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The Effects of Different Organic Pastures on Dairy Heifer Growth and Development

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THE EFFECTS OF DIFFERENT ORGANIC PASTURES ON DAIRY HEIFER

GROWTH AND DEVELOPMENT

by

Jacob A. Hadfield

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ABSTRACT

The Effects of Different Organic Pastures on Dairy Heifer Growth and Development

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Dairy heifers developed in certified organic programs, especially those utilizing pasture-based management schemes, have lower rates of gain than heifers raised in conventional production systems. This study investigates the effects that different forages in a rotational grazing system have on the development of organically raised dairy heifers. Over 3 years, 210 yearling Jersey heifers were assigned to one of nine treatments, including a conventional dry lot control (TMR) or one of eight pasture treatments. Pasture treatments included: tall fescue (TF), meadow bromegrass (MB), orchard grass (OG), perennial ryegrass (PR) and each individual grass interseeded with birdsfoot trefoil (BFT). Every 35 days, over a 105-d period, heifers were weighed, measured for hip height, and blood samples were collected to determine serum insulin-like growth factor-1 (IGF-1) and blood urea nitrogen (BUN) concentrations. Fecal samples were collected to determine fecal egg count (FEC). Heifer body weights (BW), BUN, and IGF-1 concentrations were affected by treatment ($p < 0.01$) when analyzed over time. Heifers on mixed legume-grass pastures (MIX) tended to have greater BW compared to heifers on monoculture grass pastures. Heifers receiving TMR or PR+BFT had increased BW gain ($p < 0.05$) over the 105-d period than heifers grazing TF+BFT, OG, PR, MB, or TF. Whereas, at d 105, heifers receiving TMR, PR+BFT, OG+BFT, or MB+BFT had greater ($p < 0.05$) BW than heifers receiving TF. Heifer hip-height, conception rate, and FEC were not affected ($p > 0.05$) by pasture type or treatment when analyzed over time. Whereas, heifers grazing MIX pastures had greater ($p < 0.01$) BUN compared to heifers grazing MONO pastures. These results show that the addition of BFT to pasture improves growth of replacement heifers. Grass pastures interseeded with BFT may be a sustainable option to achieve adequate growth of dairy heifers raised in a pasture scenario.

(71 pages)

PUBLIC ABSTRACT

The Effects of Different Organic Pastures on Dairy Heifer Growth and Development

Jacob A. Hadfield

Raising dairy heifers in a certified organic setting can be difficult for producers. Conventionally, heifers are raised in a confined setting, and fed a total mixed ration (TMR) that is balanced daily to contain all the needed nutrients for developing heifers. Organic producers can use a TMR in their operations, but due to high organic feed costs, many choose to raise their heifers in pasture-based systems. While pasture-based systems may lower costs, heifers on pasture commonly have lower rates of gain, which can be financially burdensome to producers. Grass-legume pastures may help improve rates of gain in heifers on pasture-based systems. In this study, yearling Jersey heifers received one of nine different treatments: eight pasture treatments or a conventional TMR control, for a 105-d period. Pasture treatments included four grass pastures: tall fescue (TF), meadow bromegrass (MB), orchard grass (OG), perennial ryegrass (PR) and four mixed pastures with each individual grass interseeded with the legume birdsfoot trefoil (BFT). To determine the effects of different pastures on heifer growth, heifers were sampled every 35 days over a 105-d period. During sampling, weight and hip-height were measured, and blood and fecal samples were taken from each heifer. Blood samples were analyzed for blood urea nitrogen (BUN), an indicator of protein status, and insulin-like growth factor-1 (IGF-1), an indicator of energy balance. Fecal samples were analyzed to determine the parasite load of each heifer. At day 105 of the study, heifers were bred, and conception rates were determined 35 days after breeding. Heifers on mixed pasture tended to have increased body weights compared to heifers on grass pastures. Heifers fed on mixed pastures had a similar weight gain to those fed a TMR, except for heifers on TF+BFT were lower. Heifers fed on mixed pastures also had higher BUN concentrations than heifers fed on grass pastures. Heifers fed grass and mixed pastures had similar IGF-1 concentrations, parasite load and conception rates. Adding the legume BFT to grass pasture helped dairy heifers grow faster and more efficiently. Interseeding grass pastures with BFT may be a sustainable method to improve growth of developing jersey heifers being raised in a pasture-based system, although additional research is needed.

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CHAPTER I

INTRODUCTION

Replacement heifer management is critical to maintain profitability in a dairy operation. However, costs of raising replacement heifers are the second largest expense incurred by dairies, only behind feed costs (Tozer & Heinrichs, 2001). To reach maximum productivity, heifers need to calve by the time they are 24 months of age (Le Cozler, Lollivier, Lacasse, & Disenhaus, 2008) and should weigh at least 65% of their mature body weight by first insemination (Patterson et al., 1992). Many producers accomplish this goal by utilizing a conventional feed system that consists of delivering a total mixed ration (TMR) in a confined area, which allows for control of nutrient intake (Endecott, Funston, Summers, & Roberts, 2012). While efficient, this method can be costly and producers welcome new strategies and alternatives to confined feeding. Current literature focuses primarily on the needs of conventional dairies. However, organic producers, who have more strict regulations and more economic costs, desire modern research aimed at finding ways to diminish expenses that do not negatively impact milk yields or quality, while still following organic requirements (McBride & Greene, 2009).

Organic dairy production has become the fastest growing segment of U.S. organic agriculture (McBride & Greene, 2009). Many producers, aiming to increase profits on organic milk sales, have converted their dairies from conventional to organic practices. Requirements for organic dairying, as established by the U.S. Department of Agriculture – Agricultural Marketing Service (USDA-AMS), state that organic producers must let cattle graze in pasture for the entire grazing season of their geographical region (USDA-AMS, 2019), during which time 30% of the ruminant's dry matter intake (DMI) must come from pasture. Organic producers meet this requirement by feeding a primarily pasture-based diet, when available, to combat high feed costs. The success of this strategy has made it a popular alternative in both organic and conventional dairies. However, producers who used the highest amount of pasture-based forage (75-100%) had the lowest net returns due to a 32% decrease in milk yield (McBride & Greene, 2009). Although pasture grazing may not provide enough energy to maintain lactating cows, it may have the capacity to develop replacement heifers. To accomplish this, pastures must provide nutrition that allows for adequate daily gains, maintain or enhance reproductive performance, and improve rumen utilization of nitrogen.

Using grass-legume mixtures in pasture could help to achieve these goals by supplying adequate amounts of herbage, energy and protein for proper heifer development. The ability of legumes to fix atmospheric nitrogen into a metabolizable form, such as ammonia, leads to an increase of nitrogen in pasture systems without having to apply fertilizers. Under optimal growing conditions and cutting management, legumes can fix 700 kg N ha^{-1} yr^{-1} (Ledgard, 2001). As optimal conditions require strict management and precise conditions, pasture legumes commonly have lower nitrogen fixation rates, BFT in pasture commonly fixes between 12-168 kg N ha^{-1} yr^{-1} and alfalfa fixes between 78-224 kg N ha^{-1} yr^{-1} (Peel, 2020). The increase of nitrogen can increase yields and nutrition quality, especially protein, in pasture forages.

Fluctuations of nutrient content during the grazing season are correlated with the growth cycle of forages (Soder & Muller, 2016). Many pasture grasses have the highest growth rate during the early spring. As temperatures rise and precipitation declines in the summer, pasture grass growth and overall plant nutrition decline as well. Legumes follow a similar pattern but tend to maintain higher growth and nutrition levels than pasture grasses during the summer (Soder & Muller, 2016). Utilizing grass-legume pasture allows producers to utilize the nutrient fluctuation across the season to their advantage. Producers can use pasture grasses for their quick growth and high nutrition in the early spring, then rely on legumes to maintain pasture nutrition levels through the summer (Soder & Muller, 2016). This system allows producers to maintain a higher quality of pasture throughout the whole grazing season when compared to using just grass monocultures alone.

Tannin containing legumes could also aid producers raising ruminants on pasture. Research has shown that legumes containing tannins can increase nitrogen utilization in the rumen, decrease the incidence of bloat, and act as a natural anthelmintic to decrease parasite load (Min, Barry, Attwood, & McNabb, 2003; Patra & Saxena, 2011). The use of legumes in grass pasture, especially those that contain tannins, could influence dairy heifer growth and reproductive performance. Previous research at Utah State University has focused on the effects that tannin-containing legumes interseeded in grass pastures can have on beef steer growth (Waldron et al., 2020). My research attempts to translate Waldron et al.'s research into the organic dairy sector, while also studying the effects that BFT, coupled with different nutritive grasses, can have on dairy heifer growth and development. The results of this research coupled with current knowledge gaps in the literature in understanding how different types of pastures impact growth, health and reproductive status of dairy heifers has led us to our objectives. The objective of this study is to determine the impacts that different organic pasture forages, have on dairy heifer growth, health and reproductive development.

The hypothesis for this study was that the provision of mixed pastures (legume and grass) would result in improved growth, health, and reproductive efficiency in developing dairy heifers when compared to heifers developed on monoculture grass pastures.

CHAPTER II

LITERATURE REVIEW

Heifer Development

Replacement heifer costs are the second highest expense, behind feed costs, on an operating dairy (Tozer & Heinrichs, 2001). On average, one-third of a U.S. dairy herd is replaced each year. The main goal of a producer when developing replacement heifers is to raise them quickly and at the lowest cost possible without causing negative effects on lifetime milk production. Using high-energy concentrates, such as grains, to increase growth rates of heifers is a common practice that producers use to decrease feed and labor costs and allow heifers to become productive more quickly. However, research has found that accelerated growth, especially during the pre-pubertal stage, can have detrimental effects on mammary gland development by inhibiting mammary gland growth, which ultimately impacts milk yield capacity during lactation (Le Cozler et al., 2008). Other studies have found that growth, independent of dietary treatment, does not result in impaired mammary development (Silva, VandeHaar, Whitlock, Radcliff, & Tucker, 2002). Although heifers fed on a conventional TMR can struggle with high rates of accelerated growth, heifers raised on pasture are more commonly affected by slower rates of gain, which can be seen in pasture-based and organic dairy systems.

Organic Dairying

Organic milk production has become the fastest growing segment of organic agriculture (McBride & Greene, 2009). Increases in pasture-based dairies, organic milk production, and increased demand for organic milk have been the main factors responsible for the growth of this sector of the dairy industry. The most recent survey stated that organic producers across all states on average receive $18.84/cwt of milk more than conventional producers (USDA - Economic Research Service (USDA-ERS), 2016). It should be noted that organic dairy systems on average have higher operating costs ($8.62/cwt of milk) than conventional systems (USDA-ERS, 2016). Even with higher operational costs, many dairy producers seeking to increase profits have transitioned from conventional to organic practices, especially smaller dairies. According to the requirements for organic dairying as established by the USDA-AMS, organic dairies are required to feed animals 100% organic feeds, and pasture must provide a minimum of 30% of a ruminant's DMI during the grazing season (USDA-AMS, 2019). Geographical region determines grazing season length, but the minimum is 120 calendar days. Antibiotic and parasiticide use are restricted to specific products and must be followed by dairy farmers to maintain organic certification (USDA-AMS, 2019). Raising replacement heifers on pasture is a challenge for organic dairy producers. High quality pasture forages must be produced to ensure proper development of dairy heifers.

Pasture-Based Grazing

Feeding a TMR in a confined setting is common practice on conventional dairies. This efficient method allows producers to control nutrient intake. Producers can estimate the feed requirements of their animals and can then deliver the correct ration to match those requirements daily. While a TMR can be given in an organic setting, the high price of organic feeds often drives organic producers to use primarily pasture in their operations, especially for their developing heifers.

When compared to a traditional TMR, grazing systems often have decreased feed expenses. However, grazing systems can present unique and challenging problems for producers. In a pasture-based system, nutrient requirements are more variable than in a confined feeding setting. Ruminant nutritional requirements may change due to environment, grazing activity, travel, and animal preference (Allison, 1985). The nutrition of forages is also affected by many environmental factors. As the grazing season persists, many forages began to acquire more indigestible fiber. An increase in forage fiber and decrease in moisture can lead to decreased voluntary intake of ruminants. Research has shown with primarily roughage diets, intake is limited due to the capacity of the rumen and by the rate of disappearance or digestion in the rumen (Allison, 1985; Balch & Campling, 1962). Higher quality forages with improved nutrition may be able to increase the voluntary intake of ruminants on pasture. However, maintaining high pasture nutritional content can be difficult for producers because of a range of factors that affect plant nutrition throughout the entirety of a grazing season. Climate, maturity, grazing intensity, plant species, and soil fertility are just a few of the many factors that affect the nutrition of plants in pastures (Waghorn & Clark, 2004). Maturity has been shown in certain grasslands to cause a decrease in protein and mineral content and an increase in fiber (Corona, Aldana, Criado, & Ciudad, 1998). Research has also shown that selective grazing can change the plant composition of a pasture, due to over grazing nutrient-rich plants, as well as change organic and mineral composition through fertilization from feces and urine (Corona et al., 1998; Georgiadis & McNaughton, 1990; Jaramillo & Detling, 1992). Multiple factors affecting plant nutrition create variation in the available nutrients found in pasture. To reach or maintain optimal nutritional level, producers must manage pastures carefully while attempting to account for these many variables.

Diminished weight gain, wool yields, and milk yields in grazing animals compared to dry-lot controls is commonly observed in grazing studies. McClure, Van Keuren, and Althouse (1994) did a multi-year grazing study comparing grass pasture, legume-mixed pasture, and a dry-lot control in the growth and carcass traits of weaned lambs. Using a rotational grazing system, lambs were separated into four treatments: orchard grass, ryegrass, alfalfa, or an all-concentrate dry-lot diet (McClure et al., 1994). Results showed that average daily gain (ADG) numbers were in the order of dry-lot > alfalfa > grasses (McClure et al., 1994). Lambs grazing alfalfa had better ($p < 0.01$) performance than those grazing grasses, which was attributed to increased ($p < 0.01$) crude protein and decreased ($p < 0.01$) neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (McClure et al., 1994). Across all years, lambs grazing on grasses had decreased ($p < 0.002$) carcass performance traits compared to alfalfa grazed lambs, except one year (1984) when lambs grazing ryegrass had no difference in carcass characteristics from their alfalfa counterparts (McClure et al., 1994). The research suggested that a difference of breed choice, cross-bred rather than Targhee, used that year may have been related to this interaction (McClure et al., 1994). Alfalfa carcasses were lighter ($p < 0.01$) than dry-lot lambs, but the alfalfa carcasses had similar ($p > 0.05$)

muscle mass to the dry-lot carcasses and had less ($p < 0.01$) fat (McClure et al., 1994).

Funston and Larson (2011) conducted a study comparing the differences in growth among grazing winter range with corn residue and dry-lot beef heifers. Dry-lot heifers had higher ($p = 0.01$) body weight (BW) gains compared with grazing heifers (Funston & Larson, 2011). Grazing heifers also had a lighter ($p = 0.02$) BW at breeding, and fewer ($p < 0.01$) had reached puberty before breeding (Funston & Larson, 2011). However, conception rates by artificial insemination did not differ among groups and final pregnancy rates did not differ (Funston & Larson, 2011). Grazing heifers had better ($p = 0.02$) ADG after breeding, attributed to compensatory gain, which resulted in a similar pre-calving BW (Funston & Larson, 2011). Overall heifers grazing winter range with corn residue saw a reduced ($p < 0.01$) cost of $45 per pregnant heifer compared with dry-lot heifers (Funston & Larson, 2011).

Another study conducted in Holstein heifers investigated the differences of pasture treatments on heifer growth (Barker et al., 1999). Three pasture treatments consisted of alfalfa and smooth bromegrass, Birdsfoot Trefoil (*Lotus corniculatus* L.;BFT) and smooth bromegrass, and a monoculture of smooth bromegrass plus nitrogen (Barker et al., 1999). The alfalfa and bromegrass treatments yielded more ($p < 0.05$) forage than the BFT treatment (Barker et al., 1999). However, alfalfa and BFT treatments resulted in increased ($p < 0.05$) heifer ADG when compared to bromegrass (0.93 and 0.97 vs. 0.83 kg/d; Baker et al., 1999).

Waldron et al. (2020) researched the effect that interseeding legumes into a grass pasture can have on beef steer growth and performance. Beef steers were divided into four pasture treatments consisting of tall fescue monoculture (with or without nitrogen fertilizer) and tall fescue in binary mixture with BFT or alfalfa (Waldron et al., 2020). Steers that grazed BFT binary mixed pastures had the largest ($p < 0.05$) overall BW gain (81.5 kg) over the 112-d grazing period. Steers on alfalfa binary mixed pastures had the next highest BW gain ($p < 0.05$; 75.2 kg), followed by tall fescue with nitrogen fertilizer ($p < 0.05$; 68.5), and tall fescue pastures without nitrogen fertilizer had the least overall BW gain ($p < 0.05$; 44.4) over the 112-d grazing period (Waldron et al. 2020). At the conclusion of the study, Waldron et al. ran an economic analysis and discovered that beef steers grazing BFT binary mixed pastures had the greatest ($p < 0.05$) net return of $1,197 USD/ha and steers grazing tall fescue monocultures without fertilizer barely broke even with a net return of only $96 USD/ha. Overall, the researchers concluded that grazing pastures planted to tall fescue mixed with alfalfa or BFT were more economically viable than fertilized or unfertilized tall fescue monoculture pastures.

Effect of Heifer Growth on Performance

Developing productive and efficient dairy cows starts with proper growth and development of replacement heifers. Physical growth is measured through BW, height (wither height [WH] or hip height [HH]), and age. Growth can also be characterized by measuring two different circulating growth factors: growth hormone (GH) and insulin-like growth factor-1 (IGF-1).

Heifer growth studies are common in both the beef and dairy industries. A review focused on beef heifer research found that rearing heifers to only 50-57% of mature BW at conception, instead of 60-65%, reduced costs and did not hinder reproduction (Endecott et al., 2012). Funston and Deutscher (2004) found that developing heifers to 53% of mature BW lowered development costs and had no difference ($p > 0.05$) on reproductive performance when compared to heifers at 58% mature BW. However, little to no research has been published on how lowering mature BW at breeding may affect longevity in the cow; research has shown in rodent and non-human primate models that lifespan can be increased by limiting caloric intake during juvenile development (Speakman & Hambly, 2007). While this research is applicable to the beef industry (Endecott et al., 2012), due to a short specific calving window and limited winter nutrition, the dairy industry does not see the same implications. Limiting caloric intake in dairy heifers increases the time of nonproductive performance of the animal, which would increase both feed and labor costs and bring a heavy financial burden to producers.

However, in a review by Heinrichs, Zanton, Lascano, and Jones (2017) it was discussed that breeding heifers at a younger age, while establishing a proper development program, has the potential to increase profits for the dairy industry. Although, research supports that dairy heifers should be 65% of mature BW at first insemination (Patterson et al., 1992). Heifers bred as early as 350-d of age had no negative effects when it came to retained placenta or calving ease, but it did result in calves weighing 1.2 kg lighter ($p < 0.05$, Lin et al., 1986). Heifers from the 350-d group had lower conception rates (38%) than their 462-d counter parts (47%, Lin et al., 1986). The 350-d breeding group also had lower ($p < 0.05$) milk yield per day during their first lactation when compared with the 462-d breeding group (14.3 and 15.3 kg, respectively; Lin et al., 1986). However, yield per day of life to end of first lactation had no difference ($p > 0.05$) between the 350-d and 462-d breeding groups (4.3 and 4.2 kg, respectively; Lin et al., 1986). Conception rates at first service and first lactation yields may be reduced due to early breeding, but diminished rearing costs and a quicker return of income gives early breeding an economic advantage over late breeding.

To shorten the unproductive time of dairy replacement heifers, many producers increase rate of gains. However, high increases in BW before puberty from high energy diets result in decreased mammary development (Le Cozler et al., 2008) due to increased mammary fat deposition. Although, Silva et al. (2002) found that heifers with higher rates of gain saw no impaired mammary development when compared to other heifers receiving the same dietary treatment with lower rates of gain. These results suggest rapid heifer BW gain may not be the cause of reduced milk yield (Silva et al., 2002). Body fatness may be a better indicator of impaired mammary development than BW (Capuco, Smith, Waldo, & Rexroad, 1995).

Growth Hormone and Insulin-Like Growth Factor-1

The hormone recognized for its essential role in the growth and development of mammals is GH (Sonntag et al., 2005). Numerous organ systems and complex processes benefit from GH and its anabolic mediator, IGF-1. Stimulation of fatty acid metabolism and amino acid uptake, as well as protein synthesis are some of the known actions of GH (Corpas, Harman, & Blackman, 1993). These actions aide GH in its role of regulating cell division and tissue growth (Sonntag, Ramsey, & Carter, 2005). Most processes that GH regulates or has a role in occur via IGF-1, excluding fatty acid metabolism. Released from the anterior pituitary, GH travels through the blood and binds to the growth hormone receptor with high affinity. Growth hormone receptors are found in tissues throughout the body and activation of these receptors leads to initiation of gene expression and subsequent activation of many different pathways involved in growth. Gene expression, synthesis and release of IGF-1 are all increased when GH receptors are activated (Xu & Sonntag, 1996).

The liver produces most of the IGF-1 that is found in the blood (Fenwick et al., 2008). As the mediator of the effects of GH, IGF-1 can initiate cell division and tissue growth throughout the body. Plasma IGF-1 levels are used as a measure of nutritional and reproductive status. Endocrine IGF-1 levels are not a predictor of reproductive events to come but could be an estimator of an animal's ability to achieve a reproductive event (Velazquez, Spicer, & Wathes, 2008). Plasma IGF-1 levels have also been found to be negatively correlated with fecal egg counts (FEC; Díaz-Torga et al., 2001, Lacau-Mengido et al., 2000). Parasites absorb nutrients from their host and can decrease feed intake (Idris, Moors, Sohnrey, & Gauly, 2012), creating a state of undernutrition in the host. Research has shown that undernutrition can uncouple IGF-1 regulation from GH (Elsasser, Rumsey, & Hammond, 1989). Studies have proposed that the somatotropic axis responds to nutrient restriction by partitioning nutrients away from muscle to the immune system (Davis, 1998; Diaz-Torga et al., 2001). A study using parasitized animals and a control group found no difference in circulating GH levels among treatments but did find animals with parasites had lower ($p < 0.05$) concentrations of circulating IGF-1

(Lacau-Mengido et al., 2000). Differences were attributed to a decrease in the production of IGF-1 at the hepatic level due to reduced feed intake. Although, a possible ivermectin effect on IGF-1 production in the animals that were treated for parasites could not be ignored (Díaz-Torga et al., 2001).

Research has indicated that GH may play a role in mammary gland development (Le Cozler et al., 2008). Sejrsen, Huber, and Tucker (1983) found that an increased plane of nutrition results in a decrease in concentration of circulating GH. Researchers also found that mammary growth was positively correlated with GH (Sejrsen & Purup, 1997). Taking this a step further, Sejrsen, Foldager, Sorensen, Akers, and Bauman (1986) administered exogenous GH and found that exogenous GH increased pubertal mammary gland growth. However, in-vitro studies found that GH does not bind to the mammary gland and does not stimulate mammary cell growth (Purup, Sejrsen, & Akers, 1995). The research suggests that GH acts through its anabolic mediator IGF-1 to initiate mammary cell growth (Sejrsen & Purup, 1997). Receptors for IGF-1 have been discovered on mammary cells, and when activated stimulate mammary cell proliferation (Sejrsen & Purup, 1997).

Tannins

Livestock performance has benefited from grass-legume pastures due to higher forage nutritive value. Introducing legumes into grass pastures can improve pasture performance (Hoveland, Hardin, Worley, & Worley, 1991; Stephenson & Posler, 1988). Legumes, when grown in mixtures, can supply nitrogen to grasses, increasing grass forage yield and decreasing the use of nitrogen fertilizer (Carlsson & Huss-Danell, 2003). The legume BFT, with its condensed tannin (CT) content, has become an area of interest in ruminant research. Moderate CT concentrations have been shown to have positive effects in grazing ruminants. In New Zealand, a study analyzing the effects of sheep grazing BFT reported increased wool growth and carcass gains compared to animals who grazed grasses, many of the differences found were attributed to CT content (Wang et al., 1996). Ramírez-Restrepo et al. (2005) reported similar findings in sheep, adding also a decrease ($p < 0.05$) in parasite burden. Lactating cows fed BFT had 42% higher milk yields than their other grazing counterparts and half of the increase in milk yield was attributed to CT (Woodward, Auldist, Laboyrie, & Jansen, 1999).

The increase in animal product yields can be explained by the ability of CT to bind with forage proteins and prevent protein degradation in the rumen. Aerts (1999) found that CT extracted from BFT protected forage proteins from microorganisms. Results showed that 400 μg CT/mL or greater was the concentration of CT needed to produce maximum inhibition of protein degradation in in-vitro studies (Aerts et al., 1999). These results were confirmed by Molan, Waghorn, Min, and McNabb (2000). As CT containing forages are masticated, CT-substrate complexes are formed that are insoluble (Jones & Mangan, 1977, Min et al., 2003). These complexes prevent substrates from being exposed to rumen microorganisms and thus allow those proteins to pass into the small intestine where they can be absorbed.

The legume BFT has gained interest because of its CT content and its ability to decrease incidence of bloat in ruminants (Patra & Saxena, 2011). Bloat occurs in ruminants when gas production levels exceed the rate of gas expelled by normal methods. Frothy bloat is the most common type of bloat among ruminants grazing legume forages (Cheng et al., 1998; Patra & Saxena, 2011). The presence of soluble proteins from forage legumes changes rumen microbial fermentation, leading to an increase in slime and gas in the rumen, which is the main symptom of frothy bloat. Soluble proteins can be precipitated by CT, which reduces bloat during grazing (McMahon et al., 2000). Chiquette, Cheng, Costerton, and Milligan (1988) found that feeding ruminants CT-containing forages reduced rumen gas production by precipitating the forage protein foam. A concentration of 5 mg CT g^{-1} DM was reported by Li, Tanner, and Larkin (1996) as the minimum concentration to consider a forage bloat-safe.

Parasite infection is a major health concern that affects grazing ruminants. Organic dairy producers are more susceptible to parasite infection due to imposed limitations on anthelmintic use. Extensive protein losses have been found in sheep with parasite infection of the abomasum and small intestine (MacRae, 1993, Minn et al., 2003). Heavy protein loss and decreased rate of gains in animals due to parasites can be a heavy financial burden to the animal industry. Natural methods, such as using forages with CT (Minn et al., 2003; Molan et al., 2000), have been suggested to help decrease parasite burden and fight anthelmintic resistance in parasites. Niezen, Robertson, Waghorn, and Charleston (1998) reported that lambs grazing high CT-containing forages, such as sulla (*Hedysarum coronarium*), had a reduction in FEC and worm burdens. Molan (2000) reported that CT can inhibit development of *Trichostrongelus colubriformis* eggs and reduce larval motility. Molan also suggested that CT may interrupt the life cycle of sheep nematodes and be able to decrease infective larvae in pasture. A recent study found that dairy heifers grazing BFT may have reduced FEC compared to control animals (Shepley, Vasseur, Bergeron, Villeneuve, & Lachance, 2015). Taken together, these findings demonstrate grazing legumes containing CT can help reduce parasite load in ruminants.

Reproduction and Grazing

Organic producers have many concerns that come with grazing their animals. Parasite load, decreased production/intake, and bloat are just a few of the problems that are associated with grazing animals. However, the concern that has the largest economic impact on dairy producers is herd fertility. It is widely known that herd fertility is one of the most important factors in dairy sustainability. Organic producers are challenged to maintain high reproductive fertility and high milk yield when nutrition management is limited.

Excessive amounts of rumen degradable protein have been shown to decrease conception rates as well as elevate urea and ammonia levels in the blood plasma (Elrod & Butler, 1993). Bruckental, Drori, Kaim, Lehrer, and Folman (1989) found lower ($p < 0.05$) pregnancy rates in dairy cattle that were fed high levels (210 g/kg CP) of crude protein via soybean meal. Elrod and Butler researched a step further and found that cows fed high amounts of rumen degradable protein in their ration had elevated ($p < 0.05$) levels of urinary urea nitrogen and plasma urea nitrogen (PUN). A change in uterine pH during the luteal phase was also noticed in the study, which suggested it may play a role in reduction of fertility found in cows fed high amounts of rumen degradable protein (Elrod & Butler, 1993). One research study found that high CP diets (20%), particularly diets high in rumen degradable protein (72.5% of CP) delayed the interval to first ovulation (Staples, Garcia-Bojalil, Oldick, Thatcher, & Risco, 1993). However, another research study showed that high CP diets (19%) had no effect on delaying ovulation, creating contradiction in the research (Gilbert, Shin, Rabuffo, & Chandler, 1996). With conflicting research, much of the literature has turned to the common metabolism end-point of both rumen degradable protein and rumen undegradable protein in cattle, which is the formation of urea (Butler, 1998). Research has shown that rumen degradable protein and rumen undegradable protein increase PUN and alter uterine pH to a similar degree (Elrod, Van Amburgh, & Butler, 1993). Researchers found that PUN varies inversely with uterine pH, making it a possible mechanism behind decreased fertility in cattle with elevated milk urea nitrogen (MUN) or PUN (Elrod et al., 1993). The results from these studies suggest that PUN levels between the range of 12 to 24 mg/dL can have direct effects on uterine function (Butler, 1998). Taken together, these findings demonstrate the decreased fertility in cattle with elevated urea nitrogen levels and the potential molecular mechanisms that cause these reproductive inefficiencies.

Common measurements of degradable protein in dairy cattle include blood urea nitrogen (BUN), PUN, and milk urea nitrogen (MUN), all three measurements report similar urea nitrogen levels in cattle and are comparable. Research has found that BUN and MUN are highly correlated with little to no differences, although MUN was found to have a 1-2 hr time lag behind BUN and was noted in the research (Butler, 1998;

Gustafsson & Palmquist, 1993). When using BUN and MUN, diurnal variations of serum and milk need to be considered as time of sampling versus time of feeding is crucial (Gustafsson & Palmquist, 1993). Rajala-Schultz, Saville, Frazer, and Wittum (2001) found that cows with MUN levels below 10.0 mg/dL were 2.4 more times likely and cows between 10 and 12.7 mg/dL were 1.4 times more likely to be pregnant than cows that had 15.4 mg/dL or higher levels of MUN at breeding. Another research study found that PUN and MUN levels in lactating cows that were > 19 mg/dL were associated with a 20% decrease in pregnancy rate after AI (Butler, Calaman, & Beam, 1996).

Pasture-based systems have also been found to adversely affect pregnancy rates (Diskin, Murphy, & Sreenan, 2006). It is hypothesized that this effect is caused by excess rumen-degradable protein (Roche et al., 2009), which is common in lush fresh forages that provide ample amounts of nutrients. The amount of rumen-degradable protein then exceeds what is needed to maintain healthy microbial populations. Research suggests that BUN levels below 15 mg/dL are ideal for proper reproductive performance, whereas BUN levels above 20 mg/dL have been shown to be detrimental to conception (Ferguson, Blanchard, Galligan, Hoshall, & Chalupa, 1988; Ferguson, Galligan, Blanchard, & Reeves, 1993; Rajala-Schultz et al., 2001). Plasma nitrogen levels in improved pasture grazing systems routinely have been reported in excess of 40 mg/dL (Kolver & Macmillan, 1994; Ordóñez et al., 2007; Roche, Petch, & Kay, 2005). Adding BFT to pasture based systems may increase BUN levels in dairy cows. However, due to the ability of CT to protect rumen degradable protein in the rumen (Jones & Mangan, 1977; Minn et al., 2003; Patra & Saxena, 2011), BFT may reduce BUN levels in heifers while still allowing the animals to consume a high-quality forage. Future research beyond is needed to determine the effects BFT can have on urea nitrogen levels in the animal and how this may affect conception rate.

CHAPTER III

MATERIALS AND METHODS

All animal experiments were conducted following procedures approved by the Institutional Animal Care and Use Committee (IACUC protocol #2777 and #10063) at Utah State University. Over a 3-year period (2016- 2018), a total of 210 yearling Jersey heifers were purchased from commercial dairies. In May of each year, 81 heifers (48 in 2016) were transported to the Intermountain Irrigated Pasture Project in Lewiston, Utah. Upon arrival, heifers began a 2-week grazing acclimation period to ensure heifers could consume forage from pastures adequately. After the 2-week transition period was completed, heifers were fasted for 12 h in preparation for sampling. As heifers were sampled at d 0, three heifers (two in 2016) were randomly assigned to each treatment (n = 9, n = 6 in 2016).

Treatments

Pasture Treatments

This study utilized eight different pasture treatments and a dry-lot TMR control. The eight pasture treatments consisted of four monoculture grass pastures and four mixed pastures. The four monoculture grasses used were: Cache Meadowbrome Grass (MB), QuickDraw Orchard Grass (OG), Amazon Perennial Ryegrass (PR), and Fawn Tall Fescue (TF). Mixed pastures consisted of one of the four monoculture grasses listed previously, mixed with BFT (MB+BFT, OG+BFT, PR+BFT, TF+BFT). All heifers on pasture had access to water and a trace mineral supplement. Pasture treatments were planted at the Intermountain Irrigated Pasture Project and were grazed for a 105-d period.

Within each block, pastures of each treatment had 0.4 ha, which was divided evenly amongst five 0.08 ha paddocks. Paddocks were separated with a single strand of poly-wire charged by a battery powered fence charger. Rotational stocking was used with a stocking period of 7-d, followed by a rest period of 28-d, such that the entire cycle was 35-d. Three full rotations occurred each year, giving heifers a total of 105-d on pasture (20 June to 13 Oct., 2016 and 17 May to 30 Aug., 2017 and 16 May to 29 Aug., 2018). At the end of each 35-d rotation cycle, heifers were gathered for sample collection before resuming the next 35-d cycle.

Pastures were fertilized twice yearly with two different organic fertilizers. Mined sodium nitrate and hydrolyzed poultry feathers were both applied on all pasture treatments in early May at a rate of 28 kg N ha^{-1} and 36.8 kg N ha^{-1} respectively. In early July, sodium nitrate was spread again over the monoculture grass treatments at the same rate, mixed pastures received no fertilizer at this time. Paddocks were irrigated one week before grazing and within a week after grazing, so that pastures received 8.89 cm of water every 14-20 days.

Pasture samples were taken pre- and post- grazing to determine yield as well as nutritional quality of the individual pasture. The in-depth analyses of these pasture samples was previously reported (Rose, 2019). However, Table 1 shows the average nutritional quality for each of the nine treatments found from the analyses of pasture samples as well as the TMR (Rose, 2019).

Table 1

Nutrient Analysis of Individual Pasture Treatments and TMR, Averaged Over 3 Years and Separated by Sampling Period

Day	Treatments[1]	CP[2]	ADF[3]	aNDF[4]	Fat	ME[5]	Ash
0 - 35	MB	9.02	39.68	61.11	2.29	2.75	10.34
	MB+BFT	13.90	37.86	57.27	2.07	2.85	9.25
	OG	8.43	37.11	60.55	2.71	2.68	11.08
	OG+BFT	12.14	36.99	57.15	2.34	2.81	10.21
	PR	8.16	30.70	47.76	2.54	3.01	11.51
	PR+BFT	16.37	30.08	42.42	2.19	3.12	10.18
	TF	8.54	36.74	57.11	2.09	2.63	13.39
	TF+BFT	16.37	30.08	42.42	2.19	2.74	10.18
	TMR	14.31	27.43	37.84	3.03	2.40	8.96
	MONO	8.54	36.06	56.63	2.41	2.77	11.58
	MIX	14.69	33.76	49.81	2.20	2.88	9.95
35 – 70	MB	9.48	43.25	63.05	2.56	2.46	11.43
	MB+BFT	16.33	36.70	53.66	2.17	2.63	11.76
	OG	9.23	39.51	63.41	3.22	2.48	12.53
	OG+BFT	13.97	36.67	56.86	2.74	2.67	12.66
	PR	8.79	35.45	55.26	2.89	2.64	13.30
	PR+BFT	16.48	33.18	45.80	2.10	2.70	11.88
	TF	8.12	40.02	61.24	2.27	2.38	15.03
	TF+BFT	13.54	36.29	54.82	2.18	2.47	14.92
	TMR	14.54	30.54	41.44	2.88	2.32	8.74
	MONO	8.91	39.55	60.74	2.74	2.49	13.07
	MIX	15.08	35.71	52.79	2.30	2.62	12.80
70 - 105	MB	11.69	40.34	59.14	3.04	2.57	12.00
	MB+BFT	17.09	34.31	51.56	2.68	2.78	12.21
	OG	11.54	35.98	59.42	3.75	2.58	13.19
	OG+BFT	14.53	34.46	54.74	3.27	2.76	13.34
	PR	12.60	33.25	51.66	3.03	2.69	13.22
	PR+BFT	19.06	30.58	41.24	2.17	2.72	12.79
	TF	9.51	37.97	58.38	2.68	2.43	15.83
	TF+BFT	14.15	34.41	52.46	2.56	2.56	15.81
	TMR	13.40	32.45	43.36	2.42	2.28	8.11
	MONO	11.34	36.88	57.15	3.13	2.57	13.56
	MIX	16.21	33.44	50.00	2.67	2.71	13.54

[1]Treatments include: Meadow bromegrass (MB), meadow bromegrass + BFT (MB+BFT) orchard grass (OG), orchard grass + BFT (OG+BFT), perennial ryegrass (PR), perennial ryegrass + BFT (PR+BFT), total mixed ration (TMR), all monoculture grass pastures combined (MONO), all grass + BFT mixed pastures combined (MIX).

[2]Crude protein.

[3]Acid detergent fiber.

[4]neutral detergent fiber (determined by amalayse).

[5]Metabolizable energy (Mcal/kg).

Dry-Lot Control

Heifers assigned to the dry lot control were fed a TMR and had access to water and a trace mineral supplement for the 105-d period of the experiment. The dry-lot control was only used in the study during years 2017 and 2018. The TMR composition by DM% for 2017 was as follows: 45% alfalfa haylage, 19% corn silage, 18% flaked corn grain, 9% beet pulp shreds, and 9% wheat straw. The composition for the TMR in 2018 by DM% is as follows: 46% corn silage, 27% flaked corn grain, 22% alfalfa hay, and 5% wheat straw. Year differences between TMR compositions were due to feed resource availability. For ease of access to feeds and feed equipment, heifers receiving the TMR were moved from the Intermountain Irrigated Pasture Project to the Caine Dairy Teaching and Research Farm in Wellsville, Utah. Control heifers were separated by block into three different pens, with three heifers per pen. Control heifers were fed to achieve average daily BW gains of 0.84 kg/d. The TMR was fed daily at 0700, and refusals were weighed, recorded, and discarded daily before feeding to determine intakes by block.

Every 7 days, TMR samples were collected and stored at -20°C. After collecting TMR samples over the 35-d period, one full grazing rotation, TMR samples were mixed and a composite sample taken. The composite sample was then sent to Cumberland Valley Analytical Services for analysis. The TMR nutrition analyses, with the pasture treatment analyses, are shown in Table 1. Every 35-d, control heifers were gathered for sample collection similar to the heifers fed pasture treatments.

Sample Collection

Samples from heifers were collected at four different time points: d 0, d 35, d 70, and d 105. All heifers were fasted for 12 h before sample collection. Weight, hip height, blood, and fecal samples were taken from each heifer at each time point. Weights were taken via an electronic scale. A regular hip-height measuring stick (Sullivan Supply) was used to determine hip height. Blood samples were collected via jugular venipuncture, using red top tubes, and could clot at room temperature for 30 min before being stored and transported in a portable cooler. Fecal samples were collected in 50 mL conical tubes, put on ice, and taken to the Utah Veterinary Diagnostic lab for analysis of FEC. Fecal samples were analyzed using the Wisconsin Sugar Flotation Test (2017) and the McMaster Egg Counting Technique (2018).

Serum Metabolite Profiling

After blood collection, tubes were stored at 4°C for 24 h. Blood samples were then centrifuged at 1000 rpm for 15 min Serum was removed from blood samples and stored at -20°C for subsequent analysis. Serum analyses included BUN and IGF-1. A commercially available colorimetric assay was used to detect BUN in duplicate (Invitrogen, Urea NitrogenBUN Colorimetric Detection Kit; ThermoFisher Scientific). Serum samples were analyzed for IGF-1 in duplicate using the Human IGF-1 Quantikine ELISA Kit (SG100; R&D Systems). This kit has been shown to have 100% cross-reactivity with bovine IGF-1 (Moriel, Cooke, Bohnert, Vendramini, & Arthington, 2012).

Reproduction

Near the end of the grazing season, heifers began a controlled internal drug-release insert (CIDR) based, fixed-time-artificial insemination protocol to evaluate the effect of different pasture treatments on heifer conception rates. Conception rate data was used for research purposes and does not follow organic certification standards. At d 100, heifers received a CIDR insert for 5 days with a 2 cc intramuscular injection of gonadotropin-releasing hormone (Fertagyl: Merck Animal Health USA). At CIDR removal, 2 cc of Prostaglandin $F_{2\alpha}$ (Estrumate: Merck Animal Health USA) was given via intramuscular injection immediately and 12 h after removal. Estrus behavior was monitored following CIDR removal. Three days following CIDR removal, heifers were artificial insemination with semen from a single bull, by a single inseminator with another intramuscular injection of gonadotropin-releasing hormone (2 cc). Single service conception rates were determined 35 d post breeding by ultrasonography. After d 105 of the study, heifers remained on treatments until 17 d post breeding. Heifers were then moved to the Caine Dairy and Teaching Research Farm for monitoring ease of estrus behavior.

Statistical Analysis

Data was analyzed by use of a randomized complete block design with nine treatments in three blocks. Individual pastures and dry-lot pens served as the experimental unit, the mean of individual heifers within the experimental unit was used. The experimental design of this grazing study was created following the grazing trial guidelines described by Fisher (2000). Treatment, block and year were included as random variables. All statistical analysis was done using SAS® version 9.4 (SAS Institute, Cary, NC). Two analyses were carried out in the dataset. Treatment was analyzed as a fixed effect, accounting for all nine treatments and all heifers across the study. Pasture type, with (MIX) or without (MONO) BFT, was also analyzed (n = 36) as a fixed effect, to determine if the presence of BFT in pasture influenced heifer growth and development. Heifers receiving TMR were eliminated from the pasture type analysis. Repeated Measures analysis was analyzed using PROC MIXED, using a compound symmetry covariance structure. Post-hoc mean comparisons with LSD adjustments were completed to determine differences between individual treatments. Significance was determined at $p \leq 0.05$ for all comparisons. All values used for tables and figures are LS-Means.

CHAPTER IV

RESULTS

Body Weight

Pasture Type

Heifer BW was affected by both pasture type*day ($p < 0.01$) and day ($p < 0.01$) (Figure 1). Moreover, heifers who grazed MIX pastures tended ($p = 0.06$) to have greater BW when compared to heifers that grazed MONO pastures (Figure 1). Pasture type had no effect on heifer BW at d 0 or d 35 ($p = 0.99$, $p = 0.17$, respectively, Figure 1), but at d 70 and d 105 the heifers grazing MIX pastures had greater ($p < 0.01$) BW when compared to heifers receiving MONO pasture (Figure 1).

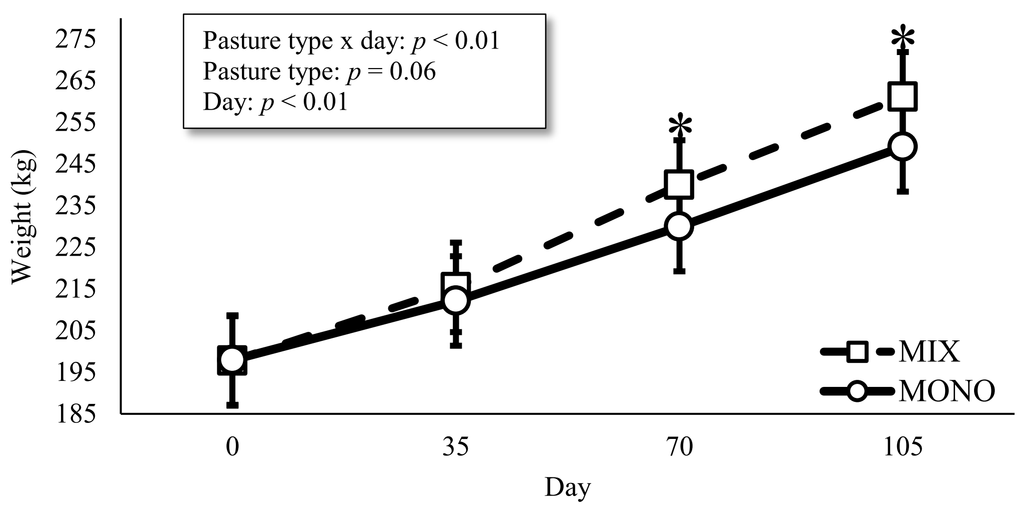

Figure 1. Effects of pasture type on heifer body weight. These data represent growth of Jersey heifers grazed in 2016, 2017 or 2018. A total of 192 heifers were used over the 3-year period with two heifers per block in year 2016 and three heifers per pasture in 2017 and 2018. Each block of heifers serves as the experimental unit with treatments being either grass only pastures (MONO, $n = 36$) or grass interseeded with Birdsfoot Trefoil (MIX, n = 36). Weights were collected every 35-d over a 105-d period and analyzed to show the effects of pasture type x day, pasture type, and day. Differences ($p < 0.05$) between pasture types within each time point are indicated with an asterisk (*).

Over the 105 d period, heifer weight gain differed ($p < 0.01$) between MONO and MIX treatments, where heifers grazing MIX pastures has improved weight gain compared to heifers grazing MONO pastures (0.60 kg/d vs. 0.49 kg/d respectively, Table 2). Taken together, these data indicate that heifers receiving MIX pastures have greater ($p < 0.05$) BW and weight gain when compared to heifers grazing MONO pastures.

Table 2

Effect of Different Pastures Treatments and Pasture Types on Heifer Average Daily Gain Over the 105-d Grazing Period

	Average daily gain (kg/d)[3]			
Treatments[1]	Day 0 - 105	Day 0 – 35	Day 35 – 70	Day 70 – 105
MB	0.50^{c}	0.29^{d}	0.60^{ab}	0.61^{ab}
MB+BFT	0.60^{ab}	0.40^{cd}	0.78^{a}	0.61^{abc}
OG	0.54^{bc}	0.50^{abc}	0.55^{bc}	0.57^{abcd}
OG+BFT	0.61^{ab}	0.47^{bc}	0.67^{ab}	0.68^{a}
PR	0.51^{c}	0.49^{abc}	0.60^{ab}	0.44^{d}
PR+BFT	0.66^{a}	0.62^{a}	0.75^{a}	0.62^{ab}
TF	0.39^{d}	0.31^{d}	0.38^{c}	0.45^{cd}
TF+BFT	0.55^{bc}	0.52^{abc}	0.61^{ab}	0.50^{bcd}
TMR	0.66^{a}	0.62^{ab}	0.72^{ab}	0.63^{ab}
SEM	0.03	0.07	0.13	0.10
Treatment[4]	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.04$
Pasture Types[2]				
Mix	0.60^{x}	0.50^{x}	0.70^{x}	0.60^{x}
Mono	0.49^{y}	0.41^{y}	0.51^{y}	0.53^{x}
SEM	0.03	0.07	0.12	0.09
Pasture Type[5]	$p < 0.01$	$p = 0.01$	$p < 0.01$	$p = 0.11$

Note. All treatments have $n = 9$, except TMR has $n = 6$, All pasture types have n = 36

[1] Treatments include: Meadow Bromegrass (MB), Meadow Bromegrass + BFT (MB+BFT) Orchard Grass (OG), Orchard Grass + BFT (OG+BFT), Perennial Ryegrass (PR), Perennial Ryegrass + BFT (PR+BFT), Total Mixed Ration (TMR). All treatments have $n = 9$, except TMR has $n = 6$.

[2] Pasture types include: Pastures with BFT (Mix) and pastures without BFT (Mono). Both mixed pastures and monoculture pastures have $n = 36$.

[3] Superscripts a, b, c, d, e, x, and y denote differences between treatments ($p < 0.05$)

[4] p value for Treatment when heifer average daily gains were analyzed. Significance was determined at $p < 0.05$

[5] p values for Pasture Type when heifer average daily gains were analyzed. Significance was determined at $p < 0.05$.

Treatment

Heifer average BW was affected by treatment x day ($p < 0.01$), treatment ($p = 0.01$), and day ($p < 0.01$). Heifers who received TMR had greater ($p < 0.05$) average BW when compared to heifers who grazed monoculture pastures (PR, OG, MB, TF) and TF+BFT. Heifers who grazed PR+BFT, MB+BFT, or OG+BFT also had greater ($p < 0.05$) average BW compared to heifers who grazed TF (Table 3). Treatment did not affect heifer average BW on d 0 ($p = 0.91$) or d 35 ($p = 0.14$, Table 3). However, treatment affected heifer average BW at d 70 ($p < 0.01$) and d 105 ($p < 0.01$, Table 3). At d 70, heifers receiving TMR had greater ($p < 0.05$) BW than heifers grazing TF, MB, PR and TF+BFT. Similarly, heifers grazing PR+BFT had greater ($p < 0.05$) average BW than heifers grazing TF and MB (Table 3). Heifers grazing mixed pastures (PR+BFT, OG+BFT, MB+BFT, TF+BFT) and PR had greater ($p < 0.05$) average BW compared to heifers grazing TF (Table 3). At d 105, heifers receiving TMR had greater ($p < 0.05$) average BW as compared to heifers grazing monoculture pastures (PR, OG, MB, TF) and TF+BFT (Table 3). Similarly, heifers grazing PR+BFT had greater ($p < 0.05$) average BW when compared to all monoculture pastures (PR, OG, MB, TF; Table 3). Heifers grazing MB+BFT and OG+BFT had greater ($p < 0.05$) average BW than heifers grazing OG, MB, and TF (Table 3). Heifers grazing TF+BFT and PR had greater ($p < 0.05$) average BW when compared to heifers that grazed TF (Table 3). Final heifer BW was significantly different ($p < 0.05$) between each individual grass monoculture and its respective mixture with BFT.

Heifer weight gain over the 105 d period was affected by treatment ($p < 0.01$).

Table 3

Effect of Different Treatments on Heifer Body Weights Over the 105-d Grazing Period

	Weight (kg)[2]				
Treatments[1]	Day-0	Day-35	Day-70	Day-105	Overall mean
MB	199	208	229^{de}	251^{de}	222^{bc}
MB+BFT	199	213	241^{abc}	262^{abc}	229^{ab}
OG	194	212	231^{cde}	251^{de}	222^{bc}
OG+BFT	198	215	238^{abcd}	262^{abc}	228^{ab}
PR	198	215	236^{bcd}	251^{cd}	225^{bc}
PR+BFT	195	217	243^{ab}	265^{ab}	230^{ab}
TF	199	210	223^{e}	240^{e}	218^{c}
TF+BFT	198	217	238^{bcd}	255^{bcd}	227^{bc}
TMR	202	225	250^{a}	271^{a}	238^{a}
SEM	9.4	9.5	12.1	10.9	10.5
Treatment x day[3]					$p < 0.01$
Treatment[3]					$p = 0.01$
Day[3]					$p < 0.01$

Note. All treatments have $n = 9$, except TMR has $n = 6$

[1] Treatments include: Meadow Bromegrass (MB), Meadow Bromegrass + BFT (MB+BFT) Orchard Grass (OG), Orchard Grass + BFT (OG+BFT), Perennial Ryegrass (PR), Perennial Ryegrass + BFT (PR+BFT), Total Mixed Ration (TMR)

[2] Superscripts a, b, c, d, & e denote differences between treatments ($p < 0.05$)

[3] p values for Treatment x Day, Treatment, and Day when heifer body weights were analyzed over time with repeated measures. Significance was determined at $p < 0.05$.

Heifers receiving TMR or grazing PR+BFT had increased ($p < 0.05$) weight gains compared to heifers grazing TF+BFT, OG, PR, MB, and TF (Table 2). Heifers grazing OG+BFT and MB+BFT had increased ($p < 0.05$) weight gains when compared to heifers grazing PR and MB (Table 2). Heifers grazing TF had the lowest ($p < 0.05$) weight gains of all pasture treatment and heifers receiving a TMR (Table 2). Overall, these data demonstrate that heifers receiving TMR or mixed pastures had greater average BW and weight gains when compared to heifers grazing monoculture pasture grasses.

Hip-Height

Pasture Type

There was no pasture type x day ($p > 0.05$) interaction or effect of pasture type ($p > 0.05$, Table 4) on heifer hip-height. However, date of measurement was significant ($p < 0.01$) with hip-height increasing over the course of the grazing season. These data indicate that although pasture treatment did not alter hip-height over time, the heifers were indeed growing over time as the trial progressed.

Treatment

Heifer hip-height was not affected by a treatment x day ($p = 0.65$) interaction, nor was a treatment ($p = 0.42$) effect observed when analyzed over time (Table 4). Final heifer hip-heights were not significantly different ($p > 0.05$) between each individual grass monoculture and its respective mixture with BFT.

Blood Urea Nitrogen

Pasture Type

Heifer BUN concentrations were affected by pasture type x day ($p < 0.01$) and pasture type ($p < 0.01$) when analyzed over time (Figure 2). However, day ($p = 0.28$) had no effect on heifer BUN concentrations (Figure 2). Heifers grazing MIX pastures had greater ($p < 0.01$) BUN concentrations compared to heifers grazing MONO pastures (Figure 2). At d 0 heifer BUN concentrations did not differ ($p = 0.20$) between pasture types (Figure 2). However, heifers receiving MIX pastures had greater BUN

Table 4

Effect of Different Pasture Treatments and Pasture Types on Heifer Hip-Height Over the 105-d Grazing Period

	Hip-Height (cm)[3]				
Treatments[1]	Day-0	Day-35	Day-70	Day-105	Overall mean
MB	111.9	113.8	115.8	117.6	114.7
MB+BFT	111.5	114.3	115.9	118.4	115.0
OG	111.8	113.2	115.6	117.8	114.6
OG+BFT	112.2	113.6	116.1	118.1	115.0
PR	112.3	113.9	115.3	118.1	114.9
PR+BFT	111.9	114.1	116.3	118.2	115.1
TF	111.8	113.6	115.1	116.2	114.2
TF+BFT	112.5	114.4	116.8	118.2	115.5
TMR	112.7	114.9	117.7	119.0	116.2
SEM	1.9	1.7	1.9	1.3	1.7
Treatment x day[3]					$p = 0.65$
Treatment[3]					$p = 0.42$
Day[3]					$p < 0.01$
Pasture types[2]					
Mix	112.0	114.1	116.3	118.2	115.2
Mono	111.9	113.6	115.4	117.5	114.6
SEM	1.8	1.7	1.9	1.3	1.6
Pasture type x day[4]					$p = 0.61$
Pasture type[4]					$p = 0.16$
Day[4]					$p < 0.01$

Note. All treatments have $n = 9$, except TMR has $n = 6$, all pasture types have $n = 36$.

[1] Treatments include: Meadow Bromegrass (MB), Meadow Bromegrass + BFT (MB+BFT) Orchard Grass (OG), Orchard Grass + BFT (OG+BFT), Perennial Ryegrass (PR), Perennial Ryegrass + BFT (PR+BFT), Total Mixed Ration (TMR). All treatments have n = 9, except TMR has n = 6.

[2] Pasture types include: Pastures with BFT (Mix) and pastures without BFT (Mono). Both mixed pastures and monoculture pastures have $n = 36$.

[3] p values for Treatment x Day, Treatment, and Day when heifer hip-heights were analyzed over time with repeated measures. Significance was determined at $p < 0.05$.

[4] p values for Pasture Type x Day, Pasture Type, and Day when heifer hip-heights were analyzed over time with repeated measures. Significance was determined at $p < 0.05$.

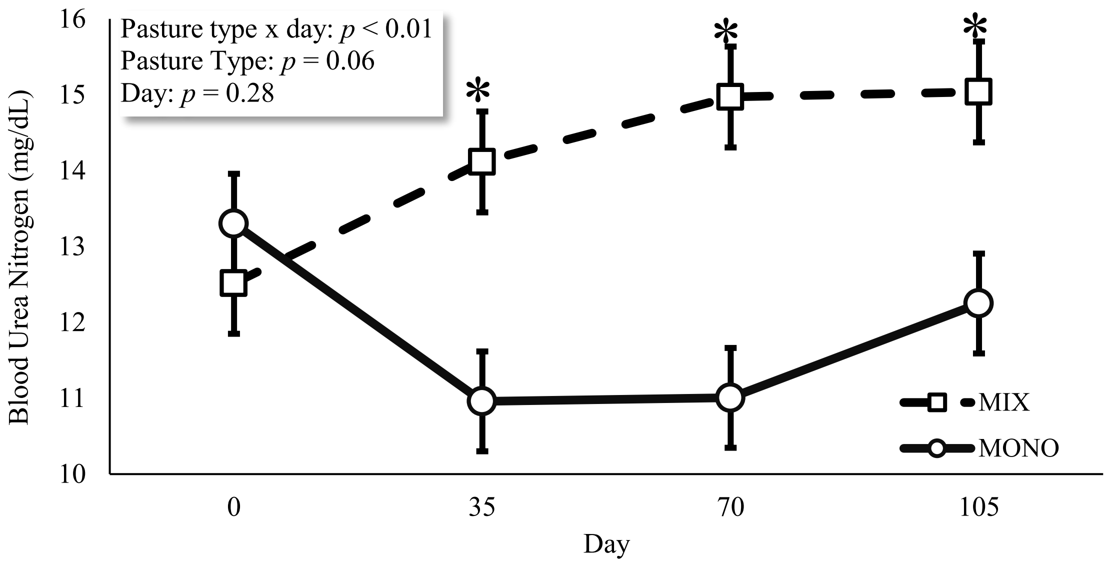

Figure 2. Effects of pasture type on Jersey heifer blood urea nitrogen concentrations grazed in 2016, 2017, or 2018. A total of 192 heifers were used over the 3-year period with two heifers per block in year 2016 and three heifers per pasture in 2017 and 2018. Each block of heifers serves as the experimental unit with treatments being either grass only pastures (MONO, $n = 36$) or grass interseeded with Birdsfoot Trefoil (MIX, $n = 36$). Blood samples were collected every 35-d over a 105-d period and were quantified and analyzed to show the effects that pasture type x day, pasture type, and day can have on heifer blood urea nitrogen concentrations. Differences ($p < 0.05$) between pasture types within each time point are indicated with an asterisk (*).

concentrations compared to heifers grazing MONO pastures at d 35 ($p < 0.01$), d 70 ($p < 0.01$), and d 105 ($p < 0.01$, Figure 2). These data indicate heifers grazing MIX pastures had greater BUN concentrations when compared to heifers grazing MONO pastures.

Treatment

Heifer BUN concentrations were not affected by a treatment x day ($p = 0.12$) interaction or a day ($p = 0.32$) effect, but treatment ($p < 0.01$) had an effect (Table 5). When analyzing treatment by overall mean it was found that heifers grazing PR+BFT had greater ($p < 0.05$) BUN concentrations when compared to heifers who grazed TF+BFT and any of the monoculture grass pastures (OG, PR, MB, TF, Table 5). Heifers receiving

Table 5

Effect of Different Pasture Treatments on Heifer Blood Urea Nitrogen (BUN) Concentrations Over the 105-d Grazing Period

	Blood urea nitrogen (mg/dL)[2]				
Treatments[1]	Day-0	Day-35	Day-70	Day-105	Overall mean
MB	12.3	9.9[c]	10.3[d]	11.6[de]	11.1[d]
MB+BFT	12.1	13.2[ab]	14.7[ab]	16.3[a]	14.0[ab]
OG	13.6	11.5[bc]	13.1[bc]	14.7[abc]	13.1[bc]
OG+BFT	12.3	14.4[a]	16.0[a]	14.9[abc]	14.4[ab]
PR	13.2	11.3[bc]	10.5[cd]	13.1[cd]	12.0[cd]
PR+BFT	12.7	15.4[a]	16.1[a]	15.8[ab]	15.0[a]
TF	13.7	10.1[c]	9.7[d]	10.2[e]	10.9[d]
TF+BFT	12.9	13.4[ab]	13.1[bc]	13.2[cd]	13.2[bc]
TMR	12.6	15.4[a]	14.9[ab]	13.3[bcd]	14.2[ab]
SEM	2.2	1.4	1.7	1.1	1.2
Treatment x day[3]					$p = 0.12$
Treatment[3]					$p < 0.01$
Day[3]					$p = 0.32$

Note. All treatments have $n = 9$, except TMR has $n = 6$

[1] Treatments include: Meadow Bromegrass (MB), Meadow Bromegrass + BFT (MB+BFT) Orchard Grass (OG), Orchard Grass + BFT (OG+BFT), Perennial Ryegrass (PR), Perennial Ryegrass + BFT (PR+BFT), Total Mixed Ration (TMR).

[2] Superscripts a, b, c, d, & e denote differences between treatments ($p < 0.05$).

[3] p values for Treatment x Day, Treatment, and Day when heifer blood urea nitrogen concentrations were analyzed over time with repeated measures. Significance was determined at $p < 0.05$.

TMR, OG+BFT, and MB+BFT had greater ($p < 0.05$) BUN concentrations than heifers who grazed PR, MB, and TF (Table 5). Heifers grazing TF+BFT and OG had greater ($p < 0.05$) BUN concentration compared to heifers who grazed MB or TF (Table 5). At d 0 ($p = 0.79$) heifer BUN did not differ between treatments (Table 5). However, at d 35 ($p < 0.01$), d 70 ($p < 0.01$), and d 105 ($p < 0.01$) heifer BUN concentrations differed between treatments (Table 5). At d 35, heifers receiving PR+BFT, OG+BFT and TMR had greater ($p < 0.05$) BUN concentrations compared to heifers grazing all monoculture treatments

(PR, OG, MB, TF, Table 5). Similarly, heifers grazing TF+BFT and MB+BFT had greater ($p < 0.05$) BUN than heifers that grazed TF and MB (Table 5). At d 70, heifers grazing PR+BFT and OG+BFT had greater ($p < 0.05$) BUN levels compared to heifers grazing monoculture treatments (OG, PR, MB, TF) and TF+BFT (Table 5). Similarly, heifers receiving TMR and MB+BFT had greater ($p < 0.05$) BUN levels compared to heifers grazing PR, MB, and TF (Table 5). Heifers grazing TF+BFT and OG had greater ($p < 0.05$) BUN than heifers grazing MB and TF (Table 5). At d 105, heifers grazing MB+BFT had greater ($p < 0.05$) BUN concentrations when compared to heifers receiving TMR, TF+BFT, PR, MB, and TF (Table 5). Similarly, heifers grazing PR+BFT had greater ($p < 0.05$) BUN concentrations than heifers grazing TF+BFT, PR, MB, and TF (Table 5). Heifers grazing OG+BFT and OG had greater ($p < 0.05$) BUN concentrations compared to heifers grazing MB and TF (Table 5). Heifers receiving TMR, TF+BFT, and PR had greater ($p < 0.05$) BUN concentrations compared to heifers who grazed TF (Table 5). Heifer BUN levels were significantly different ($p < 0.05$) between each individual grass monoculture and its respective mixture with BFT, except for OG. These data indicate that heifers receiving mixed pasture treatments as well as the TMR had overall greater BUN concentrations than heifers who grazed monoculture pasture treatments.

Insulin-Like Growth Factor-1

Pasture Type

Heifer serum IGF-1 concentrations were not affected by a pasture type*day (p =

0.14) interaction nor was a pasture type ($p = 0.87$) effect observed (Figure 3). However, day ($p < 0.01$) influenced heifer IGF-1 concentrations (Figure 3). Heifers sampled at d 105 had increased ($p < 0.05$) IGF-1 concentrations when compared to heifers sampled at any other time point (Figure 3). Heifers sampled at d 0 had increased ($p < 0.05$) IGF-1 concentrations when compared to heifers sampled at d 35 (Figure 3). These data indicate that heifer IGF-1 concentrations vary by day, but do not appear to be affected by the pasture type consumed.

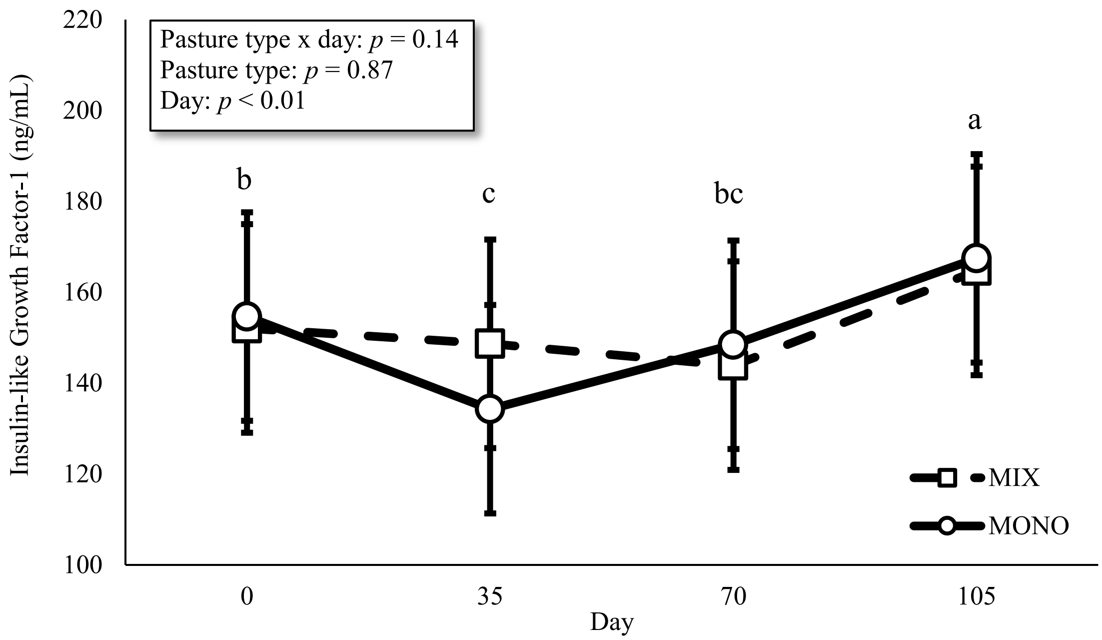

Figure 3. Effects of pasture type on heifer serum insulin-like growth factor-1 concentrations of Jersey heifers grazed in 2016, 2017, or 2018. A total of 192 heifers were used over the 3-year period with two heifers per block in year 2016 and three heifers per pasture in 2017 and 2018. Each block of heifers serves as the experimental unit with treatments being either grass only pastures (MONO, $n = 36$) or grass interseeded with Birdsfoot Trefoil (MIX, $n = 36$). Blood samples were collected every 35-d over a 105-d period and were quantified and analyzed to show the effects that pasture type x day, pasture type, and day can have on heifer insulin-like growth factor-1 concentrations. Differences ($p < 0.05$) between time points are indicated with a letter.

Treatment

Heifer IGF-1 concentrations were not affected by a treatment x day ($p = 0.23$) interaction but treatment ($p < 0.01$) and day ($p < 0.01$) were found to have an effect when analyzed over time (Table 6). When analyzing treatment by overall mean heifers receiving TMR had greater ($p < 0.05$) IGF-1 concentrations than heifers who were grazing MB+BFT, MB, OG+BFT, PR, TF, and TF+BFT (Table 6). Similarly, heifers grazing OG had greater ($p < 0.05$) IGF-1 concentrations than heifers who grazed TF or

Table 6

Effect of Different Pasture Treatments on Heifer Insulin-Like Growth Factor-1 (IGF-1) Concentrations Over the 105-d Grazing Period

	Insulin-like growth factor-1 (ng/mL)[2]				
Treatments[1]	Day-0	Day-35	Day-70	Day-105	Overall mean
MB	150.9	136.1^{bc}	149.3^{bc}	178.3^{ab}	153.7^{bcd}
MB+BFT	153.1	151.1^{b}	148.4^{bc}	172.3^{ab}	156.2^{bcd}
OG	157.4	138.2^{bc}	159.5^{ab}	184.8^{a}	159.9^{ab}
OG+BFT	158.3	148.6^{b}	146.9^{bc}	159.3^{bc}	153.3^{bcd}
PR	146.3	137.2^{bc}	147.0^{bc}	163.2^{abc}	148.4^{bcd}
PR+BFT	149.7	153.3^{ab}	151.2^{b}	184.3^{a}	159.6^{abc}
TF	164.4	121.9^{c}	139.5^{bc}	141.9^{c}	141.9^{cd}
TF+BFT	147.0	141.6^{bc}	130.0^{c}	142.3^{c}	140.2^{d}
TMR	164.2	180.3^{a}	179.4^{a}	184.2^{ab}	179.1^{a}
SEM	15.3	24.7	33.6	25.5	23.1
Treatment x day[3]					$p = 0.23$
Treatment[3]					$p < 0.01$
Day[3]					$p < 0.01$

Note. All treatments have $n = 9$, except TMR has $n = 6$.

[1] Treatments include: Meadow Bromegrass (MB), Meadow Bromegrass + BFT (MB+BFT) Orchard Grass (OG), Orchard Grass + BFT (OG+BFT), Perennial Ryegrass (PR), Perennial Ryegrass + BFT (PR+BFT), Total Mixed Ration (TMR)

[2] Superscripts a, b, c, and d denote differences between treatments ($p < 0.05$).

[3] p values and significance of Treatment x Day interaction, treatment effect and day effect (significance at $p < 0.05$).

TF+BFT (Table 6). Heifers grazing PR+BFT had greater ($p < 0.05$) IGF-1 concentration than heifers grazing TF+BFT (Table 6). When analyzing specific sampling time points by treatment, heifer IGF-1 concentrations at d 0 did not differ ($p = 0.85$) by treatment. However, at d 35 ($p = 0.02$), d 70 ($p = 0.01$), and d 105 ($p < 0.01$) heifer IGF-1 concentrations differed between treatments (Table 6). At d 35, heifers receiving TMR had greater ($p < 0.05$) IGF-1 concentrations compared to heifers who grazed monoculture pastures (PR, MB, OG, TF), MB+BFT, OG+BFT and TF+BFT (Table 6). Similarly, heifers grazing PR+BFT, MB+BFT, and OG+BFT had greater ($p < 0.05$) IGF-1 concentrations compared to heifers grazing TF. At d 70, heifers receiving TMR had greater ($p < 0.05$) IGF-1 concentrations compared to heifers grazing all other treatments except for heifers grazing OG (Table 6). Heifers grazing OG and PR+BFT had greater ($p < 0.05$) IGF-1 concentrations than heifers grazing TF+BFT (Table 6). At d 105, heifers grazing OG and PR+BFT had greater ($p < 0.05$) IGF-1 concentrations compared to heifers grazing OG+BFT, TF+BFT, and TF. Similarly, heifers receiving TMR, MB, and MB+BFT had greater ($p < 0.05$) IGF-1 concentrations in comparison to heifers receiving TF+BFT and TF (Table 6). When analyzing sampling day differences, at d 105 heifers had greater ($p < 0.05$) serum IGF-1 concentrations than at any other day. At d 0, heifers had greater ($p < 0.05$) IGF-1 concentrations than at d 35 (Table 6). Heifer IGF-1 concentrations were not significantly different ($p < 0.05$) between each individual grass monoculture and its respective mixture with BFT. Taken together, these data indicate heifers receiving TMR, OG, and PR+BFT commonly had greater IGF-1 levels than heifers receiving other treatments.

Parasite Load

Pasture Type

Fecal egg count data collected in the years 2017 and 2018 were analyzed separately as different methods to determine FEC were utilized each year. In both 2017 and 2018, a pasture type x day interaction was not found ($p = 0.88$, $p = 0.76$, respectively) nor was a pasture type effect ($p = 0.28$, $p = 0.30$, respectively) present when heifer FEC were analyzed over time (data not shown). However, heifer FEC was affected ($p < 0.01$) by day in 2017 and 2018 (Data not shown). These data indicate the presence of BFT in pasture had no effect on FEC.

Treatment

Heifer FEC were not affected ($p = 0.55$, $p = 0.93$, respectively) by a treatment x day interaction in 2017 or 2018, nor was a treatment effect ($p = 0.32$; $p = 0.61$, respectively) observed for either year. However, a day effect ($p < 0.01$) was observed for both years when analyzed over time (data not shown). These data indicate that there was no difference between treatments on FEC of the heifers either year this experiment was conducted.

Reproduction

Pasture Type

Heifer conception rate was not affected by pasture type ($p = 0.41$, data not shown). These data indicate heifers grazing either MONO or MIX pastures had no differences in conception.

Treatment

Heifer conception rate was not affected by treatment (p = 0.39, data not shown). The data indicates that heifers receiving different pasture treatments had no differences in conception rate.

CHAPTER V

DISCUSSION

A large share of the total cost of milk production is represented by the cost of development of replacement heifers (Heinrichs, 1993). To lower the cost of production it is essential to increase growth rates during heifer development. Improving pasture nutritional quality to help increase heifer growth rates may be one solution to help producers decrease overall production costs, especially among organic producers who have a requirement to utilize pasture as a feed source. The goal of the present study was to determine the impacts of different pastures on growth, health and conception rates of dairy heifers.

Waldron et al. (2020) reported that steers grazing TF+BFT had greater BW gain than those grazing TF monocultures. As such, one objective of the present study was to determine if BFT combinations with other grasses, having greater inherent nutritional quality than TF, could further improve livestock gains and be comparable to a TMR system. The present study found that on average heifers grazing MIX pastures had greater BW gain than heifers on MONO pastures (0.60 kg/d vs. 0.49 kg/d, respectively, Table 7). Similar increases in BW, milk yield, and wool growth have been found in other studies who implement BFT into grazing practices (Ramirez-Restrepo et al., 2005; Wang et al., 1996; Woodward et al., 1999). For instance, Barker (1999) conducted a similar grazing study with Holstein heifers, and found that heifers grazing legume mixed pastures had greater ADG (12-17% increase) compared to heifers grazing grass pastures. In addition to the average effect of BFT, the individual mixtures with BFT improved BW gain in all grasses compared to their respective monocultures. Nevertheless, though Rose (2019) reported that all the other grass+BFT treatments tested herein had greater ME than TF+BFT, we found no differences in heifer final BW between all other grass+BFT pastures and our benchmark treatment of TF+BFT.

Table 7

The Effect of Sampling Day on Heifer Body Weights (BW), Hip-Heights, Blood Urea Nitrogen (BUN) Concentrations, and Insulin-like Growth Factor-1 (IGF-1) Concentrations Over the 105-d Grazing Period

	Sampling day[2]					
Variables[1]	Day-0	Day-35	Day-70	Day-105	SEM	Day effect[3]
BW	198[d]	215[c]	236[b]	257[a]	10	$p < 0.01$
Hip-height	112.0[s]	114.0[r]	116.0[q]	118.0[p]	1.6	$p < 0.01$
BUN	13.0	12.7	13.0	13.7	0.5	$p = 0.32$
IGF-1	155.9[y]	145.6[y]	149.2[yz]	168.1[x]	22.5	$p < 0.01$

Note. All treatments have n = 9, except TMR has n = 6.

1 Variables include heifer Body Weights (BW, kg), Hip-Heights (cm), Blood Urea Nitrogen (BUN, mg/dL), and Insulin-like Growth Factor-1 (IGF-1, ng/mL).

2 Superscript a,b,c,d & p,q,r,s. & x,y,z - denote differences between treatments ($p < 0.05$).

3 Day Effect – The effects of different sampling days on each of the four variables.

In contrast, heifers receiving TMR had among the greatest BW gain over the 105-d period, but all MIX pastures except TF+BFT had similar BW gain to those receiving the TMR. Heifers receiving TMR were found to have greater weight gains than all grass MONO pastures (PR, OG, MB, TF). Although heifers receiving a TMR in this study had similar BW gains to heifers grazing mixed pastures, there is evidence that animals on TMR diets normally gain more than pasture fed animals. Marston, Lusby, and Wettemann (1995) found that 7-month-old heifers fed TMR diets reached puberty at 29-d younger than heifers grazing on dormant native pastures with a supplement. However, heifers grazing on pasture that were supplemented with a high-20 (20% CP) supplement had similar weights at breeding as heifers in a dry lot (Marston et al., 1995). McClure et al. (1994) found weaned lambs had improved ADG being fed a TMR diet than those consuming alfalfa or grass pastures. However, it is noted that in our study, heifers receiving the TMR did not achieve their predicted BW gains (gained 0.66 kg/d, rather than 0.84 kg/d). If the targeted TMR weight gain would have been achieved in this study, results may have been similar to the research findings in previously conducted studies. Nevertheless, our results indicate that these high-quality grasses grown in a mixture with the legume BFT resulted in overall heifer BW gain similar to those fed a TMR. From our results, we can conclude that grass-BFT mixed pastures are a sustainable alternative to feeding a TMR in a confined setting and should be considered a viable option for sustainable ruminant production on pasture.

Heifer hip-height was not affected by pasture type, but a significant day effect was found. Growing heifers were used in this study, and since these animals had not yet reached their mature height or weight, we would expect these results. Hip-height is mainly used as a measurement for producers to determine the stage of growth in heifers. Often scientific research uses more definitive and precise forms of measurements, such as BW, to determine the stage of growth in animals rather than hip-height. To our knowledge, this is the first study to report differences in hip-height of animals grazing grass or grass-legume mixed pasture. As these heifers grew through the summer their height increased, but treatment did not have an effect.

Concentrations of BUN were higher in heifers that received MIX pastures compared to those consuming MONO. As BUN is an indicator of protein intake, these results were expected since MIX pasture heifers had access to a protein rich legume and MONO pasture heifers did not. Studies done in lactating cows grazing pasture grasses have found that BUN levels stayed between 16-19 mg/dL, which is similar to our research findings (Kolver & Macmillan, 1994; Roche et al., 2005). Alternatively, concentrations of BUN found in our study were at a lower range than similar studies that utilized improved pasture-based grazing systems, by either heavily fertilizing pasture grasses or using grass-legume mixed pastures (Kolver & Macmillan, 1994; Ordóñez et al., 2007; Roche et al., 2005). Ordóñez et al. found that Holstein cows put on highly fertilized grass pastures reached BUN levels of over 60 mg/dL. Other studies have shown that lactating cattle grazing on lush pasture routinely have plasma nitrogen levels above 40 mg/dL (Roche et al., 2005, Kolver & Macmillan, 1994). The differences between BUN concentrations in ruminants from our research compared to the previously mentioned studies may be due to the effect of CT from BFT. The CT have an ability to bind protein in the rumen, helping to decrease the amount of urea present systemically (Jones & Mangan, 1977; Min et al., 2003; Patra & Saxena, 2011). However, Rose (2019) reported that CT levels among our research pastures ranged from 0.5-7.5 g CT/kg DM; whereas, Min (2003) reported that CT concentrations of 20-45 g CT/kg DM were ideal in reducing rumen forage protein degradation. It should be noted that CT from BFT could have still have had an effect on heifer BUN concentrations, but may not have led to the reduction of BUN as originally thought. The lower concentration of BUN found in this study could have also been influenced by sampling after a 12 h fast, since BUN concentrations reach their peak four to six hours postprandial (Butler, 1998). Research has shown that concentrations above 20 mg/dL of BUN may be detrimental to reproductive performance (Ferguson et al., 1988, 1993; Rajala-Schultz et al., 2001) From our results, we can conclude that heifers grazing BFT mixed pastures have higher protein intake levels than animals on grass pastures. However, even with increased levels of BUN, animals grazing BFT mixed pastures never surpassed concentrations of BUN thought to be detrimental to reproduction.

Heifers receiving TMR or a mixed pasture treatment (PR+BFT, OG+BFT, MB+BFT, TF+BFT) had increased BUN concentrations compared to heifers grazing monoculture pasture treatments (PR, OG, MB, TF). Heifers receiving a TMR had access to high quality, protein rich forage that likely lead to an increase in heifer BUN concentrations. Similarly, heifers grazing grass+BFT pastures had greater BUN concentrations than heifers grazing the respective monoculture grass, except for OG. This could be indicative of a greater inherent forage value (protein) of OG compared to the other grasses used in this study. Access to BFT lead to an increase in heifer BUN concentrations, similar to the findings of other research that has been conducted with grazing animals (Kolver & Macmillan, 1994; Roche et al., 2005). As mentioned earlier, even though these treatments increased BUN concentrations in heifers, concentrations did not reach high enough levels to be detrimental to reproduction (Ferguson et al., 1988, 1993; Rajala-Schultz et al., 2001).

Heifer IGF-1 concentrations did not differ between animals grazing MIX pastures or MONO pastures. Although heifers grazing MIX pastures had increased weight gain compared to those consuming MONO pastures, this difference in weight gain was likely not enough to cause a difference in IGF-1 production. Research has suggested that serum IGF-1 levels may be an indicator of energy balance, not necessarily an indicator of overall nutrient balance (Kolver & Macmillan, 1994). Similarities in pasture metabolizable energy content could be the main factor for a lack of differences in heifer IGF-1 concentrations. Research on ruminant IGF-1 concentration on pasture is scarce; more research is needed to determine the relationship between ruminant serum IGF-1 concentrations and pasture.

Our results indicate that IGF-1 concentrations from heifers receiving a TMR were higher than heifers who received TF throughout the study. Research has suggested that serum IGF-1 levels may be an indicator of energy balance (Kolver & Macmillan, 1994). Elsasser (1989) researched the effects that different levels of energy and protein (Low Protein, Low Energy (LPLE):1.96 ME/kg & 8% CP vs. Medium Protein, High Energy (MPHE):2.67 ME/kg & 11% CP) can have on plasma IGF-1 in steers under basal conditions. It was found that diet composition and intake influence plasma IGF-1 levels, steers receiving MPHE diets had increased (208 ng/ml vs. 105 ng/ml, $p < 0.01$) IGF-1 levels compared to steers receiving LPLE diets (Elsasser et al., 1989). However, researchers suggested that while CP may be responsible for basal IGF-1 levels, the actual IGF-1 response to CP may be more affected by the available metabolizable energy (Elsasser et al., 1989). Similarly, in a study focusing on the effects of negative energy balance on the GH axis, it was found that severe negative energy balance affected the hepatic synthesis of IGF-1 (Fenwick et al., 2008). The serum IGF-1 concentrations observed in the present study support the research findings that IGF-1 can be an indicator of energy balance (Elsasser et al., 1989, Fenwick et al., 2008, Kolver & Macmillan, 1994). The present study did not analyze energy content of the pasture samples; however, Rose (2019) reported that the TF pasture had the lowest ($p < 0.05$) total digestible nutrients (TDN) out of all pastures. The low nutritional value of TF compared to a TMR, with high energy and sugar content from concentrates, leads one to hypothesize that these two treatments differed in energy content. In addition, energy differences between heifers receiving TF and TMR can also be supported by looking at heifer ADG (0.39 kg vs. 0.66 kg respectively, Table 7) for the 105-d period, where heifers receiving TF had the lowest ADG of all treatments, and heifers receiving TMR had among the highest ADG of all treatments. The present study demonstrates that heifers that have increased growth, likely caused in part by a more energy dense diet, exhibit increased concentrations of serum IGF-1, which is likely responsible for mediating the increased growth rate observed in these animals.

The addition of BFT to pastures did not have any effect on heifer FEC in the present study. Studies have found that CT from BFT can decrease FEC in ruminants (Minn et al., 2003). Niezen (1998) found lambs grazing forages that contained CT had a reduction in FEC. Shepley (2015) suggested that BFT may reduce FEC in dairy heifers. The results of the current study do not agree with the findings of these previous studies. The differences in results may be due to the low amount of CT (0.5-7.5 g CT/kg DM) reported in our pastures by Rose (2019). In addition, it should be noted that heifers used in this study had low numbers of parasites overall, making it difficult to detect any differences in FEC. Although other research suggests animals fed BFT can reduce parasite load, our results indicate that BFT had no effect on parasite load. As such, additional research needs to be completed to determine how including BFT in a pasture may impact parasite load of developing dairy heifers.

Our results indicated that treatment did not impact heifer conception rate. Funston and Larson (2011) had similar results when comparing beef heifers that received a dry-lot diet to heifers that grazed on corn residue. Heifer final pregnancy rate was found to be not different between treatments, even though differences in BW gain and ADG were observed (Funston & Larson, 2011). Previous research has found that restricting BW gains in beef heifers leads to no difference in pregnancy rates compared to unrestricted animals (Ciccioli, et al., 2005; Gasser, Behlke, Grum, & Day, 2006). Macdonald, Penno, Bryant, and Roche (2005) found similar results with Holstein and Jersey dairy heifers. When restricting BW gains to different levels before puberty, it was found that dietary treatment had no effect on conception rates (Macdonald et al., 2005). The results of the present study agree with previously published research. While some heifers on our study exhibited decreased BW gains from consuming a less nutrient dense pasture, the differences in nutrient density between our different treatments were not significant enough to influence conception rates. It was also found that the addition of BFT does not appear to have any negative effects on reproduction. This data agrees with our reported BUN results as these values are below those that have previously been found to have a negative impact on conception (Ferguson et al., 1988, 1993; Rajala-Schultz et al., 2001).

As such, these results demonstrate that a pasture-based diet is an adequate management strategy for heifer development without having negative effects on heifer conception rate. However, more research needs to be completed in a larger group of animals to determine whether these different diets may have on conception rate.

CHAPTER VI

CONCLUSION

The current research aims to assist organic dairy producers in the Intermountain West with their pasture practices. The data presented provide a comparison between how grass pastures, mixed pastures, and a TMR effect the development of replacement dairy heifers. The results demonstrate that interseeding a legume, BFT, with pasture grasses increases heifer weight gain. Weight gains of heifers grazing mixed pastures were also similar to heifers who were fed a TMR. Results from the present study also indicate that heifers grazing BFT mixed pastures had higher BUN concentration than animals on grass pastures. Even with higher levels of BUN, animals grazing BFT mixed pastures never surpassed BUN concentrations that are known to be detrimental to reproduction. Our results also indicated that serum IGF-1 levels were commonly higher in heifers fed a TMR when compared to heifers grazing TF. Heifer parasite load, hip-height, and conception rates were not affected by the presence of BFT in pasture or any of the specific treatments. This research demonstrates that grazing heifers on grass-BFT mixed pastures may be a sustainable method to improve dairy heifer development in animals consuming pasture.

REFERENCES

Aerts, R. J., McNabb, W. C., Molan, A., Brand, A., Peters, J. S., Barry, T. N. (1999). CT from Lotus corniculatus and Lotus pedunculatus effect the degradation of ribulose 1,5-bisphosphate carboxylase (Rubisco) protein in the rumen differently *Journal of the Science of Food and Agriculture, 79,* 79-85

Allison, C. D. (1985). Factors affecting forage intake by range ruminants: A review. *Journal of Range Management*, *38*(4), 305-311. https://doi.org/10.2307/3899409

Balch, C. C., & Campling, R. C. (1962). Regulation of voluntary food intake in ruminants. *Nutrition Abstracts and Reviews*, *32*, 669–686.

Barker, J. M., Buskirk, D. D., Ritchie, H. D., Rust, S. R., Leep, R. H., Barclay, D. J., … Hartnell, G. (1999). Intensive grazing management of smooth bromegrass with or without alfalfa or birdsfoot trefoil: Heifer performance and sward characteristics. *The Professional Animal Scientist*, *15*(2), 130–135. https://doi.org/10.15232/S1080-7446(15)31741-1

Bruckental, I., Drori, D., Kaim, M., Lehrer, H., & Folman, Y. (1989). Effects of source and level of protein on milk yield and reproductive performance of high-producing primiparous and multiparous dairy cows. *Animal Production*, *48*(02), 319–329. https://doi.org/10.1017/S0003356100040319

Butler, W. R. (1998). Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. *Journal of Dairy Science, 81(9), 2533–2539*. https://doi.org/10.3168/jds.S0022-0302(98)70146-8

Butler, W. R., Calaman, J. J., & Beam, S. W. (1996). Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *Journal of Animal Science*, *74*(4), 858-865. https://doi.org/10.2527/1996.744858x

Capuco, A. V., Smith, J. J., Waldo, D. R., & Rexroad, C. E. (1995). Influence of prepubertal dietary regimen on mammary growth of holstein heifers. *Journal of Dairy Science*, *78*(12), 2709–2725. https://doi.org/10.3168/jds.S0022-0302(95)76902-8

Carlsson, G., & Huss-Danell, K. (2003). Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil,* 253, 353-372.

Cheng, K. J., McAllister, T. A., Popp, J. D., Hristov, A. N., Mir, Z., & Shin, H. T. (1998). A review of bloat in feedlot cattle. *Journal of Animal Science*, *76*(1), 299-308. https://doi.org/10.2527/1998.761299x

Chiquette, J., Cheng, K.-J., Costerton, J. W., & Milligan, L. P. (1988). Effect of tannins on the digestibility of two isosynthetic strains of Birdsfoot Trefoil (*Lotus corniculatus* L.) using in vitro and in Sacco techniques. *Canadian Journal of Animal Science*, *68*(3), 751–760. https://doi.org/10.4141/cjas88-084

Ciccioli, N. H., Charles-Edwards, S. L., Floyd, C., Wettemann, R. P., Purvis, H. T., Lusby, K. S., … Lalman, D. L. (2005). Incidence of puberty in beef heifers fed high- or low-starch diets for different periods before breeding. *Journal of Animal Science, 83(11),* 2653–2662. https://doi.org/10.2527/2005.83112653x

Corona, M. E. P., Aldana, B. R. V. D., Criado, B. G., & Ciudad, A. G. (1998). Variations in nutritional quality and biomass production of semiarid grasslands. *Journal of Range Management*, *51*(5), 570-576. https://doi.org/10.2307/4003378

Corpas, E., Harman, S. M., & Blackman, M. R. (1993). Human growth hormone and human aging. *Endocrine Reviews*, *14*(1), 20–39. https://doi.org/10.1210/edrv-14-1-20

Davis, S. L. (1998). Environmental modulation of the immune system via the endocrine system. *Domestic Animal Endocrinology*, *15*(5), 283–289. https://doi.org/10.1016/S0739-7240(98)00034-4

Díaz-Torga, G. S., Mejía, M. E., González-Iglesias, A., Formía, N., Becú-Villalobos, D., & Lacau-Mengido, I. M. (2001). Metabolic cues for puberty onset in free grazing holstein heifers naturally infected with nematodes. *Theriogenology*, *56*(1), 111–122. https://doi.org/10.1016/S0093-691X(01)00547-7

Diskin, M. G., Murphy, J. J., & Sreenan, J. M. (2006). Embryo survival in dairy cows managed under pastoral conditions. *Animal Reproduction Science*, *96*(3–4), 297–311. https://doi.org/10.1016/j.anireprosci.2006.08.008

Elrod, C. C., & Butler, W. R. (1993). Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. *Journal of Animal Science*, *71*(3), 694–701. https://doi.org/10.2527/1993.713694x

Elrod, C. C., Van Amburgh, M., & Butler, W. R. (1993). Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. *Journal of Animal Science, 71*(3), 702–706. https://doi.org/10.2527/1993.713702x

Elsasser, T. H., Rumsey, T. S., & Hammond, A. C. (1989). Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. *Journal of Animal Science*, *67*(1), 128-141. https://doi.org/10.2527/jas1989.671128x

Endecott, R. L., Funston, R. N., Summers, A. F., & Roberts, A. J. (2012). Alpharma beef cattle nutrition symposium: Implications of nutritional management for beef cow-calf systems. *Journal of Animal Science*, *90*(7), 2301–2307. https://doi.org/10.2527/jas.2011-4568

Fenwick, M. A., Fitzpatrick, R., Kenny, D. A., Diskin, M. G., Patton, J., Murphy, J. J., & Wathes, D. C. (2008). Interrelationships between negative energy balance (NEB) and IGF regulation in liver of lactating dairy cows. *Domestic Animal Endocrinology*, *34*(1), 31–44. https://doi.org/10.1016/j.domaniend.2006.10.002

Ferguson, J. D., Blanchard, T., Galligan, D. T., Hoshall, D. C., & Chalupa, W. (1988). Infertility in dairy cattle fed a high percentage of protein degradable in the rumen. *Journal of the American Veterinary Medical Association*, *192*(5), 659–662.

Ferguson, J. D., Galligan, D. T., Blanchard, T., & Reeves, M. (1993). Serum urea nitrogen and conception rate: The usefulness of test information. *Journal of Dairy Science*, *76*(12), 3742–3746. https://doi.org/10.3168/jds.S0022-0302(93)77716-4

Fisher, D. S. (2000). Defining the experimental unit in grazing trials. *Journal of Animal Science*, *77*(E-Suppl), 1-5. https://doi.org/10.2527/jas2000.00218812007700ES0006x

Funston, R. N., & Deutscher, G. H. (2004). Comparison of target breeding weight and breeding date for replacement beef heifers and effects on subsequent reproduction and calf performance. *Journal of Animal Science*, *82*(10), 3094–3099. https://doi.org/10.2527/2004.82103094x

Funston, R. N., & Larson, D. M. (2011). Heifer development systems: Dry-lot feeding compared with grazing dormant winter forage. *Journal of Animal Science*, *89*(5), 1595–1602. https://doi.org/10.2527/jas.2010-3095

Gasser, C. L., Behlke, E. J., Grum, D. E., & Day, M. L. (2006). Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. *Journal of Animal Science, 84(11), 3118–3122*. https://doi.org/10.2527/jas.2005-676

Georgiadis, N. J., & McNaughton, S. J. (1990). Elemental and fibre contents of Savanna grasses: Variation with grazing, soil type, season and species. *The Journal of Applied Ecology*, *27*(2), 623-634. https://doi.org/10.2307/2404307

Gilbert, R. O., Shin, S. T., Rabuffo, T. S., & Chandler, S. K. (1996, July). *An in vitro model for the study of bovine endometrial physiology and pathophysiology*. Paper presented at the 13th International Congress on Animal Reproduction, Sydney, Australia.

Gustafsson, A. H., & Palmquist, D. L. (1993). Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. *Journal of Dairy Science*, *76*(2), 475–484. https://doi.org/10.3168/jds.S0022-0302(93)77368-3

Heinrichs, A. J. (1993). Raising dairy replacements to meet the needs of the 21st century. *Journal of Dairy Science, 76(10), 3179–3187.* https://doi.org/10.3168/jds.S0022-0302(93)77656-0

Heinrichs, A. J., Zanton, G. I., Lascano, G. J., & Jones, C. M. (2017). A 100-year review: A century of dairy heifer research. *Journal of Dairy Science*, *100*(12), 10173–10188. https://doi.org/10.3168/jds.2017-12998

Hoveland, C. S., Hardin, D. R., Worley, P. C., & Worley, E. E. (1991). Steer performance on perennial vs. winter annual pastures in north Georgia. *Journal of Production Agriculture*, *4*(1), 24-28. https://doi.org/10.2134/jpa1991.0024

Idris, A., Moors, E., Sohnrey, B., & Gauly, M. (2012). Gastrointestinal nematode infections in German sheep. *Parasitology Research*, *110*(4), 1453–1459. https://doi.org/10.1007/s00436-011-2648-1

Jaramillo, V. J., & Detling, J. K. (1992). Small-scale heterogeneity in a semi-arid North American grassland. I. Tillering, N uptake and retranslocation in simulated urine patches. *The Journal of Applied Ecology*, *29*(1), 1-8. https://doi.org/10.2307/2404340

Jones, W. T., & Mangan, J. L. (1977). Complexes of the CT of sainfoin (Onobrychis viciifolia scop.) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. *Journal of the Science of Food and Agriculture*, *28*(2), 126–136. https://doi.org/10.1002/jsfa.2740280204

Kolver, E. S., & Macmillan, K. L. (1994). Variation in selected blood plasma constituents during the post-partum and breeding periods in dairy cows. *New Zealand Veterinary Journal*, *42*(5), 161–166. https://doi.org/10.1080/00480169.1994.35813

Lacau-Mengido, I. M., Mejía, M. E., Díaz-Torga, G. S., Gonzalez Iglesias, A., Formía, N., Libertun, C., & Becú-Villalobos, D. (2000). Endocrine studies in ivermectin-treated heifers from birth to puberty. *Journal of Animal Science*, *78*(4), 817. https://doi.org/10.2527/2000.784817x

Le Cozler, Y., Lollivier, V., Lacasse, P., & Disenhaus, C. (2008). Rearing strategy and optimizing first-calving targets in dairy heifers: A review. *Animal*, *2*(9), 1393-1404. https://doi.org/10.1017/S1751731108002498

Ledgard, S. F. (2001). Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures., *Plant and Soil 228*(1), 43–59. https://doi.org/10.1023/A:1004810620983

Li, Y.-G., Tanner, G., & Larkin, P. (1996). The DMACA-HCl protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. *Journal of the Science of Food and Agriculture*, *70*(1), 89–101. https://doi.org/10.1002/(SICI)1097-0010(199601)70:1<89::AID-JSFA470>3.0.CO;2-N

Lin, C. Y., McAllister, A. J., Batra, T. R., Lee, A. J., Roy, G. L., Vesely, J. A., … Winter, K. A. (1986). Production and reproduction of early and late bred dairy heifers. *Journal of Dairy Science*, *69*(3), 760–768. https://doi.org/10.3168/jds.S0022-0302(86)80465-9

Macdonald, K. A., Penno, J. W., Bryant, A. M., & Roche, J. R. (2005). Effect of feeding level pre- and post-puberty and body weight at first calving on growth, milk production, and fertility in grazing dairy cows. *Journal of Dairy Science, 88(9), 3363–3375*. https://doi.org/10.3168/jds.S0022-0302(05)73020-4

MacRae, J. C. (1993). Metabolic consequences of intestinal parasitism. *Proceedings of the Nutrition Society*, *52*(01), 121–130. https://doi.org/10.1079/PNS19930044

Marston, T. T., Lusby, K. S., & Wettemann, R. P. (1995). Effects of postweaning diet on age and weight at puberty and mild production of heifers. *Journal of Animal Science, 73*(1), 63-68. https://doi.org/10.2527/1995.73163x

McBride, W. D., & Greene, C. (2007). A Comparison of Conventional and Organic Milk Production Systems in the U.S. *American Agricultural Economics Association Annual Conference.,* 1-30. http://dx.doi.org/10.22004/ag.econ.9680

McClure, K. E., Van Keuren, R. W., & Althouse, P. G. (1994). Performance and carcass characteristics of weaned lambs either grazed on orchardgrass, ryegrass, or alfalfa or fed all-concentrate diets in drylot2. *Journal of Animal Science*, *72*(12), 3230–3237. https://doi.org/10.2527/1994.72123230x

McMahon, L. R., McAllister, T. A., Berg, B. P., Majak, W., Acharya, S. N., Popp, J. D., … Cheng, K.-J. (2000). A review of the effects of forage CT on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science*, *80*(3), 469–485. https://doi.org/10.4141/P99-050

Min, B., Barry, T., Attwood, G., & McNabb, W. (2003). The effect of CT on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science and Technology*, *106*(1–4), 3–19. https://doi.org/10.1016/S0377-8401(03)00041-5

Molan, A. L., Waghorn, G. C., Min, B. R., & McNabb, W. C. (2000). The effect of CT from seven herbages on Trichostrongylus colubriformis larval migration in vitro. *Folia Parasitologica*, *47*(1), 39–44. https://doi.org/10.14411/fp.2000.007

Moriel, P., Cooke, R. F., Bohnert, D. W., Vendramini, J. M. B., & Arthington, J. D. (2012). Effects of energy supplementation frequency and forage quality on performance, reproductive, and physiological responses of replacement beef heifers. *Journal of Animal Science*, *90*(7), 2371–2380. https://doi.org/10.2527/jas.2011-4958

Niezen, J., Robertson, H., Waghorn, G., & Charleston, W. A. (1998). Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. *Veterinary Parasitology*, *80*(1), 15–27. https://doi.org/10.1016/S0304-4017(98)00202-7

Ordóñez, A., Parkinson, T., Matthew, C., Holmes, C., Miller, R., Lopez-Villalobos, N., … Brookes, I. (2007). Effects of application in spring of urea fertiliser on aspects of reproductive performance of pasture-fed dairy cows. *New Zealand Veterinary Journal, 55(2), 69–76*. https://doi.org/10.1080/00480169.2007.36744

Patra, A. K., & Saxena, J. (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture*, *91*(1), 24–37. https://doi.org/10.1002/jsfa.4152

Patterson, D. J., Perry, R. C., Kiracofe, G. H., Bellows, R. A., Staigmiller, R. B., & Corah, L. R. (1992). Management considerations in heifer development and puberty. *Journal of Animal Science*, *70*(12), 4018–4035. https://doi.org/10.2527/1992.70124018x

Peel, M. (2020, March 19). *Forage legumes in pastures and successful inter-seeding* [Webinar]. https://www.youtube.com/watch?v=RWrmNBtqiUs

Purup, S., Sejrsen, K., & Akers, R. M. (1995). Effect of bovine GH and ovariectomy on mammary tissue sensitivity to IGF-I in prepubertal heifers. *Journal of Endocrinology*, *144*(1), 153–158. https://doi.org/10.1677/joe.0.1440153

Rajala-Schultz, P. J., Saville, W. J. A., Frazer, G. S., & Wittum, T. E. (2001). Association between milk urea nitrogen and fertility in Ohio dairy cows. *Journal of Dairy Science*, *84*(2), 482–489. https://doi.org/10.3168/jds.S0022-0302(01)74498-0

Ramírez-Restrepo, C. A., Barry, T. N., Pomroy, W. E., López-Villalobos, N., McNabb, W. C., & Kemp, P. D. (2005). Use of Lotus corniculatus containing CT to increase summer lamb growth under commercial dryland farming conditions with minimal anthelmintic drench input. *Animal Feed Science and Technology*, *122*(3–4), 197–217. https://doi.org/10.1016/j.anifeedsci.2005.03.009

Roche, J. R., Petch, S., & Kay, J. K. (2005). Manipulating the dietary cation-anion difference via drenching to early-lactation dairy cows grazing pasture. *Journal of Dairy Science*, *88*(1), 264–276. https://doi.org/10.3168/jds.S0022-0302(05) 72684-9

Roche, J. R., Turner, L. R., Lee, J. M., Edmeades, D. C., Donaghy, D. J., Macdonald, K. A., … Berry, D. P. (2009). Weather, herbage quality and milk production in pastoral systems. 2. Temporal patterns and intra-relationships in herbage quality and mineral concentration parameters. *Animal Production Science*, *49*(3), 200-210. https://doi.org/10.1071/EA07308

Rose, M. (2019). Herbage *characteristics affecting intake by dairy heifers grazing grass-monoculture and grass-birdsfoot trefoil* (Master's thesis). Utah State University, Logan, UT.

Sejrsen, K. (1994). Relationships between nutrition, puberty and mammary development in cattle. *Proceedings of the Nutrition Society*, *53*(01), 103–111. https://doi.org/ 10.1079/PNS19940014

Sejrsen, K., Foldager, J., Sorensen, M. T., Akers, R. M., & Bauman, D. E. (1986). Effect of exogenous bovine somatotropin on pubertal mammary development in heifers. *Journal of Dairy Science*, *69*(6), 1528–1535. https://doi.org/10.3168/jds.S0022-0302(86)80569-0

Sejrsen, K., Huber, J. T., & Tucker, H. A. (1983). Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. *Journal of Dairy Science*, *66*(4), 845–855. https://doi.org/10.3168/jds.S0022-0302(83) 81866-9

Sejrsen, K., & Purup, S. (1997). Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review. *Journal of Animal Science*, *75*(3), 828-835. https://doi.org/10.2527/1997.753828x

Shepley, E., Vasseur, E., Bergeron, R., Villeneuve, A., & Lachance, S. (2015). Short communication: Birdsfoot trefoil as a preventative treatment for gastrointestinal nematodes in pastured dairy heifers. *Canadian Journal of Animal Science*, *95*(4), 533–537. https://doi.org/10.4141/cjas-2014-169

Silva, L. F. P., VandeHaar, M. J., Whitlock, B. K., Radcliff, R. P., & Tucker, H. A. (2002). Short communication: Relationship between body growth and mammary development in dairy heifers. *Journal of Dairy Science*, 85(10), 2600–2602. https://doi.org/10.3168/jds.S0022-0302(02)74344-0

Soder, K., & Miller, L. (2016). *Pasture quality and quantity*. Retrieved from https://extension.psu.edu/pasture-quality-and-quantity

Sonntag, W. E., Ramsey, M., & Carter, C. S. (2005). Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. *Ageing Research Reviews*, *4*(2), 195–212. https://doi.org/10.1016/j.arr.2005.02.001

Speakman, J. R., & Hambly, C. (2007). Starving for life: What animal studies can and cannot tell us about the use of caloric restriction to prolong human lifespan. *The Journal of Nutrition*, *137*(4), 1078–1086. https://doi.org/10.1093/jn/137.4.1078

Staples, C. R., Garcia-Bojalil, C. M., Oldick, B. S., Thatcher, W. W., & Risco, C. A. (1993). Protein intake and reproductive performance of dairy cows: A review, a suggested mechanism, and blood and milk urea measurements. *Proceedings of the 4th Annual Florida Ruminant Nutr. Symp., 37–51.*

Stephenson, R. J., & Posler, G. L. (1988). The influence of tall fescue on the germination, seedling growth and yield of birdsfoot trefoil. *Grass and Forage Science*, *43*(3), 273–278. https://doi.org/10.1111/j.1365-2494.1988.tb02152.x

Tozer, P., & Heinrichs, A. (2001). What affects the costs of raising replacement dairy heifers: A multiple-component analysis. *Journal of Dairy Science,84*(8), 1836-1844. doi:10.3168/jds.s0022-0302(01)74623-1

U.S. Department of Agriculture – Agricultural Marketing Service (USDA-AMS). (2019). *Guidelines for organic certification of dairy livestock*. Retrieved from https://www.ams.usda.gov/sites/default/files/media/Dairy - Guidelines.pdf

U.S. Department of Agriculture – Economic Research Service (USDA-ERS). (2016). *Organic costs and returns, 2005-16*. Retrieved from https://www.ers. usda.gov/data-products/commodity-costs-and-returns/organic-costs-and-returns/.

Velazquez, M. A., Spicer, L. J., & Wathes, D. C. (2008). The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. *Domestic Animal Endocrinology*, *35*(4), 325–342. https://doi.org/10.1016/j.domaniend.2008.07.002

Waghorn, G., & Clark, D. (2004). Feeding value of pastures for ruminants. *New Zealand Veterinary Journal*, *52*(6), 320–331. https://doi.org/10.1080/00480169. 2004.36448

Waldron, B. L., Bingham, T. J., Creech, J. E., Peel, M. D., Miller, R., Jensen, K. B., …Snyder, D. L. (2020). Binary mixtures of alfalfa and birdsfoot trefoil with tall fescue: herbage traits associated with the improved growth performance of beef steers. *Grassland Science*, 66(2), 74-87. doi:DOI: 10.1111/grs.12257.

Wang, Y., Douglas, G. B., Waghorn, G. C., Barry, T. N., Foote, A. G., & Purchas, R. W. (1996). Effect of CT upon the performance of lambs grazing Lotus corniculatus and lucerne (Medicago sativa). *The Journal of Agricultural Science*, *126*(01), 87-98. https://doi.org/10.1017/S0021859600088833

Woodward, S. L., Auldist, M. J., Laboyrie, P. J., & Jansen, E. B. L. (1999). Effect of Lotus corniculatus and CT on milk yield and milk composition of dairy cows. *Proceedings of the New Zealand Society of Animal Production, 59,* 152–155.

Xu, X., & Sonntag, W. E. (1996). Moderate caloric restriction prevents the age-related decline in growth hormone receptor signal transduction. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *51A*(2), B167–B174. https://doi.org/10.1093/gerona/51A.2.B167