THE ROLE OF PREBIOTICS IN DAIRY CALF PERFORMANCE, HEALTH, AND IMMUNE FUNCTION

by

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Abstract

Rapid responses in milk production to changes in dairy cow management, nutrition, and health give producers feedback to help optimize the production and health of dairy cattle. On the contrary, a producer waits up to two years before the investments in calf growth and health are observed thru lactation. Even so, performance, health, and immune status during this time play a large role in subsequent cow production and performance.

A recent report from the USDA's National Animal Health Monitoring System estimated that 7.6 to 8.0% of dairy heifers die prior to weaning and 1.7 to 1.9% die post-weaning (2010). The cost of feed, housing, and management with no return in milk production make for substantial replacement-heifer cost. Therefore, management strategies to improve calf health, performance, and immune function are needed.

Prebiotic supplementation has gained interest in recent years as a method to improve gastrointestinal health and immune function in livestock. It has been provided that prebiotic supplementation may be most effective in times of stress or increased pathogen exposure throughout the calf's lifetime (McGuirk, 2010; Heinrichs et al., 2009; Morrison et al., 2010). Multiple studies have researched the effect of prebiotics around the time of weaning, but to the author's knowledge, none have focused on prebiotic's effects during the transition from individual housing prior to weaning to commingled housing post-weaning which may also be a time of stress or increased pathogen exposure. Therefore, a study was conducted to determine the effects of prebiotic supplementation of mannan-oligosaccharide and beta-glucan during this commingling phase. The results indicate that prebiotic supplementation alters feeding behavior, modulates neutrophil function, and increases antibody response during this time.

The purpose of industry-based research, such as studies on prebiotics and other methods to improve calf health and performance, is to provide producers with tools to advance and improve their operations. In this respect, it is beneficial to learn what producers' needs are and what they are interested in improving. An extension survey was conducted to establish priorities, need, and management practices of Kansas dairy producers. The results of the survey indicate that nearly half of the producers (49.3%) are interested in extension programs focused on calf/heifer management. Similarly, over half (54.8%) of the producers responded that they are interested in improving calf/heifer management in the next 5 years. The death loss observed as well as the results of the survey display a need and a producer desire to improve calf management, warranting research on prebiotics and further methods to continue to improve calf health and performance

Table of Contents

List of Figures

List of Tables

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Dedication

“Agriculture is our wisest pursuit because it will in the end contribute most to real wealth, good morals, and happiness”

- Thomas Jefferson

To all those who strive to make a difference and continue to improve the world of agriculture.

Chapter 1 - Literature review

Introduction

The profitability of a dairy is centered around milk production. Maintaining a steady flow of milk production on an operation involves raising healthy replacement heifers. Early dairy calf health and growth is very influential on milk production later in life. Therefore, research on methods to improve calf health and growth is warranted.

The dairy calf encounters potentially stressful situations in its first few months of life including transportation, dehorning, castration, weaning, and commingling. Stress can lead to suppression of the immune system and increase the risk of disease in the presence of a pathogen (Salak-Johnson and McGlone, 2014). The commingling phase of a dairy calf's life, the transition from individual housing to group housing, has been shown to increase the risk of bovine respiratory disease, decrease leukocyte function, and decrease average daily gain (Bach et al., 2011; Hulbert and Ballou, 2012). Consequently, research specifically involving methods to aid calves in maintaining health and growth through stressful situations, such as commingling, may be beneficial.

Prebiotics are non-digestible fibers that can directly influence the innate and adaptive immune system and effect performance measures such as intake and body weight gain (Ghosh and Mehla, 2012; Heinrichs et al., 2003); therefore, prebiotics may play a role in assisting calves through stressful situations. This thesis is designed to give an overview of the immune system of the calf as well as provide the relationship of prebiotics and the immune system and how prebiotics may play a role in aiding calves through commingling. This thesis will then provide opportunities for how to communicate industry-related research to dairy producers throughout Kansas.

Immune development of the calf

Dairy calves have almost completely developed immune systems at birth, including primary and secondary lymphoid organs and immune cells, because of their long gestation period of 280 days (Halliwell and Gorman, 1989). The first lymphoid organ to develop is the thymus which appears around 40 days post-conception (Schultz et al., 1973; Tizard, 1982). The thymus is responsible for producing thymocytes that mature to become T lymphocytes. Interestingly, the thymus reaches full maturity around 140 days post-conception and steadily decreases in size until regression at puberty (Cortese, 2009; Kushida et al., 2012).

At approximately 40 to 45 days, peripheral blood lymphocytes start circulating (Pearson et al., 1976) and starting mid-gestation, peripheral blood lymphocytes can respond to bacterial and viral mitogens (Tizard et al., 1982; Liggitt et al., 1982). Beginning one month prior to birth, the number of peripheral blood T cells decrease from approximately 60% to 30%; however, there are less B cells in the fetus than in mature calves (Chase et al., 2008; Senogles et al., 1979; Kampen et al., 2006).

The bone marrow and spleen appear around 55 days post-conception, followed by immunoglobulin (Ig) M-carrying cells and lymph nodes 5 days later (Schultz et al., 1973; Tizard, 2013). Bone marrow is a site of leukocyte, erythrocyte, and thrombocyte development and B lymphocyte maturation. Secondary lymphoid organs such as the spleen and lymph nodes facilitate antigen trapping and presentation to lymphocytes.

Even though blood lymphocytes and IgM-positive cells appear fairly early in development, antibody production does not start until approximately 130 days with serum IgG and serum IgM (Fennestad and Borg-Petersen, 1962; Tizard, 2013). During development, serum IgM is predominant over IgG (Pereira et al., 1982).

The ruminant animal has a syndesmochorial type of placentation meaning that there are five layers of tissues present between maternal and fetal circulation. This results in very little to no prenatal IgG transfer (Watson, 1980). Peyer's patches, important for B cell development in calves, and tonsils are the last to develop (Tizard, 2013).

In conclusion, the primary and secondary lymphoid organs, as well as many of the cells involved in innate and adaptive immune responses are near full in development at the time of birth. However, the dairy calf's immune system still necessitates much maturation, growth, and priming.

Immune status after birth

Despite the neonatal calf's immune system being near complete in development at birth, the number and function of immune cells are altered around the time of birth. This may be due in part to maternal and neonatal glucocorticoids around the time of birth. In addition, immune cell numbers have not yet reached mature levels at birth and the adaptive immune system is naïve in nature.

Suppression of the normal function of innate immune cells, such as chemotaxis and phagocytosis, can last up to four months (Hauser et al., 1986). This decrease in function may be related to the effects of high serum steroids such as cortisol released by the fetal adrenal gland during parturition (Fauci et al., 1976; Barrington, 2001). A study conducted by Salvemini et al. (1995), found that when the glucocorticoid dexamethasone was administered to rats, iNOS protein expression was inhibited and NO_2^- was suppressed. Nitric oxide synthase and nitrite both play a role in oxidative burst responses of leukocytes which demonstrates the suppressive potential of glucocorticoids.

As a result of immunosuppression at birth, the calves' immune system does not mature until 5 to 8 months after birth. At birth, amounts of circulating complement are less than 20% the amount of mature calves, but increase to 50% by one month of age (Firth et al., 2005; Chase et al., 2008). Adult amounts of complement are not reached until approximately 6 months of age (Cortese, 2009). The number of neutrophils decrease after birth, however the ability of the neutrophils to function increases. Neutrophils are able to respond to pathogens by one week of age, but neutrophil function does not reach full maturity until five months of age (Hauser et al., 1986). Mature amounts of T cells such as CD4+, CD8+, and TCRγδ+ are not reached until approximately 8 months of age (Cahill, 1999; Cortese, 2009). Similarly, B cell amounts increase from 4% of total lymphocytes at 1 week of age to 20% by 6 to 8 weeks of age (Kampen et al., 2006) as well as an increase in circulating IgA and IgG at this time (Husband and Lascelles, 1975).

The acquired immune system is also naïve at birth and relies on exposure to antigens. Additionally, immune responses are biased toward T helper 2 immune responses from placental production of progesterone, prostaglandin E2, and cytokines such as IL-4 and IL-10 which suppresses T helper 1 immune responses *in utero* (Morein et al., 2002). The immature, naïve, and potentially altered nature of the neonatal immune system exhibits the importance of passive transfer of maternal immune protection to protect the calf while the immune system becomes fully competent. Windeyer et al. (2014) found that greater than 20% of the BRD cases from 2,874 heifer calves may have been prevented if those calves had not had failure of passive transfer of immunity. Calves that had failure of passive transfer also had lower body weights than calves with successful passive transfer.

The impact of colostrum

Newborn calves rely crucially on adequate colostrum to aid in immunity during the time of neonatal immune development by providing maternal antibodies along with various other immune cells such as CD cells, macrophages, and neutrophils (Cortese, 2009). Researchers speculate that the neonatal gastrointestinal tract temporarily allows passage of these molecules through gaps in the tight junctions at birth. Passage of these molecules remain most efficient through 4 hours of life and decline rapidly after 12 hours until absorption is almost completely obstructed at 24 hours (Bush and Staley, 1980). Deutsch and Smith (1957) discovered the obstruction of passage when feeding calves colostrum and subsequently taking blood and urine samples. Maternal antibodies can also pass through intestinal cells via the FcRn receptor. However, epithelial cells with FcRn receptors are replaced with cells lacking an FcRn receptor typically within 48 hours providing another form of "gut closure" (Lecce and Morgan, 1962; Roopenian and Akilesh, 2007). Therefore, industry recommendation dictates that calves receive 4 L of colostrum within 4 to 6 hours of birth and an additional 2 L within 12 hours. As a result of these recommendations, the majority of dairy farmers (59.2%) hand feed colostrum within 3.3 hours after birth (NAHMS, 2010). Serum total protein can be tested for IgG concentration within 48 hours of birth using various methods including refractometry. Serum IgG concentration less than 1 g/dL is indicative of failure of passive transfer. Inadequate passive transfer can increase morbidity and mortality of dairy calves as well as decrease performance measures of body weight gain and lactation later in life (Faber, 2005). Tyler et al. (1998) conducted a 10 year study on 3,479 Holstein replacement heifers and found that calves with <4.0 g/dL serum protein concentration were at 4.6 times greater risk of mortality than calves with a serum protein concentration of ≥6.0 g/dL.

Along with colostrum's nutritive value and immunological importance, colostrum plays a role in the development of the gastrointestinal tract. Colostrum can influence the microbial population, as well as epithelial cell proliferation, migration, differentiation, and apoptosis. Moreover, colostrum may modulate digestion, absorption, motility, and protein synthesis and degradation (Sauter et al., 2004; Blum, 2006; McGuirk, 2010). Even though the importance of colostrum is well-known, it is estimated that 1 in 5 heifers (19.2%) have failure of passive transfer of immunity (NAHMS, 2010).

Relationship between the gastrointestinal tract and immunity

Despite management efforts to provide clean environments to raise calves, calves will always have some exposure to pathogens from the air, water, and feed. Fortunately, the gastrointestinal tract, the largest immunological organ of the body (McGuirk, 2010), is equipped with an elaborate immune system at the mucosal surface. One of the primary defenses of the gastrointestinal tract is to prevent pathogens from directly entering systemic circulation. This is accomplished by the physical barrier formed by the epithelial cells that line the mucosal surface and their tight junctions (Fasano and Shea-Donohue, 2005). Another protective barrier that is formed is a mucus layer. Mucus is produced from goblet cells in the epithelium of the gastrointestinal tract. In addition to providing a barrier against pathogens, the mucus layer also contains leukocytes and antimicrobial factors such as defensins, lysozyme, and secretory IgA contributed by other cells in the epithelium (Gallo and Hooper, 2012). Paneth cells in the crypts of the epithelium are responsible for producing lysozyme and α-defensins, bacteriocidal proteins that aid in the regulation of microflora (Elphick and Mahida, 2005). In the villus of the epithelium, M cells play a role in the transport of intraluminal antigens to the lymph tissue of Peyer's patches (Mabbott et al., 2013). The different cells in the epithelium have a relatively

rapid turnover rate. This continual renewal of cells prevents adhesion of harmful enteric pathogens. Similarly, peristaltic contractions of the gastrointestinal tract help flush out potentially harmful pathogens and prevent colonization. The second layer of the mucosal surface of the small intestine after the epithelium is the lamina propria. The lamina propria is continuous connective tissue that houses blood and lymph vessels, as well as various immune cells such as dendrites, lymphocytes, and macrophages (Brandtzaeg et al., 2008). The barriers, secretions, and functions of the gastrointestinal tract greatly contribute to aiding in calf health.

The role of intestinal microflora

A discussion on the impact of the gastrointestinal tract on health and immune status of the calf would not be complete without also covering the role of commensal microorganisms and their symbiotic relationship with the calf. Microflora in the intestine is one of the most densely populated microbial habitats known in the body (Gill et al., 2006). In humans and cattle, it is estimated that there are more than 10^{14} commensal microbes encompassing more than 400 different species (Ley et al., 2006). The gastrointestinal tract of the calf is devoid of flora at birth, but is colonized shortly after by the fecal and vaginal flora during delivery (Eckburg et al., 2005). The population of microflora colonizing the gastrointestinal tract is influenced by the environment and diet of the calf, the dam, and genetic background (Ozutsumi et al., 2005). Neonatal calves' microbial communities are comprised predominantly of facultative anaerobes from the environment such as *Enterobacteriaceae, Streptococcus,* and *Staphylococcus*. However, strict anaerobes, *Bifidobacterium, Bacteroides, Lactobacilli,* and *Clostridia*, dominate the gastrointestinal tract as the calf ages (Edrington, 2012; Ballou, 2015). Commensal microflora, for example *Lactobacilli* and *Bifidobacteria*, form a barrier much like the mucus layer that limits the colonization of pathogenic microorganisms in the gastrointestinal tract.

Certain commensal microorganisms may also contribute to the production of mucus and antimicrobial factors (Shahani and Ayebo, 1980). Furthermore, commensal organisms have been demonstrated to stimulate the immune system and growth of gut colonocytes and improve digestion through fermentation (Guarner and Malagelada, 2003).

An imbalance of commensal microflora to pathogenic microbes can lead to disease such as diarrhea in calves (Ishihara et al., 2000). This imbalance can be caused by several factors including diet, stress, and the environment (Guarner and Malagelada, 2003). *Salmonella* is a pathogenic microorganism that can cause fever and diarrhea when it dominates in the normal microbiome (Smith, 2002). *Clostridium* and *E. coli* are also examples of pathogenic bacteria that can cause harm to calf health.

The introduction of prebiotics

One way to improve the proliferation of commensal microflora in the gastrointestinal tract is through the use of prebiotics. Prebiotics are defined as 'non-digestible food ingredients that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health' (Gibson and Roberfroid, 1995). One of the reasons prebiotics are effective is because they are resistant to gastric acidity, absorption, and hydrolysis by enzymes produced in the gastrointestinal tract (Patel and Goyal, 2012). They are, however, fermented by intestinal microflora which promotes proliferation of commensal microorganisms. Bifidobacteria produce various glycosidases which are enzymes that hydrolyze glycosidic bonds of polysaccharides like most prebiotics, hence prebiotics' ability to be readily fermented (Russo et al., 2012). The fermentation products of prebiotics by intestinal microflora also provide benefit by immune modulation, improved energy efficiency and digestibility, and decreased intestinal pH which suppresses pathogenic bacteria (Mizota,

1996; Roodposhti and Dabiri, 2012). Prebiotics themselves have a positive influence on immune parameters in the gut-associated lymphoid tissues, secondary lymphoid tissues, and peripheral circulation (Bodera, 2008). Prebiotics may promote T Helper 1 and regulatory T cell-dependent immune responses over T helper 2 responses (Patel and Goyal, 2012). Types of prebiotics used in the livestock industry include fructooligosaccharides, galactooligosacchardies, mannan oligosaccharides, and beta glucans.

Mannan Oligosaccharides

Production and composition

Mannan oligosaccharides are short-chain, low molecular weight carbohydrate fragments of the yeast cell wall, particularly *Saccharomyces Cerevisiae*. Mannans represent approximately 30% of the cell wall weight and are found on the outer parts of the cell wall (Kollár et al., 1997). They are comprised of many α-1,2 and α-1,3 N-linked glycan side chains attached to an α-1,6 linked mannose monomer backbone (Kollár et al., 1997). To obtain these cell wall derivatives, yeast cells are lysed and the yeast culture that is obtained is centrifuged to isolate the cell wall components. The cell wall components are then washed and spray dried (Spring et al., 2000). The most important antigenic component of the cell wall are the mannans of the yeast cell surface (Ballou, 1970).

Mechanisms

One of the primary functions of mannan oligosaccharides is to provide competitive binding for gram negative bacteria. Gram negative bacteria have mannose-specific type-1 fimbriae that attach to D-mannose receptors on the epithelium of the gastrointestinal tract (Friman et al., 1996; Ofek et al., 1977). The presence of mannan oligosaccharides can provide an alternate binding site for these pathogens which then block them from colonizing the

epithelium and the complex exits the tract without causing harm (Spring, 2000). Mannan oligosaccharides have the ability to alter the composition of the intestinal flora, transport time, digestibility, absorption, and intestinal health of calves in this way (McGuirk, 2008). Improved intestinal health and the inhibition of pathogenic microbes may contribute to smaller fecal scores and fewer incidence of scours. Pathogenic bacteria produce toxins that cause intestinal hyperactivity, secretion, and diarrhea (Giannella, 1983). In addition to competitive binding, mannan oligosaccharides may also promote immune function such as phagocytosis and oxidative burst (Magalhães et al., 2008). A potential mechanism for the immunomodulatory effects of mannan oligosaccharides was described by Franklin et al (2005). The authors proposed that collectins may be responsible for this immunomodulatory function. One of the three types of collectins present in cattle are mannose-binding proteins that can bind to mannose, N-acetylmannosamine, or N-acetylglucosamine. Mannan oligosaccharides may promote the production of these mannose binding proteins. Once bound, this complex can act as an opsonin and improve phagocytosis or activate the complement system, as described in humans (Neth et al., 2002).

Beta Glucans

Production and composition

Beta glucans are other carbohydrate components of the yeast cell wall of *Saccharomyces cerevisiae*. Beta glucans are also components of fungi and cereal grains like barley and oats (McGuirk, 2010). Beta glucans are glucose polymers consisting of β-1,3 and β-1,6 linked D-glucopyranosyl units (Wang et al., 2008). They account for 50 to 60% of the yeast cell wall weight. In contrast from mannan oligosaccharides, glucans are found towards the inside of the cell wall. They provide structure and rigidity to the cell wall that allows organization of the

other cell wall components (Kollár et al., 1997). The efficacy of beta glucans may be modulated by the degree of branching, the molecular mass, and the tertiary structure (Russo et al., 2012).

Mechanisms

Beta glucans that are large in molecular weight have been found to directly affect phagocytic, cytotoxic, and antimicrobial activities of leukocytes, particularly macrophages. They also promote oxidative burst responses by helping to produce reactive oxygen and nitrogen intermediates and clear apoptotic cells by up-regulating the FS receptor (Gantner et al., 2005; Brown and Gordon, 2003). In addition to promoting innate immune responses, beta glucans increase production of proinflammatory cytokines and chemokines. Cytokines and chemokines stimulated by beta glucan-activated cells include IL-1β, IL-6, and TNF-α (Vetvicka and Yvin, 2004). These cytokines and chemokines aid in the recruitment of additional leukocytes to the site of infection.

The mechanism by which beta glucans can stimulate these immune responses is credited to the Dectin-1 receptor. The Dectin-1 receptor is expressed on monocytes, macrophages, neutrophils, dendritic cells, and splenic T cells and can recognize carbohydrates with β-1,3 and β-1,6 glucan linkages (Sonck et al., 2009). A study done in mice found that the Dectin-1 receptor has a cytoplasmic tail with an immunoreceptor tyrosine-based activation motif. When beta glucan binds to Dectin-1, the motif becomes phosphorylated which sends a signal to induce phagocytosis and respiratory burst (Brown and Gordon, 2003). On the other hand, cytokine and chemokine production may be attributed to Toll-like receptor 2 (Brown and Gordon, 2003). The authors also found that to produce TNF-α and IL-12, both Dectin-1 and Toll-like receptor 2 were required. TNF-α has many functions, one of them being to aid in the oxidative burst response of

neutrophils (Mcleish et al., 1996). IL-12 is important in stimulating production of IFN-γ and promoting the T helper 1 immune response (Manetti et al., 1993).

Effects of prebiotics on calf performance

Dairy calf performance is important for productivity later in life. Prebiotics have been shown to improve performance measures such as average daily gain, feed intake, and digestibility. Volatile fatty acid production may increase nutrient digestibility and subsequently increase feed efficiency. In a study conducted with calves fed mannan oligosaccharides, no differences in volatile fatty acid production were observed (Hill et al., 2009) and no differences were seen in dogs supplemented with fructooligosaccharides or mannan oligosaccharides (Swanson et al., 2002). However, Herfel et al. (2011) found that supplementing piglets with polydextrose, a precursor to human milk oligosaccharide, increased production of both propionic and lactic acids in a linear fashion. In this same study, concentrations of cecal *lactobacilli,* a commensal-type microflora, increased linearly with polydextrose supplementation (Herfel et al., 2011). In a separate study, calves supplemented with beta glucan had increased rumen pH and nutrient digestibility (Kim et al., 2011).

An increase in body weight gain per calf per day, feed intake per calf per day, and feed conversion efficiency were observed by Ghosh and Mehla (2012) when calves were administered 4 g/d of a mannan oligosaccharide supplement. Although feed cost per calf per day was increased with prebiotic supplementation, these costs were off-set by the increases in performance. Studies comparing prebiotic supplement to antibiotics, found no differences in overall body weight gain, feed intake, or feed efficiency, indicating that prebiotics may be a viable alternative for prophylactic antibiotic use (Donovan et al., 2002).

There is evidence that prebiotics may modulate feeding behavior, indicated by results of studies showing improved body weight gain or feed intake at certain time points throughout the trials conducted (Roodposhti and Dabiri, 2012; Quigley et al., 1997; Quigley et al., 2002). In addition, studies have shown that prebiotic-supplemented calves increase intake at a faster rate than un-supplemented calves (Heinrichs et al., 2003; Terré et al., 2006; Morrison et al., 2010).

Effect of prebiotics on calf health

In a recent survey conducted by the USDA, it was estimated that on average 7.8 ± 0.2% of dairy heifer calves die pre-weaning (NAHMS, 2010). The majority (56.5%) of these pre-weaning deaths are caused by enteric diseases such as scours and digestive problems. As previously mentioned, prebiotics such as mannan oligosaccharides that prevent attachment of pathogenic bacteria and both mannan oligosaccharides and beta glucans that improve the immune system of the calf may help in preventing these challenges.

Indeed many studies have shown an increase in normal fecal scores and a decrease in the incidence of scours with prebiotic supplementation. Pre-weaned calves fed milk replacer supplemented with mannan oligosacchardies had decreased risk of abnormal fecal scores compared with calves that received no supplement (Heinrichs et al., 2003). Dairy calves fed a yeast culture derived from *Saccharomyces cerevisiae* had more incidences of normal fecal scores and had less incidence of fever, diarrhea, and the risk of health disorders compared to controls (Magalhães et al., 2007). Calves fed a 1% brewers yeast, a form of beta glucan, had a reduced incidence of fever and reduced number of antibiotic treatments administered during the pre-weaning period (Seymour et al., 1995). Furthermore, Ghosh and Mehla (2012) reported that a mannan oligosaccharide supplementation reduced fecal coliform counts.

Effect of prebiotics on immune function

The above health outcomes may be influenced by prebiotic stimulation of the immune system. Innate immune responses, such as phagocytosis and oxidative burst, cytokine production, and antibody response may be influenced. Mice supplemented with 10% oligofructose or inulin had increased peritoneal macrophage phagocytosis and macrophage superoxide production compared to control mice (Trushina et al., 2005). Macrophage phagocytosis was also increased when calves were supplemented with β 1,4 mannobiose compared to control calves (Ibuki et al., 2010). An *in vitro* study in humans found an increase in neutrophil oxidative burst response and microbicidal activity with beta glucan supplementation (Wakshull et al., 1999). Peritoneal neutrophil respiratory burst activity and neutrophil number were also increased in mice supplemented with oat beta glucan (Murphy et al., 2007).

Researchers speculate that prebiotics may directly influence pro-inflammatory cytokines while having an indirect effect on anti-inflammatory cytokines. The secretion of pro-inflammatory cytokines such as TNF-α and IL-10 were increased in human peripheral monocytes *in vitro* when human subjects were administered either fructooligosaccharide or inulin (Capitán-Cañadas et al., 2013). Mice supplemented with beta glucans also had increased secretion of TNF- α, IL-6, and IL-1 *in vivo* compared with controls (Vetvicka and Yvin, 2004). An increase in *in vitro* lymphocyte proliferation from weanling piglets was observed in response to ConA in a dose-dependent manner with beta glucan supplementation (Wang et al., 2008).

The fermentation products produced by prebiotics such as butyrate (Nilsson et al., 2010), may indirectly effect anti-inflammatory cytokines. Butyrate is one of the most prevalent fermentation products in the rumen and has been shown to increase the production of anti-inflammatory cytokines (Shley and Field, 2002). Secretion of anti-inflammatory IL-10 was increased in elderly humans supplemented with galactooligosaccharides (Vulevic et al., 2008).

Fructooligosaccharide-supplemented rats had increased TGF-β in cecal tissue compared with control rats as well as an increase in commensal microflora counts, *Lactobacilli* and *Bifidobacteria* (Hoentjen et al., 2005).

Antibody production of IgA which plays a role in mucosal immunity and IgG, important in memory responses, may also be influenced by prebiotics. Ileal IgA concentrations from dogs supplemented with both mannan oligosaccharides and fructooligosaccharides were increased compared with control animals in a study done by Swanson et al. in 2002. Secretion of IgA in peyer's patch cells of fructooligosaccharide-supplemented mice with was increased in a dose-dependent manner compared with controls (Hosono et al., 2003). Hydrolyzed yeast-fed neonatal calves challenged with both Hog cholera, a viral pathogen, and Erysipelothrix insidiossa, a bacterium, had increased bacterial- and viral-specific IgA and IgG concentrations compared with challenged calves without supplementation (Kim et al., 2011). Increases in total IgG have also been observed. Beta glucan supplementation increased total IgG concentrations of immunosuppressed mice (Yun et al., 1997). Serum IgG concentrations were improved by 32% and 23% compared to controls in two trials involving mannan oligosaccharide-supplemented piglets (Lazarevic et al., 2010). In a third trial by the same researcher, Holstein calves fed mannan oligosaccharides had an increase in serum IgG concentrations of 39% (Lazarevic et al., 2010).

Conclusion

In the dairy industry, to be an efficient, productive, and profitable operation, quality replacement heifers are essential. Raising replacement heifers can cost between $1,200 and $1,600 (McGuirk, 2008). Therefore, excellent health and performance of these heifers is essential. Good management practices to optimize nutrition, immune status, and decrease the

risk of disease are vital. The use of prebiotics may be a viable option to increase the proliferation of commensal bacteria in the gastrointestinal tract, modulate feeding behavior, and increase immune function to optimize calf health. Research has shown prebiotic supplementation to be most beneficial in times of stress or increased pathogen exposure. Stressful time points in a dairy calves' life can include weaning, transportation, and commingling. Previous research on prebiotics has focused on weaning and transportation, however prebiotics during the commingling phase has yet to be studied.

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Chapter 2 - Immune parameters and performance of Holstein calves fed prebiotic supplementation during commingling

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Abstract

The risk of disease increases for post-weaned calves when they are transitioned from individual housing to group housing (commingling). Therefore, this study was conducted to determine if prebiotic supplementation of Mannan-oligosaccharide (MOS) and Beta-glucan (BG) assists calves in the transition from individual hutches to groups of 3. Feed intake, body weight gain, *in vivo* adaptive immune responses, and *ex vivo* innate immune responses were measured from sixty, weaned Holstein heifer calves (age 52 ± 4.0 d; 83 ± 14.7 kg BW). One week prior to commingling (-7 d), calves were randomly assigned to either a daily bolus dose of oral prebiotics (3 g; 10% MOS, 18% BG) dissolved in 15 mL of molasses or control (15 mL molasses only) for 7 weeks. Daily DMI was collected and calves were weighed weekly. Whole blood was collected via jugular venipuncture on d -7, 7, 14, and 42 relative to commingling. In addition, all calves were administered an innocuous protein injection of ovalbumin (OVA; subQ; 0.5 mg/mL) at commingling (d 0) and 28 d after commingling. All blood samples were measured for hematological measures, leukocyte function via flow-cytometry for peripheral polymorphonuclear (PMNL) phagocytosis (PG) and oxidative burst (OB) responses to heat-killed *E. Coli* (8739) and neutrophil L-selectin, as well as whole blood killing, and cytokine secretion. Plasma OVA-specific IgG and IgA were measured 2 weeks after each OVA injection. Two weeks after commingling, prebiotic-treated calves had PMNL with greater OB intensity than control calves ($P = 0.01$). Prebiotic-calves had greater primary IgG ($P = 0.04$) responses to OVA than control calves, as well as greater secondary IgA response ($P < 0.01$) than control calves. The week of commingling, prebiotic-calves had greater ADG (1.08 vs. 0.98 ± 0.063 kg/d; $P = 0.02$), as well as smaller F:G (2.62 vs. 3.58 ± 0.255; $P = 0.001$) than control calves. Prebiotic supplements improved innate and adaptive immune measures and performance during

the commingling phase which may help reduce the risk of disease and improve vaccination response in post-weaned dairy heifers.

Key words: bovine, immunology, prebiotics

Introduction

The most recent report of the USDA's National Animal Health Monitoring System noted that on average 7.8 ± 0.2% of dairy heifers die before weaning and more than half (55.2 to 57.8%) of these deaths are attributed to enteric disease (NAHMS, 2007). To reduce the risk of enteric disease transmission on dairies in the United States, approximately 74.9 ± 1.3% of heifer calves are typically housed in individual pens or hutches pre-weaning (NAHMS, 2007). Soberon et al. (2009) provided that 1 pound of gain from birth to weaning could mean 850 more pounds of milk through the calves' life. Maintaining health and minimizing the risk of disease may therefore avoid long-term consequences such as poor growth, reproductive performance, milk production, and longevity (Poulsen and McGuirk, 2009).

During commingling, calves are introduced to pen-mates for the first time and the transmission of microbiota increases. Commingled, post-weaned calves are at increased risk for bovine respiratory disease (BRD) during this time. Respiratory disease has been reported to cause 44.8 to 48.2% of deaths in post-weaned heifers (NAHMS, 2010). In addition, it has been reported that commingled calves with at least one previous case of BRD had 3.89 times greater odds of incurring BRD after grouping (Bach et al., 2011).

Hulbert and Ballou (2012) conducted a study in which calves were commingled into groups of 3 or left in their home hutches. The overall incidence of BRD after calves were commingled was 37.9%. The introduction to group housing is also associated with changes in leukocyte function. Hulbert and Ballou (2012) reported increased circulating leukocytes 3 days after commingling in Holstein-bull calves compared with their counterparts that were left in individual housing. Circulating neutrophils were also influenced by commingling, as commingled calves had less oxidative burst responses to heat-killed bacteria compared with calves left in their home hutches.

Immune challenges and the transition to group-housed feeding systems also influence feeding behavior during this phase. Calves commingled into groups of 3 tended to have less feed intake and less average daily gain than the calves left in home hutches throughout the study (Hulbert and Ballou, 2012).

Prebiotics comprised of yeast cell wall components can modulate leukocyte function and adaptive immunity (Davis et al., 2004; Murphy et al., 2007; Swanson et al., 2002; Kim et al., 2011). Seymour et al. in 1995 also found that MOS supplemented calves needed a decreased number of antibiotic treatments pre-weaning compared with control. In addition, prebiotics may help increase performance (Heinrichs et al.,2003; Terre et a l., 2007; Morrison et al., 2010). These results indicate that prebiotic supplementation of MOS and BG may be beneficial in enhancing calves' ability to defend against pathogens and maintain feed intake through stressors. Therefore, the objectives of this study were to determine if prebiotics aid calves in the transition from individual hutches to group-housed pens. We hypothesized that prebiotics would modulate circulating leukocyte function and increase antibody production against an innocuous protein.

Materials and methods

Animals, housing, and treatment

Sixty, healthy weaned Holstein heifers were enrolled in this experiment at age 52 ± 4 d SD from May to December 2014 at the Kansas State University Department of Animal Science and Industry's Dairy Unit located in Manhattan, Kansas. All animal procedures were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC protocol 3408).

Prior to weaning, calves were bottle-fed 1.8 ± 0.2 L of pasteurized milk 3 times daily at 0700, 0300, and 2300 h and were provided ad libitum fresh water and calf starter (18% CP;

Hubbard Feeds Inc.). Calf starter refusals were weighed daily. Each calf completed weaning after consuming 1.36 kg or more of starter for 3 consecutive days.

Weaned calves were randomly assigned to a daily bolus dose of either 3 g of prebiotic (Preb; 10% MOS, 18% BG) dissolved in 15 mL of molasses or control (Con; 15 mL of molasses only) for 7 weeks. After one week on treatment, all calves were commingled into groups of 3 (d 0) in straw-bedded sheds with 49 m^2 of free-space. Calves received ad-libitum access to water and calf-grower (CG), which consisted of a total mixed ration (43.5% alfalfa hay, 8.7% prairie hay, and 47.8% grain mix) top-dressed with 4 kg of calf-starter. For each pen, daily CG refusals were weighed and recorded for the calculation of feed intake and information of health observations were collected. Body weight and growth (height and girth) were measured weekly from birth through the completion of the trial.

Sampling

Just prior to treatment (d -7), calves were weighed and blood samples were collected. Nine milliliters of peripheral blood (3 mL and 6 mL with EDTA and sodium heparin, respectively; BD Vacutainer, Pulmolabs, Porter Ranch, CA) was collected via jugular venipuncture on d 7, 14, and 42 post-commingling. In addition, calves were given a subcutaneous injection of 1 mg ovalbumin (OVA; Sigma-Aldrich, St. Louis, MO) in the neck region at commingling (d 0) and d 28 post-commingling. Ovalbumin was administered with an adjuvant of 0.5 mg Quillaja Saponin (VET-SAP, Desert King International, San Diego, CA) dissolved in 1 mL of saline.

Blood and plasma analyses

Whole blood samples (EDTA) were measured for complete blood counts (CBC) using a Procyte analyzer (Idexx Laboratories, Sacramento, California) and a neutrophil:lymphocyte was

calculated from the CBC leukocyte differential. Within 1 hour of sampling, plasma (heparin) was collected after centrifugation at 2,500 x *g* and stored at -80°C until analyzes.

The bactericidal activity of whole blood (WB; heparin) against a live culture of *E. coli* 8739 was measured using methods previously described (Ballou, 2012). Briefly, blood was incubated with bacteria at a 4 to 1 ratio for 10 minutes, then cultured over tryptic soy agar plates (22091; Sigma-Aldrich, St. Louis, MO). The amount of cfu's were manually counted 24 h after incubation and the percent of cfu's eliminated by WB were calculated using controls (200 cfu's in 50 uL of RPMI).

Phagocytic and oxidative burst responses of polymorphonuclear (PMNL) cells in WB samples (heparin) to heat-killed *E. coli* (8739; labeled with propidium iodide) were analyzed by methods previously described by Hulbert et al. 2011a. Polymorphonuclear oxidative burst and phagocytosis were simultaneously analyzed using a Guava easyCyte flow cytometer (Darmstadt, Germany) and measured with FlowJo software (Ashland, OR, USA). FlowJo software (Ashland, OR, USA) was used to determine the percentage of PMNL displaying both phagocytosis and oxidative burst and within this population, the measured geometric mean fluorescence intensity (GMFI) was calculated for oxidative burst (FL-1) and phagocytosis (FL-3).

Peripheral neutrophil L-selectin (BOV2046 anti-CD62L-IgG1; Washington State University) was measured in WB samples (EDTA) using methods previously described (Hulbert et al., 2011a). Briefly, 100 uL WB was incubated in an ice bath for 1 h with 5 ug/mL primary antibody. After lysis of erythrocytes, leukocytes were incubated with a secondary antibody conjugated to FITC (goat anti-mouse IgG1-FITC; Santa Cruz Biotechnology). Using FlowJo software, the PMNL population was gated and GMFI was measured for FL-1.

The ability of leukocytes to produce TNF-α and IFN-γ were analyzed by stimulating WB with LPS (*E. coli* 0111:B4; Sigma-Aldrich, St. Louis, MO) and PHAp (L8754; Sigma-Aldrich, St. Louis, MO), respectively. Supernatant was collected and frozen at -80°C. Samples were analyzed using a commercial sandwich-based ELISA kit (Kingfisher Biotech, Saint Paul, MN, USA). The intra- and inter-assay coefficients of variation for TNF-α were 15.45% and 7.16% respectively. For IFN-γ, the intra- and inter-assay coefficient of variation were 6.45% and 6.14%.

Plasma samples collected two weeks after each OVA injection were analyzed for OVA-specific Immunoglobulins G and A (OVA-IgG; OVA-IgA) using an ELISA protocol as previously described by Yuan et al.(2015). The inter-assay coefficient of variation for OVA-IgA were 7.62. The intra-assay coefficient of variation for low concentrations of Ig was expected to be great and therefore, samples were randomly assigned to 8 plates. For OVA-IgG, the intra- and inter-assay coefficient of variation were 7.72 and 39.11.

Statistical analysis

Data were analyzed by restricted-maximum likelihood ANOVA using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). A linear, mixed model with the fixed effects of treatment and time and their interaction was fitted. The average daily gain prior to enrollment (age 0 d to enrollment) was used as a covariate in all models. The random effect was calf nested within pen. The mean model was run with unstructured, compound symmetry, and autoregressive (1) covariance structures for the within-subject measurement. The appropriate covariance structure of was chosen for each analysis based on the Schwarz-Bayesian information criterion. Degrees of freedom for *F*-tests of the fixed effects were estimated using Satterthwaite approximation. Prior to analyses, normality of the residuals was confirmed by

evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS. Least squares means (± SEM) are reported throughout. Means were subsequently separated using the sliced (SLICE) time effects for treatment × time interaction and the PDIFF function associated with generation of least squares means (±SEM). Differences were considered significant at $P \leq 0.05$ and tendency differences were considered significant at $0.05 < P \leq 0.10$.

Results

Performance

Prior to enrollment, there were no differences in body weight (49.55 vs. 48.12 ± 1.93 kg; $P = 0.46$), hip height (84.71 vs. 86.64 ± 0.54 cm; $P = 0.52$), shoulder height (82.22 vs. 84.15 ± 0.46 cm; $P = 0.37$), calf starter intake (0.49 vs. 0.46 ± 0.06 kg; $P = 0.66$), and pasteurized milk intake (4.59 vs. 4.34 ± 0.21 L; $P = 0.23$) across all treatments. There was a tendency of a treatment by week interaction for BW ($P = 0.088$; Table 2.1) and DMI ($P = 0.060$; Table 2.1). At commingling and 7 days after commingling, Con-calves had a greater BW ($P = 0.0260$; P = 0.019; Table 2.1). Preb-calves had a greater total DMI d 28 to 34 and d 35 to 41 ($P = 0.02$; $P = 0.007$; Table 2.1). Overall, ADG did not differ between treatments ($P = 0.578$; Table 2.1). Feed conversion efficiency was greater in Con-calves in individual hutches -7 to -1 d before commingling ($P = 0.002$; Table 2.1) and 7 to 13 d after commingling ($P = 0.038$; Table 2.1), whereas feed conversion efficiency was greater among Preb-calves the week of commingling (d 0 to 6) and 2 weeks after commingling (d 14 to 20; $P = 0.001$; $P = 0.042$; Table 2.1).

Blood parameters

No treatment by time interactions were observed for hematological measures. There was a treatment effect observed of less circulating monocytes in Preb- than Con-calves ($P = 0.041$; Table 2.2). Percent hematocrit, hemoglobin, erythrocytes, mean cell volume, and total

leukocyte values all increased on d 14 and 42 as calves aged ($P < 0.001$; Table 2.2). All calves had increased mean cell hemaglobin on d 42 ($P < 0.001$; Table 2.2).

No treatment, time, or treatment by time interactions were observed for WB bactericide ($P > 0.10$; Table 2.3). All calves had less TNF-α secretion from LPS-stimulated WB on d -7 (baseline; $P < 0.001$; Table 2.3). However, Con-calves had increased TNF-α concentrations d 14 and 42 after commingling compared with Preb-calves (Figure 2.2). IFN-γ from PHA-stimulated WB was increased for all calves on d 7 and 42 after commingling ($P < 0.001$; Table 2.3).

Treatment and the interaction between treatment and time did not affect the expression of neutrophil L-selectin or percent of PMNL that were positive for phagocytosis and oxidative burst ($P > 0.05$; Table 2.3). All calves had decreased neutrophil L-selectin on d 42 post-commingling ($P < 0.001$; Table 2.3) as well as decreased % PG+OB+ d 14 and 42 post-commingling ($P = 0.007$; Table 2.3). Among the PG+OB+ PMNL cells, treatment did not affect the intensity of phagocytosis ($P = 0.959$; Table 2.3) or oxidative burst ($P = 0.559$; Table 2.3) although Preb-calves had increased PMNL OB intensity on d 14 post-commingling ($P = 0.01$; Figure 2.3B).

Finally, OVA-specific IgA concentrations were influenced by time and the interaction of treatment by time. Preb-calves had an increased secondary IgA response to OVA ($P =0.038$; Figure 2.4A). No treatment or the interaction of treatment by time effects were observed for OVA-specific IgG concentrations.

Discussion

Optimal calf health is vital to a productive dairy operation. Calf health may be influenced by gastrointestinal function as well as immune function. Gastrointestinal function and immune function may be altered in times of stress or increased pathogen exposure. For example, commingling has been shown to alter immune function and performance (Hulbert and

Ballou, 2012). Therefore, prebiotic supplementation was given during the commingling phase to determine its effects on immune function and performance.

A balanced intestinal flora, immune function, motility, and transport of nutrients play a large role in gastrointestinal function (McGuirk, 2010). Prebiotics are fermented by beneficial microflora in the large intestine and colon, producing VFA's that are thought to increase energy efficiency and alter intestinal morphology (Roodposhti and Dabiri, 2012). The resulting alterations to the gastrointestinal tract may improve digestibility and therefore, increase intake and growth parameters. In a study done by Heinrichs et al. in 2003, MOS increased consumption of calf starter at a faster rate and prebiotic-calves consumed more calf starter after weaning than calves fed antibiotic. Similar results were observed by Terre et al. in 2007 when calves fed MOS in milk replacer tended to have increased intake pre-weaning and greater intake the week after weaning compared to unsupplemented calves. Improvements in weight gain, grain intake, and the conversion of feed to gain of MOS-fed calves were reported by Ghosh and Mehla (2012). Results of the current study indicate that Preb-calves had an increase in DMI d 28 to 34 and d 35 to 41. Results of these studies reflect that prebiotics may modulate feeding behavior. In a study done by Yuan et al. (2014), transition dairy cows fed enzymatically hydrolyzed yeast had no difference in DMI or water intake, however the researchers did observe an increase in meal frequency from supplemented cows.

It has also been hypothesized that prebiotics may be more effective on animals under stress or increased pathogen exposure (McGuirk, 2010; Heinrichs et al., 2009; Morrison et al., 2010). Preb-calves had less F:G during the stress of commingling in the current study which supports this hypothesis.

No effects of treatment or the interaction of treatment by time were observed for any of the hematological measures in the current study except for monocyte percent which was less in Preb-calves. All measures fell within the normal range indicating all calves remained healthy throughout the study. In the current study, measures of hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, and mean corpuscular hemoglobin concentration, as well as total leukocyte counts were all affected by time.

One of the primary functions of MOS in the gastrointestinal tract is to bind to mannose-specific fimbriae of gram negative bacteria to prevent adherence and migration into tissue to cause damage. Therefore, MOS may be playing a role before it passes into the periphery by mitigating risk and lessening the need for an immune response. In contrast, one of the predominant functions of BG is to stimulate macrophages, leading to an increase in phagocytic activity or elevated cytokine production. Capitán-Cañadas et al. (2013) suggest prebiotics can directly increase monocytes' production of pro-inflammatory cytokines by TLR4 activation. The pro-inflammatory cytokine TNF- α has the ability to increase neutrophil migration into tissue and enhances the oxidative burst capability of neutrophils. In the current study, the ability of monocytes to secrete TNF-α was less d 14 and 42 compared with Con-calves this may be attributed in part to the Preb-calves having less monocytes throughout the study. However, the oxidative burst response of Preb-calves increased throughout the study compared with Con. Increased production of TNF-α was reported in human monocytes when subjects were administered fructooligosaccharide and inulin (Capitán-Cañadas et al., 2013). There is also research to support that prebiotics have a indirect effect of increasing anti-inflammatory cytokines (Ferket, 2002; Vulevic et al., 2008). These results may be associated with the difficulty of distinguishing between the direct effects of prebiotics on the immune system and the

immunomodulatory effects of the commensal bacteria and fermentation products stimulated by prebiotics. For example, one of the VFA's, butyrate, is known to promote anti-inflammatory cytokines (Schley and Field, 2002). The current study found no differences in IFN- γ. On the contrary, Wang et al. found that lymphocyte proliferation *in vitro* in response to ConA increased linearly with increasing β-1,3/1,6-glucan supplementation in piglets (2008).

Neutrophil L-selectin is an adhesion molecule that helps peripheral neutrophils adhere to the endothelial cell wall and migrate to phagocytize and oxidatively kill pathogens. No differences were seen in L-selectin expression in the current study, similar to L-selectin measured from calves supplemented with probiotics, prebiotics, and hyperimmune dried egg protein (Ballou, 2011). Furthermore, no differences were observed for WB bactericide or phagocytosis which may relate to the excellent health of the calves throughout the study. Oxidative burst response increased throughout the trial for Preb-calves and was elevated on d 14 compared with controls. An *in vitro* study in humans found an increase in neutrophil oxidative burst response and microbicidal activity with beta glucan supplementation (Wakshull et al., 1999). Peritoneal neutrophil respiratory burst activity and neutrophil number were also increased in mice supplemented with oat beta glucan (Murphy et al., 2007).

Adaptive immunity may be influenced by prebiotics. Both OVA-specific IgG and IgA responses were increased with prebiotic treatment. Immunoglobulin A plays an important role in mucosal immunity and is present in two forms, secretory IgA and serum IgA. Secretory IgA is increased with prebiotic supplementation, especially MOS, because of IgA's type-1 fimbriae-binding receptor (Friman et al., 1996). Fecal IgA was increased with a supplement of fructooligosaccharide (FOS) plus MOS (Swanson et al., 2002) and IgA secretion from Peyer's patches and IFN-γ were dose-dependently increased by FOS (Hosono et al., 2003). In a previous

study, challenged calves supplemented with a MOS and BG product had increased bacterial- and viral-specific serum IgA production (Kim et al., 2011). Serum IgG concentrations were improved by 32% and 23% compared to controls in two trials involving mannan oligosaccharide-supplemented piglets (Lazarevic et al., 2010). In a third trial by the same researcher, Holstein calves fed mannan oligosaccharides had an increase in serum IgG concentrations of 39% (Lazarevic et al., 2010). The increase in Ig's seen may be attributed by an increase in CD4+ cells that promote humoral responses (Szymańska-Czerwińska et al., 2009).

Conclusion

The efficacy of prebiotic supplementation may be influenced by dose, environment, stress, and immune status and health of calves. Prebiotic supplementation to weaned dairy heifers during the commingling phase modulated feeding behavior, as determined by an increase in DMI from prebiotic supplementation the last 2 weeks of the trial, as well as changes in the conversion of feed to gain throughout the trial. Prebiotic supplementation during the commingling phase also has an effect on neutrophil oxidative burst response, as well as improved antigen-specific antibody response. Further research is needed to obtain more understanding of prebiotic's effects on feeding behavior and the mechanism by which prebiotics are most efficacious.

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Tables and figures

Table 2.1 Prebiotic effects on performance measures

		Treatments		Largest	*P*-values			
Item	Housing	Control	Prebiotic	SE	SLICE	Trt	Time	Trt*Time
Body weight, kg						0.694	<0.001	0.088
d -7	Ind	71.7	71.1	1.205	0.924			
d 0	Ind	77.5	75.4	1.205	0.026			
d 7	Grp	83.6	82.8	1.208	0.019			
d 14	Grp	90.4	88.9	1.210	0.213			
d 21	Grp	96.5	96.1	1.211	0.138			
d 28	Grp	103.3	103.4	1.213	0.519			
d 35	Grp	110.5	110.4	1.217	0.638			
d 42	Grp	117.2	117.6	1.221	0.440			
[2]Total DMI, kg/d						0.012	<0.001	0.043
d -7 to -1	Ind	2112.5	2124.3	63.3	0.663			
d 0 to 6	Grp	2798.4	2834.8	63.4	0.954			
d 7 to 13	Grp	3057.3	3222.9	64.2	0.185			
d 14 to 20	Grp	3407.8	3598.0	65.9	0.566			
d 21 to 27	Grp	3787.1	4009.4	66.7	0.189			
d 28 to 34	Grp	4125.7	4433.4	67.0	0.020			
d 35 to 41	Grp	4357.3	468.35	67.2	0.007			
[2]ADG, kg/d						0.578	<0.001	0.116
d -7 to -1	Ind	0.89	0.66	0.063	0.026			
d 0 to 6	Grp	0.98	1.08	0.063	0.019			
d 7 to 13	Grp	0.90	0.88	0.064	0.213			
d 14 to 20	Grp	0.97	1.03	0.064	0.138			
d 21 to 27	Grp	1.04	1.03	0.064	0.519			
d 28 to 34	Grp	0.96	1.00	0.064	0.638			
d 35 to 41	Grp	0.96	1.02	0.063	0.440			
[2,3]Feed:Gain						0.774	<0.001	0.001
d -7 to -1	Ind	2.90	4.01	0.255	0.002			
d 0 to 6	Grp	3.58	2.62	0.255	0.001			
d 7 to 13	Grp	3.29	3.95	0.255	0.038			
d 14 to 20	Grp	4.56	3.76	0.260	0.042			
d 21 to 27	Grp	4.22	4.02	0.260	0.486			
d 28 to 34	Grp	4.44	4.53	0.260	0.603			
d 35 to 41	Grp	4.84	4.88	0.260	0.882			

[1] 3 g of prebiotics (10% Mannan oligosaccharide, 18% Beta glucan) were dissolved in 15 mL of 30% molasses

[2] Measures were calculated on a per pen average after commingling (d 0)

[3] *P*-values were log transformed

Table 2.2 Prebiotic effects on hematological parameter

	Treatment[1]		Largest	Time relative to commingling, d				Largest	*P*-values		
	Control	Prebiotic	SEM	-7	7	14	42	SEM	Trt	Day	Trt*Day
Hematocrit, %	29.17	28.75	0.57	28.58^{a,b}	28.00^{a}	29.17^{b}	30.08^{b}	0.601	0.611	<0.001	0.427
Hemoglobin, g/dL	9.97	9.89	0.132	9.82^{a,b}	9.67^{a}	9.94^{a,b}	10.30^{b}	0.153	0.675	<0.001	0.706
Erythrocytes, x10^{12}/mL	8.34	8.25	0.127	8.28^{a,b}	8.09^{a}	8.33^{a,b}	8.50^{a,b}	0.136	0.619	<0.001	0.557
Mean cell volume, fL	34.80	34.81	0.358	34.34^{a,b}	34.56^{b}	34.98^{c}	35.35^{c}	0.323	0.981	<0.001	0.269
Mean cell hemoglobin, pg	11.96	12.01	0.074	11.85^{a}	11.98^{a}	11.95^{a}	12.15^{b}	0.065	0.660	<0.001	0.883
[2]MCHC, g/dL	34.46	34.54	0.298	34.61	34.73	34.26	34.39	0.248	0.844	0.055	0.082
Total leukocytes, x10^{6}	10.70	11.13	0.40	10.20^{a}	10.53^{a}	11.06^{a,b}	11.87^{b}	0.459	0.459	0.001	0.236
Neutrophil, %	33.49	35.10	1.18	35.82	34.59	33.55	33.23	1.554	0.342	0.593	0.117
Lymphocyte, %	43.99	45.37	1.20	45.71	43.84	44.29	44.88	1.401	0.424	0.544	0.297
Monocyte, %	21.54	18.87	0.90	17.69	20.72	21.39	21.03	1.186	0.041	0.095	0.472
[3]Eosinophil, %	0.904	0.705	0.161	0.809	0.916	0.794	0.699	0.179	0.467	0.616	0.258
[3]Basophil, %	0.038	0.026	0.010	0.049	0.794	0.699	0.179	0.024	0.456	0.199	0.093
Neutrophil:Lymphocyte	0.867	0.856	0.056	0.869	0.892	0.857	0.828	0.066	0.894	0.859	0.182

[a,b,c] LS Means differ $P < 0.05$

[1] 3 g of prebiotics (10% Mannan oligosaccharide, 18% Beta glucan) were dissolved in 15 mL of 30% molasses

[2] Mean Corpuscular Hemoglobin Concentration

[3] Log-Transformed *P*-values

Table 2.3 Prebiotic effects on ex vivo blood measures

	Treatment[1]		Largest	Time relative to commingling, d				Largest	*P*-values		
	Control	Prebiotic	SEM	-7	7	14	42	SEM	Trt	Day	Trt*Day
Whole blood stimulated with											
[2]Live *E. Coli* 8739, % cfu killed	80.9	76.2	2.26	77.4	79.8	77.1	79.9	3.11	0.141	0.715	0.515
[3,5]LPS, TNF-α secreted, pg/mL	1089.7	965.6	86.38	712.1[a]	1026.2[b]	1202.7[b]	1169.5[b]	105.86	0.573	<0.001	0.033
[4,5]PHA, IFN-γ secreted, pg/mL	1393.7	1608.5	246.60	798.5[a]	1333.3[b]	1963.8[b,c]	1908.8[c]	275.87	0.373	<0.001	0.936
Neutrophil											
[6]L-selectin, GMFI	105.4	104.3	3.33	118.1[a]	106.9[a]	108.8[a]	85.7[b]	4.31	0.822	<0.001	0.780
[7]PG+OB+, %	72.5	71.3	1.76	75.2[a]	73.2[a,b]	74.3[b]	65.0[c]	2.53	0.611	0.007	0.388
[8]OB+, GMFI	108.1	112.2	5.03	109.2	107.7	112.1	111.5	8.64	0.559	0.971	0.012
[9]PG+, GMFI	70.8	71.0	3.11	76.8	69.6	73.1	64.2	5.24	0.959	0.073	0.201

[a,b,c] LS Means differ $P < 0.05$

[1] 3 g of prebiotics (10% Mannan oligosaccharide, 18% Beta glucan were dissolved in 15 mL of 30% molasses

[2] After whole blood (heparin) was stimulated with live *E. Coli* 8739 for 24 h, the percent colony forming units killed were measured

[3] After whole blood (heparin) was stimulated with LPS for 24 h, supernatant TNF- α was measured

[4] After whole blood (heparin) was stimulated with PHA for 72 h, supernatant IFN- γ was measured

[5] Log Transformed *P*-values

[6] The Geometric mean fluorescent intensity (GMFI) of unstimulated, circulating neutrophils from whole blood (EDTA) was measured

[7] Whole blood (heparin) was stimulated with heat-killed *E. coli* and the percent neutrophils performing both phagocytosis (PG+) and oxidative burst (OB+) were measured

[8] Within the PG+OB+ population of neutrophils, oxidative burst (rhodamine; FL-3) GMFI was measured

[9] Within the PG+OB+ population of neutrophils, phagocytosis (heat-killed *E. Coli* 8739 labelled with propidium iodide; FL-1) GMFI was measured

Figure 2.1

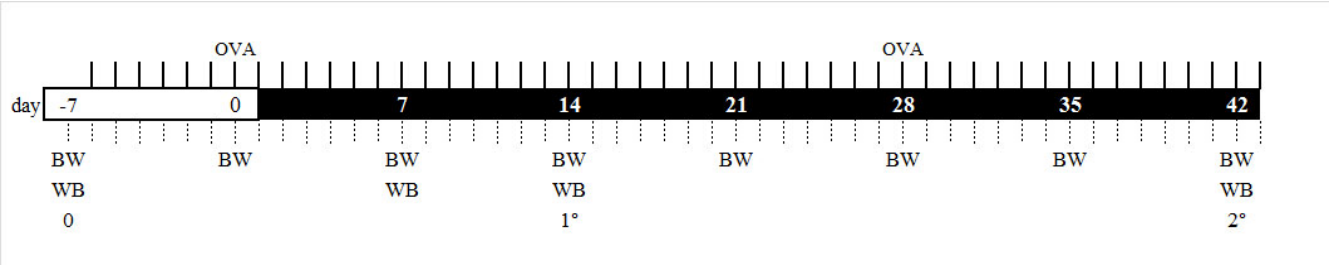


Figure 2.2 Prebiotic effects on TNF-α secretion from LPS-stimulated whole blood

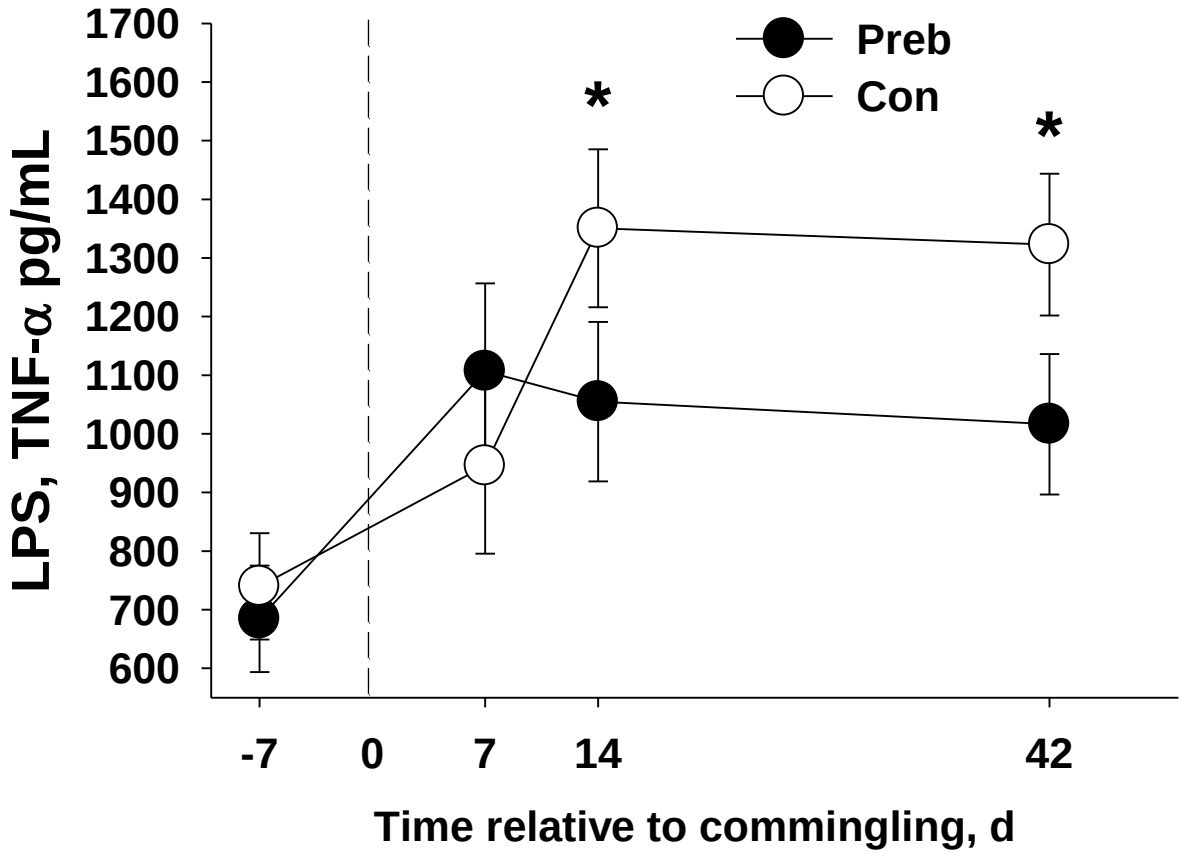


Figure 2.3 Prebiotic effects on neutrophil phagocytosis and oxidative burst response

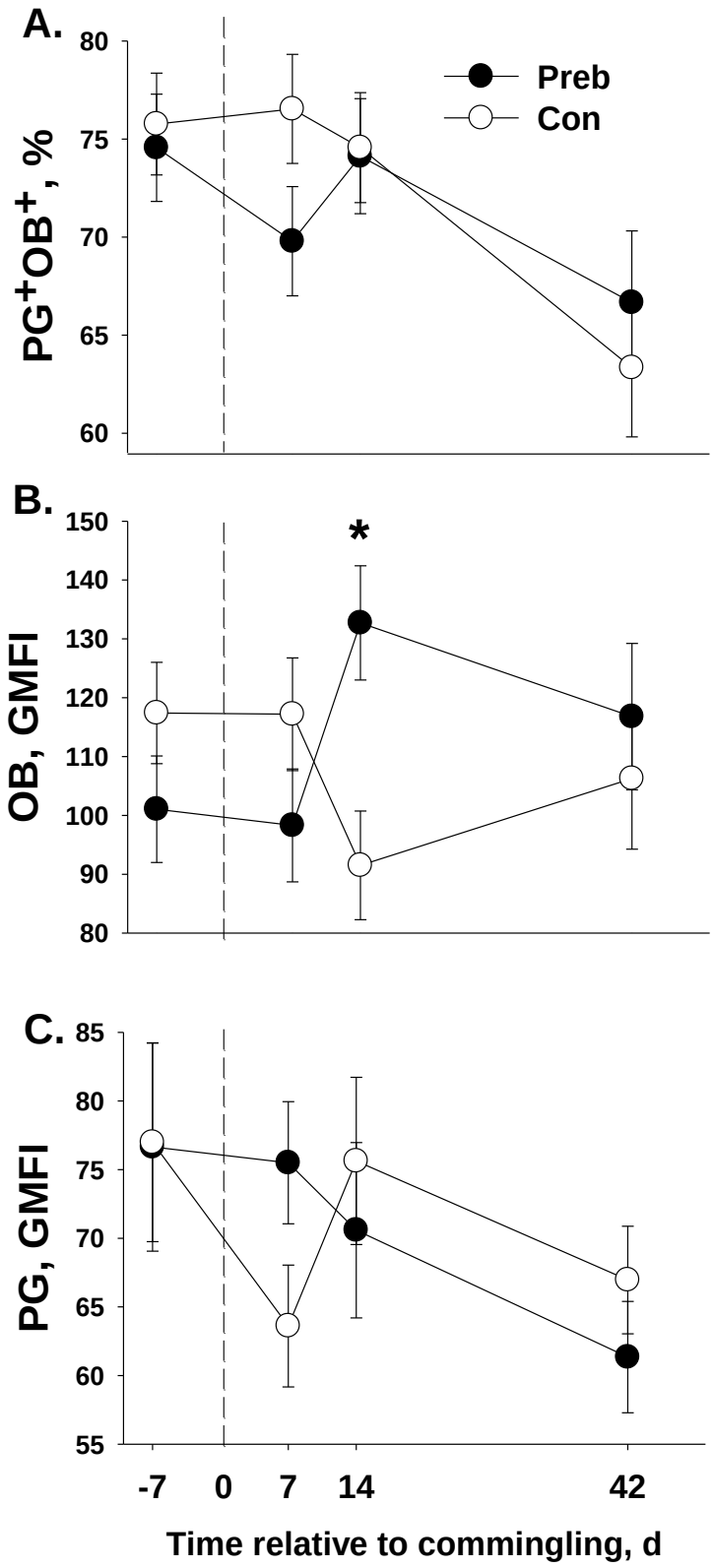
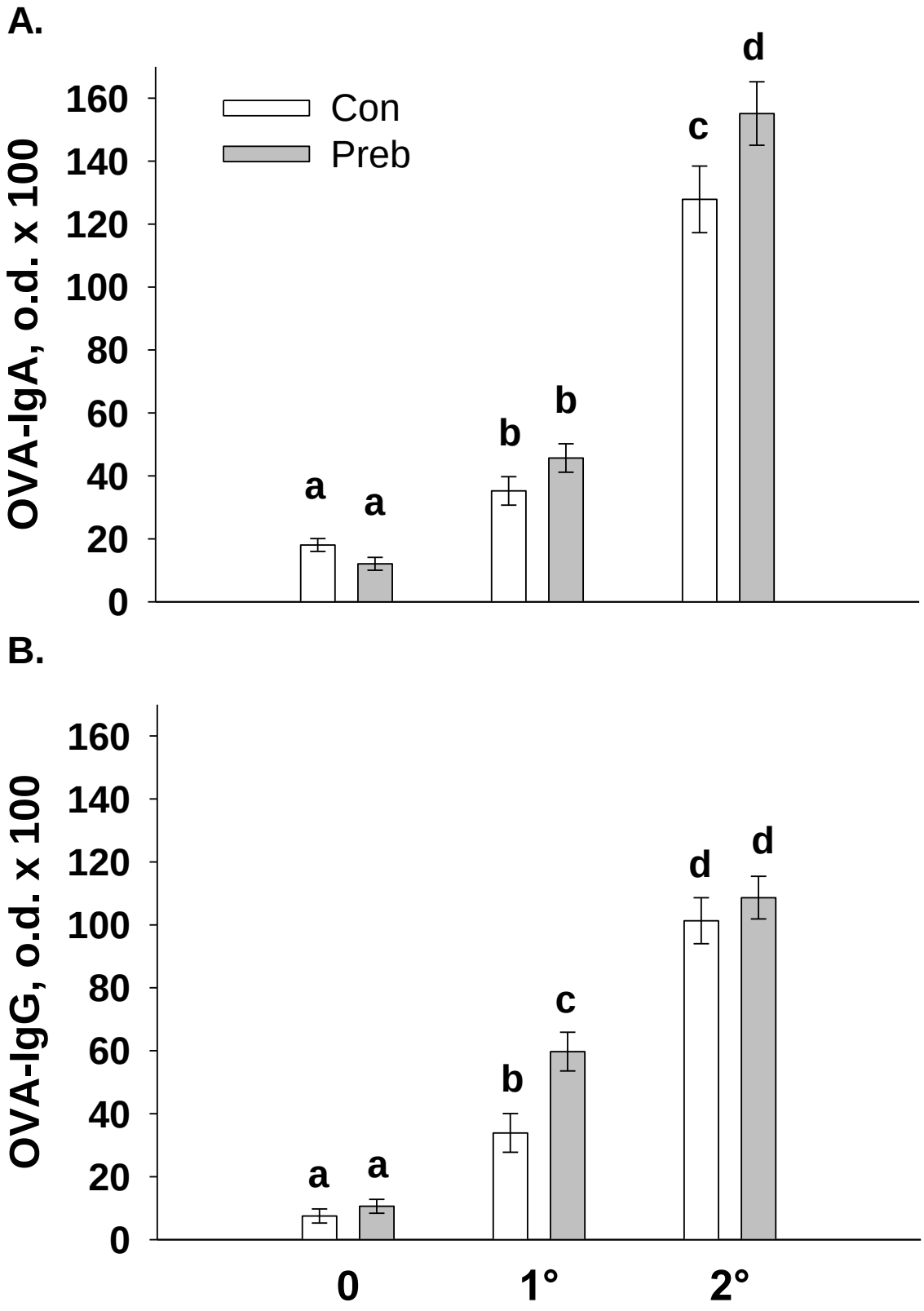


Figure 2.4 Prebiotic effects on OVA-specific IgA and IgG



Chapter 3 - Kansas dairy producer's needs survey

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Abstract

The dairy industry in Kansas has shown rapid growth within the past 20 years, expanding the diversity in size, management practices and educational interests and needs. To establish extension tools and programs most effective in aiding the continuation of growth and profitability of the dairy industry, a survey was distributed to dairy producers throughout the state. Survey results indicate that hands-on demonstrations are the preferred method of educational delivery, as well as having a preference of paper mail over e-mail or newsletters. Topics highlighted include reproduction, milk quality, cow health, and nutrition.

Keyword list: Educational delivery methods, producer needs, educational interests, extension programming, dairy management practices

Introduction

The dairy industry in the United States has witnessed much change in the last 50 years with a trend toward increased milk production per cow with a lesser number of total cows and an increased herd size with a decline in dairy farm numbers (Blayney, 2002). In 2013, Kansas exhibited the largest growth in milk production in the country with an increase of 7.3% (KDA, 2014). This is representative of a 20-year increase in number of dairy cows and milk production in Kansas by 55% and 151%, respectively (KU, 2013). An increase in milk production of 5.1% on January of 2015 from the previous year, indicates that this trend continues (NASS, 2015). These drastic changes have made the dairy industry a progressively more vital part of Kansas agriculture than in the past. However, changes in demographics, herd size, and dairy farm numbers within the state of Kansas have resulted in a large variation in farm characteristics and management practices. The diversity in management practices may warrant different priorities and needs from dairy producers depending on the region of the state. Therefore, a survey was conducted to identify areas of opportunity and need of Kansas dairy producers, and provide useful information for extension specialists and agents to tailor their programs to better serve the dairy producers throughout Kansas.

Materials and methods

A 24 question survey was mailed to 313 Kansas dairy producers throughout the state to evaluate management practices, interest of educational programs, and needs. The survey was administered on a volunteer basis with no reward incentive. Surveys were sent in a hand-addressed envelope containing the survey itself, a cover letter, and a return-addressed envelope. Producers were given a deadline that allotted 45 days for completion. Responses to the survey were anonymous.

General information about the dairy farms were asked in the survey, including herd location, size, employees' characteristics, management practices, productivity parameters, and parameters related to udder health and reproductive performance. Furthermore, respondents were asked to provide information related to training of employees, training delivery methods, and resources used to gain knowledge. In the last section of the survey, participants were asked to report their future plans.

Results

Participation

Responses were received from 81 respondents, resulting in a 25.9% response rate. Ten surveys were received blank and indicated that they were no longer dairying. One respondent indicated that they only had dairy goats. The remaining 70 responses were characterized by region according to the location or county indicated in the survey response: Northeast (n=29), Southeast (n=8), Central (n=24), and West (n=9). A distribution of responses is presented in Figure 3.1.

Herd characteristics

Both small- and large-scale dairy producers participated in the survey. A majority of respondents had a herd size of less than 250 milking dairy cows (80%). The remaining respondents had herd sizes of 251-2,000 (10%) and greater than 2,001 milking cows (10%). Respondents from herds with less than 250 milking cows were predominantly located in the Central and Northeast part of the state, (42.9 and 39.3%, respectively), followed by the Southeast (14.3%) and West (3.6%) regions (Figure 3.1). Respondents from herds with 251-2,000 milking

cows were located in the Northeast region, and all herds with more than 2,001 milking cows were located in the Western region.

Kansas dairy producers estimated an average milk production per cow per day of 64.3 ± 27.3 SD pounds, which is similar to the national average reported in 2014 of 61.0 pounds (USDA, 2015). In the survey, producers were also asked to categorize their average milk production per cow per day during winter (October to May) and summer (June to September) months. The average milk production per cow for winter and summer months was: 62.3 ± 26.2 and 66.3 ± 28.4 SD pounds, respectively (Table 3.1). Similarly, producers reported a greater somatic cell count, key indicator of milk quality, during summer than winter months (254,500 ± 124.3 vs. 222,434 ± 108.4). The yearly average somatic cell count was 238,450, which is less than the 2014 national average of 262,000 (Norman and Walton, 2014).

Producers were asked to estimate the herd's pregnancy rate in a 21-d period, a key indicator of reproductive performance of a dairy herd, during summer and winter months (Table 3.1). There was a variety of responses beyond the expected ranges of pregnancy rate, leading us to believe that some producers may not have a clear understanding how 21-day pregnancy rates are calculated. In this respect, extension tools on performance indicators may be beneficial. In accordance with the other measures, average 21-day pregnancy rate was less during the summer compared with winter (29.8 ± 19.2 vs. 23.7 ± 16.5). The decrease in performance of Kansas dairy cattle during summer months is an indication that heat stress is a major bottleneck to increase efficiency in dairy farms in this part of the United States.

Many dairy farms throughout the country employ workers that do not use English as a first language (Jenkins, 2009). Producers were asked to estimate the percent of employees that speak Spanish as their primary language to assess the need for extension programs to work

towards providing tools for translation and cultural differences. The majority of producers (81.4%) indicated that no employees from their dairy speaks Spanish as a primary language. Of the respondents that had at least one Spanish-speaking employee, 15.4% worked on a dairy less than 250 head, 30.8% worked on a dairy between 251 and 2,000 head, and 53.8% worked on a dairy greater than 2,001 head. Based on these statistics, extension educational materials to dairy employees should be in English and Spanish to ensure material is useful to a broad array of employees.

Management practices and educational needs

Training employees

Nearly half (40%) of the dairy producers responded that they do not formally train their employees (Table 3.2). Among the producers that train their employees, most of them (51.9%) only train employees at the start of employment with the remaining predominantly training their employees every 6 months of more frequently. Dairies predominantly use experienced employees to train new employees. When looking at the combinations of resources used to train employees, 15 responded that they only use experienced employees (27.3%) and 6 responded that they use all resources in combination (10.9%). Extension agents and specialists may be able to aid dairy producers that do not formally train their employees frequently by creating written protocols for employees to reference, as well as providing tools to assist producers in conducting training sessions.

Management meetings

Farm management meetings can be a very valuable practice to help farms operate in a more effective manner and become more profitable. Management meetings can be used to revisit farm goals, ensure goals are being achieved, discuss problems and ideas, and to help

resolve conflicts. The majority (65.7%) of dairy producers that responded to the survey do not conduct farm management meetings (Table 3.3). This might be an area of opportunity for extension agents and specialists to promote meetings with tools such as discussion topics, meeting tips, and conflict resolution advice.

Of the dairies that responded they do conduct management meetings, the most predominant group of people included are managers, owners, and workers with combinations of all three being a popularly noted option. Other dairies have also included veterinarians and nutritionists. Industry professionals, extension personnel, veterinarians, and farm staff all provide their own perspectives and ideas conducive to great meeting opportunities that could be taken more frequent advantage of.

Delivery methods/resources

The advancement of technology has created many different avenues to deliver information. For extension personnel, this aids in distributing information to a vast expanse of people. However, narrowing down which delivery methods are most effective would be beneficial. In this survey, producers have indicated that hands-on demonstrations are the preferred method of educational delivery (Table 3.4). In addition, paper mail or newsletters and on-farm presentations are also desired. Surprisingly, among the less preferred methods are webinars, websites, and e-mails. When asked specifically about the frequency of use of extension websites, 50% said they never frequent the websites and nearly 40% of the producers that responded, dictated that they rarely use extension in any form (Table 3.4). Therefore, making extension websites more user-friendly may promote more frequent use. Sending information provided on the websites by paper mail or newsletters as well as the website, may also benefit producers. Of the producers that do use extension, the most predominant use is to

get help answering questions or problem solving and to gather new information (Table 3.4). Close to 25% of producers indicated that they are planning to expand in the next 5 years, but only 17% currently use extension for help in expansion. There may be an opportunity to help producers make decisions related to expansion such as rearing additional heifers, purchase of heifers, and facility expansion. Consistently, 38-50% of producers say they never use extension resources, which is slightly decreased from the findings in the statewide survey conducted in 2007 that showed 63% never used extension (Boone et al.). Knowing which areas producers would like to improve and would benefit from education or training could help expand the use of extension. Producers exhibited the most interest in topics pertaining to reproduction, cow health, milk quality, and nutrition.

Transition cow management

The transition period is a time of vital importance in mitigating metabolic disease and various challenges related to calving that can consequently affect cow health, reproductive performance, milk production, and longevity in the herd. Proper management and housing peripartum can greatly reduce the risk of these challenges. Mature cows and heifers housed together in close-up pens may exhibit competition at the feed-bunk which could result in problems through parturition. More than half (54.3%) of Kansas dairy producers that responded to the survey separate cows and heifers during the close-up period (Table 3.5). Another management practice used during calving is to move cows and heifers to a maternity pen to calve in a secluded and clean environment that is more accessible to farm employees for care and assistance. Results indicate that only 32.9% move cows and heifers to maternity pens. Whereas maternity pens are not required, it does heighten the need to keep pens clean and keep careful attention from employees to ensure a safe, clean environment for both cows and calves.

Most Kansas cities average temperatures greater than 60 degrees for 7 months of the year (US Climate data, 2015). Dairy cows are said to start experiencing heat stress at a temperature as low as 65 degrees Fahrenheit (Thomas, 2012). Heat stress in dairy cattle can have a significant economic impact. It has been estimated that heat stress causes $5 to $6 billion annual loss in milk production and performance in the United States (Spiers et al., 2004). Decreases in milk production and pregnancy rate accompanied by greater somatic cell counts in the summer months depicts that the effects of heat stress are impacting dairy farms in Kansas. This places much importance on heat abatement strategies such as the use of sprinklers, fans, and shade for cattle. Only 18.6% and 34.3% of survey respondents use these heat abatement strategies on dry cows and fresh cows, respectively, which presents opportunities for improvement.

Reproductive management

Consistently, it has been indicated in the results of this survey that reproductive management is a topic that dairy producers would like to improve and obtain more information on. For extension agents and specialists to be most efficient in providing this information, detailed information about reproductive management practices were evaluated. Many producers (80%) use visual heat detection to identify cows in estrus (Table 3.6). Thirty-six percent of the producers responded that they use chalk or paint as tools to determine whether cows are in estrus and 11.4% use accelerometers or pedometers.

Only 8.6% of Kansas producers use breeders from artificial insemination (AI) companies; therefore, most producers would directly benefit from on-farm presentations or demonstrations on reproductive topics that they would be able to apply on their own operations. Hibbs et al. noted that adult learners prefer to be actively involved in the learning process and apply their learning to current situations rather than auditory learning only (2014). A presentation topic that

may be useful would be on various options for timed-AI protocols seeing that 51.4% of producers use this management practice. Effective and efficient reproductive performance is key in operating a profitable dairy. There is a very substantial amount of research encompassing reproductive tools and management practices to aid in accomplishing the utmost performance.

Future plans

The purpose of this survey was to help industry stakeholders, extension agents and extension specialists format forthcoming programs to fit producers' needs. Gaining knowledge of the up-coming challenges and changes Kansas dairy producers will be facing, could positively influence the appropriateness of programs developed. To gather perspective on the outlook of the dairy industry in the next 5 years, producers were asked about their current five-year plan. The most prevalent response from producers was that they currently do not have any plans for the next 5 years (Table 3.7). Expansion and passing the farm down to a successor ranked among the other top choices. In this respect, decision making tools, information about raising replacement heifers, buying youngstock, and passing down the farm may be of interest to producers.

Producers were also asked about their future plans to improve facilities in the next 5 years. More than 40% of producers would like to improve their parlor, housing for youngstock and lactating cows, as well as waste management facilities. The most popular facility of interest to improve was feed facilities (63.3%). Kansas State already has great resources available to producers about free-stall housing, however tools specifically for parlor improvements and feed facilities may be valuable.

Popular management areas producers would like to improve in the next year included milk quality, cow health, and cow nutrition with reproduction being the most popular (Table 3.7). It is interesting to note that milk quality is among the top management areas to improve

according to producers' responses, however parlor management was the least likely area to be improved in the next year, an area that plays a large role in milk quality. A milk quality extension program conducted in Wisconsin, Milk Money, was estimated to have an impact of increasing milk production (3 kg/day), decreasing bulk tank SCC (43,000 cells/mL), and decreasing clinical mastitis rate (1.52%; Hohmann & Ruegg, 2012). This is an indication that extension programs can be successful in helping producers improve certain management areas of dairy farms. Generally, reproduction, milk quality, cow health, and nutrition are areas that receive much attention. Fascinatingly, more than half of the responding dairy producers indicated that they strive to improve record keeping and risk management. Employee management and training was also indicated as an area of interest to improve.

Conclusions and Implications

In the evaluation of this survey, it is evident that dairy farms range widely in size, production, management practices, and needs. Farms will continue to change with many planning to expand, pass down to successors, and make improvements in management practices and facilities.

The success of dairy farms have a far greater impact than may be realized. In 2013, the value of milk produced by Kansas dairy farmers increased the Kansas economy by approximately $131 million and added 482 local jobs (Kansas Livestock Association). It was also noted that for every dollar spent by dairy farms, three to five dollars was returned to the community through various purchases (Kansas Livestock Association). A study done on the impact of on-farm visits to Vermont dairy farmers indicated that 43% of surveyed dairy farmers felt on-farm extension visits increased their profitability by more than $500 (Calderwood, 1997). Therefore, it is very important that extension agents and specialists are effective in aiding dairy

farmers in producing a high quality, safe product at a profitable margin in accordance with the core values of extension: integrity, communication, scholarship, leadership, and inclusion.

Future extension programs may be most influential focusing on topics such as reproduction, nutrition, milk quality, and animal health with hands-on demonstrations, on-farm presentations, and paper mail or newsletters. However, other measures of distributing information continue to be essential.

Topics of education such as heat abatement strategies, transition cow management, record management, reproductive management, and tools for expansion, parlor and feed facility improvement would be of interest to producers. Farms may also benefit from programs on employee training and conducting management meetings.

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Tables and figures

Figure 3.1 Participant demographics

Table 3.1 Herd performance

	Mean (SD) (n=68)	**Range**
Milk production (lb/cow/d)		
Winter	66.3 (28.4)	18-93
Summer	62.3 (26.2)	21-90
Somatic cell count (x1,000)		
Winter	222.4 (108.4)	100-500
Summer	254.5 (125.9)	110-450
21-day pregnancy rate (%)		
Winter	29.8 (19.2)	10-90
Summer	23.7 (16.5)	4-80

Table 3.2 Employee training

How often do you train your employees?	**% of total** (n=55)
At the start of employment only	51.9
Every 6 months of more frequently	42.6
Once a year	5.6
Every 2 years	0
What resources do you use to train employees?	**% of total** (n=55)
Experienced employees	54.5
I don't formally train my employees	40.0
Extension education programs	25.5
Veterinarian or nutritionist	25.5
Allied industry	18.2

Table 3.3 Farm management meetings

Do you conduct farm management meetings on a regular basis?	**% of total (n=70)**
No	65.7
Once a month or less	27.1
Every other month	0
Every 6 months	2.9
Once a year	1.4
Who is included in the meetings?	**% of total (n=24)**
Owners	91.7
Managers	62.5
Workers	50.0
Veterinarian	33.3
Nutritionist	33.3

Table 3.4 Extension resources

Which of the following educational delivery methods do you prefer?	**% of total** (n=70)
Hands-on demonstrations	61.4
Paper mail/newsletters	44.3
On-farm presentations	42.9
Conferences	34.3
E-mails	12.9
Webinars	10.0
Websites	10.0
Radio programs	4.3
How often do you use extension websites?	
Never	50.0
Once a month	15.7
Once every 6 months	18.6
Once a year	15.7
How have you used extension resources?	
Questions and problem solving	40.0
I rarely use extension	38.6
New information	37.1
Upcoming events	21.4
Expansion of my operation	17.1
How often do you contact K-State county or district extension agents or specialists?	
Never	40.0
Once a month	5.7
Once every 6 months	31.4
Once a year	22.9
What program topics would you most benefit from attending?	**% of total** (n=67)
Reproduction	68.7
Cow health	58.2
Milk quality	53.7
Nutrition	50.7
Calf/heifer management	49.3
Lameness	47.8
Cow comfort	46.3
Transition cow management	40.3
Waste management	25.4
Technology	22.4
Record management	22.4
Employee leadership skills	20.9

Table 3.5 Transition cow management

Which of the following apply to your transition cow management program?	**% of total responses** (n=69)
Heifers and cows are housed separately in the close-up pen	54.3
First lactation and mature cows are housed separately in the fresh pen	18.6
Cows and heifers are moved to a maternity barn/pen as soon as they demonstrate physical signs of labor	32.9
Shades, fans, and sprinklers are used to reduce the effects of heat stress on dry cows	18.6
Shades, fans, and sprinklers are used to reduce the effects of heat stress on fresh cows	34.3

Table 3.6 Reproductive management

Which of the following practices apply to your reproductive program?	**% of total responses** (n=69)
Visual heat detection	80.0
Chalk or paint as heat detection aids	35.7
Accelerometers/pedometers as heat detection aids	11.4
Use breeders from AI companies only	8.6
Utilization of sexed semen in dairy heifers	38.6
Utilization of sexed semen in dairy cows	7.1
Utilization of beef semen in dairy heifers	4.3
Utilization of beef semen in dairy cows	12.9
Timed artificial insemination protocols	51.4
Any kind of bull breeding	44.3

Table 3.7 Future plans

General 5-year plan	**% of Total** (n=72)
No plan	44.4
Expand	23.6
Pass down to successor	15.3
Sell	8.3
Implement robots or time saving technology	6.9
Downsize	1.4
Facilities to Improve in the next 5 years	**% of Yes responses**
Feed facilities (including equipment)	63.3
Housing for calves	47.7
Housing for lactating cows	47.6
Parlor	44.7
Waste management	43.2
Housing for heifers	41.9
Housing for transition cows (dry/fresh)	37.1
Automatic feeders (calves)	3.0
Management Areas to Improve in the next year	**% of Yes responses**
Reproduction	80.4
Milk quality	68.9
Cow health	65.9
Cow nutrition	59.5
Transition cow management	56.1
Calf/heifer management	54.8
Waste management	54.5
Risk management	53.8
Record keeping	51.2
Employee management/training	42.9
Parlor management	39.5

Chapter 4 - Overall conclusions

In concluding both studies, it becomes evident that dairy farming is a very diverse industry. Exemplified by examining varying effects of prebiotic supplementation and the many different management strategies and philosophies, facilities, and practices identified by the dairy producer survey.

Classes that were vital to the completion of these projects and educational training for a master's degree included nutritional physiology and immunology among others. Furthermore, the graduate experience and education utilized to complete this research and master's degree was greatly enhanced by attending extension-led producer seminars and clinics and on-farm extension-led employee training as well as visiting dairy farms throughout Kansas.

When conducting producer-based research, such as research projects on feed additive products like prebiotics, it is especially important to keep in mind the goals and values, management strategies, and resources of dairy farms in the industry. For a product to be beneficial to the industry, it must be something that producers will use and therefore, it is essential to know producer wants and needs and what producers deal with on a daily basis to justify use of a product. Additionally, once a product or protocol has been tested and proven beneficial it is important to be able to communicate this to producers. Therefore, the research trial on the effects of prebiotics and the dairy producer survey tied together very nicely.

Prebiotics are not a replacement for essential management practices such as timely administration of colostrum along with quality and quantity of colostrum, clean environments for calf rearing, and vaccination protocols. However, previous research on prebiotics and the current study have indicated that prebiotics may be able to improve the effects of these management practices. Previous research has shown that prebiotics may enhance the benefits of passive

transfer of maternal immunity and decrease the incidence and severity of scours. Other aspects of prebiotic efficacy may be influenced by environment, immune status, and stress of cattle. Multiple research trials have indicated that prebiotics administered near the stress of weaning may influence calves to increase intake at a faster rate than non-supplemented calves. The current research trial on prebiotics also noted a benefit in average daily gain and feed conversion the week of commingling which could be stressful for some calves. Another useful avenue for prebiotic supplementation may be in adaptive immune response and possibly vaccine efficacy. Earlier studies in conjunction with the current trial have found increases in IgG as well as both serum and secretory IgA responses.

It may be beneficial to conduct future research on prebiotic effects on the efficacy of vaccines used in on-farm protocols. This could be very useful in that vaccine efficacy is hindered by the presence of maternal antibodies up until approximately 6 months of age in dairy calves. Another idea for future projects would be to conduct research on the use of prebiotics prior to administration of antibiotics or in conjunction with antibiotics. The role of antibiotics is to rid the body of bacteria, both commensal and pathogenic. Prebiotics administered with antibiotics or prior to antibiotics may benefit calves' intestinal tract by replenishing the commensal bacteria and helping to fight disease.

Included in the Kansas dairy producer survey was a question on which program topics producers would most benefit from. Cow health was the second most popular answer and nutrition was the fourth most popular answer. This indicates that prebiotics, a feed additive that could positively affect performance and calf health, is a topic that dairy producers may be interested in learning about.

In conducting the Kansas dairy producer survey, we also found that dairy producers predominantly prefer hands-on demonstrations as a method of receiving education and new information, followed by information received by means of paper mail or newsletters and on-farm presentations. Therefore, an additional idea for future research would be to conduct on-farm research trials. This could help develop further information on how prebiotics work on industry operations, show producers first-hand the benefits prebiotics can elicit, and promote word of mouth communication on the idea of prebiotics.

One of the variables studied in many of these research trials that has shown inconsistent results is neutrophil function. One study that may be advantageous in learning more about prebiotic effects on neutrophil function would be to study neutrophil function of calves challenged with an infectious agent. It may also be beneficial to study prebiotic effects on neutrophil function on a large-scale calf ranch or commercial dairy farm. Other variables that would be of interest on a large scale would be body weight gain, feed intake, and feed efficiency, as well as severity and incidence of scours, and overall health. Many studies have included scours as a variable of interest when studying pre-weaned dairy calves. Additionally, many studies have researched the effects of prebiotics on incidence and severity of bovine respiratory disease in beef calves, however it may be interesting to study the incidence and severity of bovine respiratory disease in dairy calves.

In conclusion, prebiotics may be a viable option to improve gastrointestinal health, performance, and immune function. Prebiotics can influence health and performance parameters by stimulating commensal microflora, preventing adhesion of pathogenic bacteria, and increasing digestibility through fermentation. Furthermore, immune parameters may be improved by both mannan oligosaccharides and beta glucans. Mannan oligosaccharides can

improve immune function by promoting mannose binding proteins that can opsonize, phagocytize, and activate the complement system. Beta glucans influence immune function by activating macrophages, enhancing oxidative burst responses, and producing proinflammatory cytokines and chemokines.

Dairy producers have shown an interest in improving calf and heifer management, health and nutrition. Therefore, prebiotics have great potential in the industry. Further research on potential effects of prebiotics, mechanisms, and prebiotics' role in large-scale dairy operations is merited.

Appendix A - Kansas Dairy Producer Need's Survey

Kansas Dairy Producer Survey

Dairy and herd characterisitcs

Q1 What region and county is your dairy located in?

- ❍ Northeast _____
- ❍ Southeast _____
- ❍ Central _____
- ❍ Northwest _____
- ❍ Southwest _____

Q2 What is the average number of milking cows in your herd?

- ❍ Less than 250 _____
- ❍ 251-500 _____
- ❍ 501-1,000 _____
- ❍ 1,001-2,000 _____
- ❍ 2,001-4,000 _____
- ❍ 4,001+ _____

Q3 What is the average milk production (lbs/cow/day) in your herd?

In winter (October-May):

In summer (June-September):

Q4 What is the average somatic cell count (ie. 175,000) in your herd?

In winter (October-May):

In summer (June-September):

Q5 What is the average 21-day pregnancy rate in your herd?

In winter (October-May):

In summer (June-September):

Q6 What percentage of employees speak Spanish as their primary language on your dairy?

- ❍ 0%-Nobody
- ❍ 1% - 20%
- ❍ 21% - 49%
- ❍ 50% - 100%

General management practices, educational, and training information

Q7 Approximately how often do you train your employees?

- ❍ At start of employment only
- ❍ Every six months or more frequently
- ❍ Once a year
- ❍ Every 2 years

Q8 Which of the following resources do you rely on to train your employees? (Check all that apply)

- ❍ Allied Industry
- ❍ Extension educational programs
- ❍ Experienced employees at my dairy
- ❍ Veterinarian or nutritionist
- ❍ Other _____
- ❍ I don't formally train my employees

Q9 Which of the following education delivery methods do you prefer? Check all that apply)

- ❑ Radio programs
- ❑ Hands-on demonstrations
- ❑ Conferences
- ❑ Webinars
- ❑ Websites
- ❑ Paper mail or newsletters
- ❑ E-mails
- ❑ On-farm presentations
- ❑ Other _____

Q10 Do you conduct farm management meetings on a regular basis?

- ❍ No
- ❍ Once a month or less
- ❍ Every other month
- ❍ Every 6 months
- ❍ Once a year

Q11 If you answered yes to the previous question, who is included at the meetings? (Check all that apply)

- ❍ Managers
- ❍ Owners
- ❍ Workers
- ❍ Veterinarian
- ❍ Nutritionist
- ❍ Other: _____

Q12 How often do you use various university extension websites?

- ❍ Never
- ❍ Once a month
- ❍ Once every six months
- ❍ Once a year

Q13 How have you used extension resources?

- ❍ Upcoming events
- ❍ Expansion of my operation
- ❍ Questions and problem solving
- ❍ New information
- ❍ I rarely use extension

Q14 How often do you contact K-State county or district extension agents, or state extension specialists?

- ❍ Never
- ❍ Once a month
- ❍ Once every six months
- ❍ Once a year

Q15 Which of the following educational program topics would you or your employees mostly benefit from attending? (Check all that apply)

- ❑ Nutrition
- ❑ Reproduction
- ❑ Milk Quality
- ❑ Cow health
- ❑ Cow comfort
- ❑ Lameness
- ❑ Technology
- ❑ Record management
- ❑ Employee leadership skills
- ❑ Transition Cow Management
- ❑ Calf/Heifer Management
- ❑ Waste Management

Q16 Which of the following practices apply to your transition cow management program (3 weeks before and 3 weeks after calving)? (Check all that apply)

- ❑ Heifers and cows are housed separately in the close-up pen (approximately 21 days before calving)
- ❑ First lactation and mature cows are housed separately in the fresh pen
- ❑ Cows and heifers are moved to a maternity barn/pen as soon as cows demonstrate physical signs of labor
- ❑ Shades, fans, and sprinklers (all three) are used to reduce the effects of heat stress on dry cows
- ❑ Shades, fans, and sprinklers (all three) are used to reduce the effects of heat stress on fresh cows

Q17 Which of the following practices apply to your reproductive program? (Check all that apply)

- ❑ Visual heat detection
- ❑ Utilization of chalk or paint as heat detection aids
- ❑ Utilization of accelerometers or pedometers as heat detection aids
- ❑ Utilization of breeder(s) from AI companies (do NOT have in-house breeders).
- ❑ Utilization of sexed semen in dairy heifers
- ❑ Utilization of sexed semen in dairy cows
- ❑ Utilization of beef semen in dairy heifers
- ❑ Utilization of beef semen in dairy cows
- ❑ Timed artificial insemination protocols
- ❑ Any kind of bull breeding

Plan for the future

Q18 Check the box(es) that most closely match your 5 year plan:

- ❑ Downsize
- ❑ Expand
- ❑ Sell dairy
- ❑ Pass down to successor
- ❑ Implement robots or other labor saving technology
- ❑ No plans at this time

Q19 Which ***facilities*** do you plan to improve in the next 5 years? (Check all that apply)

_____Parlor

_____ Housing for calves

_____ Housing for heifers

_____ Housing for lactating cows

_____ Housing for transition cows (dry/fresh)

_____ Waste management

_____ Feed Facilities (including equipment)

_____ Automatic feeders (calves)

Q20 Which ***management*** areas do you plan to improve in the next year? (Check all that apply)

_____ Record Keeping

_____ Employee Management & Training

_____ Cow Nutrition

_____ Waste Management

_____ Parlor Management

_____ Transition Cow Management

_____ Calf/Heifer Management

_____ Reproduction

_____ Milk Quality

_____ Cow health

_____ Risk management

Q21 How do you think K-State Research and Extension should help support the dairy industry in the state?

Q22 What are some of your concerns about your operation?

Q23 What are some of your concerns about the Kansas dairy industry?

Q24 What are some of your concerns about the US dairy industry?