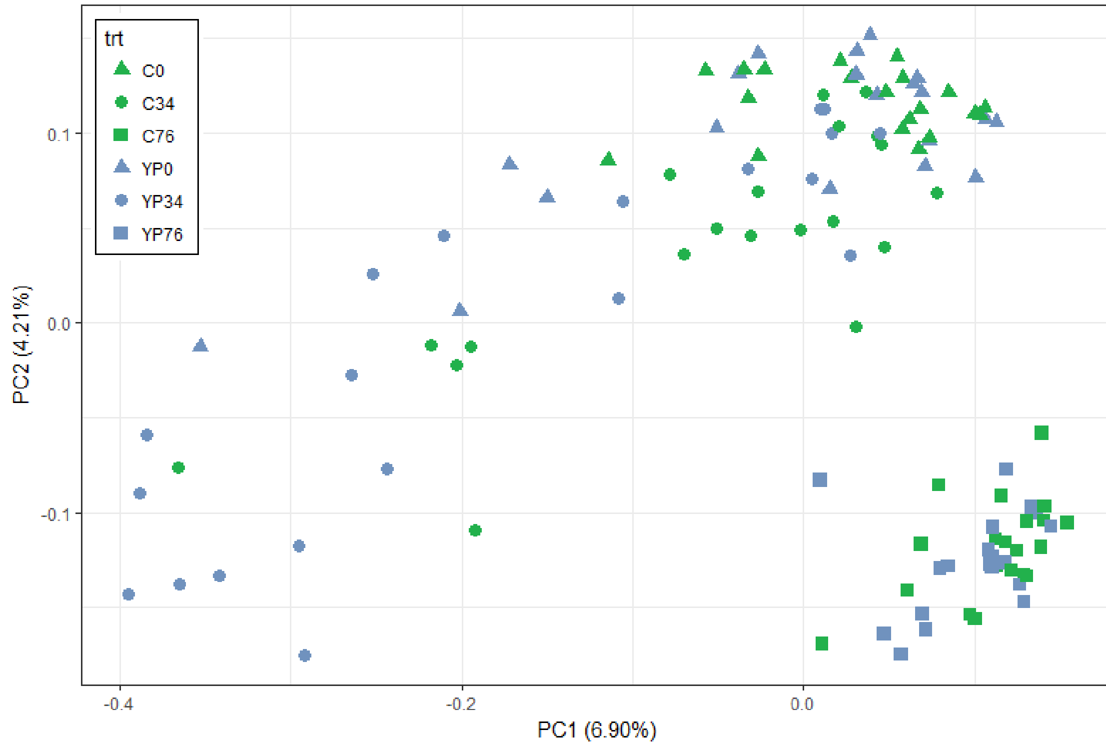


B.

Figure 3. Beta diversity measures for rumen microbiome. Yeast product calves were supplemented with 3 g of live yeast and 1 g of yeast cell wall for 35 d prior to weaning and through a 42-d backgrounding period. A. Jaccard dissimilarity index B. Bray-Curtis Index.



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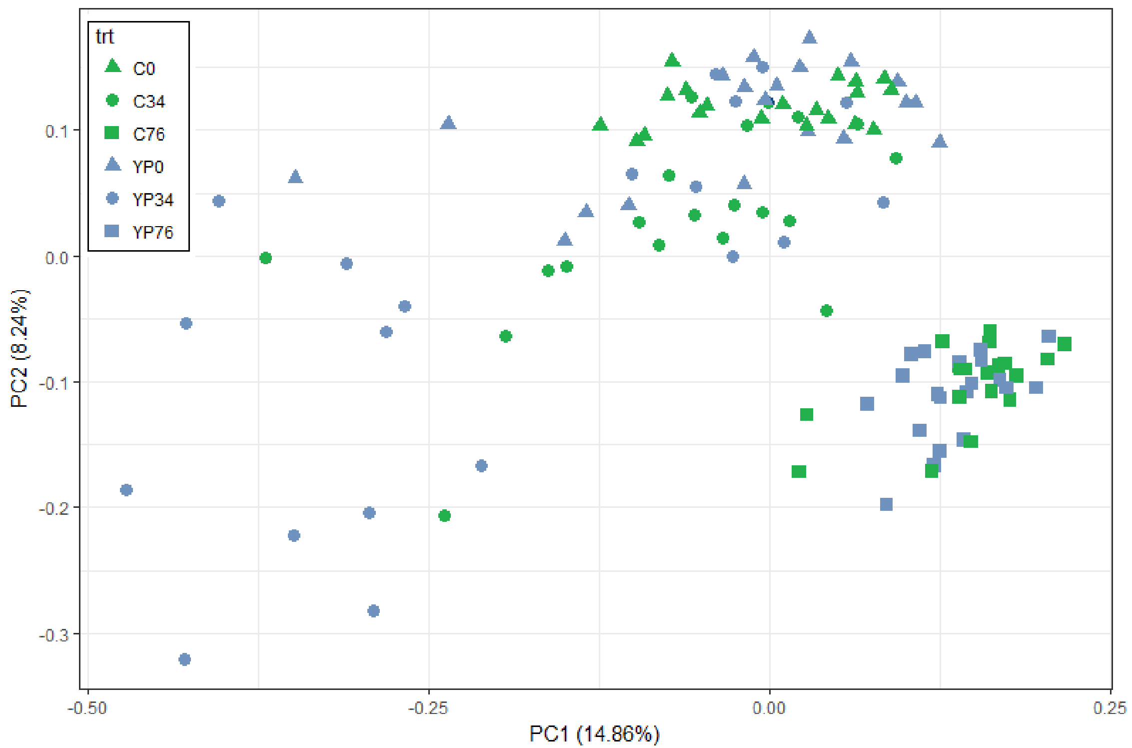


B.

Figure 4. Beta diversity measures for fecal microbiome. Yeast product calves were supplemented with 3 g of live yeast and 1 g of yeast cell wall for 35 d prior to weaning and through a 42-d backgrounding period. A. Jaccard dissimilarity index B. Bray-Curtis Index.

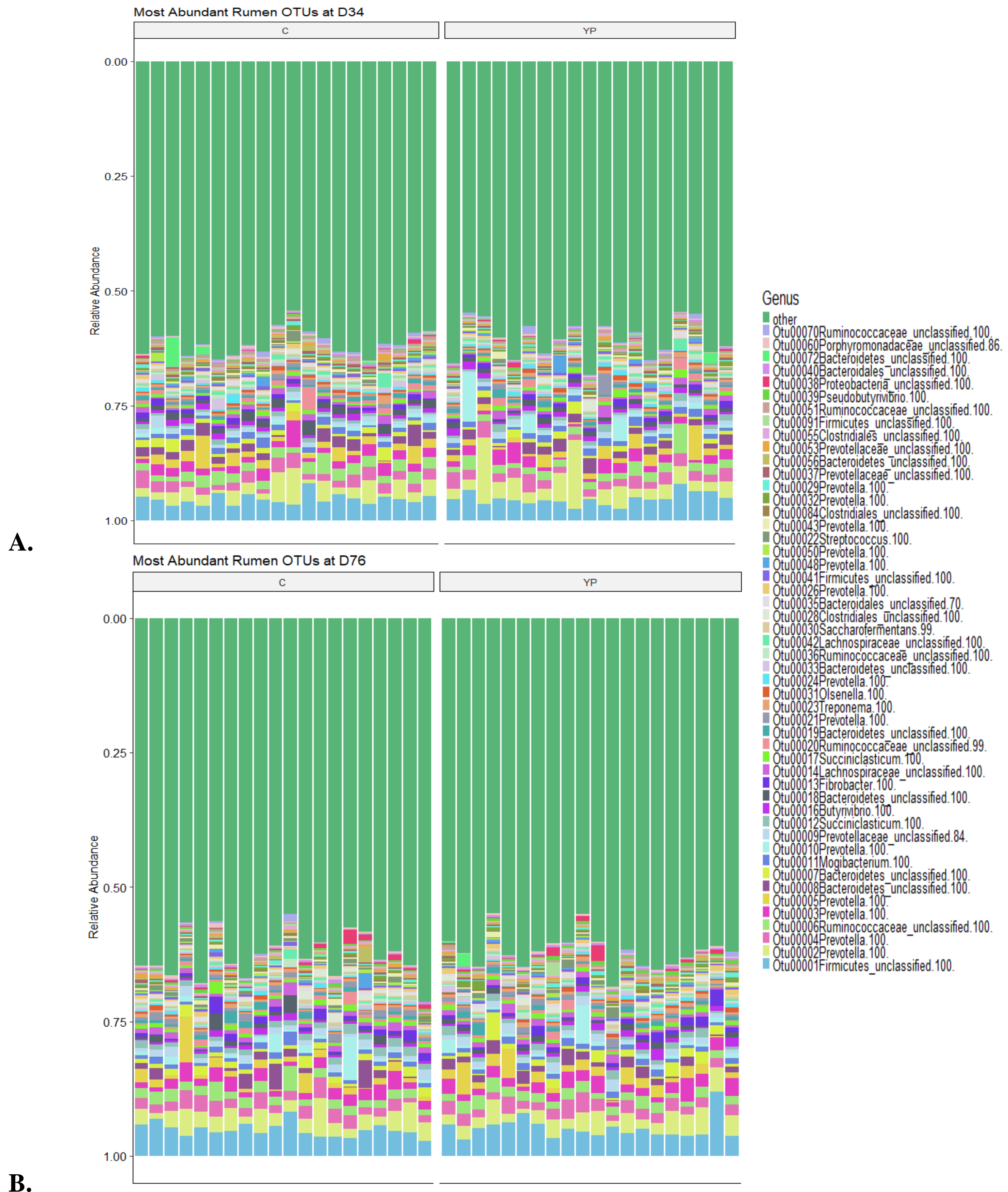


Figure 5. Relative abundance of the top 50 OTU present in the rumen microbiome on each day of sample collection. C = Control and YP = Yeast product.

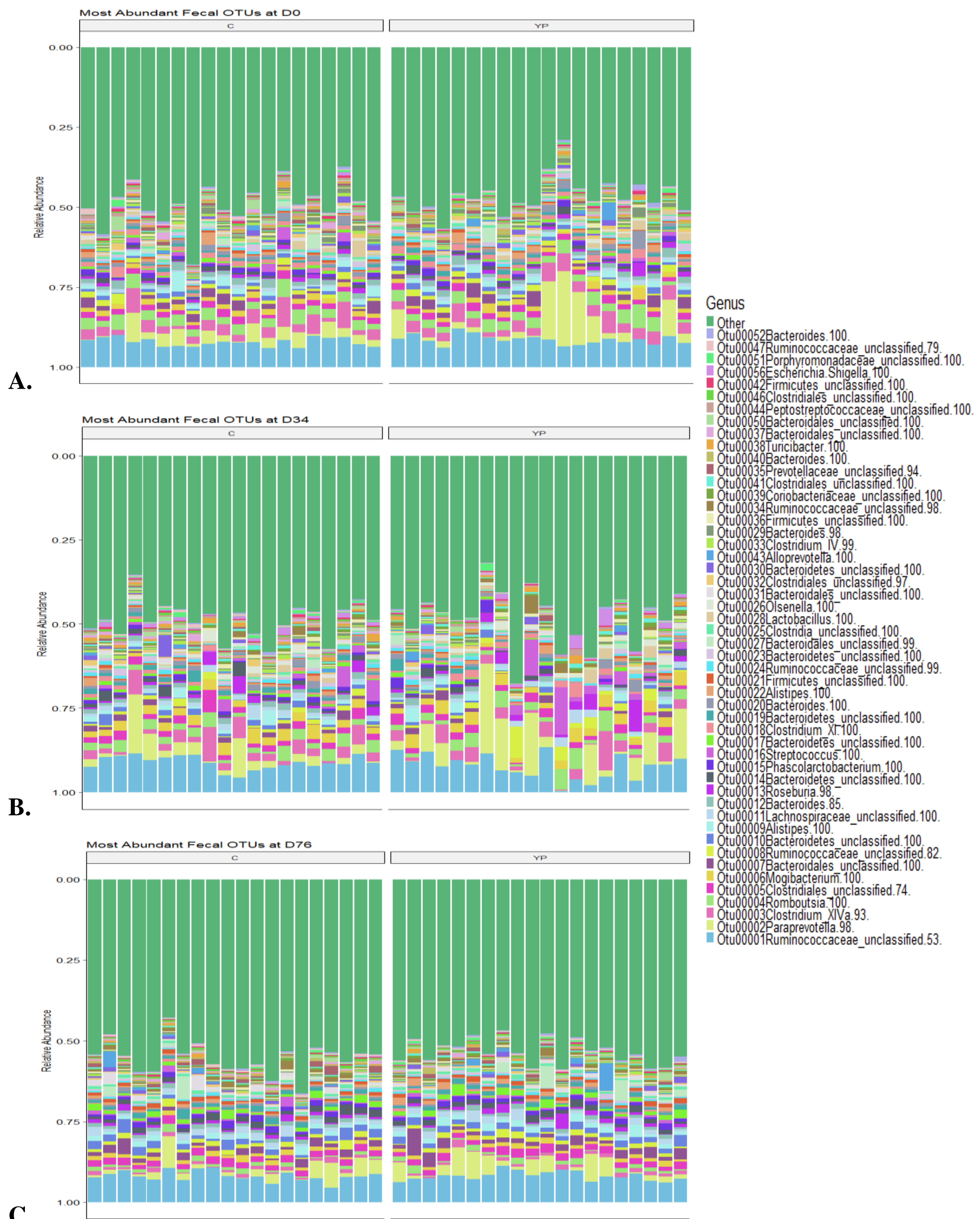


Figure 6. Relative abundance of the top 50 OTU present in the fecal microbiome on each day of sample collection. C = Control and YP = Yeast product.

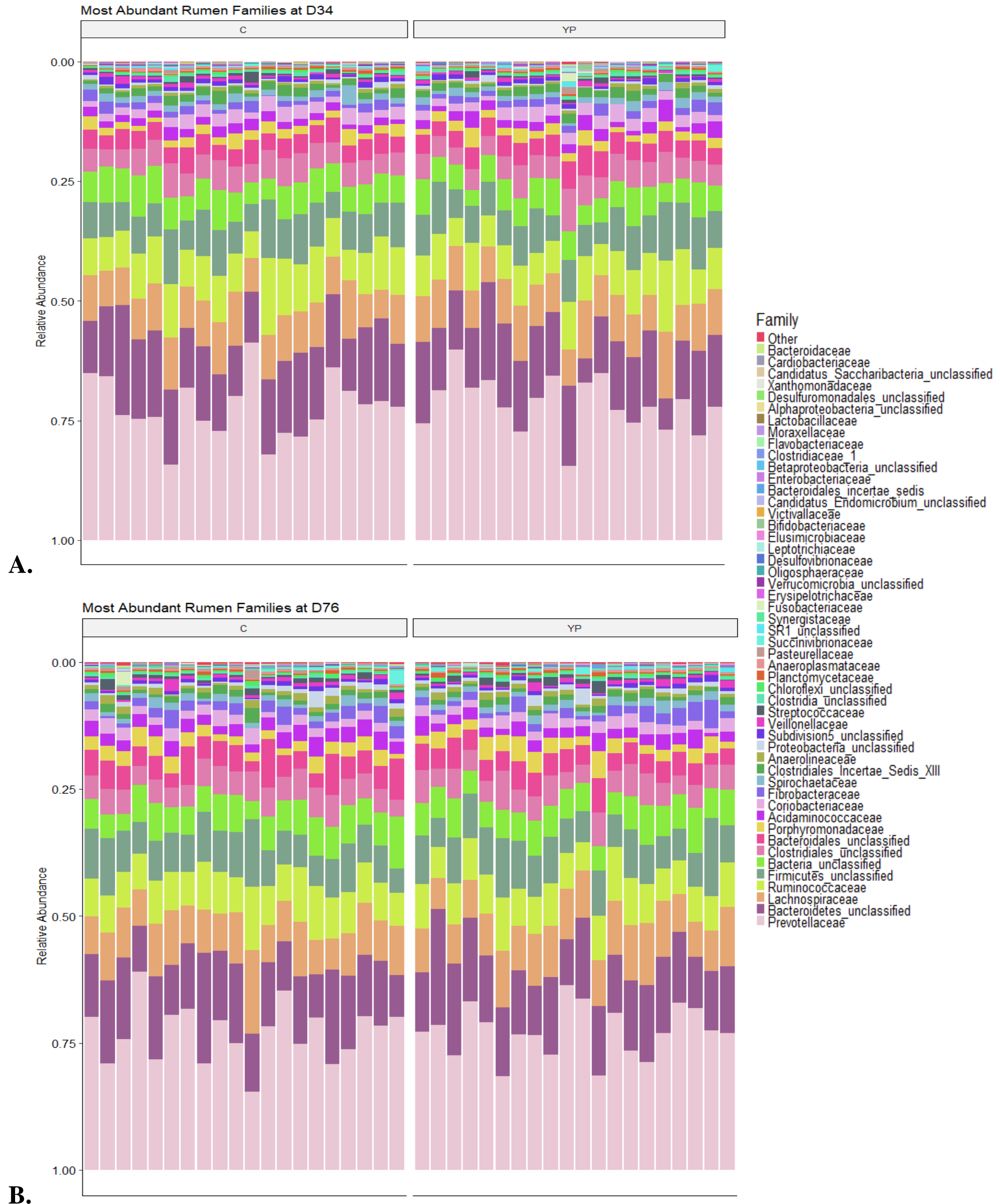


Figure 7. Relative abundance of the top 50 families present in the rumen microbiome on d 34 and 76. C = Control and YP = Yeast product.

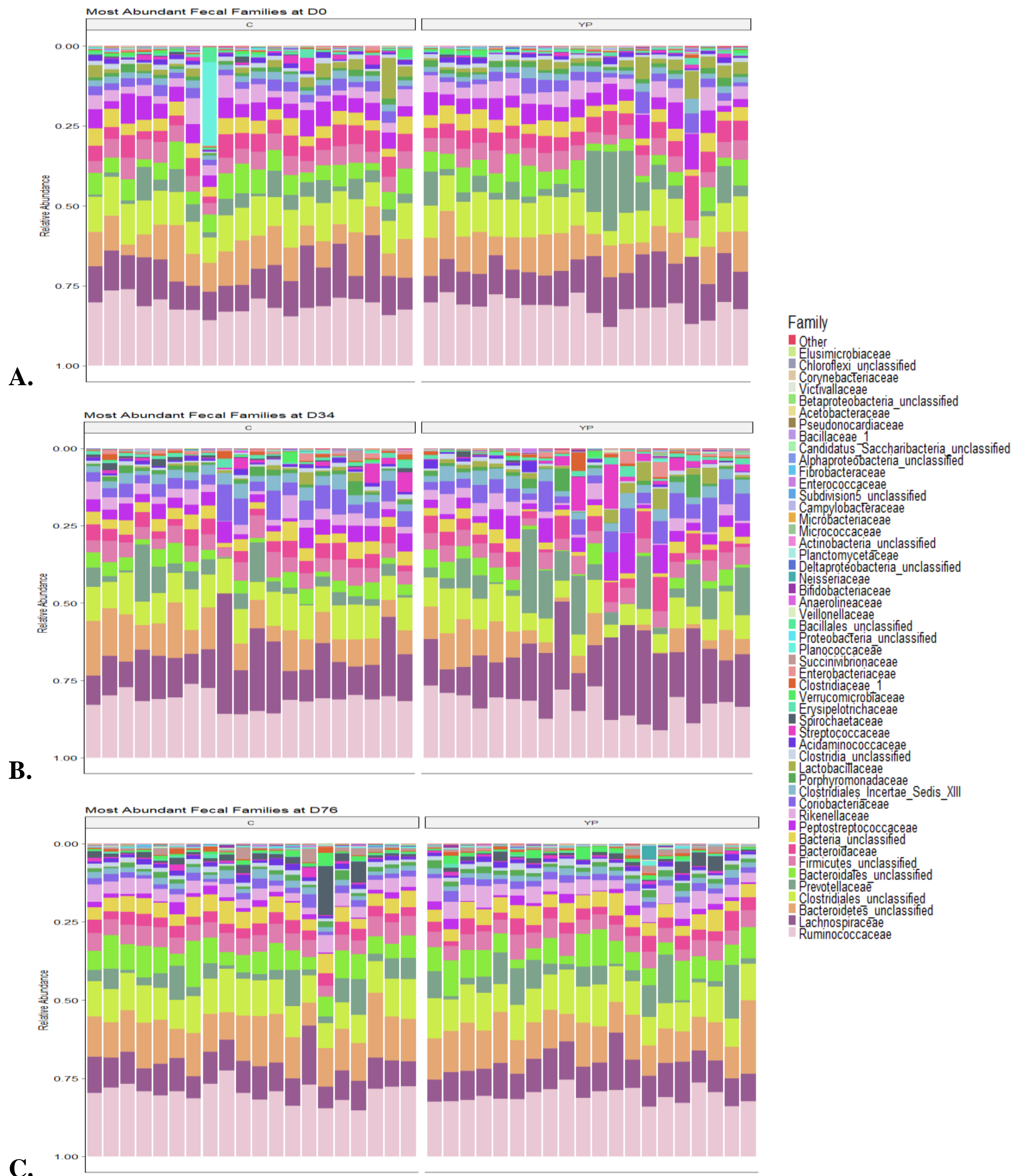
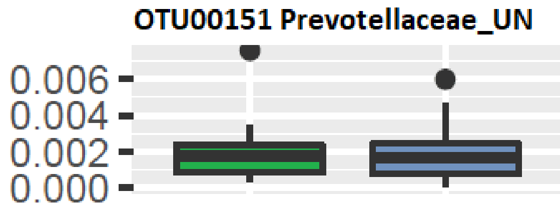
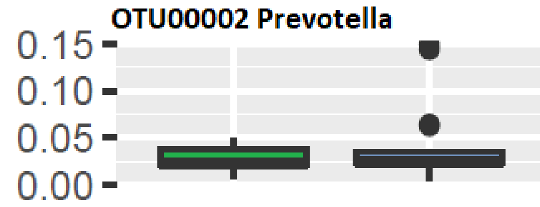


Figure 8. Relative abundance of the top 50 families present in the fecal microbiome on d 0, 34, and 76. C = Control and YP = Yeast product.

A.





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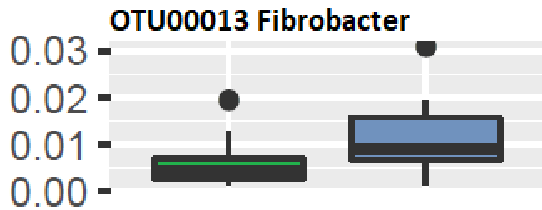

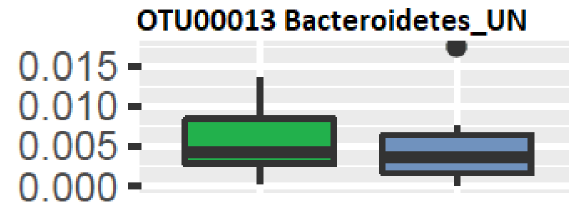

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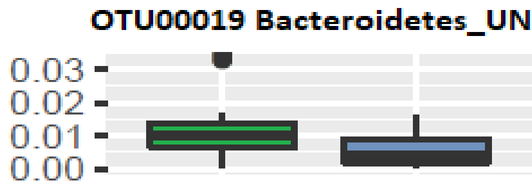

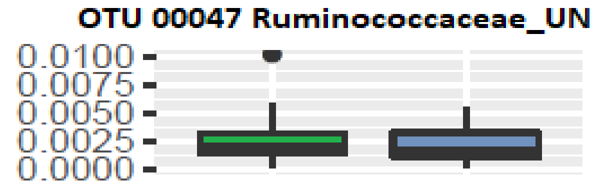

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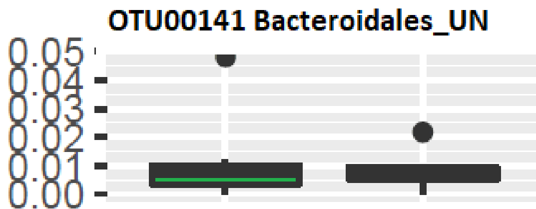

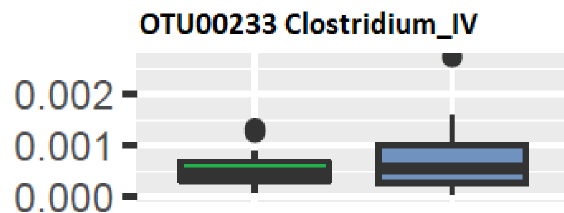

Figure 9. A. The top two predictors of dietary treatment for rumen samples on d 34. B. The top two predictors of dietary treatment for rumen samples on d 76. C. The top two predictors of dietary treatment for fecal samples on d 34. D. The top two predictors of dietary treatment for fecal samples on d 76. Control is designated by the green boxes and yeast product (YP) by the blue boxes.

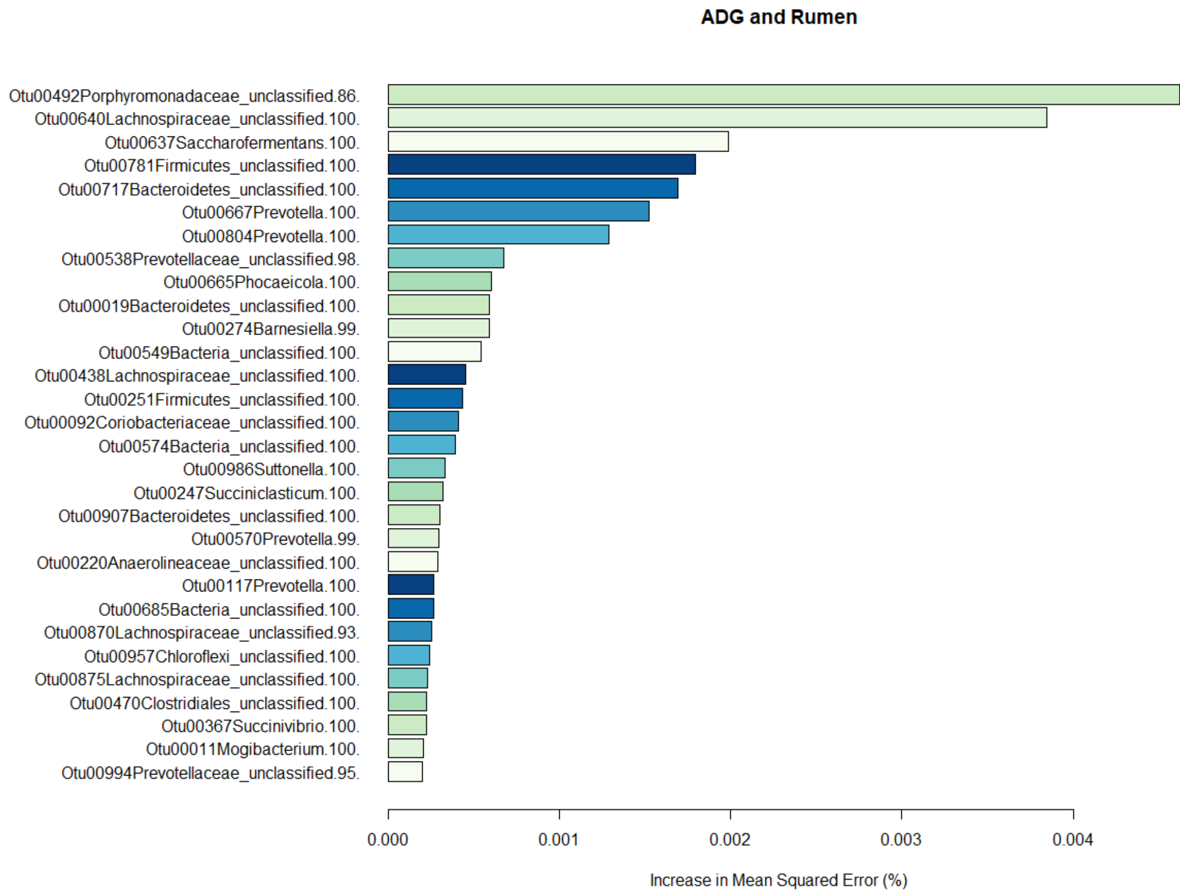

Figure 10. The top 30 OTU for predicating ADG based on the difference in relative abundance of OTU in the rumen on d 34 and 76.

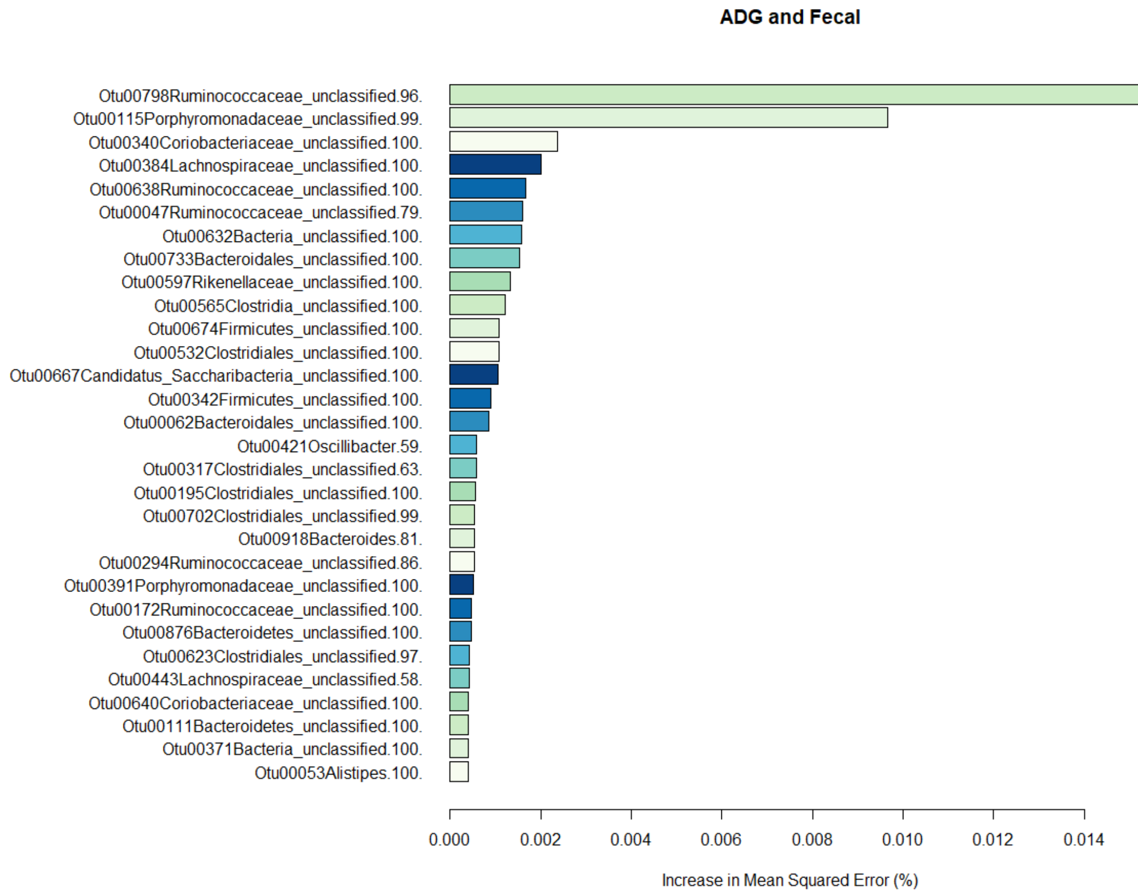

Figure 11. The top 30 OTU for predicating ADG based on the difference in relative abundance of OTU in the feces on d 34 and 76.

CHAPTER VI

Effects of yeast product supplementation during late gestation and subsequent effects on calf health and growth performance

ABSTRACT

Late gestation cows (n = 97) were stratified based on parity, sire of fetus, and body weight (**BW**) and assigned randomly to pasture (5 pastures/treatment). Pastures were assigned randomly to treatment: 1) supplement without yeast (**CON**) or, 2) supplement with yeast products (**YP**). Cows were supplemented 0.9 kg/d corn gluten pellets and 0.45 kg/d ground corn. The YP was supplied in ground corn at a target intake of 8 g YP/d (6 g live yeast and 2 g yeast cell wall; Phileo Lesaffre Animal Care, Milwaukee, WI). At parturition, a colostrum and blood sample were collected from a subset of cows (n = 30, 3 cows/pasture) and calf BW were recorded. Supplementation ended on d 85, 22 d after the last calf was born. Birth weights of calves were not different ($P = 0.72$). However, BW on d 85 was greater ($P = 0.01$) in calves whose dams were supplemented YP (76 kg) compared to CON (69 kg). Cows that were supplemented YP had a lower ($P = 0.03$) neutrophil:lymphocyte at h 0 and 48 after parturition. Similarly, calves on YP had a lower ($P = 0.02$) neutrophil:lymphocyte at h 48. Colostrum IgA ($P = 0.43$) and IgG ($P = 0.62$) concentrations were not impacted by YP. Additionally, serum IgA ($P = 0.68$) and IgG ($P = 0.33$) concentrations in calves at h 24 and 48 after parturition were not different due to treatment. There was no treatment effect ($P = 0.77$) observed for blood urea nitrogen. There was a treatment × time interaction ($P = 0.04$) for serum non-esterified fatty acid serum concentrations with cows on YP having a greater concentration at h 48 compared to CON at h 0 or 48. Supplementation of YP increased calf BW on d 85 and reduced neutrophil:lymphocyte 48 h after birth, potentially indicating a reduction in stress compared to CON.

Keywords: Colostrum, late gestation cows, yeast product

INTRODUCTION

At birth the calf relies on the transfer of antibodies in colostrum to defend against infection and foreign pathogens. Therefore, adequate intake of colostrum is extremely critical for the health of the calf (Besser and Gay, 1994). Factors that affect transfer of immunoglobulins include but are not limited to the quality and quantity of colostrum, timing of consumption, breed and parity of the dam (Weaver et al., 2000). According to Perino (1997), 10 to 25% of calves do not receive adequate transfer of immunoglobulins at birth. Beef calves that do not receive proper transfer of immunoglobulins have an increased risk of morbidity prior to weaning and in the feedlot (Wittum and Perino, 1995). Nutritional status of the cow can affect the quality of colostrum (Hough et al., 1990) and further influence the performance of their offspring (Funston et al., 2010; Radunz et al., 2012).

Yeast products are naturally occurring feed additives that are used to promote health and increase growth performance in livestock. Feeding yeast products has been extensively studied in the receiving period where it can improve dry matter intake (**DMI**) and average daily gain (**ADG**; Finck et al., 2014; Ponce et al., 2012). But research determining the effects of yeast prior to the receiving period is limited in beef calves. In the swine and dairy industry, more work has been conducted to evaluate the effectiveness of yeast supplementation prior to parturition and in the neonate. Dairy calves provided yeast had improved ADG and DMI, with a decrease in the incidence of scours (Magalhães et al., 2008; Lesmeister et al., 2004; Seymour et al., 1995). In late gestation sows fed live yeast, there was a greater concentration of IgG reported in the colostrum that led to a greater concentration of circulating IgG in piglets (Jang et al., 2013). Seeing how there have been benefits of providing yeast during this stage of production, the hypothesis of the current experiment was that similar effects would be observed in beef calves.

Thus, the goal of this study was to evaluate the effects of supplementing yeast to beef cows prior to parturition and the subsequent effects on calf performance and immunity.

MATERIALS AND METHODS

Animals and diet

All experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (protocol # 18020). Ninety-seven late gestation cows (initial BW = 560 ± 57 kg; parity ≥ 2) that were confirmed bred by artificial insemination were stratified based on parity, sire of fetus, BW, and previous treatment and assigned randomly to pasture (10 pastures; 9 or 10 cows/pasture). Pastures were assigned randomly to 1 of 2 experimental treatments: 1) concentrate supplement without yeast (**CON**), or 2) concentrate supplement with yeast product (**YP**; 8 g of YP/d). On d -1 cows were weighed, vaccinated for reproductive diseases including bovine viral diarrhea virus (**BVDV**; Virashield 6 VL 5, Elanco Animal Health, Greenfield, IN), ear notched to test for cows persistently infected with bovine viral diarrhea virus (**PI-BVDV**), and confirmed pregnant by rectal palpation. Ear notches were sent to Cattle Stats (Oklahoma City, OK) and all cows were reported negative for PI-BVDV. Cows were held overnight in holding pens with access to hay and water. The following day, cows were moved to their assigned 2.4 ha pastures with access to automatic waterers and concrete bunks.

Supplementation with dietary treatment began on d 0, approximately 45 d prior to the anticipated calving date. Cows were fed 1.4 kg of the supplement/d at approximately 0800. Supplement consisted of 0.9 kg/d corn gluten pellets and 0.5 kg/d top-dress (99% ground corn and 1% fat). Yeast product was delivered in top-dress and was formulated to provide 8 g of YP/d (6 g of live yeast and 2 g of yeast cell wall; Phileo Lesaffre Animal Care, Milwaukee, WI). Cows had access to a free choice mineral supplement (Fortigraze; Livestock Nutrition Center,

Memphis, TN). Once forage availability began to decline, hay was offered as a large round bale for ad libitum intake. All pens were offered hay on the same day based on the unit manager's decision. Any orts were removed, weighed, and subsampled and samples were stored frozen. Additionally, forage samples were collected every 28 d for nutrient analysis. Supplementation ended on d 85, 22 d after the last calf was born, and at this time cow-calf pairs were commingled and grazed available forage until weaning at an average age of 210 d.

Calving

At birth, calves were weighed, tagged with unique individual identification, tattooed, castrated via banding (if necessary), and ear notched for PI-BVDV analysis. Ear notches were sent to Cattle Stats (Oklahoma City, OK) every Monday until the last calf was born and all calves came back negative for PI-BVDV. Cows were assigned a calving ease score (1 = no assistance, 2 = assisted, easy, 3 = assisted, very difficult, 4 = caesarean delivery, and 5 = breech birth, abnormal presentation) and a calf thriftiness score (1 = nursed immediately, calf was healthy at birth, 2 = nursed on own, but took time, 3 = required some assistance to suckle, 4 = died shortly after birth, and 5 = dead on arrival).

Colostrum and blood sampling

A subsample of cows (n = 30, 3 cows/pasture) were moved closer to handling facilities on d 36 for further data collection. From each pen, 1 cow from parity 2, 4, and 5 or 6 was selected for the subsample. Additionally, to be eligible for subsample the fetus had to be sired by an Angus bull. Cows were housed on 0.4 ha pastures (n = 6, 3 pens/treatment) with access to an automatic waterer and a concrete bunk. Cows continued to be fed their appropriate dietary treatment; however, feeding time was switched to 1900 to promote daytime calving.

After the calf had nursed, both cow and calf were removed from their pasture and brought to the handling facilities. Further data collection from cows included: 1) a pooled colostrum sample from all four quarters and 2) a blood sample via jugular venipuncture at 0 and 48 h after calving. Calves were bled at 24 and 48 h after birth via jugular venipuncture. All blood samples from the 24 and 48 h collections were obtained twice a day, at 0800 and again at 1500. If calves were born between midnight and 1200, then cows and calves were bled at the 0800-collection time and if calves were born between 1200 and midnight then cows and calves were bled at 1500. The 0 h blood sample was collected as soon as cows and calves were brought in from the pasture. Cow-calf pairs were held in holding pens until after the 48 h blood collection, at which time they were moved to their appropriate 2.4 h pasture.

Body weight, Blood, and Colostrum Samples

Cow BW were collected on d -1, 0, 84, 85, and 257 (weaning); while calf BW were collected at birth, d 84, 85, and 257 (weaning). Consecutive BW were recorded and averaged together at the beginning and end of the study. At calving, blood collected from cows (n = 30) at 0 and 48 h were analyzed for complete blood cell count, immunoglobulin G (**IgG**), immunoglobulin A (**IgA**), BVDV Type 1a titers, haptoglobin (**Hp**), blood urea nitrogen (**BUN**), and non-esterified fatty acids (**NEFA**). Blood samples from calves (n = 30) at 24 and 48 h were analyzed for IgG, IgA, and BVDV Type 1a titers. Additionally, a complete blood cell count was evaluated from calves at 48 h. On d 84 (1 d before the end of dietary supplementation), all calves (n = 90) were bled via jugular venipuncture for IgG, IgA, BVDV Type 1a titers, and complete blood count.

Whole blood was collected via jugular venipuncture for complete blood cell analysis in 6 mL vacuum tubes containing EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ) and

processed on an automated analyzer (HemaVet HV950; Drew Scientific, Miami Lakes, FL). Blood for the remaining parameters was collected in a 10 mL plain vacuum tube (Becton, Dickinson and Company), centrifuged at 3,000 g for 20 min, and stored at -20°C until further analysis. Serum IgG, IgA, and Hp were assessed using a commercially available ELISA kit (Immunology Consultants Laboratory Inc., Portland, OR). Serum BUN were analyzed by colorimetric determination (Teco Diagnostics, Anaheim, CA). Serum NEFA concentrations were determined using an enzymatic colorimetric method (Wako Pure Chemical Industries, Ltd., Mountain View, CA). Lastly, serum was shipped on dry ice to the Iowa State University Veterinary Diagnostic Laboratory for BVDV Type 1a titer analysis.

Two aliquots of colostrum (n = 30) were saved for proximate analysis and determination of IgA and IgG concentrations. Colostrum intended for proximate analysis was mixed with a preservative and stored at room temperature and sent to Mid-South Dairy Records (Springfield, MO) every week until the end of calving. Samples were analyzed for percent fat, protein, lactose, solids, and somatic cell count. The remaining colostrum was stored in a 50 mL sterile conical tube and kept at -20°C until further laboratory analysis could be performed. A commercially available ELISA kit (Immunology Consultants Laboratory Inc.) was used to determine the concentration of colostrum IgG and IgA.

Diet Analysis

Forage and supplement samples were composited and dried in a forced air oven. Dried samples were ground via a Willey Mill (Thomas Scientific, Swedesboro, NJ) through a 1 mm screen. Ground samples were analyzed for CP (Rapid Combustion Method, Elementar Americas, Inc., Mt. Laurel, NJ), NDF, and ADF (ANKOM Techonolgy Corp., Fairport, NJ; Vogel et al., 1999). Mineral samples were prepared by wet ashing and analyzed by inductively coupled

plasma spectroscopy (Model 3560, Applied Research Laboratory, Sunland, CA) at the University of Arkansas Altheimer Laboratory (Fayetteville, AR). Nutrient analysis are presented in Table 1.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) with pen set as the experimental unit for all variables. Cow performance and colostrum data contained treatment as the only variable in the model; while, blood parameters were run as a repeated measure and the model included treatment, h, and the treatment by h interaction. Blood measurements for calves were run as repeated measure with treatment, h, and treatment by h interaction included in the model and contained sex as a random effect. The statistical model for calf performance included treatment with sex as a random effect. Date of birth was considered for calf performance on d 85 but was non-significant and thus, removed from the random statement. Normality was tested for every variable and data were transformed if necessary. Significance was declared at $P \leq 0.05$ with tendencies at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The calving season lasted for a total of 56 d using cows confirmed bred by artificial insemination. The average calf age was similar between treatments, on d 85 calves averaged 40 and 42 d old for CON and YP, respectively. All cows received a calving ease score of 1; 3 cows received a calf thriftiness score > 1 but this was not different ($P = 0.93$) between the treatments. During the first 85 d 7 cows were removed from the study. Three from the CON treatment; 1 cow was open, 1 had a calf that died shortly after birth (part of the subsample), and 1 calf was lame. Colostrum and blood samples were collected from the cow who lost her calf shortly after birth and were included in the data analysis; once samples were collected the cow was removed from the study and a blood sample from h 48 was not obtained. On the YP treatment, 2 cows had

twins and the other 2 cows aborted their calf. This resulted in a total of 90 cow-calf pairs (44 on YP and 46 on CON) on d 85. After treatment administration stopped, while grazing in a single group, 5 more cow-calf pairs (all on YP treatment) were removed from the study, 1 calf was diagnosed with calf diphtheria, 1 cow had a foot abscess, and 3 cows died from bloat. This left 85 cow-calf pairs (39 on YP and 46 on CON) at weaning. Additionally, the only morbidity recorded was 1 calf (on the CON diet) was treated during the duration of the study for an ulceration on the eye.

Cow and calf performance

Calf birth weight was not different ($P = 0.72$) between the CON and YP treatment. There was a 7 kg weight advantage ($P = 0.01$) in YP calves on d 85; as well as an increase ($P = 0.04$) in ADG from birth to d 85. However, at time of weaning there was no difference ($P = 0.68$) in calf BW. Similarly, ADG from d 85 to weaning was not impacted ($P = 0.14$) by dietary treatment; nor was there a difference ($P = 0.61$) in ADG from birth to weaning. Other studies evaluating the effects of yeast during late gestation have not followed calves past the time of parturition but several studies have evaluated the effectiveness of yeast products on growth performance when fed to the neonatal calf. A few studies found that adding yeast to the diet of young dairy calves increased DM intake and improved BW (Lesmeister et al., 2004; Galvão et al., 2005), but others have reported no improvement on growth performance when yeast was provided (Seymour et al., 1995; Magalhães et al., 2008). In swine, adding active dry yeast to the sow's diet during late gestation improved weaning weight of their piglets (Jurgens et al., 1997).

One hypothesis behind the improvement of growth performance seen in young calves is the ability of yeast to stimulate bacterial growth and development in the rumen. Young ruminants fed live yeast had a greater abundance of cellulolytic bacteria in the rumen which is

indication of a more developed rumen (Chaucheyras-Durand and Fonty, 2001; Jami et al., 2013). Furthermore, adding yeast to the diet of young ruminants can improve rumen morphology by enhancing papillae length and width (Lesmeister et al., 2004; Xiao et al., 2016) both of which are associated with improved rumen function and development. While parameters measuring rumen development were not identified in the current study, previous work on rumen development could in part explain the 7 kg weight advantage in calves on d 85.

Cow BW was not affected (*P* = 0.44; Table 2) at the end of the 85-d period and there was no difference (*P* = 0.67) in cow weight at time of weaning. Providing yeast culture to dairy cows did not affect cow performance pre-partum but it did improve cow BW by 23.4 kg during the first 42 d postpartum (Dann et al., 2000). Dann et al. (2000) further reported an increase in DMI during the first 42 d of lactation for cows fed yeast culture. These findings have not been consistent in dairy cows as others (Nocek and Kautz, 2006; Robinson, 1997) have reported no effect on cow performance when fed yeast products

Colostrum

One cow (CON) in the subsample did not let down her milk; thus, 29 colostrum samples were available for analysis (Table 3). Colostrum IgG from cows on both the CON and YP diet were within the recommended range of 50 to 100 mg/mL (Larson et al., 1980). The concentration of colostrum IgA (*P* = 0.43) and IgG (*P* = 0.62) were not different between CON and YP. Like the current study, immunoglobulin concentrations (IgG_1, IgG_2, IgA, and IgM) in colostrum were not different in cows provided mannan oligosaccharides (**MOS**) during the last 3 wk of gestation (Franklin et al., 2005). In swine, feeding yeast during gestation increased colostrum IgG (Jang et al., 2013).

Similar to immunoglobulin concentrations, there was no difference due to treatment in the proximate analysis of the colostrum. The percentage of fat in colostrum was not impacted (*P* = 0.20) by dietary treatment. But, supplemental YP numerically increased the percentage of fat in colostrum from 4.7% in CON to 6.4% in YP, respectively. Percentage of protein (*P* =0.73) and percentage lactose (*P* = 0.27) also did not differ between treatments. Yeast had no affect on the milk components of dairy cows during early gestation (Dann et al., 2000; Robinson. 1997). Jurgens et al. (1997) found that supplemental yeast increased the total amount of solids and percentage of CP in the milk of sows. These findings have been reported in milk rather than colostrum, which could explain the differences observed between studies.

Blood Parameters

Haptoglobin is an acute phase protein secreted by the liver during periods of inflammation and infection (Baumann and Gauldie, 1994). As seen in this study, serum Hp concentrations were not affected (*P* = 0.25; Table 4) by dietary treatment which has been observed previously in dairy cows (Zaworski et al., 2014). Although, there was an effect ($P <$ 0.01) of time on serum Hp with h 48 being greater compared to h 0. When the innate immune system is stimulated it elicits an increase in acute phase proteins in cows near parturition (Nightingale et al., 2015). Therefore, parturition elevates Hp levels in the subsequent week following parturition (Crawford et al., 2005; Humblet et al., 2006).

While yeast did not effect the acute phase response as measured by serum Hp concentrations, yeast did alter blood cell composition in cows. The percentage of neutrophils was greater (*P* = 0.03; Table 5) in CON compared to YP which was accompanied by a tendency for a greater (*P* = 0.08) lymphocyte percentage in cows fed YP compared to CON. This gave rise to a reduction (*P* = 0.03) in the neutrophil to lymphocyte ratio (**N:L**) in cows supplemented YP. The

N:L ratio is used as a tool to measure the level of stress. A reduced N:L ratio indicates less stress, which would suggest that cows provided YP prior to parturition had reduced stress at time of calving.

Providing YP in the diet did not affect serum BUN concentrations ($P = 0.77$; Table 6); while time of blood collection did influence ($P < 0.01$) BUN concentrations with h 48 having elevated concentrations of circulating BUN compared to h 0. Comparatively, Zaworski et al. (2014) reported greater urea N concentrations 48 h after birth in cows fed yeast. As with BUN concentrations, serum NEFA concentrations did not differ ($P = 0.42$) due to dietary treatment. Which agrees with serum NEFA concentrations in dairy cows fed yeast prior to parturition (Nocek and Kautz, 2006). There was a treatment by time interaction ($P = 0.04$) with cows on YP having a greater concentration at h 48 compared to CON at h 0 or 48 and YP at h 0. Heifer calves experiencing an immune challenge showed potential for yeast to alter blood metabolites (Burdick Sanchez et al., 2014). Therefore, it was hypothesized that adding YP to the diet could impact energy metabolism at time of calving in beef cows, but that was not the case in the current study.

At 48 h after birth, calves supplemented YP had a reduction ($P = 0.04$; Table 7) in the percentage of neutrophils along with an elevated ($P = 0.02$) percentage of lymphocytes. There was no difference ($P \geq 0.15$) in the percentage of monocytes, eosinophils, or basophils due to dietary treatment at h 48. These results differ from those reported by Kim et al. (2011) who found that adding yeast to calf starter did not impact the proportion of leukocytes. Calves from cows on the YP diet had a reduced ($P = 0.02$) N:L compared to CON. With a lower N:L ratio 48 h after calving it is believed that calves supplemented YP experienced less stress at birth compared with calves on the CON treatment. But this advantage was not maintained ($P = 0.22$; Table 8) on d 85. When calves are born, neutrophils make up a greater proportion of white blood

cells compared to lymphocytes (Tennant et al., 1973). Tennant et al. (1973) reported that after d 5, neutrophil concentration decreases while the concentration of lymphocytes increase; making the N:L more similar to adult cattle. While there was no treatment effect observed on d 85, calves hematology could have changed with age. Additionally, the influence of YP could have been less effective at this time due to the lower stress level of calves 22 d after the last calf was born.

Passive immunity (Table 9) from dam to calf was not altered due to YP supplementation. There was no treatment effect on circulating IgA (P = 0.56; Table 6) or IgG (P = 0.51) in cows. At time of birth both serum IgA (P = 0.68) and serum IgG (P = 0.33) concentrations did not differ due to treatment. On d 85, a similar result was observed for serum IgG concentrations; on the contrary, there was a tendency for serum IgA to be greater (P = 0.07) in YP calves compared with CON. Franklin et al. (2005) reported reduced serum IgA concentrations 24 h after birth in calves whose dams were supplemented MOS during late gestation. Contrary, Jang et al. (2013) found greater serum IgG 24 h after birth in piglets when the sow was fed yeast. It is important to note that failure of passive transfer occurs when calves are not provided adequate volumes of colostrum and is identified when serum IgG is < 10 g/L (Tyler et al., 1996; Weaver et al., 2000). Therefore, passive transfer was sufficient in calves when dams regardless of diet.

The humoral immune response was not affected by YP supplementation as evident by the results for BVDV Type 1a titers (Table 10) observed in both cow and calf serum. The BVDV Type 1a antibody titers did not differ (P = 0.32) in cows due to dietary treatment. Franklin et al. (2005) reported no difference in rotavirus neutralization titers in cows but found a tendency for elevated titers in calves when their dams were supplemented with MOS. Likewise, serum antibody titers in calves against BVDV Type 1a were not affected (P = 0.44) by dam’s diet; nor

was there a difference (P = 0.66) on d 85. In agreement, Magalhães et al. (2008) did not observe differences in humoral immune response when calves were fed yeast culture from 2 to 70 d of age.

Implications

Feeding YP at 8 g/d to beef cows during the last 45 d of gestation had a beneficial influence on both calf performance and immunity. Calves from dams fed YP had greater BW on d 85 of the study; however, this weight advantage was not seen at weaning. Both cows and calves had a smaller N:L at time of parturition, indicating a reduction in stress at time of calving. Supplementation of YP did not influence passive transfer of immunoglobulins. Serum and colostrum IgG and IgA concentrations were not different between cows supplemented YP or CON; nor did serum IgA and IgG differ in their calves. Additionally, the humoral response, as measured by antibody titer to BVDV Type 1a, was not affected by dietary treatment of dam. Overall, there were advantages to providing YP to cows prior to parturition; but a long-term effects of YP supplementation on calf performance was not evident.

LITERATURE CITED

Baumann, H., and J. Gauldie. 1994. The acute phase response. Immunol. Today 15:74-80.

Besser, T. E., and C. C. Gay. 1994. The importance of colostrum to the health of the neonatal calf. Vet. Clin. North Am. Food Anim. Pract. 10:107-117.

Burdick Sanchez, N. C., T. R. Young, J. A. Carroll, J. R. Corley, R. J. Rathmann, and B. J. Johnson. 2014. Yeast cell wall supplementation alters the metabolic responses of crossbred heifers to an endotoxin challenge. Innate Immunity 20:104-112.

Chaucheyras-Durand, F. and G. Fonty. 2001. Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive *Saccharomyces cerevisiae* CNCM I-1077. Reprod. Nutr. Dev. 41:57-68.

Crawford, R. G., K. E. Leslie, R. Bagg, C. P. Dick, and T. F. Duffield. 2005. The impact of controlled release capsules of monensin on postcalving haptoglobin concentrations in dairy cattle. Can. J. Vet. Res. 69:208-214.

Dann, H. M., J. K. Drackley, G. C. McCoy, M. F. Hutjens, and J. E. Garrett. 2000. Effects pf yeast culture (Saccharomyces cerevisiae) on prepartum intake and postpartum intake and milk production of Jersey cows. J. Dairy Sci. 83:123-127.

Finck, D. N., F. R. B. Ribeiro, N. C. Burdick, S. L. Parr, J. A. Carroll, T. R. Young, B. C. Bernhard, J. R. Corley, A. G. Estefan, R. J. Rathmann, and B. J. Johnson. 2014. Yeast supplementation alters the performance and health status of receiving cattle. Prof. Anim. Sci. 30:333-341.

Franklin, S. T., M. C. Newman, K. E. Newman, and K. I. Meek. 2005. Immune parameters of dry cows fed mannan oligosaccharide and subsequent transfer of immunity to calves. J. Dairy Sci. 68:766-775.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: implications for beef cattle production. J. Anim. Sci. 88(13 Suppl):E205-215.

Galvão, K. N., J. E. P. Santos, A. Coscioni, M. Villasenor, W. M. Sischo, and A. C. B. Berge. 2005. Effect of feeding live yeast products to calves with failure of passive transfer on performance and patterns of antibiotic resistance in fecal *Escherichia coli*. Reprod. Nutr. Dev. 45:427-440.

Hough, R. L., F. D. McCarthy, H. D. Kent, D. E. Eversole, and M. L. Wahlberg. 1990. Influence of nutritional restriction during late gestation on production measures and passive immunity in beef cattle. J. Anim. Sci. 68:2622-2627.

Humblet, M. F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, F. Rollin, and J. M. Godeau. 2006. Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clinic. Pathol. 35:188-193.

Jami, E., A. Israel, A. Kotser, and I. Mizrahi. 2013. Exploring the bovine rumen bacterial community from birth to adulthood. ISME J. 7:1069-1079.

Jang, Y. D., K. W. Kang, L. G. Piao, T. S. Jeong, E. Auclair, S. Jonvel, R. D'Inca, and Y. Y. Kim. 2013. Effects of live yeast supplementation to gestation and lactation diets on reproductive performance, immunological parameters, and milk composition in sows. Livestock Sci. 152:167-173.

Jurgens, M. H., R. A. Rikabi, and D. R. Zimmerman. 1997. The effect of dietary active dry yeast supplement on performance of sows during gestation-lactation and their pigs. J. Anim. Sci. 75:593-597.

Kim, M. H., J. K. Seo, C. H. Yun, S. J. Kang, J. Y. Ko, and J. K. Ha. 2011a. Effects of hydrolyzed yeast supplementation in calf starter on immune responses to vaccine challenge in neonatal calves. Animal 5:953-960.

Larson, B. L., H. L. Heary, and J. E. Devery. 1980. Immunoglobulin production and transport by the mammary gland. J. Dairy Sci. 63:665-671.

Lesmeister, K. E., A. Heinrichs, and M. T. Gabler. 2004. Effects of supplemental yeast (*Saccharomyces cerevisiae*) culture on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. J. Dairy Sci. 87:1832-1839.

Magalhães, V. J., F. Susca, F. S. Lima, A. F. Branco, I. Yoon, and J. E. P. Santos. 2008. Effect of feeding yeast culture on performance, health, and immunocompetance of dairy calves. J. Dairy. Sci. 91:1497-1509.

Nightingale, C. R., M. D. Sellers, and M. A. Ballou. 2015. Elevated plasma haptoglobin concentrations following parturition are associated with elevated leukocyte responses and decreased subsequent reproductive efficiency in multiparous Holstein dairy cows. Vet. Immunol. Immunopathol. 164:12-23.

Nocek, J. E. and W. P. Kautz. 2006. Direct-fed microbial supplementation on ruminal digestion, health, and performance of pre- and postpartum dairy cattle. J. Dairy Sci. 89:260-266.

Perino, L. J. 1997. A guide to colostrum management in beef cows and calves. Vet. Med. 92:75-82.

Ponce, C. H., J. S. Schutz, C. C. Elrod, U. Y. Anele, and M. L. Galyean. 2012. Effects of dietary supplementation of a yeast product on performance and morbidity of newly received beef heifers. Prof. Anim. Sci. 28:618-622.

Radunz, A. E., F. L. Fluharty, A. E. Relling, T. L. Felix, L. M. Shoup, H. N. Zerby, and S. C. Loerch. 2012. Prepartum dietary energy source fed to beef cows: II. Effects on progeny

postnatal growth, glucose tolerance, and carcass composition. J. Anim. Sci. 90:4962-4974.

Robinson, P. H. 1997. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to diets postpartum. J. Dairy Sci. 80:1119-1125.

Seymour, W. M., J. E. Nocek, and J. Siciliano-Jones. 1995. Effects of a colostrum substitute and of dietary brewer's yeast on the health and performance of dairy calves. J. Dairy Sci. 78:412-420.

Tennant, B., D. Harrold, M. Reina-Guerra, J. W. Kendrick, and R. C. Laben. 1973. Hematology of the neonatal calf: erythrocyte and leukocyte values of normal calves. Cornell Vet. 64:4-5.

Tyler, J. W., D. D. Hancock, S. M. Parish, D. E. Rea, T. E. Besser, S. G. Sanders, and L. K. Wilson. 1996. Evaluation of 3 assays for failure of passive transfer in calves. J. Vet. Intern. Med. 10:304-307.

Vogel, K. P., J. F. Pederson, S. D. Masterson, and J. J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276-279.

Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern. Med. 14:569-577.

Wittum, T. E., and L. J. Perino. 1995. Passive immune status at postpartum hour 24 and long-term health and performance of calves. Am. J. Vet. Res. 56:1149-1154.

Xiao, J. X., G. M. Alugongo, R. Chung, S. Z. Dong, S. L. Li, I. Yoon, Z. H. Wu, and Z. J. Cao. 2016. Effects of *Saccharomyces cerevisiae* fermentation products on dairy calves: Ruminal fermentation, gastrointestinal morphology, and microbial community. J. Dairy Sci. 99:5401-5412.

Zaworski, E. M., C. M. Shriver-Munsch, N. A. Fadden, W. K. Sanchez, I. Yoon, and G. Bobe. 2014. Effects of feeding various dosages of *Saccharomyces cerevisiae* fermentation product in transition dairy cows. J. Dairy Sci. 97:3081-3098.

APPENDIX

Table 1. Nutrient analysis of diet and forages consumed during the first 85 d.

	Topdress carrier				
	CON[1]	YP[2]	Corn Gluten	Forage	Bermudagrass Hay
DM	92.7	93.5	96.0	36.2	88.6
CP	11.4	8.8	23.1	13.0	11.3
ADF	4.4	3.4	13.5	37.0	36.4
NDF	44.6	49.4	40.9	65.4	66.5
Ash	2.5	1.4	6.8	8.3	9.8
P	0.4	0.3	1.3	0.3	0.4
K	0.5	0.4	2.0	2.3	2.8
Ca	0.3	0.02	0.01	0.4	0.7
Mg	0.1	0.1	0.5	0.2	0.2
Na	0.2	0.1	0.4	0.2	0.3
S	0.1	0.008	0.1	0.009	0.018
Fe	269.4	70.4	275.4	89.7	286.4
Mn	52.4	2.9	17.1	94.5	70.1
Zn	92.2	30.0	64.5	45.1	34.0
Cu	25.5	4.3	5.6	6.5	8.7

[1]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[2]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.

Table 2. Effect of yeast product supplemented during late gestation on cow and calf growth performance.

	Treatment			
	CON[1]	YP[2]	SEM	*P*-Value
Cow performance, kg				
D 0 BW	560	561	2.4	0.95
D 85 BW[3]	548	544	3.7	0.44
Weaning BW	513	517	6.2	0.67
Calf performance, kg				
Birth Wt	32	31	1.1	0.72
D 85 BW[3]	69	76	1.7	0.01
Weaning Wt	215	212	5.3	0.68
ADG birth to d 85	0.9	1.1	0.05	0.04
ADG d 85 to wean	0.84	0.78	0.03	0.14
ADG birth to wean	0.86	0.84	0.02	0.61

[1][1]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[2]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.
[3]Day 85 BW was taken 22 d after the last calf was born. The average age for control on d 22 was 40 d and the average age for YP on d 22 was 42 d ($P = 0.28$).

Table 3. Effect of YP supplemented during late gestation on colostrum composition.[1]

	Treatment			
	CON[2]	YP[3]	SEM	*P*-Value
Milk Proximate Analysis				
Fat, %	4.7	6.4	0.86	0.20
Protein, %	10.5	11.0	1.0	0.73
Lactose, %	2.3	2.8	0.3	0.27
Solids, %	12.3	13.4	1.1	0.49
Somatic cell count, cells x 10^3/mL	963	1,460	295	0.27
Immunoglobulins, mg/mL				
IgA	10.5	8.9	1.33	0.43
IgG	96.9	106.8	13.64	0.62

[1]n = 27; 14 cows on YP and 13 cows on control.
[2]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[3]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.

Table 4. Effect of YP supplemented during late gestation on cow plasma haptoglobin (ng/mL) concentrations.[3]

	Treatment			*P*-Value		
	CON[1]	YP[2]	SEM	Treatment	Time	Treatment × time
H 0	3,390	4,357	6.68	0.25	<0.01	0.97
H 48	16,147	18,314	6.82			

[1]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[2]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.
[3]Haptoglobin concentrations were transformed using the square root to improve normality. Means were back transformed from normal data. SEM values were obtained from the transformed data

Table 5. Effect of YP supplemented to pregnant beef cows during late gestation on the concentration and proportion of white blood cells in cows.

	CON[1]		YP[2]					
	H 0[4]	H 48[5]	H 0[4]	H 48[5]	SEM	Treatment	Time	Treatment × time
Concentration, K/µl[3]								
White blood cells	9.70	6.61	12.58	7.82	0.13	0.17	<0.01	0.69
Neutrophil	4.42	2.89	4.79	2.90	0.13	0.76	<0.01	0.76
Lymphocyte	2.20	2.21	3.39	3.10	0.17	0.09	0.77	0.74
Monocyte	0.33	0.30	0.54	0.39	0.18	0.06	0.26	0.56
Eosinophil	1.89	0.80	2.48	0.82	0.19	0.36	<0.01	0.58
Basophil	0.28	0.10	0.25	0.12	0.34	0.90	0.02	0.68
Proportions, %[3]								
Neutrophil	45.8	43.8	38.1	37.1	0.07	0.03	0.64	0.90
Lymphocyte	22.5	33.7	26.9	39.7	0.09	0.08	<0.01	0.94
Monocyte	3.4	4.5	4.3	5.0	0.08	<0.01	0.03	0.50
Eosinophil	19.6	12.3	19.7	10.5	0.12	0.38	<0.01	0.59
Basophil	2.9	0.9	2.0	1.0	0.34	0.75	<0.01	0.48
Neutrophil:lymphocyte ratio	2.03	1.30	1.42	0.94	0.14	0.03	<0.01	0.92

[1]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat

[2]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.

[3]White blood cell concentrations and proportions were log transformed to improve normality. Means were back transformed from normal data. SEM values obtained from transformed data.

[4]At h 0 blood was collected from 30 cows.

[5]At h 48 blood was collected from 29 cows; 1 cow lost her calf from h 0 to 48

Table 6. Effect of YP supplemented during late gestation on cow blood metabolites at h 0 and 48.[1]

	Treatment			*P*-Value		
	CON[2]	YP[3]	SEM	Treatment	Time	Treatment × time
Blood urea N, mg/dL						
H 0	6.7	6.3	0.51	0.77	<0.001	0.81
H 48	9.1	9.0	0.52			
Nonesterified fatty acids, µEq/L[4]						
H 0	369	312	0.10	0.42	0.07	0.04
H 48	359	490	0.10			

[1]n = 30 at h 0 and n = 29 at h 48.

[2]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat

[3]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.

[4]Nonesterified fatty acid concentrations were log transformed to improve normality. Means reported from back transformed data. SEM values from transformed data

Table 7. Effect of YP supplemented during late gestation on the concentration and proportion of white blood cells in calves at 48 h.[1]

	Treatment			
	CON[2]	YP[3]	SEM	*P*-Value
Concentrations, K/µL[4]				
White blood cells	5.69	4.36	0.10	0.13
Neutrophils	3.55	2.41	0.04	0.01
Lymphocytes	1.12	1.27	0.20	0.84
Monocytes	0.19	0.19	0.21	0.97
Eosinophils	0.39	0.28	0.31	0.48
Basophils	0.02	0.02	0.21	0.93
Proportions, %[4]				
Neutrophils	63.3	56.6	0.03	0.04
Lymphocytes	22.4	28.9	0.06	0.02
Monocytes	3.5	4.4	0.10	0.15
Eosinophils	7.5	5.0	0.27	0.32
Basophils	0.3	0.3	0.16	0.98
Neutrophil:lymphocyte ratio	2.83	1.96	0.09	0.02

[1]n = 29 calves; 15 calves on YP and 14 calves on control.
[2]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[3]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.
[4]White blood cell concentrations and proportions were log transformed to improve normality. Means were back transformed from normal data. SEM values obtained from transformed data

Table 8. Effect of YP supplemented during late gestation on the concentration and proportion of white blood cells in calves on d 85.[1]

Item[2]	Treatment		SEM	*P*-Value
	CON[2]	YP[3]		
Concentrations, K/µL[4]				
White blood cells	9.14	9.28	0.60	0.87
Neutrophils	3.57	3.91	0.38	0.53
Lymphocytes	4.42	4.08	0.23	0.30
Monocytes	0.40	0.40	0.03	0.98
Eosinophils	0.72	0.85	0.10	0.36
Basophils	0.03	0.04	0.01	0.63
Proportions, %[4]				
Neutrophils	38.5	40.4	2.23	0.56
Lymphocytes	49.7	46.8	2.24	0.35
Monocytes	4.3	4.3	0.44	0.94
Eosinophils	7.5	8.4	0.77	0.43
Basophils	0.3	0.3	0.05	0.73
Neutrophil:lymphocyte ratio	0.83	1.01	0.10	0.22

[1]Day 85 BW was taken 22 d after the last calf was born.
[2]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[3]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.
[4]Data obtained from 23 calves on control and 30 calves on YP.

Table 9. Effect of YP supplementation on cow and calf serum IgG and IgA concentrations.[1]

	Treatment			*P*-Value		
	CON[2]	YP[3]	SEM	Treatment	Time	Treatment x Time
Cow IgA, mg/ml[4]						
H 0	0.85	0.96	0.17	0.56	0.34	0.88
H 48	0.75	0.87	0.18			
Cow IgG, mg/ml[4]						
H 0	19.3	21.6	0.11	0.51	0.62	0.64
H 48	19.3	18.8	0.11			
Calf IgA, mg/ml[5]						
H 24	4.7	5.0	0.18	0.68	0.05	0.37
H 48	3.9	3.1	0.18			
D 85	0.07	0.10	0.10	0.07	NA	NA
Calf IgG, mg/ml[5]						
H 24	26.8	26.2	0.16	0.33	<0.01	0.40
H 48	74.5	55.4	0.16			
D 85	10.6	11.9	0.07	0.26	NA	NA

[1]IgG and IgA concentrations log transformed to improve normality. Means back transformed and SEM values from transformed data.
[2]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat
[3]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.
[4]At h 0 blood collected from 30 cows. At h 48 blood was collected from 29 cows for analysis. One calf died between h 0 and 48.
[5]At h 24 and 48 blood collected from 29 calves. At D 85 blood collected from 90 calves

Table 10. Effect of YP supplemented during late gestation on BVD type 1 titers in cows and calves.[1]

	Treatment			*P*-Value		
	Control[2]	YP[3]	SEM	Treatment	Time	Treatment × time
Cow BVD type 1a titers[4]						
H 0	6.8	6.0	0.45	0.32	0.66	0.91
H 48	6.5	5.8	0.46			
Calf BVD type 1a titers[5]						
H 24	7.1	6.5	0.43	0.44	0.26	0.76
H 48	7.6	7.3	0.43			
D 85	6.1	5.9	0.19	0.66	N/A	N/A

[1]BVD type 1 titers were log2 transformed.

[2]CON=Control, topdressed on supplement at 0.5 kg/d consisting of 99% ground corn and 1% fat

[3]YP=Yeast product, topdressed on supplement at 0.5 kg/d to provide 6 g live yeast and 2 g yeast cell wall.

[4]At h 0 blood collected from 30 cows. At h 48 blood was collected from 29 cows for analysis. One calf died between h 0 and 48.

[5]At h 24 and 48 blood collected from 29 calves. At d 85 blood collected from 90 calves

To: Beth Kegley
FR: Craig Coon
Date: November 4th, 2016
Subject: IACUC Approval
Expiration Date: May 5th, 2019

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your personnel additions of Elizabeth Palmer to protocol # 16068 *Influence of Commercial Yeast Products in Diets for Beef Cattle New to the Feedlot Environment*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond May 5th, 2019 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/aem

To:	Beth Kegley
FR:	Craig Coon
Date:	March 13th, 2017
Subject:	IACUC Approval
Expiration Date:	March 12th, 2018

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **17060:** *Combination of live yeast and yeast cell wall in creep-feed on calf health and performance*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond March 12th, 2018 you can submit a modification to extend project up to 3 years, or submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Beth Kegley, Jeremy Powell, Peter Hornsby, Doug
Galloway, Jase Ball, Toby Lester, and Elizabeth Palmer. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines

involving animal subjects. CNC/aem

17060

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To:	Beth Kegley
Fr:	Craig Coon
Date:	September 7th, 2017
Subject:	IACUC Approval
Expiration Date:	August 14th, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18020:** *Immune function in beef calves when dams are supplemented with a combination of live yeast and yeast cell wall during late gestation and calves are supplemented after weaning*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond August 14th, 2020 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Beth Kegley, Jeremy Powell, Toby Lester, Pete Hornsby, Jase Ball, Doug Galloway, Jana Reynolds, and Elizabeth Palmer. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines

involving animal subjects. CNC/tmp

18020