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Evaluating the Influence of the Inclusion of Liquid Brewers Yeast during Gestation on Ewe and Lamb Performance, Colostrum and Milk Quality, and Transfer of Passive Immunity in Lambs

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EVALUATING THE INFLUENCE OF THE INCLUSION OF LIQUID BREWERS YEAST DURING GESTATION ON EWE AND LAMB PERFORMANCE, COLOSTRUM AND MILK QUALITY, AND TRANSFER OF PASSIVE IMMUNITY IN LAMBS

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The microbrewery industry has experienced exponential growth since 2005. As a result, the by-products associated with this industry are becoming increasingly available. One of these by-products is liquid brewers yeast, which is produced in excess during the production of beer. For livestock producers that have access to microbreweries, a potentially economical way to feed their flock would to be repurpose the by-product that would otherwise be disposed. Similarly, microbreweries would benefit from repurposing the liquid brewers yeast as livestock feed, resulting in less waste and a more sustainable beer production system.

Researchers have evaluated the effect of liquid brewers yeast supplementation in other species, especially cattle. Literature pertaining to its influence on performance measures, colostrum quality, and the transfer of passive immunity has been inconsistent. Furthermore, while research has investigated the use of liquid brewers yeast in cattle, there is currently limited research on feeding liquid brewers yeast to small ruminants. Due to the lack of literature published, small ruminant producers may be hesitant to utilize liquid brewers yeast in their sheep diets, even though there is an increasing availability of the potentially valuable feed ingredient.

The objective of this research was to evaluate the effects of liquid brewers yeast on late gestation ewe and lamb performance, colostrum quality, and the transfer of passive immunity. In

the first experiment, from 4 weeks prior to expected lambing to 4 weeks post-lambing, ewes were group housed and fed either a control diet, or the control diet with liquid brewers yeast included at a rate of 32 g of DM/hd/d. Findings of this research indicated no significant effect of liquid brewers yeast supplementation on ewe body weight (BW; $P = 0.31$), body condition score (BCS; $P = 0.97$), and F:G ratio at start of experiment ($P = 1.00$), prior to lambing ($P = 0.82$), post lambing ($P = 0.94$), and at the end of experiment ($P = 0.79$). Additionally, treatment did not have a significant effect on colostrum or milk quality at 2- or 4-wk post lambing ($P = 0.22$, $P = 0.64$, $P = 0.13$, respectively) or lamb ADG ($P = 0.77$).

In the second experiment, ewes received an increased rate of 60 g of DM/hd/d of liquid brewers yeast. All ewes in this experiment were immunologically challenged with a parainfluenza-3 vaccine and antibody titer levels were monitored throughout the experiment. The supplementation of liquid brewers yeast did not result in significant differences in antibody titer levels (195 for control group and 122 for treatment group; $P = 0.30$) or colostrum IgG concentrations between control (30.4% Brix) and treatment (24.6% Brix) ewes ($P = 0.21$).

Results from these two studies suggest there are no improvements in animal performance, colostrum quality, or offspring immune status with the rate of liquid brewers yeast provided. However, these findings also suggest that the inclusion of liquid brewers yeast in late gestation diets of sheep does not negatively affect the parameters evaluated. Additional research is warranted to identify what level of liquid brewers yeast supplementation may have a beneficial effect on sheep and lamb performance, colostrum and milk quality, and the transfer of passive immunity in lambs.

KEYWORDS: colostrum, feed efficiency, immunity, liquid brewers yeast, milk quality, sheep

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INTRODUCTION AND BACKGROUND

In the U.S., there has been exponential growth in the number of microbreweries over the last several years. With an increase in microbreweries, comes an increase in availability of their associated by-products. The by-product liquid brewers yeast comes from the fermentation step of beer production and is produced in excess during this process. Microbreweries have additional expenses tied to the disposal of the excess yeast because of costs associated with discharging the yeast to sewage systems or shipping product for further processing to dried brewers yeast.

The most common strain of yeast utilized in beer production is *Saccharomyces cerevisiae*. This strain of yeast is composed of mannan oligosaccharides, which are responsible for improvements in immune function and cell-to-cell recognition, and 1,3-beta-glucans, which are responsible for productivity, immunity, and physical strength of the cell. In addition, yeast contains a variety of proteins, vitamins, and minerals, which has been shown to benefit livestock.

Liquid brewers yeast has been the focus of numerous animal nutrition research projects, in a variety of species, due to the potential nutritional benefit it provides. Many of the original studies evaluated liquid brewers yeast from large commercial breweries, which is a more consistent product due to the large batch sizes produced at these facilities. The liquid brewers yeast from today's microbreweries is much less consistent due to the smaller batch sizes and variety of beers being brewed. The composition of the associated liquid brewers yeast varies considerably.

Much of the animal research supplementing liquid brewers yeast has been done in ruminants. However, published research on the effects of liquid brewers yeast on small ruminants is limited. Some studies have shown benefits in feeding liquid brewers yeast, while

others have shown no effect, suggesting more research is warranted. The objective of this Master's Thesis was to determine the effect of microbrewery liquid brewers yeast inclusion on sheep and lamb performance, colostrum and milk quality, and transfer of passive immunity in lambs.

CHAPTER II

LITERATURE REVIEW

Brewery Industry

The first fermented beverage was reported in the seventh millennium B.C. in China (Cabras and Higgins, 2016). However, from 7000 BC to the 1800s, there was great variation in the ingredients utilized, the fermentation processes employed, and the amount of alcohol produced (Cabras and Higgins, 2016). Methods to prevent spoilage of final product were also not well understood. Scientists today report that the phosphoric acid released from barley during processing assisted in decreasing the spoilage rate (Farag et al., 2019). Otherwise, beer would quickly go flat after production, especially Ancient Egyptian beers.

Until the nineteenth century, there was no 'standardized' beer product (Cabras and Higgins, 2016). However, with technological advances and the development of refrigeration and pasteurization in the 1900s, beer brewing became more standardized in its process. The knowledge of the fermentation process improved, including controlling the environment, the type of fermentation, and the yeast cultures used. Beer production increased, however, the brewery industry faced challenges when beer production and consumption decreased due to the World Wars. These historical events, along with the Great Depression and the Dust Bowl, forced brewers to source less expensive ingredients to produce beer (Cabras and Higgins, 2016). Consequently, the use of corn and rice significantly decreased and was replaced by malted barley, which currently remains as the most common grain source for U.S. beer production.

From post-WWI to 1980s, the market effect of supply and demand resulted in a decrease in the number of independent breweries, as smaller brewers were bought out by larger breweries. By the early 1980s, there were four major companies that accounted for 75% of the beer market

in the U.S. They were Anheuser-Busch, Miller Brewing Company, Coors Brewing Company, and Pabst. In 1980, there were about 92 micro and craft breweries in the United States (Cabras and Higgins, 2016). Craft breweries are small, independently owned breweries that account for 3% of the U.S. annual sales. A microbrewery is a craft brewery that produces less than 15,000 barrels of beer per year. Specifically, the Brewers Association classifies a craft brewery as a microbrewery if it is selling 75% or more of their product off-site (Brewers Association, 2022).

From 1985 to 1995, the number of microbreweries in the United States doubled (Lynn et al., 2009). In 1998, microbrewery businesses accounted for 2.6% of shares in the U.S. beer market, with 420 microbreweries. In 2005, the microbrewery population in the U.S. started to grow at an increasing rate (Moore and McLaughlin, 2016). By 2013, with 1,492 microbreweries, the microbrewery business accounted for 7.8% share of the U.S. beer market (Moore and McLaughlin, 2016). By 2014, there were 2,116 microbreweries in the U.S. (Cabras and Higgins, 2016). Research from 2016 reports that in a five-year period, the number of microbrewers had more than tripled in number in the U.S. (Callen and Hait, 2018). In 2018, the number of microbreweries in the U.S. was 4,518 (Brewers Association, 2022).

There are several by-products associated with beer production. The increased growth of the microbrewery business created more production of and an increase in availability of these various by-products. With by-products becoming more available, research such as that which is presented in the following chapters provides insight in helping microbreweries find an outlet for their liquid brewers yeast.

Brewery Process

There is not one single brewing process that all U.S. microbreweries utilize but rather several variations of the general fermentation process. This is especially true regarding

microbreweries, as many create unique products by modifying one or more steps of the process. Generally, the process starts in a malt house where barely (most common), wheat, oats, or rye are germinated to fully ripened grains. During germination, the starches in the grains are converted to sugars by enzymes within the plant. Then, these grains are kilned or toasted, which will contribute to final flavor and color of the beer. Once dried, the product is considered malt (English, 2017). The malt is then ground, where specialized dry milling techniques work to expose the center of the seed, maximizing carbohydrate and sugar availability. This process can vary and is dependent on the grain used, as the difficulty of hull removal varies by grain (Morvant, 2014; McHugh, 2017).

Next is the mashing process where the milled product is transferred to a large vessel called a mash tun where it will be mixed with water. To produce high quality beer, the water calcium, magnesium, and pH levels must be within proper ranges to begin to hydrolyze the sugars. Interestingly, because of this, water quality has a significant impact on the quality of the final brewing product (English, 2017). As the starch hydrolysis enzymes from the plant convert starches to small fermentation sugars (a process called saccharification), the temperature of the mash must be closely monitored as too high of temperatures can deactivate these enzymes. After two to three hours of mashing, the end product is transferred into a vessel called a lauter tun. The spent grains settle to the bottom of the vessel and the wort (liquid portion) drains through the spent grains which act as a filter. To ensure fermentable sugars are extracted from the grains, an additional process called sparging occurs. During sparging, water is added gradually back to the vessel of spent grains. It remains there for a short period of time before the wort is separated from the spent grains again in the lautering process (Morvant, 2014; English, 2017; McHugh,

2017). At this point, breweries often source spent brewers grains to local livestock producers that utilize it as a feed source for their herds. Spent brewers grains will be further discussed later.

The next step in the beer production process is boiling, which occurs in the brew kettle. In this step, hops, which create bitterness to balance the sugary flavor, are added to the wort, and boiled for one to two hours. In addition to balancing the sweetness, boiling also sterilizes the wort. After boiling, the mixture is transferred to a whirlpool that serves as a filter to remove the hops. To prevent oxidation and production of undesired flavors, the wort is cooled quickly. The wort is transferred to a large vessel and yeast is added to begin the fermentation process. As the wort ferments, the yeast absorbs sugars and converts them into ethanol (or alcohol) and carbon dioxide (Morvant, 2014; English, 2017; McHugh, 2017). The strain of yeast added and the temperature at which the wort ferments can produce different beer types. The yeast *Saccharomyces cerevisiae* is utilized in warm fermentations to make ale beers while *Saccharomyces pastorianus* and *Saccharomyces carlsbergensis* are utilized in cool fermentations to make lager brews (Jaeger et al., 2020). After the primary fermentation, the yeast absorbs undesired flavors created by fermenting in the conditioning stage, which can take a week up to several months. The temporarily inactive yeast, called liquid brewers yeast, is removed from the fermentation vessel following conditioning (Morvant, 2014; English, 2017; McHugh, 2017).

The final processes for beer production are pasteurization and packaging. To prevent further yeast activity and alcohol production, the beer is pasteurized by heating and cooling for a 56 min period. The beer is transferred to cans, bottles, or kegs for shipment or wholesale (McHugh, 2017; Karatas et al., 2022).

Brewery By-Products

By-products from the brewery industry come from various parts of the beer production process and have been evaluated for a variety of uses. The size of the brewery often dictates how the by-products are used. Larger commercial breweries that produce large quantities of a consistent product have been more successful in identifying an outlet for their by-products compared to smaller craft breweries that produce smaller quantities of more variable products. The primary by-products include brewers grains, spent hops, and brewers yeast.

Breweries have found an outlet for the by-product of brewers grain as it has been successfully utilized as livestock feed. Spent hops have been repurposed to farmlands as a form of reconditioning of the soil. Brewers yeast by-products have been dried and utilized in livestock feed. For smaller breweries, the economic barrier associated with drying the liquid brewers yeast is often too large, not making it a sustainable form of disposal (Ingledew and Burton, 1979). As a result, brewers yeast has been disposed of through sewage systems. Canadian brewers were of the first to investigate alternative disposal methods, as cost of sewage disposal was high (Steckley et al., 1979).

Wet Brewers Grains

Wet brewers grains (WBG) or spent brewers grains are brewery by-products that have been incorporated in livestock feeds for several years. This by-product is filtered out as a high-moisture residual after the mashing process and formation of wort (Mussato et al., 2006). This by-product commonly consists of spent cereal grains of barley, wheat, rice, and corn.

Practical incorporation of this product in livestock diets is complicated due to its variation in nutritional and physical composition. The nutritional profile of WBG includes high crude protein (24.9 – 34.2% DM), crude fiber (8.3 – 15.7% DM), and total digestible nutrients

(TDN; 76 – 86% DM), while containing a moderate concentration crude fat (7.6 – 10.7% DM; Thomas et al., 2010). It contains low calcium and potassium with a ratio of 1:1, where the National Research Council recommends 7:1 for ruminants. The variability of WBG is due to the type of beer brewed (i.e., ingredients and yeast utilized), and further varies from one brewery to another (Thomas et al., 2010; Hueze et al., 2015; Parmenter, 2017). Due to variation in nutrient content of WBG, the animal's requirements for optimal performance may not be met and additional supplementation, particularly in the form of minerals, is likely required to balance the diet (Parmenter, 2017). This can have an economic impact on producers and may influence their decision to utilize WBG in their diets.

Another special consideration associated with utilizing WBG in diets is its transportation and storage due to its high water content of approximately 74% (Thomas et al., 2010). Since the product is high in water, transportation of WBG can be challenging and expensive. If the distance traveled from brewer to farm is greater than 200 miles, there are economical losses associated with transporting WBG (Thomas et al., 2010). Wet brewers grains are typically stored in an on-farm bunker silo or a plastic silage bag. After five to seven days, the product will begin to spoil due to its high moisture content. This results in increased mold growth, decreased moisture content, and decreased palatability (Thomas et al., 2010). Increases in environmental temperature above 20°C for more than six days or above 35°C for more than one day results in spoiling and loss of nutrients (Wang et al., 2014; Kitaw et al., 2022). Storage of WBG becomes further complicated during cold temperatures as freezing of the product commonly occurs, preventing accurate weighing and mixing of the ingredient in a ration (Parmenter, 2017). Inability to utilize this ingredient due to environmental conditions can result in an economic loss to the producer.

Finally, WBG have been shown to decrease dry matter intake in ruminants. Due to the structural volume of plant water held in its cell wall, consumption of WBG creates a greater filling effect compared to silage or fresh forages alone (Thomas et al., 2010). Dry matter intake decreases by 0.2 lb/100 lb of body weight for every 10 % increase of moisture content in the diet in cattle. The recommended incorporation of 30 to 50 pounds of WBG in a cow's diet per day contains 7.8 to 13 pounds of dry matter (Balch and Campling 1962; Van Soest 1982; Allen 1996; Thomas et al., 2010). Despite a decrease in DMI, WBG it has been found to be a valuable source of nutrients when incorporated into ruminant diet with other feedstuffs.

Spent Hops

During the boiling process of brewing beer, hops are added to create bitterness to balance the sugary taste. After a few hours the spent hops are filtered out through a whirlpool (Morvant, 2014). Due to its bitterness, it is not utilized as a feed additive in livestock. This by-product has a high nitrogen profile that can serve as a fertilizer. It can also be used in gardens as a mulch to keep down the weeds (Karlovic et al., 2020).

Brewers Yeast

Liquid brewers yeast is also a by-product of the brewing industry. Yeast is used in the beer production process to convert sugars from grains into alcohol. As yeast cells grow and multiply through the utilization of nutrients in the wort, they metabolize sugars and produce alcohol, carbon dioxide, and multiple flavor compounds (Jaeger et al., 2022). Yeast is utilized in the fermentation process and is collected at the end of this step (Hertrampf et al., 2000). In *Saccharomyces cerevisiae* strain batches, the thousands of yeast cells present undergo yeast flocculation in which the cells form into flocs, separate from the wort, and rise to the surface of the fermentation vessel (Verstrepen et al., 2003; Jaeger et al., 2020). The wort is collected via the

bottom of the fermentation vessel, leaving the yeast flocs, which are collected separately. A small amount of this yeast can be used in the next fermentation batch and, usually, the excess is discarded.

Through the fermentation process, yeast yields five times more yeast than the required amount for fermenting subsequent batches of beer, making it the second largest by-product of the brewing process (Crawshaw 2004; Huige 2006; Colpo et al., 2021). Breweries can reuse a portion of the excess yeast in subsequent batches. The capability of yeast to ferment does not change from first time use to reuse. However, the impact of the reused yeast is highly dependent on the yeast strain, the wort the yeast was previously used in, and company policy (Kordialik-Bogacka and Diowksz, 2013). The limit for the number of times a yeast culture could be reused has not yet been defined but it is not unusual for breweries to reuse yeast up to twenty times (Powell et al., 2003).

Other than reusing the by-product, there are various methods for disposing of the excess yeast. Research in 1950 stated that yeast by-products were usually sent to food processing plants, where they were repurposed to provide vitamins and meat flavorings (Schneider, 1950). Another method of disposal of brewers yeast was discharging into local sewage systems. Canadian brewers were of the first to investigate alternative disposal methods, as the cost of sewage maintenance was high when brewers yeast was discharged into local sewer systems (Steckley et al., 1979). A final disposal method involves a complete moisture removal to produce a dry brewers yeast product. The advantage of this process is that the product has longer shelf life and can more easily be incorporated into animal feeds compared to the liquid brewers yeast. However, for smaller breweries, this method presents an economic barrier as they do not produce a large enough quantity of a consistent product to warrant further processing to produce dried

brewers yeast. Thus, drying is not an economical means of disposal for microbreweries (Ingledew and Burton, 1979).

Yeast Saccharomyces cerevisiae

As the most common yeast strain used in beer production, the cell wall of *Saccharomyces cerevisiae* is composed of mannan oligosaccharides (Utsugi et al., 2002; Linneen et al., 2014). The yeast cell wall consists of two layers. The inner layer makes up approximately 50-60% of the cell wall dry weight and is composed of chitin and 1,3-beta-glucans that are responsible for the physical strength of the cell. The outer layer is responsible for cell-to-cell recognition and is composed of mannoproteins, which are proteins that bond to carbohydrate molecules in the mannan oligosaccharides (Klis et al., 2002).

In addition to being a source of nutrients, *Saccharomyces cerevisiae* has been characterized as a natural immunostimulator in livestock because of the mannans and glucose polymers in the cell wall. The mechanism has been described as a stimulation of immunocompetent cells, where the intercellular defense mechanisms are activated by mannans and glucans (Milewski et al., 2013; Zaleska et al., 2015). Mannan oligosaccharides have been found to be responsible for the improvement in immune function and reduction of invading pathogens in swine (Newman and Newman, 2001; O'Quinn et al., 2001) and bovine (Srinivasan et al., 1999; Newman, 1994). This is due to mannan stimulating immune cells to fight current infection or prevent infection to the host against mannan-containing pathogens (Srinivasan et al., 1999; Newman, 1994). Research also reports that beta-glucans have shown a similar boost to the immune function and an increased resistance to invading pathogens (Utsugi et al., 2002; Volman et al., 2008). In 2008, Magalhães and others found yeast products had a positive effect on dairy calf survival and enhancement of calf health. However, the components of yeast culture and their

respective mechanisms were unknown. In more recent research, beta-glucans have been shown to improve productivity and immunity in the transition Holstein cow, as well as increase immunoglobulin levels in cow colostrum (Xia et al., 2021). In dogs, the supplementation of beta-glucans significantly improved biological and immunological conditions, like increasing phagocytic activity of blood monocytes and neutrophils and antibody responses (Vetvicka and Oliveira, 2014). In contrast, in broiler chickens, a study evaluated the effects of yeast culture on immunomodulatory functions, measured as serum IgG concentrations, found no significant impact (Gao et al., 2008). The lack of consistent results suggests there is a need for additional research on the effect of yeast supplementation.

Additionally, yeast is high in proteins, vitamins, polyphenols, and antioxidants (Jaeger et al., 2020). *Saccharomyces cerevisiae* is high in minerals like iron, manganese, zinc and copper, and an excellent source of selenium (Yamada et al., 2005; Halasz and Laszity, 1991). With the nutritional value yeast provides, researchers have evaluated its effectiveness as a nutritional supplement for livestock.

As mentioned previously, liquid brewers yeast can be dried to yield dried brewers yeast. Dried brewers yeast can be provided as live yeast cultures (serving as a probiotic) or yeast extract, which is obtained by separating yeast cell walls from the cell interior (serving as a prebiotic; Dobicki et al., 2007). Researchers report dried brewers yeast has immunomodulatory properties when supplemented to ewes. This mechanism is described as a stimulation on the humoral and cellular defense against pathogens (Milewski and Zaleeska, 2011). The ability to incorporate dried brewers yeast into animal diets depends on transportation, storage, and mixing capabilities. However, for smaller breweries, this method is not economical viable because not

enough product is being produced to account for costs associated with sending the product for drying.

Sheep Immunity

Ewes provided good nutrition are more likely to produce more vigorous lambs. Especially in the last trimester, when fetal growth/development is most rapid, adequate ewe nutrition is important for the support of lamb growth and lamb survivability (Charrani et al., 1991; Binns et al., 2002; Nielsen et al., 2013; Barkley, 2016). Ruminants have placental barriers that prevent the passage of maternal antibodies through the placenta. Since lambs do not receive the transfer of passive immunity in utero, they are born with no protective level of immunity (Ahmad et al., 2000; Tizard et al., 1992; Borghesi et al., 2014; Poitras et al., 1986). The opportunity for the transfer of passive immunity does not occur until lambs suckle their dam and absorption of colostrum antibodies is achieved (Doaa et al., 2009). The most critical period of a lamb's life is their first 24 to 48 h. If lambs acquire adequate immunity levels via colostrum, the failure of transfer of passive immunity can be prevented and therefore increasing lamb survivability (Esser et al., 1989; Doaa et al., 2009; Barkley, 2016). The absorption of maternal antibodies effects the health of lambs in that the inadequate absorption may lead to lambs developing an infectious disease (Vihan, 1986; Hodgson et al., 1992; Doaa et al., 2009). Since the dam has developed her own immunity to environmental pathogens, it is crucial that she nurses her offspring to prevent lambs from having to fight these pathogens utilizing their own, underdeveloped immune system. For example, at birth a pathogenic microorganism invasion can overcome a lamb's health, but not necessarily affect the dam since she has the immunity to fight the invasion (Banks, 1982; Ahmad et al., 2000). For this reason, the transfer of passive immunity is especially important and essential and is achieved only through colostrum intake.

The majority of antibodies or immunoglobulins (Ig) in colostrum and serum are in the form of immunoglobulin G (IgG). In colostrum, IgG account for 90% of total antibodies and 80% of antibodies in serum (Hatfield et al., 2000). These Ig are especially important in the development of offspring immunity. The absorption of Ig occurs via pinocytosis in the small intestine. These Ig are transported to lymphatic tissue, where they enter the circulatory system to facilitate neonate immunity. Inadequate absorption of maternal Ig can have negative effects on offspring health and productivity (Seymour et al., 1995; Bettencourt, 2021). Literature states that there is a positive correlation between low concentrations of serum Ig and development of infectious diseases that result in lamb mortalities (Vihan, 1986; Hodgson et al., 1992; Doaa et al., 2009). Within the first 24 h of parturition, the colostrum IgG concentration begins to decrease drastically (Borghesi et al., 2014). The failure of transfer of passive immunity has a dramatic impact on neonatal lamb mortality in that the survival of the lamb is dependent on the immunity it acquires through its dam's colostrum in the first 24 to 48 h after birth (Esser et al., 1989; Doaa et al., 2009).

Published research has looked at multiple methods to measure IgG (as a measure of colostrum quality), but the best method has yet to be established. The following paragraphs will explain the current methods, along with the challenges that come with each.

Measuring Immunity

The ability to accurately measure IgG concentration in colostrum is essential for proper management during parturition. Quantification of colostrum IgG concentrations allows producers to gauge colostrum quality and have a better understanding of whether enough IgG were available to successfully achieve transfer of passive immunity (Ishler and Schurman, 2016).

Researchers have reported that > 50 g IgG/L is the minimum rate accepted for good quality colostrum (Quigley et al., 2013).

A survey from the U.S. Department of Agriculture, reports that 13% of bovine producers evaluate the quality of maternal colostrum regularly, and 56% of these producers evaluate the quality by visual inspection (National Animal Health Monitoring System, 2007). Although high quality colostrum has been commonly characterized as being available in high volumes or as very thick and creamy, its appearance alone is not an accurate representation of IgG content (Ishler and Schurman, 2016). While colostrum viscosity ("thick and creamy") has long been utilized as an indicator of colostrum IgG content; visually inspecting the flow of the product has not been proven to be an accurate measure of IgG content. Ahmann et al. (2021) attempted to correlate colostrum viscosity with IgG content but was unable to do so.

Radial immunodiffusion (RID) is an analytical method that has been utilized to measure IgG in maternal colostrum. In RID, the colostrum is placed on an antibody-containing agarose gel plate and the antigens diffuse into the gel. Based on the precipitate rings produced, researchers are able to quantify the amount of antigen in the sample (Ahmann et al., 2021). Research has found that this method is not always accurate because the standards used in calibration were derived from serum IgG, and the percent of serum IgG and colostrum IgG are not equivalent (Quigley et al., 2013). Other studies also report that the RID method can be time consuming, expensive, and can be prone to errors in the analysis of IgG concentrations (Fleenor and Stott, 1981; Quigley et al., 2013). For example, the temperature of the colostrum can affect its protein composition, and thereby affect IgG values measured using RID (Fleenor and Stott, 1981; Quigley et al., 2013; Ahmann et al., 2021).

Another method utilizes the colostrometer. The colostrometer is a hydrometer that has been utilized to estimate IgG concentrations by measuring the specific gravity of colostrum. This mechanism uses a calibrated color-coded scale indicating three levels of Ig concentration in colostrum. The levels are green (indicating >50 mg/mL of Ig), yellow (indicating 20 to 50 mg/mL of Ig), and red (indicating <20 mg/mL of Ig; Ahmann et al., 2021). The colostrum sample is placed in a cylinder container. A glass hydrometer is inserted and allowed to float freely. The level of IgG content can be read directly from the hydrometer. However, some studies report the fat content and temperature of the sample affect the accuracy of the reading. The ideal temperature for greatest accuracy in the colostrometer reading is 22°C. The IgG concentrations are overestimated if temperature is below 0°C and underestimated if temperature is above 35 °C (Ishler and Schurman, 2016; Godden et al., 2019). Researchers have developed calculations for correcting Ig concentrations based on colostrum temperature (Ishler and Schurman, 2016). This correction calculation takes more time by producers on farm, making it a less practical option. The hydrometer and cylinder are readily available to farmers; however, this equipment is fragile, not making it practical for common on-farm use (Ishler and Schurman, 2016). This method has been found to be inexpensive (less than $100 for colostrometer and measuring cylinder) and provide rapid readings (Mechor et al., 1991; Mechor et al., 1992; Quigley et al., 2013; Ishler and Schurman, 2016).

Another method that has been validated in estimating IgG concentrations in maternal colostrum of sheep, horses, and cattle utilizes the Brix refractometer (Quigley et al., 2013). A refractometer only requires a few drops of colostrum on the prism of the device to provide an estimate of IgG content as a Brix % value. The Brix value is a measure of sugar. Since the concentration of Ig in colostrum influences the density of the sample, a Brix refractometer can be

utilized as an indirect tool for measuring Ig concentrations (Ahmann et al., 2021). The instrument uses an LED light source and a lens beneath the prism to make the reading possible through the refractive index. This index measures light behavior as it passes through the sample. A linear image sensor then assesses how the light is refracted and reflected and uses that information to determine a concentration and density. The density is affected by the temperature of the sample. To account for temperature variations, calibration is recommended routinely using a standard sucrose solution provided by manufacturer, which is a 50%-part sucrose and 50% part distilled or deionized water, as well as, maintaining solution at constant temperature in a warm water bath (Hanna Instruments, 2014; Ishler and Schurman, 2016; Godden et al., 2019). After the reading, the sample must be wiped from the lens, and prism should be cleaned of any residue that may affect future readings. This instrument is less affected by temperature of the sample and durable for on farm usage which may be why it is preferred over other methods (Ishler and Schurman, 2016; Godden et al., 2019).

Colostrum and Serum IgG

The Brix percentage (Brix %) threshold for good quality colostrum has not been determined in sheep, however the Brix values have been found to be related to IgG in colostrum. The minimum threshold for bovine colostrum quality is ≥18-23% Brix, which is equivalent to IgG of ≥50 g/L of colostrum (Ishler and Schurman, 2016; Godden et al., 2019). To understand the thresholds for colostrum quality, the Brix % has been interpreted in g of IgG per liter of colostrum from cattle. Excellent quality consists of ≥25.0 g of IgG/L or ≥9.4% Brix, good quality is 18.0-24.9 g of IgG/L or 8.9-9.3% Brix, fair quality is 10.0-17.9 g of IgG/L or 8.1-8.8% Brix, and poor quality is <10.0 g of IgG/L or <8.1% Brix (Bielmann et al., 2010; Quigley et al., 2013; Chigerwe and Hagey, 2014; Bartier et al., 2015; Godden et al., 2019).

Variability of IgG concentration in maternal colostrum is not uncommon and has been found in multiple published works (Gullikensen et al., 2008; Bielmann et al., 2010; Morrill et al., 2012; Quigley et al., 2013). This variation results from multiple factors, including the number of offspring carried and dam nutrition. Researchers found that as litter size grew from single-bearing to twin-bearing to triplet-bearing ewes, the IgG concentrations in colostrum decreased; 55.80, 53.88, and 39.56 g IgG/L respectively (Dwyer and Morgan, 2006). During the final 4 weeks of gestation, the ewe requires increased energy, protein, mineral, and vitamins. Feeding 100% and 120% of energy requirements produces greater amounts and quality of colostrum (Bailey and Neary, 2019). Additionally, restricting energy in late gestation ewes reduced quantity of colostrum produced, which could negatively affect lamb immunity (Barkely, 2016). By providing higher levels of nutrients, IgG concentrations can be increased, thus improving quality of colostrum (Bailey and Neary, 2019; Barkley, 2016; Nielsen et al., 2013).

Milk Quality while on Liquid Brewers Yeast

The research published in evaluating the effect of liquid brewers yeast on milk butterfat and protein is inconsistent and needs more investigation, especially in small ruminants. Research in 1998, found that feeding dry active yeast from 30 d before lambing to 2 wk post-lambing had no influence on milk yield (Wohlt et al., 1998). Similarly, Ali-Haimoud-Lekhal and others found no effect of yeast supplementation on milk production in late lactation dairy cows (1999). However, Desnoyers and others reported that yeast supplementation in two experiments with dairy goats and 47 experiments with dairy cows, resulted in an increased milk yield by 1.2g/kg of BW (2009). Other research that found yeast increased milk production in dairy cattle (Robinson, 2002) and dairy goats (Abd El-Ghani, 2004; Stella et al., 2007).

Published work has evaluated the effect of liquid brewers yeast supplementation on milk production, milk fat, and protein content. A meta-analysis evaluating yeast supplementation on milk production in dairy cows found that milk fat content tended to increase ($P = 0.07$) but milk protein content was unaffected by yeast supplementation ($P = 0.7$; Desnoyers et al., 2009).

Performance Measures while on Liquid Brewers Yeast

Previous research evaluating the impact of liquid brewers yeast on diet performance (DMI, ADG, CP and ADF digestibility) is inconsistent and requires more investigation. Much of the research evaluating the effects of liquid brewers yeast is done in other ruminants. Research dating back to 1998, found that feeding dry active yeast at 10 g/d or 20g/d from 30 d before calving to 2 wk post-lambing had no effect on cow DMI, CP digestibility, or ADF digestibility (Wohlt et al., 1998). Studies evaluating the effects of *Saccharomyces cerevisiae* yeast on buffalos and dairy cows and heifers, found no effects on DMI (Ghazanfar et al., 2015; Rossow et al., 2017; Anjum et al., 2018; Perdomo et al., 2020). Recent findings in dairy cow research found that yeast did not influence BW and BCS (Perdomo et al., 2020). Yeast supplementation in dairy cows increased ($P = 0.04$) dry matter intake (DMI) by 0.44 g/kg of BW (Desnoyers et al., 2009). In weaned beef steers, researchers found that yeast supplements increased DMI but had no effect on ADG (Finck et al., 2014).

Additionally, research determining the effect of liquid brewers yeast on animal performance (BW and BCS) is not consistent and warrants more investigation, especially in small ruminants. Multiple studies have evaluated the effects of mannan oligosaccharides, a component of *Saccharomyces cerevisiae*. In studies evaluating yeast effects on maternal BW, the data is typically reported in periods before parturition or after since there is not an accurate method to account for amniotic fluid, placenta, and other parturition-related weight changes.

There was no influence on cow BW in a study feeding mannan oligosaccharide to dairy cows 30 d prior to parturition (Franklin, 2005). From calving to 30 d of lactation, researchers report a tendency for cow BW to increase ($P = 0.10$) in dairy cows supplemented with mannan oligosaccharides (Linneen et al., 2014). Research feeding mannan oligosaccharides to beef cows from 60 d prior to calving through 30 d post calving found no influence ($P > 0.36$) in BCS. However, the cows supplemented with mannan oligosaccharides obtained a greater ($P = 0.05$) ability to maintain BCS from the 30 d prior to parturition through weaning (Linneen et al., 2014).

Lamb Performance while on Yeast

Research evaluating the influence of feeding *Saccharomyces cerevisiae* yeast mixed with selenium and chromium to lambs for a 56-d feeding experiment found no improvement nor negative effects on ADG (Hernández-García et al., 2015). Similarly, a study reported no influence on lamb ADG for ram lambs that were fed olive cake and *Saccharomyces cerevisiae* yeast mixed for 63 d (Obeidat, 2017). On the contrary, research evaluating the effects of supplementing *Bacillus licheniformis* and *Saccharomyces cerevisiae* to lambs for 66 d, reported a greater ADG than control lambs (Jia et al., 2018). Additional research that evaluated yeast culture containing *Saccharomyces cerevisiae* fed to lambs for 74 d did improve ADG and feed to gain ratio (Haddad and Goussous, 2005).

There is a lack of consistent information regarding the effects of liquid brewers yeast on lamb performance, especially beyond basic growth parameters (BW and ADG). Additional research in the area of yeast supplementation and lamb immunity is needed.

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CHAPTER III

EXPERIMENT 1: IMPACT OF LIQUID BREWERS YEAST FEED ADDITIVE ON LATE GESTATION EWE PERFORMANCE

Abstract

Liquid brewers yeast is a by-product of the brewery industry. A commercial-sized brewery may have economical means for forwarding the wet by-product to a secondary industry for drying to produce dried brewers yeast. For microbreweries, the cost associated with further processing makes it difficult, and often not an option. While research has investigated the use of liquid brewers yeast in cattle, there is currently limited research feeding liquid brewers yeast to small ruminants. In this experiment thirty-one ewes were synchronized and bred via natural mating. Ewes were stratified by weight into two feeding groups: one fed a traditional diet (CON), and the other fed a traditional diet supplemented with liquid brewers yeast (at a rate of 32 g DM/hd/d; LBY). Water and grass hay was offered ad libitum to all ewes. The trial period was 60 days; 30 days prior to-parturition through 30 days post-parturition. Each of the following measures was used to assess the effect treatment had on ewe performance: feed efficiency, body condition score (BCS), and offspring ADG. Additionally, colostrum and milk samples at 2- and 4-wk post-parturition were collected to evaluate the effect of liquid brewers yeast on colostrum and milk quality. Data were analyzed used PROC MIXED (SAS 9.4). The daily grain ration, including the liquid brewers yeast was consumed completely. Treatment did not have a significant effect on ewe BW, BCS, and F:G ratio at start of experiment, prior to lambing, post lambing, and end of experiment ($P = 0.31$, $P = 0.97$, $P = 1.00$, $P = 0.82$, $P = 0.94$, $P = 0.79$, respectively). Additionally, treatment did not have a significant effect on colostrum or milk quality at 2- or 4-wk post lambing ($P = 0.22$, $P = 0.64$, $P = 0.13$, respectively). Finally, results

show that treatment had no effect on lamb ADG ($P = 0.77$). These results indicate that the addition of liquid brewers yeast to the ewes' diet during late gestation did not significantly improve, but nor did it negatively affect, ewe performance for any of the measures evaluated.

KEYWORDS: feed efficiency, lambing parameters, liquid brewers yeast, milk quality

Introduction

The U.S. sheep industry has seen drastic shifts over the last few years. The American Sheep Industry Association (ASIA) conducts regular surveys of sheep producers in an attempt to get a better understanding of sheep producer numbers. From 1989 to 2010, the number of small-scale operations of sheep increased by 5% (Trinidad, 2019). In 2010, 64% of the sheep industry was said to be made of producers with 1-100 head of sheep or small-scale operations. By 2014, small operations of 20-99 ewes made up 73.1% of all sheep operations (Trinidad, 2019; USDA, 2014). Small-scale operations are not large enough to take advantage of the economics of scale and often face tight profit margins.

An increase in production efficiency can be measured using various performance measures that have been shown to be affected by environmental, handling and welfare, reproductive, and feeding practices. Feed costs account for approximately 60-70% of total cost of production. Feeding practices differ for a variety of reasons, including geographical location, feed availability, sheep breeds, and individual flock needs. Past literature has evaluated the use of by-products, specifically brewery by-products, as an animal feed source (Perdomo et al., 2020; Miyazawa et al., 2007; Desnoyers et al., 2009). Cost efficiency and availability of product play a large role in the variability of on-farm use of brewery by-products in livestock feeds.

For producers that have access to microbreweries, an economical way to feed their flock would be to repurpose by-products that would otherwise be disposed of. From 2012 and 2016,

the total number of microbreweries in the U.S. increased from 880 to 2,802 (Callen and Hait, 2018). By 2018, there were 4,518 microbreweries in the U.S. (Brewers Association, 2022). This increase in microbreweries has created an increased availability of locally sourced liquid brewers yeast for livestock producers. With the expansion of microbrewery industry, there comes an increase in availability of microbrewery by-products. One by-product that has been proposed as a potential option for animal supplementation is brewers yeast.

The most common strain of yeast utilized in beer production is *Saccharomyces cerevisiae* (Utsugi et al., 2002; Linneen et al., 2014). Yeast contains a variety of nutrients, including proteins, vitamins, and minerals, which have been reported to be beneficial in livestock diets (Jaeger et al., 2020). Yeast cells also contain mannan oligosaccharides and beta-glucans, which have been shown to have beneficial effects on animal performance (Linneen et al., 2014). Linneen et al. (2014) reported that cows supplemented with mannan oligosaccharides, which came from the cell wall of *Saccharomyces cerevisiae* yeast, tended to maintain BW and BCS better than those who were not supplemented. A meta analysis evaluating the effect of *Saccharomyces cerevisiae* yeast supplementation on milk yield and quality in ruminants reported very little influence or inconsistent effects (Desnoyer et el., 2009; Ali-Haimoud-Lekhal et al., 1999; Robinson, 2002; Sauvant et al., 2004). Other studies have evaluated the effects of *Saccharomyces cerevisiae* yeast supplementation fed to calves at various ages and durations and found inconsistent findings on ADG (Alugongo et al., 2017; Quigley et al., 1992; Yan et al., 2005; Magalhaes et al., 2005; Lesmeister et al., 2004). There is little research evaluating offspring ADG when liquid brewers yeast is fed to the dam. This area warrants more investigation.

There is also limited research evaluating the use of brewers yeast in sheep diets. This potential new feed source could benefit the sheep industry by reducing feed costs as it is an inexpensive source of nutrients (Romero-Huelva et al., 2017).

The objective of this experiment was to evaluate the impact of liquid brewers yeast feed supplement on late gestation ewe and lamb performance. We hypothesized that feeding liquid brewers yeast would increase performance measures such as BW and BCS, ewe feed efficiency, colostrum and milk quality, and offspring ADG. Findings from this experiment can provide producers knowledge on the potential value of liquid brewers yeast, from microbreweries, for their livestock. It can also give microbreweries useful information regarding a potential marketing opportunities for their by-products.

Materials and Methods

Experimental Design

All animals and procedures for this project were in accordance with the Illinois State University's Institutional Animal Care and Use Committee (IACUC) approval (Protocol # 2019-1136). Suffolk x Dorset, pregnant ewes (n=31, 1 to 6 yrs of age) were utilized to investigate the effects of feeding liquid brewers yeast on ewe feed efficiency, milk quality, and lamb growth at the Illinois State University farm.

Prior to starting treatment diets, ewes were group housed and maintained on a traditional grain/hay diet formulated to meet the nutritional requirement of early to mid- gestation. To maximize the likelihood all ewes would be bred, ewes were split into two groups and synchronized for estrus utilizing the following standard protocol. On d 0, Eazi-Breed CIDRs (Zoetis, Parsippany, NJ) were inserted vaginally. At d 10 post-CIDR insertion, 1 mL of cloprostenol sodium (Estrumate; Merck Animal Health, Madison, NJ) was administered. Two

days after Estrumate administration, CIDRs were removed and 240 IU of equine chorionic gonadotropin and 120 IU of human chronic gonadotropin (PG600; Merck Animal Health, Madison, NJ) was administered to all ewes. Approximately 24 h after PG600 injection, both groups of ewes were turned in with one of two Suffolk rams for natural mating. The rams were removed 10 d later.

At approximately 60 d of gestation, ewes were weighed, stratified by weight, and assigned to one of two treatments. Ewes were offered a traditional gestation/lactation diet (control; CON) or the traditional gestation/lactation diet supplemented with liquid brewers yeast at an average rate of 32g DM/head/d (LBY; Table 1 and 2). At start of treatment diets, the average BW for CON groups were 102.4 ± 1.8 kg (n=6) and 90 ± 1.7 kg (n=9) with the average age being 2.9 ± 0.2 yrs of age, respectively. The average BW for LBY groups were 102.5 ± 1.9 kg (n=7) and 90.4 ± 1.4 kg (n=9) with the average age being 3.5 ± 0.2 yrs of age, respectively. Ewes had ad libitum access to grass hay (Table 2) and water. Ewes were group housed in 4.8 x 11 m pens with an attached 4.8 x 12.8 m earthen lot. Ewes were offered their assigned diets daily (0.85 kg of grain DM and 4.15 kg of hay DM per head per day). The dry matter content of the liquid brewers yeast varied from batch to batch. As a result, with each new batch of liquid brewers yeast, the amounts offered were recalculated to ensure 32 g of DM was consumed per day. Ewes readily consumed the feed containing liquid brewers yeast despite the variation in dry matter content of the two diets. This rate of liquid brewers yeast supplementation was selected based on cattle research conducted by Wohlt et al. (1998) that showed feeding dry active yeast at 10 g/d or 20g/d from 30 d before calving to 2 wk post-calving had was no effect on cow DMI, milk yield, CP digestibility, or ADF digestibility. It was hypothesized that feeding a greater amount of liquid brewers yeast (32 g DM/hd/d) might yield positive performance results.

Data Collection

Orts were recorded on a daily basis to calculate weekly feed disappearance of hay, grain, and/or liquid brewers yeast. Once a week, from 4 wk prior to lambing to 4 wk post-lambing, ewe BW and body condition score (BCS; by a single trained technician) was recorded to evaluate the effects liquid brewers yeast supplementation had on ewe feed efficiency. The feed efficiency data was divided into two periods to accurately account for any weight loss that was associated with the fetus or parturition: 1) start of treatment to wk prior to lambing (period 1) and 2) wk post-lambing to end of treatment (period 2).

Ewes were moved to individual pens (1.2 m x 2.4 m) at 3 d prior to expected lambing date. Lamb birth weight and BW were recorded at 1, 2, 3, and 4 wk of age for each lamb. To evaluate effects of treatment on milk quality, at 2- and 4-wk post-lambing colostrum and milk samples were collected via hand milking. A sample of colostrum and milk from each ewe was sent for analysis of protein and butterfat (Check Mark Dairy Laboratory, Ithaca, NY). Ewes and their lambs remained in individual pens until lambs were approximately 6 d of age for close monitoring of health. Then, ewes and their lambs were returned to their original group-housed pens to continue dietary treatments.

Statistical Analysis

A predetermined significance level was set a $P < 0.05$. Since BW data was normally distributed, the mixed procedure (PROC MIXED) of SAS 9.4 with ante-dependence repeated measures was used to evaluate the effect treatment diet had on ewe BW and BCS. The explanatory variables were treatment, time, and treatment by time. The model included the main effects of supplementation (CON or LBY). Each ewe BW and BCS were included as a response variable. Additionally, PROC MIXED with ante-dependence repeated measures was utilized to

evaluate the effect liquid brewers yeast had on ewe feed efficiency, described as a feed to gain ratio. The feed efficiency data was divided into two periods; 1) start of treatment to wk prior to lambing and 2) wk post-lambing to end of treatment. The explanatory variables were treatment, time, and treatment by time.

The PROC MIXED with ante-dependence repeated measures was also used to evaluate the effects of treatment diet on colostrum and milk quality (butterfat, and protein) of each ewe at different time points. The model included the main effect of supplementation (CON or LBY). The explanatory variables were treatment, time, and treatment by time. Finally, PROC MIXED was used to evaluate the effects treatment diets had on lamb ADG from birth to 4 wk of age. The model included the main effects of supplementation (CON or LBY). One lamb from the CON group and two lambs from the LBY group died before 1 wk of age, and therefore were removed from this analysis.

Results and Discussion

Feed Intake

All ewes consumed their complete daily grain ration, including all of the liquid brewers yeast (32 g DM/d). The liquid brewers yeast rate of inclusion was chosen from investigating previous literature. Wohlt et al. (1998) showed that feeding yeast at 10 g/d or 20 g/d from 30 d prepartum until 4 wk postpartum did not affect cow performance. Additionally, Yan and others (2005) fed liquid brewers yeast at a rate of 20 g/d to calves.

In the current experiment, all ewes consumed 5% of their BW daily as hay and 2% as grain. This consumption rate falls over the 2-5% range for average DMI as percent of BW for sheep (Kerr, 2018).

Ewe Weight Performance

BW and BCS were measured every 7 d for all ewes on the experiment. There were no significant effects of liquid brewers yeast supplementation on ewe BW ($P = 0.32$, Table 3) or BCS ($P = 0.96$, Table 4). These findings agree with research conducted by Perdomo and others (2020) that reported *Saccharomyces cerevisiae* yeast supplementation had no influence on BW and BCS in dairy cows. Similarly, other research reported that cows supplemented with 0.01 kg/d of mannan oligosaccharides from the cell wall of *Saccharomyces cerevisiae* yeast tended to maintain BW and BCS better than those who were not supplemented (Linneen et al., 2014). In the current experiment, feed efficiency was evaluated using a calculated feed to gain ratio (F:G), which is often reported as a feed conversion ratio. In research where lambs were supplemented with 10 g/kg *Saccharomyces cerevisiae* yeast, there was no effect on feed conversion ratio (Khadem et al., 2007). On the contrary, other researchers reported that 2% supplemental active dry yeast as a percentage of dry matter intake in calves enhanced feed conversion ratio (Chaucheyras et al., 2008). For the present experiment, liquid brewers yeast had no influence on F:G in period 1 ($P = 0.49$) or period 2 ($P = 0.41$, Table 5).

In conclusion, the fact that no significant differences were seen in any of these measures suggests that the level of the nutrition offered was not different between the treatment groups.

Milk Quality

The research published in evaluating the effect of liquid brewers yeast on milk butterfat and protein is inconsistent. A meta-analysis evaluating the effect of *Saccharomyces cerevisiae* yeast supplementation on milk production in dairy cows found that milk fat content tended to increase with supplementation ($P = 0.07$) but milk protein was unaffected ($P = 0.7$; Desnoyers et al., 2009). In the present experiment, liquid brewers yeast supplementation did not significantly

influence colostrum quality, milk quality at 2 wk post lambing, and milk quality at 4 wk post lambing ($P = 0.22$, $P = 0.64$, $P = 0.13$, respectively). Additionally, the butterfat and protein content of colostrum ($P = 0.82$, $P = 1.00$, respectively), milk at 2 wk,($P = 1.00$, $P = 0.97$, respectively) and milk at 4 wk ($P = 0.63$, $P = 1.00$, respectively) were not significant between treatments (Table 6). Further investigation with a higher rate of inclusion of liquid brewers yeast may be the reason why there was no differences in quality of colostrum or milk. However, these result indicate that the rate of inclusion for the current experiment, did not have negative implication on these factors.

Lamb Performance

Adequate ewe nutrition during gestation and after lambing is especially important for the support of lamb growth (Nielsen et al., 2013; Barkley, 2016). There is limited research evaluating the effects of *Saccharomyces cerevisiae* yeast supplementation on ADG in offspring and most studies involved calves. Research reported that yeast (Saccharomyces cerevisiae) culture given to calves at 2% of DM intake, improved ADG by 15.6% (Lesmeister et al., 2004). On the contrary, researchers evaluating the effects of *Saccharomyces cerevisiae* in the first 70 d of age in calves reported no differences in BW gain (Magalhães et al., 2008). In the present experiment, there was no influence of liquid brewers yeast on lamb ADG at any time point between 1 and 4 wk of age ($P = 0.77$; Table 7). Lamb ADG ranged from 0.37 to 0.32 kg (LBY) and 0.36 to 0.29 kg (CON) during the first 4 wk of age. These ADG ranges are typical for Dorset/Suffolk lambs, where ADG is 0.30 and 0.37 kg, respectively (Kennedy, 2019). However, research that evaluated dried yeast culture containing *Saccharomyces cerevisiae* fed to lambs did improve ADG and feed to gain ratio when fed 3 g/d AF (Haddad and Goussous, 2005). The potential reason no differences were seen in lamb performance in the current experiment may be

due to the degree the yeast was incorporate into their dams diet, as well as not being incorporated into lamb diets.

Applications

The objective of this experiment was to investigate the effect of liquid brewers yeast on ewe feed efficiency, colostrum and milk quality, and offspring ADG. The inclusion of liquid brewers yeast did not improve nor negatively impact BW/BCS and feed efficiency throughout late gestation of the ewes. Additionally, it did not effect milk quality or lamb growth. While no significant improvements in ewe performance were observed, liquid brewers yeast still may be a valuable feedstuff. However, previous research and the present experiment results suggest a need for more evaluation regarding the optimal inclusion rate of liquid brewers yeast in small ruminant diets.

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Tables

Table 1. Late Gestation/Lactation Diet – Ingredient Composition

Ingredient	**% Intake***
Cracked Corn	9.20
Whole Oats	12.50
Beet Pulp	1.80
Molasses	0.30
Sheep Concentrate[a]	6.80
Hay	69.30
Total	100.00

*Diet ingredients reported as percentage of total diet (DM)

[a] Nutrient profile in Table 2

Table 2. Sheep Concentrate Profile

Item	Amount
Dry Matter (%)	80.3
Nutrient Content (% DM)	
Crude protein (%)	16.3
ADF (%)	36
aNDF (%)	59
NFC (%)	14.7
TDN (%)	57
Ca (%)	1.1
P (%)	0.51
Magnesium (%)	0.23
Potassium (%)	3.04
Sodium (%)	0.001
Iron (ppm)	897
Zinc (ppm)	41
Copper (ppm)	12
Manganese (ppm)	80
Molybdenum (ppm)	3.7

Table 3. Late Gestation/Lactation Diet and Liquid Brewers Yeast – Nutrient Profile

	Component of Diet		
Item	**Grain**	**Hay**	**Yeast**
Dry Matter (%)	89.08	83.48	13.47
Nutrient Content (% DM)			
Crude protein (%)	17.06	11.93	36.73
Ash (%)	-	-	4.81
ADF (%)	8.88	39.87	-
aNDF (%)	15.42	66.23	-
TDN (%)	81.8	56.00	87.67
Ca (%)	1.28	0.35	0.20
P (%)	0.52	0.41	1.22
Magnesium (%)	0.18	0.21	0.20
Potassium (%)	0.79	2.40	1.22
Sodium (%)	0.30	0.003	0.07
Sulfur (%)	0.43	0.23	0.41
Iron (ppm)	304.8	219.2	41.3
Zinc (ppm)	202	22.7	43.5
Copper (ppm)	10.2	6.3	19.07
Manganese (ppm)	129	29.5	7.8
Molybedenum (ppm)	3.94	5.8	2.32

Table 4. Ewe Body Weight (kg)

	CON[a]	SE	LBY[a]	SE	*P*-value
Overall					
TRT					0.32
Time					<0.01
TRT*Time					0.04
Weekly					
1	99.3	2.2	100.4	2.2	0.73
2	99.7	2.0	103.5	2.0	0.19
3	102.5	2.1	107.5	2.0	0.09
4	105.3	2.0	108.1	1.9	0.33
5	103.8	2.0	107.4	1.9	0.21
6	90.2	2.6	94.4	2.5	0.24
7	88.2	2.9	89.2	2.8	0.79
8	84.7	2.9	88.5	2.8	0.34
9	82.9	2.7	87.3	2.6	0.25
10	81.7	2.8	84.3	2.7	0.50

Table 5. Ewe Body Condition Score

	CON	SE	LBY	SE	P-value
Overall					
TRT					0.96
Time					<0.01
TRT*Time					0.05
Weekly					
1	3	0.1	4	0.1	0.43
2	3	0.1	3	0.1	0.66
3	3	0.1	3	0.1	0.92
4	4	0.1	3	0.1	0.45
5	4	0.1	3	0.1	0.11
6	3	0.2	3	0.1	0.04
7	3	0.1	3	0.1	0.17
8	3	0.1	3	0.1	0.43
9	3	0.1	3	0.1	0.53
10	2	0.1	3	0.1	0.62

Table 6. Ewe Feed Efficiency, Described as a Feed to Gain Ratio

	Treatment			
Time	**CON[a]**	**LBY[a]**	**SE**	***P*-value**
Period 1[b]	-3.5	5.4	6.65	0.49
Period 2[c]	-12.3	-7.03	4.36	0.41

[a] Values are reported as feed:gain ratio

[b] Period 1: Start of treatment to one week prior to lambing

[c] Period 2: One week post-lambing to end of experiment

Table 7. Ewe Colostrum and Milk Quality, as Butterfat % and Protein %.

		Treatment				
Item	**Typical Industry Average**	**CON**	**SE**	**LBY**	**SE**	***P*-value**
Colostrum						
Butterfat (%)	13.20	14.20	1.14	12.24	1.11	0.82
Protein (%)	14.19	18.07	1.13	17.38	1.10	1.00
Milk at 2 Weeks Post-Lambing						
Butterfat (%)	5.90	8.60	0.42	8.87	0.41	1.00
Protein (%)	5.50	4.56	0.07	4.48	0.07	0.97
Milk at 4 Weeks Post-Lambing						
Butterfat (%)	5.90	9.43	0.49	8.38	0.47	0.63
Protein (%)	5.50	4.34	0.11	4.37	0.11	1.00

Table 8. Lamb ADG by Weekly Age

Time (Weeks of age)	CON[a]	SE[a]	LBY[a]	SE[a]	P-value
1	0.37	0.02	0.36	0.02	1.00
2	0.34	0.03	0.33	0.02	1.00
3	0.36	0.03	0.33	0.02	1.00
4	0.32	0.03	0.29	0.02	1.00

CHAPTER IV

EXPERIMENT 2: EFFECT OF THE INCLUSION OF LIQUID BREWERS YEAST DURING GESTATION ON COLOSTRUM QUALITY AND TRANSFER OF PASSIVE IMMUNITY IN LAMBS

Abstract

Fourteen Suffolk x Hampshire, pregnant ewes were utilized to evaluate the effects of liquid brewers yeast on colostrum quality and the transfer of passive immunity in their offspring. Ewes were randomly assigned to one of two treatments: late gestation diet (CON) or late gestation diet supplemented with liquid brewers yeast at an average rate of 60 g DM/d (LBY). Ewes were fed treatment diets 45 d prior to expected lambing until 3 d post lambing. Ewes received parainfluenza type 3 (PI3) vaccines 32 and 18 d prior to expected lambing date; blood was collected prior to vaccination, 5 d prior to expected lambing, and within 6 h postpartum. Blood was collected from lambs (n=22) at 3 d of age. Ewe and lamb serum was evaluated for anti-PI3 antibody titers. Colostrum, collected within 4 h postpartum, was analyzed for colostrum IgG and nutrient composition. Data were analyzed using PROC MIXED (SAS 9.4). All ewes tested negative for PI3 prior to vaccination and positive after vaccination. All lambs tested positive for antibody titers for PI3 with no differences in antibody titer levels between treatments (195 for CON and 122 for LBY; $P = 0.30$). Colostrum IgG concentrations did not differ between CON (30.4% Brix) and LBY (24.6% Brix) ewes ($P = 0.21$). These results indicate that, although there were numerical differences, the addition of liquid brewers yeast to the ewes' diet during late gestation did not significantly improve colostrum quality or transfer of passive immunity in lambs.

KEYWORDS: colostrum quality, immunity, liquid brewers yeast, sheep

Introduction

For the past 40 years, pre-weaning lamb mortality rates have remained relatively constant and high at 15-20% globally (Dwyer et al., 2016). Sheep producers rely on lamb survivability as an investment for profit on their operations. As a result, there has been a need for the development of novel and effective strategies to reduce neonatal loss to improve welfare, production, and income (Flinn et al., 2020). Of all sheep operations in the U.S., 73.1% are small operations with 20-99 ewes on farm (Trinidad, 2019; USDA, 2014). With smaller flock sizes in the U.S., the loss of a single lamb can be detrimental for overall farm profitability and productivity.

In general, major management factors affecting lamb survival may include the ewes' nutrition and immune status and the lambs' colostrum intake (Binns et al., 2002; Charrani et al., 1991). Ewes provided with good nutrition are more likely to produce more vigorous lambs and improved quality and quantity of colostrum. Adequate nutrition is especially important for the support of lamb growth and colostrum production in the last trimester (Barkley, 2016; Nielsen et al., 2013).

The ewe's immune health, specifically maternal immunoglobulins (Ig), are responsible for providing the lambs defense against neonatal diseases (Ahmad et al., 2000). However, placental barriers present in ruminants prevents the passage of maternal Ig through the placenta (Borghesi et al., 2014; Poitras et al., 1986). As a result, lambs are born with no protective level of immunity (Ahmad et al., 2000; Tizard et al., 1992). Instead, transfer of passive immunity via the absorption of Ig is achieved through colostrum intake (Doaa et al., 2009). Lamb survival rates increase drastically if lambs receive adequate colostrum within the first 48 h of life, which is the most critical period of a lamb's life (Barkley, 2016). Of the total Ig concentrations in colostrum

and serum, approximately 90% and 80%, respectively, are in the form of Immunoglobulins G (IgG; Hatfield et al., 2000). Inadequate absorption of maternal Ig can have negative effects on health and productivity (Seymour et al., 1995). Literature states that there is a positive correlation between low concentrations of serum Ig and development of infectious diseases that result in lamb mortalities (Vihan, 1986; Hodgson et al., 1992; Doaa et al., 2009). The failure of transfer of passive immunity has a drastic impact on neonatal lamb mortality in that the survival of the lamb is dependent on the immunity it acquires through its dam's colostrum in the first 24 to 48 h after birth (Esser et al., 1989; Doaa et al., 2009). In a study evaluating the effects of colostrum Ig level on lamb DMI, researchers found that DMI increases for lambs receiving high Ig colostrum from birth to 4 d compared to those receiving low Ig colostrum (Nocek et al., 1984). Research utilizing dairy calves has also shown that calves with insufficient Ig within 48 h of birth are not immunologically capable of handling a pathogenic invasion that commonly occurs when the fetus leaves the sterile environment of the uterus (Robison et al., 1988; Banks, 1982; Ahmad et al., 2000). As a result of these and similar studies, there is a need for ongoing research investigating nutritional programs, environmental factors, and flock management practices that could improve immunity and colostrum quality in different species.

Liquid brewer's yeast is a by-product of the brewing industry. This liquid by-product is collected at the end of the fermentation process of beer production (Hertrampf et al., 2000). The microbrewery industry has experienced exponential growth since 2005. In 2013, the number of microbreweries in the U.S. reached 2,768 and accounted for 7.8% of the U.S. beer market compared to 2.6% in 1998 (Moore and McLaughlin, 2016). In 2016, the number of breweries had increased to 2,802 (Callen and Hait, 2018). As microbrewery business continues to demonstrate a steady increase, liquid brewers yeast is becoming increasingly available.

Yeast is high in proteins, vitamins, minerals, polyphenols, antioxidants, beta-glucans and mannoproteins (Jaeger et al., 2020). The brewers yeast cells are composed of mannan oligosaccharides, which many authors reported improve immune function and reduce invading pathogens (Newman and Newman, 2001; O'Quinn et al., 2001; Srinivasan et al., 1999; Newman, 1994; Zaleska et al., 2015). Yeast products have been found to improve dairy calf survival and enhance calf health, and therefore improved farm profitability, although the mechanism is not clearly established (Magalhães et al., 2008). Numerous *in vitro* and *in vivo* studies across species have demonstrated the immune modulating properties of beta-glucans (Volman et al., 2008; Vetvicka and Oliveira, 2014). Xia et al. (2021) reported that beta-glucans improve immunity and increase Ig levels in cow colostrum when supplemented 21 d before expected calving through 21 d post calving.

Limited published research in small ruminants in this area, paired with the increasing availability of liquid brewers yeast as a byproduct of the microbrewing industry, suggests there is a need for research investigating liquid brewers yeast supplementation effects on small ruminants, especially sheep. Therefore, the objective of this experiment is to investigate the effects of liquid brewers yeast on the transfer of passive immunity from ewe to offspring and on ewe colostrum quality. We hypothesized that feeding liquid brewers yeast would improve the ewes' transfer of passive immunity to their lambs. Additionally, we anticipated colostrum quality to increase with liquid brewers yeast supplementation.

Materials and Methods

Experimental Design

All animals and procedures for this project were in accordance with the Illinois State University's Institutional Animal Care and Use Committee (IACUC) approval (Protocol # 2020

– 1170). Fourteen Suffolk x Hampshire, pregnant ewes (2 to 4 yrs of age) were utilized to investigate the effects of liquid brewers yeast on colostrum quality and the transfer of passive immunity in their offspring.

Prior to starting treatment diets, ewes were housed in groups and maintained on a traditional grain/hay diet formulated to meet the nutritional requirements of early to mid-gestation. Ewes were split into three groups and synchronized for estrus utilizing the following protocol. On d 0, Eazi-Breed CIDRs (Zoetis, Parsippany, NJ) were inserted vaginally. At d 10 (group 1), 11 (group 2), and 12 (group 3) post-CIDR insertion, 1 mL of cloprostenol sodium (Estrumate; Merck Animal Health, Madison, NJ) was administered. Two days after Estrumate administration, CIDRs were removed and 240 IU of equine chorionic gonadotropin and 120 IU of human chronic gonadotropin (PG600; Merck Animal Health, Madison, NJ) was administered to each respective group. Approximately 24 h after PG600 injection for each group, ewes were turned in with a single Dorset ram for natural mating. Since the ewes had been divided into 3 groups, ewes were turned in with the ram over 3 consecutive days. The ram was removed 10 d later.

All of the ewes exposed to the ram were bred and maintained their pregnancy until birth. At approximately 100 d of gestation, ewes were allotted to one of two treatment groups based on BW and age. Treatment groups were offered a traditional gestation/lactation diet (control; CON) or the traditional gestation/lactation diet supplemented with liquid brewers yeast at an average rate of 60 g DM/head/d (LBY; Table 1, 1a, and 2). The average BW for CON was 103.11 ± 2.73 kg with the average age being 3.57 ± 0.30 yrs of age. The average BW for the LBY was also 103.11 ± 3.32 kg with the average age being 3.43 ± 0.30 yrs of age. Ewes had *ab libitum* access to grass hay (Table 2) and water. Ewes were housed individually in 1.2 x 2.4 m pens and fed the

assigned diet daily. Orts were recorded and used to monitor feed disappearance of grain and/or liquid brewers yeast.

Data Collection

To initiate an immune challenge, ewes were vaccinated with Parainfluenza-3 (PI3; Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza 3-Respiratory Syncytial Virus Vaccine, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). This vaccine was selected as it is not a common pathogen in sheep and it has been used to successfully elicit immunological challenges in other studies utilizing ewes (Reffett et al., 1988; Redden et al., 2010; Daniels et al., 2000). Ewe blood was collected via jugular venipuncture prior to vaccination to ensure ewes did not have circulating PI3 antibodies. At 32 d prior to expected lambing date, ewes received the PI3 vaccination. The PI3 booster vaccine was administered 15 d later. Blood was also collected at 17 d and 5 d prior to expected lambing date (Figure 1). Serum was harvested from collected blood samples and frozen (-20°C) for later analysis.

At time of lambing, colostrum was collected within 4 h postpartum, and a blood sample was collected from each ewe within 6 h postpartum. To determine IgG concentrations, colostrum was evaluated using a refractometer (Hanna Instruments, Woonsocket, RI). A sample of colostrum from each ewe was sent for protein analysis by Kjeldahl AOAC 21st ed. 991.22/991.20/998.06 and butterfat analysis by Mojonnier/Ether Extraction, AOAC 21st ed. 989.05 (Check Mark Dairy Laboratory, Ithaca, NY). Also, at the time of parturition, data were collected on lamb birth weight, number of lambs born, number of lambs born alive, and lamb sex. All lambs received 30 mL of colostrum from their dams via stomach tube to ensure colostrum intake within the first 4 h of life. Ewes and their lambs remained in individual pens until the lambs reached 3 d of age. At d 3 postpartum, a blood sample from each lamb was

collected via jugular venipuncture. Serum was harvested and subsequently frozen at -20°C for later analysis. All samples of serum from ewes and lambs were sent for analysis of anti-parainfluenza type 3 titers by virus neutralization, as an indicator of transfer of passive immunity (Texas A&M Veterinary Medical Diagnostic Laboratory, Canyon, TX).

Statistical Analysis

A predetermined significant difference level was set at $P \leq 0.05$ and tendencies were determined to be between $P > 0.05$ and ≤ 0.10. Since antibody data was not normally distributed, sera data were transformed using the square root transformation. The mixed procedure (PROC MIXED) of SAS 9.4 with autoregressive repeated measures was used to evaluate the effects treatment diets had on ewe anti-PI3 titer levels at each time point. The model included the main effects of supplementation (CON or LBY). Each ewe anti-PI3 titer level was included as a response variable for each time point (prior to vaccination, at booster vaccination, 5 d prior to expected lambing, and post-lambing). The explanatory variables were treatment, time, and treatment by time interaction.

The PROC MIXED procedure was also used to evaluate the effects of treatment diet on colostrum quality (Brix %, butterfat, and protein). The model included the main effect of supplementation (CON or LBY). The effect of treatment on the number of lambs born, number of lambs born alive, and lamb birth weight was evaluated using PROC MIXED. The model included the main effects of supplementation (CON or LBY). Similar to ewe serum analyses, PROC MIXED was used to evaluate the effects treatment diets had on lamb anti-PI3 titer levels. The model included the main effect of supplementation (CON or LBY) and random effect of ewe. Each lamb anti-PI3 titer level was included as a response variable.

Results and Discussion

Feed Intake

All ewes completely consumed their daily grain ration, including all of the liquid brewers yeast (60 g DM/d). This rate of intake was selected based on previous research and a pilot experiment conducted at ISU. Wohlt et al. (1998) showed that feeding dry active yeast at 10 g/d or 20 g/d from 30 d prepartum until 4 wk postpartum did not affect cow DMI, milk yield, CP digestibility or ADF digestibility. In a pilot experiment at ISU, pregnant ewes were fed liquid brewers yeast at a rate of 30g DM/d, which did not affect ewe BW, BCS, DMI, or lamb birth weight. In an attempt to elicit a beneficial response, ewes in this experiment receive 60 g DM yeast/d.

Ewe Performance

The immune challenge model of using the Bovine Parainfluenza-3 Virus (PI3) vaccine in sheep investigations has been validated by others (Reffett et al., 1988; Redden et al., 2010; Daniels et al., 2000). In the present experiment, all ewes tested negative prior to vaccination (at approximately -32 d) and positive for PI3 after vaccination, demonstrating the vaccination challenge in the ewes was successful. It is common for the range of ewe titer levels to vary within a herd. During the present experiment, ewe titer level ranged from antibody titer of <4 to ≥256. Within each treatment group, the titer levels of ewes at booster vaccination, 5 d prior to expected lambing, and post-lambing were not different ($P < 0.01$, Table 3). This indicates that the immune challenge consistently elicited an immunological response in both groups of ewes. However, there was no significant difference in ewe titer level due to treatment ($P = 0.88$) or a treatment by time interaction ($P = 0.75$).

Published research has shown considerable variability in IgG concentrations of maternal colostrum (Gullikensen et al., 2008; Bielmann et al., 2010; Morrill et al., 2012; Quigley et al., 2013). Quigley et al. (2013) evaluated the comparison of radial immunodiffusion (RID) and turbidimetric immunoassay (TIA) methods of IgG analysis; the range of IgG in first-milking maternal colostrum was 7.1 to 159 g IgG/L (measured by RID, n = 183) and 6.9 to 139.9 g IgG/L (measured by TIA, n = 183). In cow colostrum, the concentrations of IgG evaluated using a colostrometer varied from 3 to 154 g of IgG/L (Løkke et al., 2016). The Brix refractometer has been validated as a means of estimating the IgG concentration in maternal colostrum in sheep, horses, and cattle (Quigley et al., 2013). This instrument is less affected by temperature and made of more durable material for on-farm usage (Godden et al., 2019). In recent years, researchers have found that a Brix percent of 18-23% is the approximate minimum value of good quality colostrum, which is equivalent to IgG ≥ 50 g/L (Bielmann et al., 2010; Quigley et al., 2013; Chigerwe and Hagey, 2014; Bartier et al., 2015; Godden et al., 2019). Godden and others (2019) reported on research with calves and described colostrum quality based on IgG content: excellent (≥25.0 g IgG/L, ≥9.4% Brix), good (18.0-24.9 g IgG/L, 8.9-9.3% Brix), fair (10.0-17.9 g IgG/L, 8.1-8.8% Brix), and poor (<10.0 g IgG/L, <8.1% Brix). Studies also report the minimum threshold for adequate colostrum quality was 50 g of IgG/L in cows, 20 g of IgG/L in caprine, and is unknown in sheep (Argüello et al., 2005; Castro et al., 2007; Kessler et al., 2021).

In the present experiment the averages for colostrum IgG concentrations were 30.4% Brix (CON) and 24.6% Brix (LBY; Table 4), which correlates to an average of >50 g IgG/L in colostrum IgG concentrations for both ewe groups. Brix data was not significantly different between treatments ($P = 0.21$, SE = 3.06). Research evaluating the effects of breed and litter size on colostrum IgG concentrations found that Blackface and Suffolk ewes ranged from 39.29 –

51.26 g IgG/L and 47.73 – 60.86 g IgG/L, respectively (Dwyer and Morgan, 2006). As litter size grew from single-bearing to twin-bearing to triplet-bearing ewes, the mean IgG concentrations in colostrum decreased; 55.80, 53.88, and 39.56 g IgG/L respectively (Dwyer and Morgan, 2006). This research on IgG concentrations in colostrum correlates with present experiment findings showing no failure for transfer of passive immunity with concentrations above >50 g IgG/L.

Just as colostrum IgG concentrations are important to lamb survival, it is also vital that the lambs receive the high level of nutrients typically found in colostrum to help maintain their body temperature in the first 24 h of life (Dwyer and Morgan, 2006). The butterfat and protein percentage in ewe colostrum can vary. Research reports fat and protein concentrations in multiple breeds of sheep to range 1.1 to 24.8% in fat and 7.3 to 30.5% in protein content. This research notes that variation is primarily due to breed and litter size (Kessler et al., 2018). Research evaluating the effects of breed and litter size on fat content and protein content in colostrum found that Blackface and Suffolk ewes had an average fat of 16.53% and 13.26% and protein of 15.54% and 14.19%, respectively (Dwyer and Morgan, 2006). As litter size grew from single-bearing to twin-bearing and then to triplet-bearing ewes, the mean fat content increased (12.91%, 15.05%, and 16.93%, respectively) and the mean protein content showed no trend (15.22%, 17.21%, and 12.17%, respectively; Dwyer and Morgan, 2006). In the current experiment, CON ewes averaged 11.1% butterfat and 18.4% protein while, LBY ewes averaged 9.9% butterfat and 14.5% protein, both similar to what has previously been reported. There were no significant differences in butterfat ($P = 0.57$) or protein ($P = 0.19$) content between CON and LBY colostrum (Table 4).

Published literature evaluating the effect of liquid brewers yeast on butterfat and protein is inconsistent. In agreement with the present experiment findings, researchers evaluating

lactating Jersey cows offered 0, 15, or 30% wet brewers grains (WBG) or 30% WBG plus liquid brewers yeast. They found milk protein content was less for cows receiving 15% WBG (3.90%) or 30% WBG (3.78%) compared to those receiving 0% WBG (3.95%) and even lower for cows receiving WBG plus liquid brewers yeast (3.63%; West et al., 1994). West et al. (1994), suggest that increasing dietary WBG was associated with a decrease in milk protein content, but the mechanism behind the decrease is unknown. However, Perdomo et al. (2020) found that increasing the dosage of live yeast offered to lactating Holstein cows, increased milk protein yield content but did not affect milk fat concentrations. Research that evaluated the effects of *Saccharomyces cerevisiae* dried brewers yeast on lactating ewes found a significantly higher fat content on d 70 of lactation than ewes not supplemented with yeast. They also found that at d 28 of lactation, there were no significant effects on fat content (Zaleska et al., 2015). This research suggests that the effect of yeast on milk fat may require longer time periods of supplementation. In the present experiment, ewes were evaluated at d 0 of lactation or lambing and were supplemented for approximately 45 d pre-lambing. Perhaps starting yeast supplementation earlier could demonstrate significant effects on colostrum fat concentrations. However, in the present experiment, yeast activity was not measured to account for number of live yeast so a direct comparison to these studies cannot be made.

When excluding treatment, we found that butterfat content demonstrated a tendency (P = 0.06) to increase with number of lambs carried. Ewes carrying a single lamb (7.65%) produced less butterfat than ewes carrying two (11.79%, P = 0.04) or three lambs (12.63%, P = 0.04). Ewes carrying two or three lambs were not significantly different (P = 0.70). In agreement with these findings, Kessler et al. (2018) reported that fat content in colostrum of twin and triplet bearing ewes were significantly higher than singleton bearing ewes. Additionally, we found that

protein increased ($P = 0.04$) in ewes with increasing number of lambs carried. The colostrum protein was significantly lower in ewes carrying a single lamb (14.02%) compared to those carrying three lambs (23.02%, $P = 0.02$). Similarly, colostrum protein was lower in ewes carrying two lambs (15.31%) compared to ewes carrying three lambs (23.02%; $P = 0.03$). However, colostrum protein in ewes carrying a single lamb (14.02%) was not different from ewes carrying two lambs (15.31%, $P = 0.64$). In some accordance with these findings, published research reports that protein concentrations tended to be higher in twin and triplet bearing ewes than in singleton bearing ewes (Kessler et al., 2018). The ewe is responsible for supplying neonatal lambs with sufficient energy to maintain body temperature which may explain the higher protein and fat concentration in colostrum of twins and triplet bearing ewes compared to singleton bearing ewes (Kessler et al., 2018).

Lamb Performance

Post-lambing, all ewes and lambs tested positive for PI3, therefore lambs did successfully receive the transfer of passive immunity from their dams. The average lamb anti-PI3 titers at 3 d of age in CON and LBY ewes were antibody titers of 195 and 122, respectively ($P = 0.30$, Table 3). To quantify the transfer of passive immunity, lamb serum antibody titers at 3 d of age were evaluated. Lamb serum samples ranged from antibody titers of 8 to ≥256. This variation in initial serum antibody titers is also reported in other studies that evaluated IgG concentrations of lambs, calves, and humans. The large range of initial serum antibody titers in calves has been reported as due to natural variation in maternal colostrum content. The ranges reported for PI3 vaccinated calves were 8 to 1024 at 2 d of age when measured by a virus neutralization test (Kirkpatrick et al., 2019), and 5 to 187 at 2 d age (Chamorro et al., 2014) and 8 to 2048 at 0 d of age (Fulton et al., 2004) when measured using an ELISA test. Additionally, research evaluating titer levels of

calves 28-69 d of age with maternal antibodies for PI3 found ranges measured by a virus neutralization test to be <2 to 480 (Kaeberle et al., 1998). Published research on peak IgG concentrations has been reported after 7 wk of age in lambs (Klobasa and Werhahn, 1989) and between 4 to 8 wk of age (Gershon et al., 2013) in humans. For the present experiment, it is unknown if the variation is due to treatment since lamb anti-PI3 titer were solely collected at 3 d of age. Further research needs to evaluate lamb blood IgG concentration after birth and through peak concentrations to determine if dam consumption of liquid brewers yeast has a longer term effect on offspring IgG concentrations.

Research recommends lamb colostrum intake to be 180 – 210 mL/kg of BW in their first 18 h of life (Mellor and Murray, 1986) and 10% of their BW in their first 24 h of life (Morrical et al., 1995; Alves et al., 2015). Although the threshold in colostrum IgG concentration to avoid failure of passive transfer is unknown, research suggests that serum IgG concentrations in lambs should be between 6 and 16 g/L (Massimini et al., 2006), while others report 15 g/L or 30g/L (Kessler et al., 2018; Alves et al., 2015). Research has shown that a minimum of 100 g of IgG is recommended to achieve acceptable levels of serum IgG in calves, however levels of 150 to 200 g of IgG increases passive immunity and is more reliable in preventing failure for passive transfer (Godden et al., 2009). A subsequent study by Godden and others (2019), related colostrum intake to calf BW; calves are recommended to be fed 10 – 12% of BW of colostrum at first feeding. Studies have reported that the suggested IgG intake for lambs in their first 24 h of life is 4 g of IgG/kg of BW (Castro et al., 2007; Rodríguez et al., 2009; Hernández-Castellano et al., 2014). Lambs in the present experiment weighed 4.99 ± 1.22 kg (n=13, CON) and 4.54 ± 0.97 kg (n=13, LBY); to consume the recommended 4 g of IgG/kg of BW they would need to consume approximately 19.96 g of IgG and 18.16 g of IgG, respectively, in their first 24 to 48 h

of life. In the present experiment, all lambs were ensured 30 mL of maternal colostrum, providing approximately 0.54 g IgG by 4 h of life. Based on blood serum data collected at 72 h of life, our findings suggests that lambs did receive the recommended amounts via suckling at 24 to 48 h of life.

The birth weight of lambs from CON and LBY ewes were not affected by treatment (4.99 and 4.54 kg, respectively; $P = 0.31$; Table 5). These findings agree with previous research reporting no significant differences in BW of lambs at 2 d of age reared from ewes supplemented with *Saccharomyces cerevisiae* or beta-glucan products (Zaleska et al., 2015).

Applications

The objective of this experiment was to investigate the effects of liquid brewers yeast on the transfer of passive immune from ewe to offspring and on ewe colostrum quality. The inclusion of liquid brewers yeast did not improve nor negatively affect colostrum quality or the transfer of passive immunity from ewe to offspring. While no significant improvements were observed, liquid brewers yeast still may be a valuable feedstuff. Additional research needs to be conducted to identify the optimal inclusion rate needed to maximize colostrum quality and offspring immune function in small ruminants.

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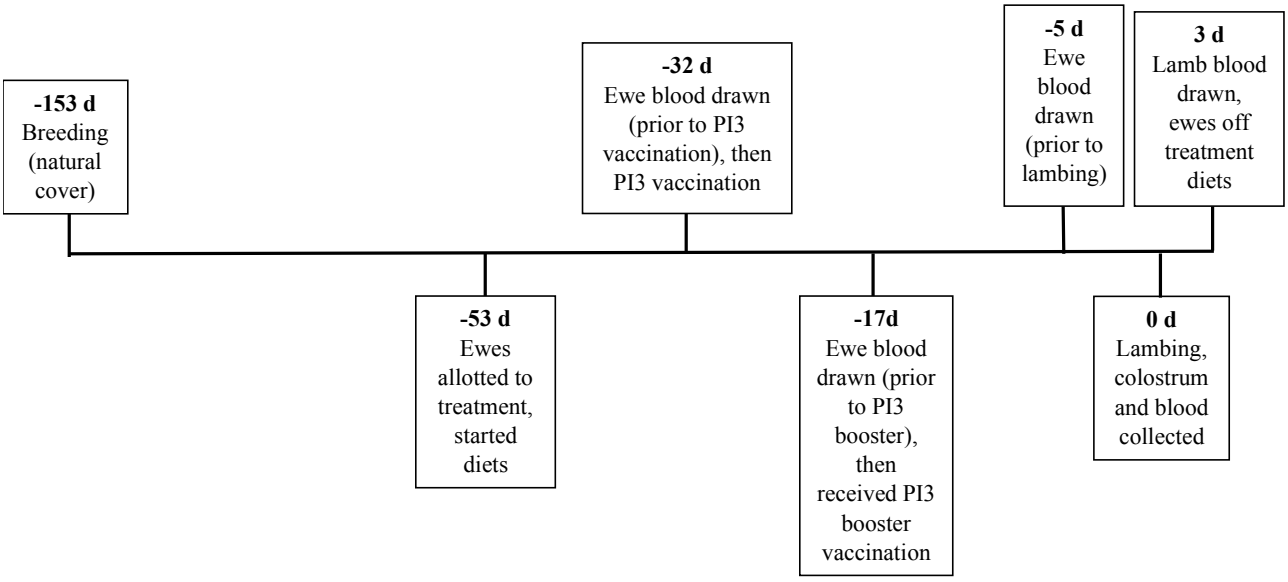
Figures

Figure 1. Experiment 2 Timeline



Tables

Table 1. Late Gestation/Lactation Diet – Ingredient Composition

Ingredient	**Inclusion**[a]
Cracked Corn	9.20
Whole Oats	12.50
Beet Pulp	1.80
Molasses	0.30
Sheep Concentrate[b]	6.80
Hay	69.30
Total	100.00

[a] Diet ingredients reported as percentage of total diet, as-fed basis

[b] Nutrient profile in Table 2

Table 2. Sheep Concentrate Profile

Item	Amount
Dry Matter (%)	80.3
Nutrient Content (% DM)	
Crude protein (%)	16.3
ADF (%)	36
aNDF (%)	59
NFC (%)	14.7
TDN (%)	57
Ca (%)	1.1
P (%)	0.51
Magnesium (%)	0.23
Potassium (%)	3.04
Sodium (%)	0.001
Iron (ppm)	897
Zinc (ppm)	41
Copper (ppm)	12
Manganese (ppm)	80
Molybedenum (ppm)	3.7

Table 3. Late Gestation/Lactation Diet and Liquid Brewers Yeast – Nutrient Profile

	Component of Diet		
Item	**Grain**	**Hay**	**Yeast**
Dry Matter (%)	86.35	86.35	8.93
Nutrient Content (% DM)			
Crude protein (%)	16.6	14.77	47.5
Crude fat (%)	-	-	10.3
Ash (%)	-	-	8.49
ADF (%)	3.93	38.17	-
aNDF (%)	8.58	56.92	-
TDN (%)	84.75	57.33	90.33
Ca (%)	1.43	0.67	0.38
P (%)	0.54	0.42	1.44
Magnesium (%)	0.17	0.23	0.21
Potassium (%)	0.83	2.76	1.19
Sodium (%)	0.33	0.03	0.15
Sulfur (%)	-	-	0.4
Iron (ppm)	252	568	115
Zinc (ppm)	253	33	32
Copper (ppm)	13	9	31
Manganese (ppm)	159	51	9
Molybdenum (ppm)	3	2	1

Table 4. Ewe and Lamb Serum Titer Levels

Time	CON[a]	SE[a]	LBY[a]	SE[a]	P-value[b]
Ewe Data					
Prior to vaccination	4	0.8	4	0.8	1.00
At booster vaccination	45	0.8	50	0.8	0.77
Prior to Lambing	235	0.8	221	0.8	0.71
Post-lambing	183	0.8	201	0.8	0.59
Lamb Data					
At 3 d of age	195	1.4	122	1.6	0.30

[a] Values are reported actual titer level estimates

[b] P-values are reported from square root transformation of ewe or lamb titer data

Table 5. Colostrum Quality

Item	CON	LBY	SEM	*P*-value
Colostrum Analysis				
IgG Colostral Concentration (% Brix)	30.4	24.6	3.1	0.21
Butterfat (%)	11.06	9.92	1.36	0.57
Protein (%)	18.46	14.54	1.99	0.19

Table 6. Lamb Body Weight

Item	CON	LBY	SEM	*P*-value
Birth Weight (kg)	4.99	4.54	0.68	0.31

CHATER V

SUMMARY AND SYNOPSIS

The effects of liquid brewers yeast supplementation on sheep or lamb performance remains unclear. With the increased availability of liquid brewers yeast, it is of interest to determine the value and application of it as a feed ingredient in sheep diets. The research presented here was explored in an attempt to fill in gaps in the literature regarding the use of liquid brewers yeast in sheep diets. The findings in these experiments did not find clear benefits to the supplementation at the current inclusion rates, however, there were also no negative effects.

In the first experiment, there were no effects on ewe BW, BCS, or F:G ratio through the 8 weeks (4 weeks prior to expected lambing and 4 weeks post lambing) of the feeding trial utilizing liquid brewers yeast. Since these data were only recorded within the last trimester of gestation, which majority of lamb growth occurs in this time period, it is likely that BW, BCS, and F:G ratio were influenced by other factors. Liquid brewers yeast supplementation had no effect on colostrum or milk quality. With the variation in research findings based on liquid brewers yeast supplementation in dairy cattle, these results were not out of line. Additionally, this research demonstrated no effects of dam liquid brewers yeast supplementation on lamb ADG for the 4 weeks following birth. Most research reporting lamb or calf ADG were reported where lambs or calves were supplemented with liquid brewers yeast directly; even so results were inconsistent.

In the second experiment, liquid brewers yeast did not significantly improve the transfer of passive immunity, as measured by lamb antibody titers for PI3. Previous research suggests there is considerable variation in ewe colostrum antibody concentrations. As a result, the amount

of antibodies transferred via colostrum consumption can differ greatly. The supplementation of liquid brewers yeast also had no influence on colostrum quality which could be a result of duration, quantity, or consistency of supplementation.

As the microbrewery population grows, the availability of liquid brewers yeast as a by-product increases. The incorporation of this yeast in sheep diets requires more research, however, current research shows no detrimental effects. The research efforts to identify a sustainable use of this by-product should allow both producers and microbreweries to establish a mutual beneficial service.