Association of acute-phase proteins with feed intake in transition dairy cows, and factors affecting feed quality and digestibility

by

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B.S., Kansas State University, 2010
M.S., Michigan State University, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Production performance of livestock is dependent upon adequate feed intake to meet metabolic demands, yet variations in health, metabolism, feed quality and feed digestibility can greatly influence an animal's ability to consume and digest feedstuffs in order to reach their full potential. Using a large dataset in the first study, we analyzed plasma alpha-1-acid glycoprotein (AGP), an acute-phase protein, in 434 transition dairy cows to determine its association with dry matter intake (DMI), common blood inflammatory and metabolic biomarkers, and transition cow health disorders with an overarching goal of evaluating AGP as a diagnostic tool. The plasma AGP concentration increased after parturition to a peak at 14 d postpartum. There was a strong negative association between AGP and DMI, metritis, retained placenta, hyperketonemia and haptoglobin in the postpartum period, but overall diagnostic ability of AGP was marginal based on receiver operating characteristic analysis. Nonetheless, the ease of quantifying plasma AGP and the lack of association with metabolic biomarkers suggest it may be a useful tool to evaluate transition status in dairy cows. In the second study, a novel high-protein corn product (HPCP) was fed to high-producing, mid-lactation dairy cows and compared with canola and soybean meals, and all diets were balanced for lysine and methionine requirements. The HPCP decreased milk and milk component yields through reduced total tract apparent dry matter and crude protein digestibility, potentially due to Maillard product formation. However, confirming previous research, the canola meal performed similarly to soybean meal when lysine and methionine requirements were met. Finally, forage quality is of utmost importance in ruminant diets, and harvesting practices influence the quality of the forage delivered to the animals. In the third study, an innovative round hay baler equipped with knives that cut the hay as it enters the baling chamber was employed, reducing the particle size and potentially facilitating easier

incorporation of hay into total mixed rations. In this study, pre-cutting alfalfa hay bales increased bale weight and density, but also slightly, but significantly, increased neutral- and acid-detergent fiber and lignin. When processing the pre-cut bales in a mixer wagon, time to reduce particle size was greater compared with tub-grinding normal bales, but less shrink occurred. The determining factor for impacts of pre-cutting hay bales is evaluation of the chemical composition of the processed hay which could be fed to livestock, and data analysis is currently underway. The fourth project seeks to understand factors impacting silage hygiene that potentially have health implications for ruminants. Soil contains numerous micro-organisms, including *Clostridia spp.*, which is implicated in enteric diseases in ruminants. The study evaluates whether drive-over silage piles stored on soil introduce soil from equipment during the packing process, which may inadvertently introduce pathogens. Of primary interest, P increased for outer layers on soil pads, and Fe was greater at lower vs. medium elevations, both of which could be indicative of soil contamination. Overall, outer layers of silage had reduced nutritive quality, increased ash and mineral content, and decreased digestibility. Evidence for an effect of pad type on microbiological aspects was marginal or limited by low detectability. Overall, the work in this dissertation demonstrates the association of inflammatory biomarkers on dry matter intake, and the importance of digestibility and high-quality harvest methods on feed quality and digestibility for optimum animal performance.

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Chapter 1 - Literature Review: Mechanisms of feed intake control during disease

INTRODUCTION

Maximizing feed intake in production animals is a common goal to foster optimum production and health of livestock. However, incidence of disease is inevitable and challenges our ability to maintain the desired production level. In particular, transition dairy cows are prone to depressed intake linked to metabolic factors and high prevalence of disease incidence. The mechanisms controlling the hypophagic response in dairy cows during disease are complex and should be appreciated within context of the natural adaptive mechanisms the animal encounters to successfully fight off infection. Signaling from the immune system acts both centrally and peripherally to enact a cascade of events that guides the animal to decrease feed intake, and our understanding of this response in many cases must be extrapolated from intense work conducted in laboratory species. While the mechanisms controlling feed intake in normal circumstances are complex, the role of dietary, physical, endocrine, metabolic, cerebral and neuronal components in domestic ruminants have been reviewed thoroughly (Baile and McLaughlin, 1987; Allen, 2000, 2014; Roche et al., 2008; Allen et al., 2009; Kuhla, 2020). Therefore, this review will focus on the processes responsible for the hypophagia realized during disease. Specifically, the role of inflammatory markers, leptin, and calcium will be emphasized before transitioning to evaluate their potential link to hypophagia in peripartum dairy cows.

THE IMMUNE SYSTEM AND FEED INTAKE

Maintaining feed intake during periods of disease is a challenge for humans (Scrimshaw, 1991), other mammals (Spurlock et al., 1997), and even insects (Ayres and Schneider, 2009). Depression of feed intake, defined as anorexia or hypophagia, can be manifested during a variety of diseases (Plata-Salamán, 1996a). Anorexia during disease appears to be a natural mechanism of host defense that benefits the host in combating pathogens. In a classic study by Murray and Murray (1979), mice were infected with *Listeria monocytogenes*. One group was fed *ad libitum* while the other was force-fed to match non-infected controls. The force-fed animals experienced a 93% mortality rate and a mean survival time of less than half that of infected animals fed *ad libitum*. Indeed, even animals that were starved for a short period prior to infection had greater survival than those fed *ad libitum* pre-infection (Wing and Young, 1980; Brown et al., 2009). In a similar manner, mice infected with *L. monocytogenes* experienced a 100% mortality rate when gavaged with food or glucose, and the researchers determined that the culprit for the death during infection was glucose utilization (Wang et al., 2016). Conversely, in the same paper, food gavage in mice infected with influenza virus survived infection when gavaged with food while phosphate-buffered saline-gavaged mice did not survive, which demonstrates a distinct difference between survival responses and food intake for bacterial and viral infections. Even so, additional evidence suggests that survival during feed restriction varies depending on the bacterial pathogen (Ayres and Schneider, 2009). All this evidence suggests that hypophagia during disease is an important response with a variety of outcomes.

More detailed evidence for the role of the immune system on feed intake is demonstrated by the ability of various factors beyond bacterial infection to induce hypophagia, including vaccination, parasitic infection (Crompton, 1984) and other experimental immune challenges.

Dry matter intake (DMI) is reduced 1 to 2 d post-immunization in beef heifers (Rodrigues et al., 2015) and vaccination of day-old chicks reduced feed intake over the first 12 weeks of life (Lee and Reid, 1977). During this time, the immune system is adapting to the antigen or adjuvant that is introduced into the body, as is demonstrated by the increase in cytokines and acute phase proteins indicative of immune system activation (Rodrigues et al., 2015). Furthermore, exogenous immune stimulants in the form of sheep red blood cells and dextran administered to chicks induced anorexia (Klasing et al., 1987). An innovative study by Greer et al. (2005) demonstrated immune system involvement in the depression in feed intake during parasitic infection since infected sheep decreased intake compared with animals that were both infected and immunosuppressed. This effect was only evident in young sheep who lacked immunocompetency, but there was no effect on intake in mature ewes who had sufficient time to establish a natural immunity (Greer et al., 2005). Further, the concentration of porcine reproductive and respiratory syndrome virus in plasma was negatively associated with feed intake in growing pigs (Greiner et al., 2000).

Reducing feed intake during an immune response is a paradoxical response, because energy requirements actually increase during immune activation. Immune challenges ranging from vaccination to sepsis increase metabolic cost to the organism from 15 to 57%, depending on the type of insult (Lochmiller and Deerenberg, 2000), and immune activation alters the site of use for both absorbed and stored nutrients (Colditz, 2008). In fact, demand for glucose and amino acids increases rapidly following lipopolysaccharide (LPS) administration, resulting in drastic drops in plasma concentrations of amino acids and eventually glucose (Waggoner et al., 2009b; Kvidera et al., 2016). These changes, coupled with the energy requirements of the immune system, alter performance. For example, ewes selected for high somatic cell score and

mastitis susceptibility experienced greater free fatty acid (FFA) and beta-hydroxybutyrate (BHB) concentrations during energy restriction suggesting they were diverting more energy towards the immune system, and coincidentally these ewes experienced exacerbated weight loss during an intramammary immune stimulation challenge (Bouvier-Muller et al., 2016). Beef heifers vaccinated with *Manheimia haemolytica* had reduced average daily gain and feed efficiency despite no evidence of difference in DMI over a 15 d period (Arthington et al., 2013). Despite the drop in plasma concentrations of amino acids, immune system activation increases the dietary amino acid requirement necessary to achieve protein deposition in growing animals (Sandberg et al., 2007; Kim et al., 2012) and maximum antibody production (Bhargava et al., 1970). Some cytokines have even been implicated in increasing the resting metabolic rate (Tsigos et al., 1997)

To further demonstrate the metabolic demand of the immune system, we can turn to models of germ-free animals or animals fed antibiotics to increase efficiency. Antibiotics administered to chickens increased weight gain and enhanced gain:feed ratios (Feighner and Dashkevicz, 1987), and antibiotics fed to pigs increased average daily gain, feed intake and gain:feed ratios at certain times of the feeding period, but effects also depend on the antibiotic type (Foster et al., 1987; Bosi et al., 2011). Additionally, antibiotics reduced IgM concentration, corroborating an effect on the humoral immune system that likely allowed for the greater performance due to decrease energy demands by the immune system (Bosi et al., 2011). To further add to this, animals raised in a germ-free environment have greater body weight gain despite similar feed consumption (Wostmann et al., 1983; Furuse and Yokota, 1985) presumably due to less activation and energy use by the immune system. Pigs raised in an unclean environment compared with highly hygienic facilities exhibited decreased gain and feed

efficiency despite similar feed intake, along with evidence of increased immune activation (Williams et al., 1997a; b).

Overall, this paradoxical situation of reducing feed intake during an immune response that increases the energy needed is an intriguing situation. In the next portion of this review we will uncover specific physiological mechanisms that occur during acute and subacute disease to induce hypophagia.

CYTOKINE REGULATION OF FEED INTAKE

Cytokines are produced by macrophages, lymphocytes, hepatocytes, neuronal cells, bone marrow stromal cells, and a vast array of cell types in the central nervous system (Curfs et al., 1997). Cytokines are key signaling agents that potentiate the immune response (serving a multitude of functions), but can also play an anti-inflammatory role depending on the cytokine that is produced (Dinarello, 2000; Opal and DePalo, 2000). Numerous cytokines are produced during the acute phase response, but several cytokines are most classically associated as biomarkers of inflammation, such as tumor-necrosis factor-α, the interleukins, and interferons to name a few (Curfs et al., 1997). Wong and Pinkney (2004) conducted an exhaustive review of numerous cytokines and their effect on feeding behavior, so we will cover some of the most commonly known cytokines that are most relevant to feeding behavior during disease in production animals.

Hypophagia during disease or infection is typically referred to as a hallmark 'sickness behavior.' Our discussion about feeding behavior as a result of immune activity and specific cytokine effects is somewhat confounded by the other behavioral aspects of disease that directly impact food acquisition, and thus requires acknowledgement. Somnolence and sleeping is a

common product of immune activation (Johnson et al., 1993; Warren et al., 1997; Dafny, 1998; Larson and Dunn, 2001) along with a decrease in total locomotor activity (Plata-Salamán, 1994), exercise and exploratory behavior (Harden et al., 2006; Teeling et al., 2007), social exploration (Bluthé et al., 2000), and sexual behavior (Teeling et al., 2007). Johnson (2002) reviews that this overall reduction in locomotor activity is actually an altered motivational state and not a mere consequence of weakness or lack of ability. As we discuss specific actions of cytokines and hormones that are increased during an acute-phase response, some of which may have pleiotropic effects, it is important to recognize that the mechanistic impacts on feed intake may also be compounded by other behavioral changes that the animal experiences during this motivational state to decrease locomotor activity.

Interleukin-1

Interleukin-1 (IL-1) is produced primarily by macrophages (Curfs et al., 1997), but can also be produced in the brain (Giulian et al., 1986) and other locations. Two isoforms of IL-1 are produced; IL-1α and IL-1β. Interleukin-1 β is the secreted form while IL-1α is associated with the membrane (Curfs et al., 1997). Numerous studies have been conducted to analyze the effects of IL-1 administration on feed intake, with responses observed in multiple species. Both human and murine isoforms of IL-1 reduced feed intake in mice (Kiuger and Vander, 1985) with human isoforms also affecting rats (Plata-Salamán, 1991a). In production animals, IL-1 administration induced hypophagia in chicks, goats and beef calves (Klasing et al., 1987; van Miert et al., 1992b; Godson et al., 1995; Tachibana et al., 2017); however, the effects of recombinant IL-1β administration in non-lactating dairy cows was transient with no effect being seen until the second d of administration, combined with immediate return to normal levels of intake post-treatment (Goff et al., 1992). Other studies have also reported quite rapid return to normal feed

intake after removal of treatment (Kent et al., 1994) and even compensation for previously reduced feed intake (Plata-Salamán, 1991a). Additionally, heat treatment of IL-1 to denature the cytokine ameliorates its effect on feed intake further demonstrating a direct role of IL-1 (Kiuger and Vander, 1985; Klasing et al., 1987). From an observational perspective, cows with greater IL-1β concentration over the transition period had lesser feed intake (Trevisi et al., 2015).

Interestingly, IL-1 appears to affect feed intake in a dose-dependent manner. Subcutaneous administration of recombinant IL-1β in beef calves caused inappetence with increasing dose (Godson et al., 1995). Both intraperitoneal and intracerebroventricular (i.c.v.) injections of IL-1 isoforms impacted the food consumed by mice and rats in a dose-dependent manner (Plata-Salamán et al., 1988; Kent et al., 1994; Plata-Salamán, 1994; Plata-Salamán and Borkoski, 1994; Swiergiel et al., 1997) by decreasing both meal size and duration, but not meal frequency (Plata-Salamán, 1994). In fact, i.c.v. infusions of IL-1β as low as 0.5 ng decreased food intake (Plata-Salamán, 1991a), although the effect was transient compared with higher doses (Plata-Salamán et al., 1988; Plata-Salamán and Borkoski, 1994). This may suggest that degree of anorexia caused by disease may depend on the severity of disease.

The site of administration of IL-1 also appears to provide clues into the site of its action. At least 100 ng of intraperitoneal (i.p.) IL-1β were required to reduce feed intake in mice (Swiergiel et al., 1997), whereas only 0.5 to 10 ng of i.c.v. IL-1β were capable of eliciting hypophagia in rats (Plata-Salamán et al., 1988, 1997; Plata-Salamán, 1991a; Plata-Salamán and Borkoski, 1994). Continuous intravenous (i.v). infusions of 20 μg/kg in rats decreased feed intake during a 4 to 6-d infusion period, which may more accurately reflect an inflammatory disease response compared with boluses typically administered in experimental settings (Ling et al., 1996, 1997). This difference suggests that the most potent action of IL-1 occurs in the

central nervous system, and it is capable of crossing the blood-brain barrier to provide peripheral signaling to the central nervous system (Threlkeld et al., 2010).

Other more targeted studies conducted during this period of exploration of cytokine influence on feeding behavior explored the hypothalamus as a potential site of action of IL-1. IL-1 has a high affinity for receptors in the ventromedial hypothalamus (Farrar et al., 1987; Katsuura et al., 1988), a region of the brain that regulates feeding behavior (King, 2006). Intrahypothalamic injection of 5 ng IL-1α decreased both feed and water intake independently (Chance and Fischer, 1992) and injection of similar quantities of IL-1β into the ventromedial nucleus decreased feed intake (Kent et al., 1994). Kuriyama et al. (1990) evaluated whether specific types of glucose-sensing neurons could be responsible for changes in feed intake due to cytokine action. They found that IL-1β acts directly on the neurons in the ventromedial hypothalamus that respond to increases in glucose concentration and would normally signal a positive energy status, but not in neurons which decrease activity during a fall in glucose concentration. Additionally, others have demonstrated that IL-1β inhibits activity of lateral hypothalamic glucose-sensitive neurons, whereas it excited glucoreceptor neurons in the ventromedial hypothalamus (Oomura, 1988). These mechanisms provide a clearer picture for the intersection between metabolism and immune function through their action on the central nervous system.

Downstream action from central nervous system activation by IL-1 could be through a variety of mechanisms. For example, IL-1 increases plasma insulin concentrations (Lang and Dobrescu, 1989), and ultimately IL-1 increases insulin secretion from the pancreas by direct signaling through the central nervous system (Cornell, 1989; Cornell and Schwartz, 1989). In

ruminants, plasma insulin concentration is negatively related with DMI (Bradford and Allen, 2007).

Despite this antagonism of IL-1 on feed intake, certain treatments can reverse its effects on anorexia. An IL-1 receptor antagonist, which is a naturally occurring protein that serves as a negative feedback mechanism, and a IL-1 receptor ligand binding domain have been shown to attenuate the feed intake drop due to IL-1β and lipopolysaccharide injection (Klasing and Korver, 1997; Swiergiel and Dunn, 1999). Additionally, dexamethasone, an anti-inflammatory product, also eliminated the effects of IL-1 administration (Plata-Salamán, 1991a).

Interleukin-6

There has been considerably less focus on interleukin-6 (IL-6) as an inhibitor of feed intake; nonetheless, the limited information that exists suggests it may play a role similar to IL-1β, although its effects seem to be less intensive. Interleukin-6 administered i.c.v. dose dependently reduced intake in rats (Plata-Salamán, 1996b), but had no effect in chicks (Tachibana et al., 2017). Knock-out mice that do not produce IL-6 were not subject to feed intake perturbations when subjected to local or systemic inflammatory insults (Fattori et al., 1994; Kozak et al., 1997). Additionally, some cytokines have been implicated in cachexia during tumor growth, and a model of infecting mice with tumor cells centrally caused cachexia and hypophagia except in the case of mice receiving an IL-6 antibody which partially ameliorated the hypophagic response (Negri et al., 2001).

Tumor necrosis factor-α

Tumor necrosis factor-α (TNF-α) is a cytokine produced primarily by macrophages and to a certain extent by other immune cells (Beutler and Cerami, 1989; Plata-Salamán, 1991b). Astrocytes located in the brain produce TNF-α upon stimulation by LPS, interferon-gamma, IL-

1β, and certain viruses (Lieberman et al., 1989; Chung and Benveniste, 1990). Infection can also upregulate expression of TNF-α in specific parts of the brain, including the arcuate nucleus of the hypothalamus (Breder et al., 1994). Furthermore, TNF-α is produced in the adipose tissue and its production increases with greater adipose tissue deposition (Daniel et al., 2001).

The most extensive evidence for the effects of TNF-α on feed intake is in mice and rats and suggests that its action on intake are similar to that of IL-1β, with some subtle differences. The most dramatic reductions in food intake occur during i.c.v. infusions with a dose-dependent response up to 5 µg eliciting up to a 50% reduction in food intake in rodents (Plata-Salamán et al., 1988, 1997; Bodnar et al., 1989; Fantino and Wieteska, 1993; Romanatto et al., 2007). Interestingly, micro-doses as small as 0.005 to 0.05 µg/kg of body weight administered i.c.v. dose dependently reduced feed intake in swine for up to 8 hours (Warren et al., 1997). Peripheral administration of TNF-α via intraperitoneal injection shows mixed results, most likely due to a dosing effect. Fantino and Wieteska (1993) showed no effect on feed intake of 4 µg of i.p. TNF-α, which had successfully induced hypophagia via i.c.v. administration. However, when the intraperitoneal dose was greatly increased to 75 µg/kg or above, intake declined 20-50% (Tracey et al., 1988; Mccarthy, 2000). Curiously, an intravenous dose of only 30 µg/kg did not affect feed intake during the first 2 d of infusion, but it did slightly depress intake on d 3 of infusion (Yang et al., 1994). Increasing the rate to 100 µg/kg decreased feed intake over a six-day continuous infusion (Ling et al., 1997), and these continuous infusion study designs may be the most representative of biology due to the continuous nature of the infusion.

Tumor necrosis factor-α has also been shown to influence feed intake in ruminants. Subcutaneous daily injection of TNF-α at varying stages of lactation decreased intake 15-30% at dosage rates of 1.5 to 3.0 µg/kg (Kushibiki et al., 2003; Bradford et al., 2009; Yuan et al., 2013),

and i.v. bolus of 4 µg/kg depressed intake over a 3 hr period in dwarf goats (van Miert et al., 1992a). However, 5 µg/kg i.v. bolus in dairy heifers (Kushibiki et al., 2002) and a continuous infusion of 2 µg/kg/d directly into adipose tissue of mature lactating cows did not alter dry matter intake (Martel et al., 2014). This lack of response at a higher rate may be a factor of the continuous nature of the infusion similar to the delayed response noted during i.v. infusion in mice (Yang et al., 1994).

The effects of TNF-α can further be isolated by innovative studies that ameliorate the effects of the cytokine after it has been administered. Heat inactivation of TNF-α prior to administration eliminates its effects entirely (Plata-Salamán et al., 1988) demonstrating that the intake depression in that study was not from the i.c.v. infusion procedure alone. Furthermore, administering an antibody specific for TNF-α ameliorated the decline in feed intake seen in mice not receiving the antibody (Tracey et al., 1988). Pentoxifylline, which is known to inhibit TNF-α production, prevented hypophagia during LPS administration, but not when TNF-α was co-administered (Porter et al., 2000), and a TNF-α binding protein tended to attenuate LPS-induced hypophagia in another instance (Swiergiel and Dunn, 1999). This is an important consideration, because LPS administration is known to cause a thorough inflammatory response involving several inflammatory markers capable of contributing to hypophagia. Furthermore, others have shown that administering a TNF-α receptor inhibitor eliminates feed intake reductions during cachexia or tumor implantation (Torelli et al., 1999; Steffen et al., 2008).

There is evidence to suggest that the mode of action of TNF-α on feed intake reduction may occur both centrally and peripherally. The firing rate of neurons in the ventromedial hypothalamus, a key site for the global regulation of feed intake (King, 2006), is inhibited by TNF-α in a dose dependent manner, but adding the anti-inflammatory sodium salicylate

maintains the firing rate (Katafuchi et al., 1997). While one study employing an incredibly high i.v. dose indicated that the feed intake reduction by TNF-α was perhaps through decreased gastric emptying (Patton et al., 1987), others have shown no change in gastric emptying despite declining feed intake during i.p. administration (Mccarthy, 2000). These differences may be a result of site of action, but TNF-α can cross the blood-brain barrier in both directions (Bodnar et al., 1989; Gutierrez et al., 1993).

Interferons

Interferons (IFN) are primarily produced by innate immune cells, such as dendritic cells and macrophages (Perry et al., 2005; Ali et al., 2019). Similar to IL-1β and TNF-α, IFN appear to reduce feed intake after both central (Plata-Salamán, 1992; Turrin et al., 1998) and peripheral administration (Segall and Crnic, 1990; Langstein et al., 1991; Crnic and Segall, 1992) in rats and mice. Also similar to other cytokines, the effect of IFN is dose dependent (Segall and Crnic, 1990; Plata-Salamán, 1992), and its effects can be partially ameliorated by antibodies specific for IFN (Crnic and Segall, 1992). While there has been very limited work evaluating IFN effects on feed intake in ruminants, one study in dwarf goats showed a transient depression of feed intake 2 to 3 h after i.v. administration (van Miert et al., 1992b).

In a similar nature to the other more thoroughly investigated cytokines, the central effect of IFN appears to be through activation of glucose-responsive neurons in the hypothalamus (Kuriyama et al., 1990; Dafny, 1998). Interestingly, neuropeptide Y, a powerful potentiator of feed intake produced in the central nervous system, can transiently counteract the hypophagic effects of IFN when both are infused i.c.v. (Turrin et al., 1998).

Synergistic effects of cytokines

While most of the cytokines discussed thus far have been evaluated individually, the reality during an inflammatory response is that multiple cytokines will be elevated. This prompts the question whether the effects of these cytokines may be additive. Due to their strong hypophagic properties individually, the most common combination for testing synergistic responses is IL-1β and TNF-α. In most of these cases, the combination of IL-1β and TNF-α administered i.v., i.p, or i.c.v at rates previously shown to have an effect on feed intake exacerbated the degree of hypophagia compared with the individual cytokine (Yang et al., 1994; Van Der Meer et al., 1995; Grunfeld et al., 1996; Sonti et al., 1996). The exception to this was when Sonti et al. (1996) used the same rate for i.p administration as they did with i.c.v administration, which further validates the sensitivity of the central nervous system to lower doses of cytokines as we discussed previously.

This synergistic effect may partially be due to how these cytokines potentiate the production of other cytokines. For example, TNF-α stimulates production of IL-1 in mononuclear cells (Dinarello et al., 1986), and IL-1 may be required for the release of IL-6 (Miller et al., 1997; Luheshi et al., 1999). Despite the very clear evidence that IL-1β reduces feed intake centrally and peripherally, IL-1β knockout mice and regular mice both had massive reductions in food intake during LPS administration, demonstrating that there are more factors than just IL-1β in feed intake during an inflammatory response (Kozak et al., 1995). In another case, blocking corticotropin-releasing hormone during IL-1 infusion partially rescued feed intake reductions (Uehara et al., 1989). Further, ibuprofen used as an anti-inflammatory agent did not alleviate hypophagia induced by LPS and IL-6, but it did when TNF-α was administered alone at higher doses (Mccarthy, 2000), clearly demonstrating cascading effects of LPS and IL-6 to

potentiate other mechanisms of feed intake reduction. However, the increase in IL-1ra during LPS infusion also demonstrate potential feedback mechanisms preventing uncontrolled upregulation of cytokines (Gabellec et al., 1995). An interesting note about all of these studies is that when a cytokine is administered in a way that alters feed intake, the other cytokines that are known to have a hypophagic effect are generally not measured, which makes the true effect of one cytokine alone difficult to interpret.

OTHER MOLECULES AFFECTING FEED INTAKE

Leptin

Leptin is a hormone produced by adipose tissue that was first discovered in the mid-1990's, and it acts on the hypothalamus to reduce feed intake (Zhou and Rui, 2013). The discovery of leptin occurred after the peak of research evaluating cytokine effects on feed intake was conducted, creating a natural bridge to investigate potential synergy. Early work showed that LPS increased leptin adipose mRNA (Grunfeld et al., 1996; Sarraf et al., 1997) and plasma leptin (Francis et al., 1999, 2000), and decreased feed intake in a dose-dependent manner (Grunfeld et al., 1996). Digging a little deeper, they further demonstrated that TNF-α and IL-1 increased leptin mRNA expression in adipose tissue (Grunfeld et al., 1996) and circulating leptin concentration, with TNF-α having a more pronounced effect (Sarraf et al., 1997). Others began to elucidate a more vivid role of TNF-α using mice that have macrophages which are insensitive to LPS with the recognition that macrophages are key producers of TNF-α. Finck et al. (1998) showed that LPS did not increase plasma TNF-α or leptin in these endotoxin-insensitive mice, but TNF-α alone did increase plasma leptin in insensitive and control mice. This group went on to show that the TNF receptor p55 in adipose tissue is required for the production of TNF-α-

stimulated leptin (Finck and Johnson, 2000). Additionally, this TNF-α-mediated leptin secretion may be partially mediated by insulin (Medina et al., 2002), although insulin's effects on leptin secretion can continue when TNF receptors are blocked (Finck and Johnson, 2000).

While the primary role of leptin is a feedback mechanism for feed intake, there is evidence that it may potentiate the actions of cytokines to reduce feed intake, too. Leptin induces expression of IL-1β in the mouse brain, including in mice lacking leptin receptors (Hosoi et al., 2002) suggesting potential homology between receptors. Further, administration of a leptin antibody reduces the expression of IL-1β and IL-1ra in the hypothalamus (Sachot et al., 2004). Others have demonstrated a role for the soluble IL-1 receptor in regulating the rise in leptin due to LPS (Francis et al., 1999, 2000). Blocking the IL-1 receptor with an antagonist ameliorates leptin's hypophagic effects, and mice lacking the Il-1 receptor are not sensitive to leptin-induced hypophagia (Luheshi et al., 1999). Some work has suggested that leptin-deficient mice have greater mortality during infection (Faggioni et al., 1999; Mancuso et al., 2002), but this effect may not be due to a lack of reduction in feed intake as noted in our initial discussion of the interaction between the immune system and feed intake, but rather the role leptin plays in enhancing immunocompetency (Lord et al., 1998; Johnson and Finck, 2001; Lago et al., 2008).

However, as we have seen with several of the cytokines, leptin is not essential for LPS-induced hypophagia (Faggioni et al 1997), and it appears there may be some differences in response among species. In the limited work conducted in ruminants regarding this topic, TNF-α or LPS have failed to increase plasma leptin concentration (Daniel et al., 2001; Soliman et al., 2001; Waldron et al., 2003a) except for a tendency to increase the peak leptin concentration after a challenge (Daniel et al., 2001). Another exception was that Rodrigues et al. (2015) increased plasma leptin through vaccination of beef heifers. The general lack of effect of inflammation on

plasma leptin in ruminants may be due to their constant exposure of LPS from ruminal bacteria and dietary changes (Khiaosa-ard and Zebeli, 2018). For example, chronic exposure of LPS in rats inhibited vagal afferent signaling by leptin (de La Serre et al., 2015) which may be required for peripheral signaling to induce hypophagia. Furthermore, even in healthy ruminants, leptin's effects on feed intake are limited to animals in long-term positive energy balance (Henry et al., 2001; Morrison et al., 2001; Ehrhardt et al., 2016), and experimental effects seem to be mostly observed after supraphysiological central administration (Henry et al., 2001; Morrison et al., 2001; Foskolos et al., 2015) with minor transient or no effects during peripheral administration (Morrison et al., 2002; Ehrhardt et al., 2016).

In pigs, TNF-α did not increase adipose leptin mRNA expression in vitro, and LPS decreased leptin mRNA *in vivo* in pigs (Leininger et al., 2000). Multiple studies have reported a decrease in leptin mRNA expression and protein production during in vitro culture of adipocytes from humans and rodents (Yamaguchi et al., 1998; Medina et al., 1999; Zhang et al., 2000). Due to these discrepancies, others have questioned the role of TNF-α to induce leptin-specific hypophagia, suggesting it may only be an adaptation during an acute response (Johnson and Finck, 2001).

Acute-Phase Proteins

In the animal sciences, acute-phase proteins have largely been investigated to understand the immunological response or state in an animal. The relationship between acute-phase proteins and feed intake is typically inverse during the initial immune activation (Pfeffer et al., 1993; Fisher et al., 1996; Kvidera et al., 2017; Horst et al., 2019), although this is not always the case (Arthington et al., 2013). Very limited work has been conducted to elucidate whether acute-phase proteins possess a direct role in hypophagia, with that work focusing on alpha-1-acid

glycoprotein (AGP). Alpha-1-acid glycoprotein is produced primarily in the liver during an acute-phase response (Hochepied et al., 2003). Infusion of AGP into the central nervous system decreased feed intake in mice (Bellinger and Mendel, 1990), and similar results were obtained from an elegant and convincing study using i.p. and i.v. infusions (Sun et al., 2016). Sun et al. (2016) provided evidence that the mechanism of AGP-induced hypophagia was through AGP binding the hypothalamic leptin receptor and activating the intracellular JAK2-STAT3 pathway. This spurred a flurry of recent work in ruminants seeking a mechanistic link between AGP and feed intake. There was no evidence of hypophagia in sheep subjected to i.c.v. infusion of bovine AGP (Gregg et al., 2019), and bovine AGP did not activate STAT3 signaling through the bovine leptin receptor *in vitro* (McGuckin et al., 2020). The compelling evidence for an effect of AGP in mice but not in ruminants is in some ways similar to the diverging effects of TNF-α on leptin secretion in rodents and ruminants. This suggests different physiological systems that control feed intake during disease among species. Overall, the possibility that an acute-phase protein can independently affect feed intake opens a new realm of possibilities for understanding mechanisms of hypophagia during disease.

Gastro-intestinal motility and gastric emptying effects on feed intake

Disease and inflammation have a marked impact on gastro-intestinal mobility and secretion that have profound impacts on the animal's ability to consume food and are directly and indirectly coordinated with the previous responses discussed in this review. Rumination and rumen motility are necessary to reduce particle size for passage through the rumen and promote additional feed consumption. In a number of studies, administration of endotoxin (van Miert et al., 1992a; Plaza et al., 1997; Jacobsen et al., 2005; Tsuchiya et al., 2012), IL-1β (van Miert et

al., 1992b) and TNF-α (Hermann et al., 1999, 2003) reduce gastro-intestinal motility in rodents and small ruminants. In ruminants, the decrease in rumen contraction frequency and amplitude caused by LPS (van Miert et al., 1992b; a; Plaza et al., 1997) may be the culprit for decreasing the rumen content passage rate (Gilliam et al., 2009; Waggoner et al., 2009a; b; Lippolis et al., 2017). Endotoxin and IL-1β also inhibit gastric secretion and delay gastric emptying (Uehara et al., 1990; Robert et al., 1991; Jennings et al., 1995). It must be pointed out, however, that the effects of an immune response on passage rate and gastric function are likely confounded with reduced intake and a direct assertion of the ability of cytokines or other immune factors to mediate these responses should be interpreted cautiously.

Aside from the potentially confounding effects of hypophagic immune factors and feed intake that are difficult to separate *in vivo*, there is some evidence of a direct effect of cytokines on the gastrointestinal tract. The action of TNF-α in gastric motility inhibition occurs in the brainstem's dorsal vagal complex (Hermann and Rogers, 1995; Hermann et al., 1999, 2003) and locally as illustrated by *in vitro* work (Montuschi et al., 1993). The dose-dependent peripheral action of Il-1β on gastric motility (Montuschi et al., 1993) may be through inhibition of cholinergic acetylcholine release from myenteric neurons in circular smooth muscle (Cao et al., 2004). Administration of an IL-1ra both i.p. and i.c.v. restores some gastric (Tsuchiya et al., 2012) and recticulo-rumen motility (Plaza et al., 1997).

Another factor potentially responsible for a portion of the hypophagia experienced during inflammation is the reduction in blood calcium. Calcium is required for smooth muscle contraction, and experimentally binding blood calcium to subclinical hypocalcemic concentrations reduces ruminal and abomasal contractions, contraction amplitude, and feed intake (Daniel, 1983; Jorgensen et al., 1998; Hansen et al., 2003; Martinez et al., 2014).

Additionally, rumination rate is inversely related to plasma calcium in cows with subclinical and clinical hypocalcemia (Goff et al., 2020) with rumination serving as an indicator of rumen motility. Dry matter intake is rapidly recovered when blood calcium concentrations return to normal (Hansen et al., 2003; Goff et al., 2020)

From the point of view of disease, interesting studies in dairy cows have shown that plasma free and total calcium concentration decline during an endotoxin challenge (Waldron et al., 2003b; Jacobsen et al., 2005; Al-Qaisi et al., 2020; Horst et al., 2020) to concentrations often considered sub-clinically hypocalcemic (Martinez et al., 2018). Nonetheless, rumen contraction frequency and amplitude (van Miert, 1987) and dry matter intake (Horst et al., 2020) are not necessarily rescued when calcium is administered during an endotoxin challenge. Additionally, feed intake is still impaired in cows that do not fall into the subclinical hypocalcemic category during an endotoxin challenge (Zebeli et al., 2011) and returning dry matter intake to pre-challenge levels typically lags behind the timepoint at which normocalcemia is achieved by a period of up to several days (Waldron et al., 2003a; b; Horst et al., 2020). While hypocalcemia has very clear effects of reducing ruminal motility and feed intake, it seems its effects are transitory and that cytokine action during an inflammatory response may have a more powerful and long-lasting role in altering motility and intake.

IMPLICATIONS IN DAIRY CATTLE

The transition period in dairy cattle, defined as 3 wk prepartum to 3 wk postpartum, is a challenging time during which the cow encounters a myriad of potential metabolic and infectious diseases. After parturition, there is a considerable spike in a variety of cytokines and positive acute phase proteins (Jafari et al., 2006; Graugnard et al., 2012; Akbar et al., 2015; Yuan et al.,

2015; McCarthy et al., 2016; McGuckin et al., 2020), and while the degree of increase in inflammatory markers depends on quantity and types of disease insults encountered, even apparently healthy cows experience a spike postpartum (Qu et al., 2014). The increase in inflammatory markers in apparently healthy animals postpartum may be tied to naturally occurring tissue damage during parturition, but a multitude of factors may be responsible for postpartum inflammation (Bradford et al., 2015). In general, four key areas are considered to potentially contribute to postpartum inflammatory conditions pertinent to transition cow hypophagia, including 1) uterine health, 2) mammary health, 3) rumen health, and 4) lipid mobilization (Kuhla, 2020).

Postpartum uterine diseases, such as metritis, arise through a complicated balance between periparturient immune depression and bacterial invasion in the reproductive tract manifesting itself several weeks postpartum (Leblanc, 2012). Clinical endometritis may cause an increase in systemic inflammatory markers (Foley et al., 2015), although cows that experience metritis may have elevated inflammatory markers for weeks before calving (Dervishi et al., 2016). Prevalence of mastitis is the highest in postpartum cows compared with any other timepoint in the lactation curve due to a variety of factors (Pyörälä, 2008). Mammary infection can create systemic inflammation evident in increases of hepatic mRNA abundance and corresponding circulating cytokines and positive-acute phase proteins (Eckersall et al., 2001, 2006; Vels et al., 2009). Further, postpartum dairy cows are subjected to a diet change at parturition that includes greater starch concentration and perhaps fermentability which can induce ruminal acidosis. Ruminal acidosis damages the epithelial lining of the rumen and allows LPS to translocate into the bloodstream, creating a systemic immune response (Plaizier et al., 2009, 2012; Zebeli et al., 2015). In fact, a recent study in postpartum dairy cows noted in

particular that highly fermentable starch sources in high starch diets elicited greater haptoglobin and TNF-α responses (Albornoz et al., 2020). Finally, transition dairy cows are subject to impressive mobilization of body fat reserves that circulates as FFA (Akbar et al., 2015; Bernabucci et al., 2017). There is speculation that these free fatty acids are capable of activating the toll-like receptor 4 (TLR-4) on monocytes and macrophages, which then activates nuclear factor-κβ and subsequent transcriptional upregulation of cytokine production (Sordillo et al., 2009; Ingvartsen and Moyes, 2017). Coincidently, saturated fatty acids *in vitro* activate microglial TLR-4 and subsequent cytokine production centrally (Wang et al., 2012). The etiology of all four of these diseases in the transition dairy cow is complex and incompletely understood to date. The implications of the increase in circulating inflammatory markers and their causative agents on production factors important to dairy profitability are great. For example, early postpartum cows with elevated plasma haptoglobin concentration have increased disease incidence and reduced liver function (Bertoni et al., 2008; Abuajamieh et al., 2016), and haptoglobin is negatively associated with milk production (Huzzey et al., 2015; Albornoz et al., 2020) and negatively associated with the likelihood of conception by 150 DIM (Huzzey et al., 2015).

The likelihood of cows encountering one or multiple cases of the inflammatory conditions described during the transition period is high and underscores the potential ability of the immune response as a potentiator of hypophagia in the transition period. Dairy cows experience a marked decline in feed intake during transition, with a nadir at or slightly after parturition despite an increase in energy demand for milk synthesis. Maximizing feed intake during this time is key to promoting a successful lactation, but cows with a greater inflammatory state during this time may have lower intake than their healthy peers. For example, cows with

greater circulating concentrations of IL-1β during the dry period had lesser feed intake and plasma calcium over the transition period (Trevisi et al., 2016). Cows that have ketosis, metritis, or a combination of multiple diseases have reduced DMI compared with healthy cows (Huzzey et al., 2007; Leblanc, 2012; Schirmann et al., 2016) through altered patterns of feeding behavior. Sick cows spend less time feeding and consume feed at a slower rate during the meal (Huzzey et al., 2007; Schirmann et al., 2016). Others have derived estimates for the quantity of feed intake reduction due to a variety of diseases, with the periparturient diseases we have discussed accounting for up to 71 kg of DMI loss before resolving the insult (Bareille et al., 2003). Furthermore, increasing energy demand through greater production capabilities may reduce the ability of the periparturient animal to fight off infection (Kahn et al., 2003; Houdijk et al., 2006; Kidane et al., 2010). To further exacerbate the compounding issues of diseases in the transition period, it is possible that production of hypophagic cytokines from immune cells during the transition period is hyperactive compared with other parts of the lactation cycle based upon *in vitro* evidence (Sordillo et al., 1995). Additionally, the plane of nutrition during the disease insult may be a contributing factor to the degree of hypophagia (Kyriazakis, 2010).

With the recognition of the increased inflammatory status in the transition dairy cow, several research groups have administered non-steroidal anti-inflammatory drugs to cows to potentially alleviate stress in the postpartum period. Initial results created excitement about the possibility of increased lactational milk yield (Farney et al., 2013b), but positive milk production effects have not been replicated in other studies (Shwartz et al., 2009; Carpenter et al., 2018). Non-steroidal anti-inflammatory drugs given to postpartum cows fail to alter (Farney et al., 2013b; Pascottini et al., 2020) or even decrease DMI (Shwartz et al., 2009; Carpenter et al., 2018) which is a surprising response considering the protection from hyophagia in late-lactation

cows challenged with mastitis and treated with anti-inflammatory agents (Yeiser et al., 2012). While these anti-inflammatory drugs may have reduced cytokines and acute-phase proteins in limited situations (Farney et al., 2013a; Pascottini et al., 2020), sodium salicylate unexpectedly increased both pro- and anti-inflammatory genes and TNF-α in adipose tissue (Takiya et al., 2019) demonstrating that inflammation in the transition cow is a complex biological state.

Attempts have been made to model infection-induced hypophagia, mostly focusing on parasitic infections. Sandberg et al. (2006) highlight the variable characteristics of infection-induced hypophagia, including 1) the lag time after infection, 2) rate of relative feed intake reduction, 3) quantity of reduction, 4) duration of the reduction, and 5) rate of relative feed intake recovery. These stages may be more applicable to instances in later stages of lactation than for the peripartum dairy cow considering the complex timing of infection occurring in transition dairy cows and the occurrence of inflammation in animals appearing healthy. Development of models to predict DMI during parasitic infection have been created, but *in vivo* studies are generally lacking to support the model (Laurenson et al., 2011) and likely foreshadow the difficulty of developing similar models in transition dairy cows due to the complexity and multiplicity of insults may occur.

CONCLUSION

Suppression of feed intake during disease and infection is a counterintuitive approach to assist the host in fighting the infection and preserving the life of the animal compared to situations where food intake is maintained. Cytokines produced by immune cells, such as IL-1β, IL-6, IFN, and TNF-α play a critical role in actively reducing feed intake, with the most potent action occurring through central action. The effect of other molecules, such as leptin and acute-phase proteins, is less clear and perhaps transient in nature. In transition dairy cows, acute and

sub-acute inflammation from infection, dietary changes and metabolic alterations is common and has detrimental impacts on production performance and DMI. The multiplicative effects of transition cow disorders make it difficult to predict DMI. Further work should seek mechanistic avenues for determining the effects of specific events on DMI in transition dairy cows.

REFERENCES

Abuajamieh, M., S.K. Kvidera, M.V.S. Fernandez, A. Nayeri, N.C. Upah, E.A. Nolan, S.M. Lei, J.M. DeFrain, H.B. Green, K.M. Schoenberg, W.E. Trout, and L.H. Baumgard. 2016. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. Res. Vet. Sci. 109:81–85. doi:10.1016/j.rvsc.2016.09.015.

Akbar, H., T.M. Grala, M. Vailati Riboni, F.C. Cardoso, G. Verkerk, J. McGowan, K. Macdonald, J. Webster, K. Schutz, S. Meier, L. Matthews, J.R. Roche, and J.J. Loor. 2015. Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. J. Dairy Sci. 98:1019–1032. doi:10.3168/jds.2014-8584.

Al-Qaisi, M., S.K. Kvidera, E.A. Horst, C.S. McCarthy, E.J. Mayorga, M.A. Abeyta, B.M. Goetz, N.C. Upah, D.M. McKilligan, H.A. Ramirez-Ramirez, L.L. Timms, and L.H. Baumgard. 2020. Effects of an oral supplement containing calcium and live yeast on post-absorptive metabolism, inflammation and production following intravenous lipopolysaccharide infusion in dairy cows. Res. Vet. Sci. 129:74–81. doi:10.1016/j.rvsc.2020.01.007.

Albornoz, R.I., L.M. Sordillo, G.A. Contreras, R. Nelli, L.K. Mamedova, B.J. Bradford, and M.S. Allen. 2020. Diet starch concentration and starch fermentability affect markers of inflammatory response and oxidant status in dairy cows during the early postpartum period. J. Dairy Sci. 103:352–367. doi:10.3168/jds.2019-16398.

Ali, S., R. Mann-Nüttel, A. Schulze, L. Richter, J. Alferink, and S. Scheu. 2019. Sources of type I interferons in infectious immunity: Plasmacytoid dendritic cells not always in the driver's seat. Front. Immunol. 10. doi:10.3389/fimmu.2019.00778.

Allen, M.S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624. doi:10.3168/jds.S0022-0302(00)75030-2.

Allen, M.S. 2014. Drives and limits to feed intake in ruminants. Anim. Prod. Sci. 54:1513–1524. doi:10.1071/AN14478.

Allen, M.S., B.J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. doi:10.2527/jas.2009-1779.

Arthington, J.D., R.F. Cooke, T.D. Maddock, D.B. Araujo, P. Moriel, N. Dilorenzo, and G.C. Lamb. 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. J. Anim. Sci 91:1831–1837. doi:10.2527/jas2012-5724.

Ayres, J.S., and D.S. Schneider. 2009. The Role of Anorexia in Resistance and Tolerance to Infections in Drosophila. PLoS Biol 7:1000150. doi:10.1371/journal.pbio.1000150.

Baile, C.A., and C.L. McLaughlin. 1987. Mechanisms controlling feed intake in ruminants: A review. J. Anim. Sci. 64:915–922.

Bareille, N., F. Beaudeau, S. Billon, A. Robert, and P. Faverdin. 2003. Effects of health disorders on feed intake and milk production in dairy cows. Livest. Prod. Sci. 83:53–62. doi:10.1016/S0301-6226(03)00040-X.

Bellinger, L.L., and V.E. Mendel. 1990. The Effects of Semi-and HPLC-Purified Human Satietin and Alpha-1-Glycoprotein on Ingestion and Body Weight. Brain Res. Bull. 25:941–947.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2017. Influence of Body Condition Score on Relationships Between Metabolic Status and Oxidative Stress in Periparturient Dairy Cows. J. Dairy Sci. 88:2017–2026. doi:10.3168/jds.S0022-0302(05)72878-2.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of Inflammatory Conditions on Liver Activity in Puerperium Period and Consequences for Performance in Dairy Cows. J. Dairy Sci. 91:3300–3310. doi:10.3168/jds.2008-0995.

Beutler, B., and A. Cerami. 1989. The biology of cachectin/TNF - A primary mediator of the host response. Annu. Rev. Immunol. 7:625–655. doi:10.1146/annurev.iy.07.040189.003205.

Bhargava, K.K., R.P. Hanson, and M.L. Sunde. 1970. Effects of Methionine and Valine on Antibody Production in Chicks Infected with Newcastle Disease Virus. J. Nutr. 100:241–248.

Bluthé, R.-M., S. Laye, B. Michaud, C. Combe, R. Dantzer, and P. Parnet. 2000. Role of interleukin-1β and tumour necrosis factor-α in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. Eur. J. Neurosci. 12:4447–4456.

Bodnar, R.J., G.W. Pasternak, P.E. Mann, D. Paul, R. Warren, and D.B. Donner. 1989. Mediation of Anorexia by Human Recombinant Tumor Necrosis Factor through a Peripheral Action in the Rat. Cancer Res. 49:6280–6284.

Bosi, P., G. Merialdi, S. Scandurra, S. Messori, L. Bardasi, I. Nisi, D. Russo, L. Casini, and P. Trevisi. 2011. Feed supplemented with 3 different antibiotics improved food intake and decreased the activation of the humoral immune response in healthy weaned pigs but had differing effects on intestinal microbiota 1. J. Anim. Sci 89:4043–4053. doi:10.2527/jas.2010-3311.

Bouvier-Muller, J., C. Allain, F. Enjalbert, G. Tabouret, D. Portes, C. Caubet, C. Tasca, G. Foucras, and R. Rupp. 2016. Response to dietary-induced energy restriction in dairy sheep divergently selected for resistance or susceptibility to mastitis. J. Dairy Sci. 99:480–492. doi:10.3168/jds.2015-9785.

Bradford, B., K. Yuan, J. Farney, L. Mamedova, and A. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650. doi:10.3168/jds.2015-9683.

Bradford, B.J., and M.S. Allen. 2007. Depression in feed intake by a highly fermentable diet is related to plasma insulin concentration and insulin response to glucose infusion. J. Dairy Sci. 90:3838–3845. doi:10.3168/jds.2007-0086.

Bradford, B.J., L.K. Mamedova, J.E. Minton, J.S. Drouillard, and B.J. Johnson. 2009. Daily Injection of Tumor Necrosis Factor-α Increases Hepatic Triglycerides and Alters Transcript Abundance of Metabolic Genes in Lactating Dairy Cattle. J. Nutr. 139:1451–1456. doi:10.3945/jn.109.108233.

Breder, C.D., C. Hazuka, T. Ghayurt, C. Klug, M. Huginint, K. Yasuda, M. Tengii, and C.B. Saper. 1994. Regional induction of tumor necrosis factor α expression in the mouse brain after systemic lipopolysaccharide administration. Proc. Natl. Acad. Sci. USA 91:11393–11397.

Brown, A.E., J. Baumbach, P.E. Cook, and P. Ligoxygakis. 2009. Short-Term Starvation of Immune Deficient Drosophila Improves Survival to Gram-Negative Bacterial Infections. PLoS One 4:e4490. doi:10.1371/journal.pone.0004490.

Cao, W., L. Cheng, J. Behar, C. Fiocchi, P. Biancani, and K.M. Harnett. 2004. Proinflammatory cytokines alter/reduce esophageal circular muscle contraction in experimental cat esophagitis. Am. J. Physiol. - Gastrointest. Liver Physiol. 287:G1131–G1139. doi:10.1152/ajpgi.00216.2004.

Carpenter, A., C. Ylioja, L. Mamedova, K. Olagaray, and B. Bradford. 2018. Effects of early postpartum sodium salicylate treatment on long-term milk, intake, and blood parameters of dairy cows. J. Dairy Sci. 101:1437–1447. doi:10.3168/jds.2017-13057.

Chance, W.T., and J.E. Fischer. 1992. Aphagic and adipsic effects of interleukin-1. Brain Res. 568:261–264.

Chung, I.Y., and E.N. Benveniste. 1990. Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta.. J. Immunol. 144:2999–3007.

Colditz, I.G. 2008. Immunity to gastrointestinal nematode infections. Parasite Immunol. 30:63–70.

Cornell, R.P. 1989. Central interleukin 1-elicited hyperinsulinemia is mediated by prostaglandins but not autonomies. Am. J. Physiol. 257:R839–R846.

Cornell, R.P., and D.B. Schwartz. 1989. Central administration of interleukin 1 elicits hyperinsulinemia in rats. Am. J. Physiol. Integr. Comp. Physiol. 256:R772–R777.

Crnic, L.S., and M.A. Segall. 1992. Behavioral effects of mouse interferons-α and -γ and human interferon-α in mice. Brain Res. 590:277–284. doi:10.1016/0006-8993(92)91106-O.

Crompton, D.W.T. 1984. Influence of parasitic infection on food intake. Proc. Fed. Am. Soc. Exp. Biol. 43:239–245.

Curfs, J.H.A.J., J.F.G.M. Meis, and J.A.A. Hoogkamp-Korstanje. 1997. A Primer on Cytokines: Sources, Receptors, Effects, and Inducers. Clin. Microbiol. Rev. 10:742–780.

Dafny, N. 1998. Is interferon-α a neuromodulator?. Brain Res. Rev. 26:1–15. doi:10.1016/S0165-0173(97)00029-5.

Daniel, J.A., T.H. Elsasser, C.D. Morrison, D.H. Keisler, B.K. Whitlock, B. Steele, D. Pugh, and J.L. Sartin. 2001. Leptin, tumor necrosis factor-α (TNF), and CD14 in ovine adipose tissue and changes in circulating TNF in lean and fat sheep. J. Anim. Sci. 2590–2599.

Daniel, R.C.W. 1983. Motility of the Rumen and Abomasum During Hypocalcaemia. Can J Comp Med 47:276–280.

Dervishi, E., G. Zhang, D. Hailemariam, S.A. Goldansaz, Q. Deng, S.M. Dunn, and B.N. Ametaj. 2016. Alterations in innate immunity reactants and carbohydrate and lipid metabolism precede occurrence of metritis in transition dairy cows. Res. Vet. Sci. 104:30–39. doi:10.1016/j.rvsc.2015.11.004.

Dinarello, B.Y.C.A., J.G. Cannon, S.M. Wolff, H.A. Bernheim, B. Beutler, A. Cerami, I.S. Figari, M.A. Palladino, and J. V O'Connor. 1986. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J. Exp. Med. 163:1433–1450.

Dinarello, C.A. 2000. Proinflammatory Cytokines. Chest 118:503–508.

Eckersall, P.D., F.J. Young, C. McComb, C.J. Hogarth, S. Safi, J.L. Fitzpatrick, A.M. Nolan, A. Weber, and T. McDonald. 2001. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. Vet. Rec. 148:35–41. doi:10.1136/vr.148.2.35.

Eckersall, P.D., F.J. Young, A.M. Nolan, C.H. Knight, C. McComb, M.M. Waterston, C.J. Hogarth, E.M. Scott, and J.L. Fitzpatrick. 2006. Acute phase proteins in bovine milk in an experimental model of Staphylococcus aureus subclinical mastitis. J. Dairy Sci. 89:1488–1501. doi:10.3168/jds.S0022-0302(06)72216-0.

Ehrhardt, R.A., A. Foskolos, S.L. Giesy, S.R. Wesolowski, C.S. Krumm, W.R. Butler, S.M. Quirk, M.R. Waldron, and Y.R. Boisclair. 2016. Increased plasma leptin attenuates ptive metabolism in early lactating dairy cows. J. Endocrinol. 229:145–157. doi:10.1530/JOE-16-0031.

Faggioni, R., G. Fantuzzi, C. Gabay, A. Moser, C.A. Dinarello, K.R. Feingold, and C. Grunfeld. 1999. Leptin deficiency enhances sensitivity to endotoxin-induced lethality. Am. J. Physiol.

- Regul. Integr. Comp. Physiol. 45:R136-142.

Fantino, M., and L. Wieteska. 1993. Evidence for a Direct Central Anorectic Effect of Tumor-Necrosis-Factor-Alpha in the Rat. Physiol. Behav. 53:477–483.

Farney, J.K., L.K. Mamedova, J.F. Coetzee, B. Ku Kanich, L.M. Sordillo, S.K. Stoakes, J.E. Minton, L.C. Hollis, and B.J. Bradfor. 2013a. Anti-inflammatory salicylate treatment alters the metabolic adaptations to lactation in dairy cattle. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 305:110–117. doi:10.1152/ajpregu.00152.2013.

Farney, J.K., L.K. Mamedova, J.F. Coetzee, J.E. Minton, L.C. Hollis, and B.J. Bradford. 2013b. Sodium salicylate treatment in early lactation increases whole-lactation milk and milk fat yield in mature dairy cows. J. Dairy Sci. 96:7709–7718. doi:10.3168/jds.2013-7088.

Farrar, W.L., P.L. Kilian, M.R. Ruff, J.M. Hill, and C.B. Pert. 1987. Visualization and characterization of interleukin 1 receptors in brain. J. Immunol. 139:459–463.

Fattori, E., M. Cappalletti, P. Costa, C. Sellitto, L. Cantoni, M. Carelli, R. Faggioni, G. Fantuzzi, P. Ghezzi, and V. Poli. 1994. Defective Inflammatory Response in Interleukin 6-deficient Mice. J. Exp. Med. 180:0–7.

Feighner, S.D., and M.P. Dashkevicz. 1987. Subtherapeutic Levels of Antibiotics in Poultry Feeds and Their Effects on Weight Gain, Feed Efficiency, and Bacterial Cholyltaurine Hydrolase Activity. Appl. Environ. Microbiol. 53:331–336.

Finck, B.N., and R.W. Johnson. 2000. Tumor necrosis factor (TNF)-α induces leptin production through the p55 TNF receptor. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 278:537–543. doi:10.1152/ajpregu.2000.278.2.r537.

Finck, B.N., K.W. Kelley, R. Dantzer, and R.W. Johnson. 1998. In vivo and in vitro evidence for the involvement of tumor necrosis factor-α in the induction of leptin by lipopolysaccharide. Endocrinology 139:2278–2283. doi:10.1210/endo.139.5.6012.

Fisher, A.D., M.A. Crowe, E.M. O'Nuallain, M.L. Monaghan, J.A. Larkin, P. O'Kiely, and W.J. Enright. 1996. Effects of Cortisol on In Vitro Interferon-γ Production, Acute-Phase Proteins, Growth, and Feed Intake in a Calf Castration. J. Anim. Sci. 75:1041–1047.

Foley, C., A. Chapwanya, J.J. Callanan, R. Whiston, R. Miranda-CasoLuengo, J. Lu, W.G. Meijer, D.J. Lynn, C. O' Farrelly, and K.G. Meade. 2015. Integrated analysis of the local and systemic changes preceding the development of post-partum cytological endometritis. BMC Genomics 16:1–20. doi:10.1186/s12864-015-1967-5.

Foskolos, A., R.A. Ehrhardt, S.M. Hileman, A. Gertler, and Y.R. Boisclair. 2015. Insensitivity of well-conditioned mature sheep to central administration of a leptin receptor antagonist. Animal 9:1852–1858. doi:10.1017/S1751731115001159.

Foster, A.G., D.H. Baker, T.R. Cline, G.L. Cromwell, T.L. Veum, R. Alva-Valdes, and G.F. Ericsson. 1987. Effect of efrotomycin on gain and feed efficiency for pigs from weaning until market weight. J. Anim. Sci. 65:877–880.

Francis, J., P.S. MohanKumar, S.M.J. MohanKumar, and S.K. Quadri. 1999. Systemic administration of lipopolysaccharide increases plasma leptin levels: Blockade by soluble interleukin-1 receptor. Endocrine 10:291–295. doi:10.1007/BF02738628.

Francis, J., S.M.J. MohanKumar, and P.S. MohanKumar. 2000. Correlations of norepinephrine release in the paraventricular nucleus with plasma corticosterone and leptin after systemic lipopolysaccharide: blockade by soluble IL-1 receptor. Brain Res. 867:180–187.

Furuse, M., and H. Yokota. 1985. Effect of the gut microflora on chick growth and utilisation of protein and energy at different concentrations of dietary protein. Br. Poult. Sci. 26:97–104.

Gabellec, M.M., R. Griffais, G. Fillion, and F. Haour. 1995. Expression of interleukin 1α, interleukin 1β and interleukin 1 receptor antagonist mRNA in mouse brain: regulation by bacterial lipopolysaccharide (LPS) treatment. Mol. Brain Res. 31:122–130.

Gilliam, G.G., B.C. Graham, J.W. Waggoner, K.A. DeAtley, L. Chen, S.L. Lodge-Ivey, and C.A. Loest. 2009. Rumen fermentation and passage rate of growing steers exposed to an endotoxin. Pages 431–434 in Proceedings of the Western Section of American Society of Animal Science.

Giulian, D., T.J. Baker, L.N. Shih, and L.B. Lachman. 1986. Interleukin 1 of the central nervous system is produced by ameboid microglia. J. Exp. Med. 164:594–604.

Godson, D.L., M.E. Baca-Estrada, A.G. Van Kessel, P.A. Hughes, M.A. Morsy, J. Van Donkersgoed, R.J. Harland, D.E. Shuster, M.J. Daley, and L.A. Babiuk. 1995. Regulation of Bovine Acute Phase Responses by Recombinant Interleukin-1B. Can. J. Vet. Res. 59:249–255.

Goff, J., M. Kehrli, and M. Daley. 1992. Physiologic effects of administration of interleukin 1β in cows. Am. J. Vet. Res. 53:1983–1987.

Goff, J.P., A. Hohman, and L.L. Timms. 2020. Effect of subclinical and clinical hypocalcemia and dietary cation-anion difference on rumination activity in periparturient dairy cows. J. Dairy Sci. 103:2591–2601. doi:10.3168/jds.2019-17581.

Graugnard, D.E., M. Bionaz, E. Trevisi, K.M. Moyes, J.L. Salak-Johnson, R.L. Wallace, J.K. Drackley, G. Bertoni, and J.J. Loor. 2012. Blood immunometabolic indices and polymorphonuclear neutrophil function in peripartum dairy cows are altered by level of dietary energy prepartum. J. Dairy Sci. 95:1749–1758. doi:10.3168/jds.2011-4579.

Greer, A.W., M. Stankiewicz, N.P. Jay, R.W. McAnulty, and A.R. Sykes. 2005. The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal

parasite Trichostrongyulus conubriformis on food intake and utilization in both immunologically naive and competent sheep. Anim. Sci. 80:89–99.

Gregg, B.A., P.A. Parker, K.M. Waller, L.G. Schneider, M. Garcia, B. Bradford, J.A. Daniel, and B.K. Whitlock. 2019. Effects of central and peripheral administration of an acute-phase protein, α-1-acid-glycoprotein, on feed intake and rectal temperature in sheep. J. Anim. Sci. 97:4783–4791. doi:10.1093/jas/skz336.

Greiner, L.L., T.S. Stahly, and T.J. Stabel. 2000. Quantitative relationship of systemic virus concentration on growth and immune response in pigs. J. Anim. Sci. 78:2690–2695. doi:10.2527/2000.78102690x.

Grunfeld, C., C. Zhao, J. Fuller, A. Pollock, A. Moser, J. Friedman, and K.R. Feingold. 1996. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters: A role for leptin in the anorexia of infection. J. Clin. Invest. 97:2152–2157. doi:10.1172/JCI118653.

Gutierrez, E.G., W.A. Banks, and A.J. Kastin. 1993. Murine tumor necrossi factor alpha is transported from blood to brain in the mouse. J. Neuroimmunol. 47:169–176.

Hansen, S.S., P. Nörgaard, C. Pedersen, R.J. Jörgensen, and L.S.B. Mellau. 2003. The Effect of Subclinical Hypocalcaemia Induced by Na2EDTA on the Feed Intake and Chewing Activity of Dairy Cows. Vet. Res. Commun. 27:193–205.

Harden, L.M., S. Poole, and H.P. Laburn. 2006. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behavior. Physiol. Behav. 89:146–155. doi:10.1016/j.physbeh.2006.05.016.

Henry, B.A., J.W. Goding, A.J. Tilbrook, F.R. Dunshea, and I.J. Clarke. 2001. Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. J. Endocrinol. 168:67–77. doi:10.1677/joe.0.1680067.

Hermann, G., and R.C. Rogers. 1995. Tumor Necrosis Factor-Alpha in the Dorsal Vagal Complex Suppresses Gastric Motility. Neuroimmunomodulation 2:74–81.

Hermann, G.E., C.A.M.Y. Tovar, and R.C. Rogers. 1999. Induction of endogenous tumor necrosis factor-α: suppression of centrally stimulated gastric motility. Am. J. Physiol. Integr. Comp. Physiol. 276:R59–R68.

Hermann, G.E., C.A.M.Y. Tovar, and R.C. Rogers. 2003. LPS-induced suppression of gastric motility relieved by TNFR:Fc construct in dorsal vagal complex. Am. J. Physiol. - Gastrointest. Liver Physiol. 70808:G634–G639.

Hochepied, T., F.G. Berger, H. Baumann, and C. Libert. 2003. Alpha-1-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties.

Horst, E.A., E.J. Mayorga, M. Al-Qaisi, M.A. Abeyta, B.M. Goetz, H.A. Ramirez Ramirez, D.H. Kleinschmit, and L.H. Baumgard. 2019. Effects of dietary zinc source on the metabolic and immunological response to lipopolysaccharide in lactating Holstein dairy cows. J. Dairy Sci. 102:11681–11700. doi:10.3168/jds.2019-17037.

Horst, E.A., E.J. Mayorga, M. Al-Qaisi, M.A. Abeyta, S.L. Portner, C.S. McCarthy, B.M. Goetz, S.K. Kvidera, and L.H. Baumgard. 2020. Effects of maintaining eucalcemia following immunoactivation in lactating Holstein dairy cows. J. Dairy Sci.. doi:10.3168/jds.2020-18268.

Hosoi, T., Y. Okuma, and Y. Nomura. 2002. Leptin regulates interleukin-1β expression in the brain via the STAT3-independent mechanisms. Brain Res. 949:139–146. doi:10.1016/S0006-8993(02)02974-8.

Houdijk, J.G.M., F. Jackson, R.L. Coop, and I. Kyriazakis. 2006. Rapid improvement of immunity to Teladorsagia circumcincta is achieved through a reduction in the demand for protein in lactating ewes. Int. J. Parasitol. 36:219–227. doi:10.1016/j.ijpara.2005.09.014.

Huzzey, J.M., S. Mann, D. V. Nydam, R.J. Grant, and T.R. Overton. 2015. Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows. Prev. Vet. Med. 120:291–297. doi:10.1016/j.prevetmed.2015.04.011.

Huzzey, J.M., D.M. Veira, D.M. Weary, and M.A.G. Von Keyserlingk. 2007. Prepartum behavior and dry matter intake identify dairy cows at risk for metritis. J. Dairy Sci. 90:3220–3233. doi:10.3168/jds.2006-807.

Ingvartsen, K.L., and K. Moyes. 2017. Nutrition, immune function and health of dairy cattle. Animal 7:112–122. doi:10.1017/S175173111200170X.

Jacobsen, S., T. Toelboell, and P.H. Andersen. 2005. Dose dependency and individual variability in selected clinical , haematological and blood biochemical responses after systemic lipopolysaccharide challenge in cattle. Vet. Res. 36:167–178. doi:10.1051/vetres.

Jafari, A., D.G. V Emmanuel, R.J. Christopherson, J.R. Thompson, G.K. Murdoch, J. Woodward, C.J. Field, and B.N. Ametaj. 2006. Parenteral administration of glutamine modulates acute phase response in postparturient dairy cows. J. Dairy Sci. 89:4660–4668. doi:10.3168/jds.S0022-0302(06)72516-4.

Jennings, G., P.G. Lunn, and M. Elia. 1995. The effect of endotoxin on gastrointestinal transit time and intestinal permeability. Clin. Nutr. 14:35–41. doi:10.1016/S0261-5614(06)80008-7.

Johnson, R.W. 2002. The concept of sickness behavior: a brief chronological account of four key discoveries. Vet. Immunol. Immunopathol. 87:443–450.

Johnson, R.W., S.E. Curtis, R. Dantzer, and K.W. Kelley. 1993. Central and Peripheral Prostaglandins Are Involved in Sickness Behavior in Birds. Physiol. Behav. 53:127–131.

Johnson, R.W., and B.N. Finck. 2001. Tumor necrosis factor α and leptin: Two players in an animal's metabolic and immunologic responses to infection. J. Anim. Sci. 79:E118. doi:10.2527/jas2001.79e-supple118x.

Jorgensen, R.J., N.R. Nyengaard, S. Hara, J.M. Enemark, and P.H. Andersen. 1998. Rumen Motility During Induced Hyper- and Hypocalcaemia. Acta Vet. Scand. 39:331–338.

Kahn, L.P., M.R. Knox, S.W. Walkden-Brown, and J.M. Lea. 2003. Regulation of the resistance to nematode parasites of single- and twin-bearing Merino ewes through nutrition and genetic selection. Vet. Parasitol. 114:15–31. doi:10.1016/S0304-4017(03)00099-2.

Katafuchi, T., S. Baba, K. Motomura, K. Motomura, S. Baba, K. Ota, T. Hori, R.I. Comp, H. Tnfs, and T.N. Factor. 1997. Differential effects of tumor necrosis factor-a and -p on rat ventromedial hypothalamic neurons in vitro. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 272:R1966–R1971.

Katsuura, G., P.E. Gottschall, and A. Arimura. 1988. Identification of a high-affinity receptor for interleukin-1 beta in rat brain. Biochem. Biophys. Res. Commun. 156:61–67.

Kent, S., F. Rodriguez, K. Kelley, and R. Dantzer. 1994. Reduction in Food and Water Intake Induced by Microinjection of Interleukin-1β in the Ventromedial Hypothalamus of the Rat. Physiol. Behav. 56:1031–1036.

Khiaosa-ard, R., and Q. Zebeli. 2018. Diet-induced inflammation: From gut to metabolic organs and the consequences for the health and longevity of ruminants. Res. Vet. Sci. 120:17–27. doi:10.1016/j.rvsc.2018.08.005.

Kidane, A., J. Houdijk, S. Athanasiadou, B. Tolkamp, and I. Kyriazakis. 2010. Nutritional sensitivity of periparturient resistance to nematode parasites in two breeds of sheep with different nutrient demands. Br. J. Nutr. 104:1477–1486. doi:10.1017/S0007114510002503.

Kim, J.C., B.P. Mullan, B. Frey, H.G. Payne, and J.R. Pluske. 2012. Whole body protein deposition and plasma amino acid profiles in growing and/or finishing pigs fed increasing levels of sulfur amino acids with and without Escherichia coli lipopolysaccharide challenge. J. Anim. Sci 90:362–365. doi:10.2527/jas53821.

King, B.M. 2006. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. Physiol. Behav. 87:221–244. doi:10.1016/j.physbeh.2005.10.007.

Kiuger, M.J., and A.J. Vander. 1985. Suppression of food intake during infection: is interleukin-1 involved?. Am. J. Clin. Nutr. 42:1179–1182.

Klasing, K., and D. Korver. 1997. Leukocytic Cytokines Regulate Growth Rate and Composition Following Activation of the Immune System. J. Anim. Sci. 75:58–67. doi:10.2527/animalsci1997.75Supplement_258x.

Klasing, K.C., D.E. Laurin, R.K. Peng, and A.M. Fry. 1987. Immunologically Mediated Growth Depression in Chicks: Influence of Feed Intake, Corticosterone and Interleukin-1. J. Nutr. 117:1629–1637.

Kozak, W., V. Poli, D. Soszynski, C.A. Conn, L.R. Leon, and M.J. Kluger. 1997. Sickness behavior in mice deficient in interleukin-6 during turpentine abscess and influenza pneumonitis. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 272:R621–R630. doi:10.1152/ajpregu.1997.272.2.r621.

Kozak, W., H. Zheng, C.A. Conn, D. Soszynski, L.H.T. van der Ploeg, and M.J. Kluger. 1995. Thermal and behavioral effects of lipopolysaccharide and influenza in interleukin-. Am J Physiol Regul Integr Comp Physiol 269:R969–R977.

Kuhla, B. 2020. Review: Pro-inflammatory cytokines and hypothalamic inflammation: Implications for insufficient feed intake of transition dairy cows. Animal 14:S65–S77. doi:10.1017/S1751731119003124.

Kuriyama, K., T. Hori, T. Mori, and T. Nakashima. 1990. Actions of Interferonα and Interleukin-1β on the Glucose-Responsive Neurons in the Ventromedial Hypothalamus. Brain Res. Bull. 24:803–810.

Kushibiki, S., K. Hodate, H. Shingu, T. Hayashi, E. Touno, M. Shinoda, and Y. Yokomizo. 2002. Alterations in lipid metabolism induced by recombinant bovine tumor necrosis factor-alpha administration to dairy heifers. J. Anim. Sci. 80:2151–2157. doi:10.2527/2002.8082151x.

Kushibiki, S., K. Hodate, H. Shingu, Y. Obara, E. Touno, M. Shinoda, and Y. Yokomizo. 2003. Metabolic and Lactational Responses during Recombinant Bovine Tumor Necrosis Factor-α Treatment in Lactating Cows. J. Dairy Sci. 86:819–827. doi:10.3168/jds.S0022-0302(03)73664-9.

Kvidera, S.K., M.J. Dickson, M. Abuajamieh, D.B. Snider, M.V.S. Fernandez, J.S. Johnson, A.F. Keating, P.J. Gorden, H.B. Green, K.M. Schoenberg, and L.H. Baumgard. 2017. Intentionally induced intestinal barrier dysfunction causes inflammation, affects metabolism, and reduces productivity in lactating Holstein cows. J. Dairy Sci. 100:4113–4127. doi:10.3168/jds.2016-12349.

Kvidera, S.K., E.A. Horst, E.J. Mayorga, M.V. Sanz Fernandez, and L.H. Baumgard. 2016. Technical note: A procedure to estimate glucose requirements of an activated immune system in steers. J. Anim. Sci. 94:4591–4599. doi:10.2527/jas2016-0765.

Kyriazakis, I. 2010. Is anorexia during infection in animals affected by food composition?. Anim. Feed Sci. Technol. 156:1–9. doi:10.1016/j.anifeedsci.2010.01.001.

de La Serre, C.B., G. de Lartigue, and H.E. Raybould. 2015. Chronic exposure to Low dose bacterial lipopolysaccharide inhibits leptin signaling in vagal afferent neurons. Physiol. Behav. 139:188–194. doi:10.1016/j.physbeh.2014.10.032.

Lago, R., R. Gomez, F. Lago, J. Gomez-Reino, and O. Gualillo. 2008. Leptin beyond body weight regulation-Current concepts concerning its role in immune function and inflammation. Cell. Immunol. 252:139–145. doi:10.1016/j.cellimm.2007.09.004.

Lang, C.H., and C. Dobrescu. 1989. Interleukin-1 induced increases in glucose utilization are insulin mediated. Life Sci. 45:2127–2134.

Langstein, H.N., G.M. Doherty, D.L. Fraker, C.M. Buresh, and J.A. Norton. 1991. The Roles of γ-Interferon and Tumor Necrosis Factor α in an Experimental Rat Model of Cancer Cachexia. Cancer Res. 51:2302–2306.

Larson, S.J., and A.J. Dunn. 2001. Behavioral Effects of Cytokines. Brain. Behav. Immun. 15:371–387.

Laurenson, Y.C.S.M., S.C. Bishop, and I. Kyriazakis. 2011. In silico exploration of the mechanisms that underlie parasite-induced anorexia in sheep. Br. J. Nutr. 106:1023–1039. doi:10.1017/S0007114511001371.

Leblanc, S.J. 2012. Interactions of Metabolism, Inflammation, and Reproductive Tract Health in the Postpartum Period in Dairy Cattle. Reprod. Domest. Anim. 47:18–30. doi:10.1111/j.1439-0531.2012.02109.x.

Lee, K., and L.S. Reid. 1977. The Effect of Marek's Disease Vaccination and Day-Old Debeaking on the Performance of Growing Pullets and Laying Hens. Poult. Sci. 56:736–740.

Leininger, M.T., C.P. Portocareero, C.A. Bidwell, M.E. Spurlock, and K.L. Houseknecht. 2000. Leptin Expression Is Reduced with Acute Endotoxemia in the Pig: Correlation with Glucose, Insulin, and Insulin-like Growth Factor-1 (IGF-1). J. Interf. Cytokine Res. 20:99–106.

Lieberman, A.P., P.M. Pitha, H.S. Shin, and M.L. Shin. 1989. Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proc. Natl. Acad. Sci. USA1 86:6348–6352.

Ling, P.R., J.H. Schwartz, and B.R. Bistrian. 1997. Mechanisms of host wasting induced by administration of cytokines in rats. Am. J. Physiol. - Endocrinol. Metab. 272:E333–E339. doi:10.1152/ajpendo.1997.272.3.e333.

Ling, P.R., J.H. Schwartz, M. Jeevanandam, J. Gauldie, B.R. Bistrian, P. Ling, J.H. Ra, and J.-A. Schwartz. 1996. Metabolic changes in rats during a continuos infusion of recombinant interleukin-1. Am. J. Physiol. Endocrinol. Metab. 270:E305–E312.

Lippolis, K.D., R.F. Cooke, K.M. Schubach, R.S. Marques, and D.W. Bohnert. 2017. Effects of intravenous lipopolysaccharide administration on feed intake, ruminal forage degradability, and liquid parameters and physiological responses in beef cattle. J. Anim. Sci. 95:2859–2870. doi:10.2527/jas2017.1502.

Lochmiller, R.L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity?. OIKOS 88:87–98.

Lord, G.M., G. Matarese, J.K. Howard, R.J. Baker, S.R. Bloom, and R.I. Lechler. 1998. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. Nature 394:897–901.

Luheshi, G.N., J.D. Gardner, D.A. Rushforth, A.S. Loudon, and N.J. Rothwell. 1999. Leptin actions on food intake and body temperature are mediated by IL-1. Proc. Natl. Acad. Sci. 96:7047–7052. doi:10.1073/pnas.96.12.7047.

Mancuso, P., A. Gottschalk, S.M. Phare, M. Peters-Golden, N.W. Lukacs, and G.B. Huffnagle. 2002. Leptin-Deficient Mice Exhibit Impaired Host Defense in Gram-Negative Pneumonia. J. Immunol. 168:4018–4024. doi:10.4049/jimmunol.168.8.4018.

Martel, C.A., L.K. Mamedova, J.E. Minton, M.L. Jones, J.A. Carroll, and B.J. Bradford. 2014. Continuous low-dose infusion of tumor necrosis factor alpha in adipose tissue elevates adipose tissue interleukin 10 abundance and fails to alter metabolism in lactating dairy cows. J. Dairy Sci. 97:4897–4906. doi:10.3168/jds.2013-7777.

Martinez, N., R.M. Rodney, E. Block, L.L. Hernandez, C.D. Nelson, I.J. Lean, and J.E.P. Santos. 2018. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses. J. Dairy Sci. 101:2563–2578. doi:10.3168/jds.2017-13740.

Martinez, N., L.D.P. Sinedino, R.S. Bisinotto, E.S. Ribeiro, G.C. Gomes, F.S. Lima, L.F. Greco, C.A. Risco, K.N. Galvão, D. Taylor-Rodriguez, J.P. Driver, W.W. Thatcher, and J.E.P. Santos. 2014. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. J. Dairy Sci. 97:874–887. doi:10.3168/jds.2013-7408.

Mccarthy, D.O. 2000. Tumor Necrosis Factor Alpha and Interleukin-6 Have Differential Effects on Food Intake and Gastric Emptying in Fasted Rats. Res. Nurs. Health 23:222–228.

McCarthy, M., T. Yasui, M. Felippe, and T. Overton. 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J. Dairy Sci 99:680–700. doi:10.3168/jds.2015-9694.

McGuckin, M.M., S.L. Giesy, A.N. Davis, M.A. Abyeta, E.A. Horst, S.S. Samii, Y. Zang, W.R. Butler, L.H. Baumgard, J.W. Mcfadden, and Y.R. Boisclair. 2020. The acute phase protein orosomucoid 1 is upregulated in early lactation but does not trigger appetite-suppressing STAT3 signaling via the leptin receptor. J. Dairy Sci. 103:4765–4776. doi:10.3168/jds.2019-18094.

Medina, E.A., K.L. Erickson, K.L. Stanhope, and P.J. Havel. 2002. Evidence that tumor necrosis factor-alpha-induced hyperinsulinemia prevents decreases of circulating leptin during fasting in rats. Metabolism. 51:1104–1110. doi:10.1053/meta.2002.34699.

Medina, E.A., K.L. Stanhope, T.M. Mizuno, C. V Mobbs, F. Gregoire, N.E. Hubbard, K.L. Erickson, and P.J. Havel. 1999. Effects of tumor necrosis factor alpha on leptin secretion and gene expression: relationship to changes of glucose metabolism in isolated rat adipocytes. Int. J. Obes. 23:896–903.

Van Der Meer, M.J.M., C.G.J. Fred Sweep, G.J. Pesman, G.F. Borm, and A.R.M.M. Hermus. 1995. Synergism between IL-1β and TNF-α on the activity of the pituitary- adrenal axis and on food intake of rats. Am. J. Physiol. - Endocrinol. Metab. 268:E551–E557. doi:10.1152/ajpendo.1995.268.4.e551.

van Miert, A.S.J.P.A.M. 1987. Fever, anorexia and forestomach hypomotility in ruminants. Vet. Res. Commun. 11:407–422.

van Miert, A.S.J.P.A.M., C.T.M. van Duin, and T. Wensing. 1992a. Fever and acute phase response induced in dwarf goats by endotoxin and bovine and human recombinant tumour necrosis factor alpha. J. Vet Pharmacol. Ther. 15:332–342.

van Miert, A.S.J.P.A.M., F. Kaya, and C.T.M. van Duin. 1992b. Changes in Food Intake and Forestomach Motility of Dwarf Goats by Recombinant Bovine Cytokines (IL-1β, IL-2) and IFN-γ. Physiol. Behav. 52:859–864.

Miller, A.J., S.J. Hopkins, and G.N. Luheshi. 1997. Sites of action of IL-1 in the development of fever and cytokine responses to tissue in Inflammation in the rat. Br. J. Pharmacol. 120:1274–1279.

Montuschi, P., P. Preziosi, and P. Navarra. 1993. Interleukin-1α and tumor necrosis factor inhibit rat gastric fundus motility in vitro. Eur. J. Pharmacol. 233:303–304.

Morrison, C.D., J.A. Daniel, B.J. Holmberg, J. Djiane, N. Raver, A. Gertler, and D.H. Keilser. 2001. Central infusion of leptin into well-fed and undernourished ewe lambs: Effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinol. 168:317–324. doi:10.1677/joe.0.1680317.

Morrison, C.D., R. Wood, E.L. Mcfadin, N.C. Whitley, and D.H. Keisler. 2002. Effect of intravenous infusion of recombinant ovine leptin on feed intake and serum concentrations of GH, LH, insulin, IGF-1, cortisol, and thyroxine in growing prepubertal ewe lambs. Domest.

Anim. Endocrinol. 22:103–112.

Murray, M.J., and A.B. Murray. 1979. Anorexia of infection as a mechanism of host defense. Am. J. Clin. Nutr. 593–596.

Negri, D.R.M., D. Mezzanzanica, S. SAcco, M. Gadina, F. Benigni, L. Cajola, G. Finocchiaro, P. Ghezzi, and S. Canevari. 2001. Role of cytokines in cancer cachexia in a murine model of intracerebral injection of human tumours. Cytokine 15:27–38.

Oomura, Y. 1988. Chemical and Neuronal Control of Feeding Motivation. Physiol. Behav. 44:555–560.

Opal, S.M., and V.A. DePalo. 2000. AntiInflammatory Cytokines. Chest 117:1162–1172.

Pascottini, O.B., S.J. Van Schyndel, J.F.W. Spricigo, M.R. Carvalho, B. Mion, E.S. Ribeiro, and S.J. LeBlanc. 2020. Effect of anti-inflammatory treatment on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. Sci. Rep. 10:1–9. doi:10.1038/s41598-020-62103-x.

Patton, J.S., P.M. Peters, J. Mccabe, D. Crase, S. Hansen, A.B. Chen, and D. Liggitt. 1987. Development of Partial Tolerance to the Gastrointestinal Effects of High Doses of Recombinant Tumor Necrosis Factor-a in Rodents. J. Clin. Invest. 80:1587–1596.

Perry, A.K., G. Chen, D. Zheng, H. Tang, and G. Cheng. 2005. The host type I interferon response to viral and bacterial infections. Cell Res. 15:407–422. doi:10.1038/sj.cr.7290309.

Pfeffer, A., K.M. Rogers, L. O'Keeffe, and P.J. Osborn. 1993. Acute phase protein response, food intake, liveweight change and lesions following intrathoracic injection of yeast in sheep. Res. Vet. Sci. 55:360–366. doi:10.1016/0034-5288(93)90108-R.

Plaizier, J.C., E. Khafipour, S. Li, G.N. Gozho, and D.O. Krause. 2012. Subacute ruminal acidosis (SARA), endotoxins and health consequences. Anim. Feed Sci. Technol. 172:9–21. doi:10.1016/j.anifeedsci.2011.12.004.

Plaizier, J.C., D.O. Krause, G.N. Gozho, and B.W. Mcbride. 2009. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. Vet. J. 176:21–31. doi:10.1016/j.tvjl.2007.12.016.

Plata-Salamán, C.R. 1991a. Dexamethasone Inhibits Food Intake Suppression Induced by Low Doses of Interleukin-1β Adminsitered Intracerebroventricularly. Brain Res. Bull. 27:737–738.

Plata-Salamán, C.R. 1991b. Immunoregulators in the Nervous System. Neurosci. Biobehav. Rev. 15:185–215.

Plata-Salamán, C.R. 1992. Interferons and central regulation of feeding. Am. J. Physiol. - Regul.

Integr. Comp. Physiol. 263:R1222–R1227.

Plata-Salamán, C.R. 1994. Meal Patterns in Response to the Intracerebroventricular Administration of Interleukin-1β in Rats. Physiol. Behav. 55:727–733.

Plata-Salamán, C.R. 1996a. Anorexia During Acute and Chronic Disease. Nutrition 12:69–78.

Plata-Salamán, C.R. 1996b. Anorexia induced by activators of the signal transducer gp 130. Neuroreport 7:841–844.

Plata-Salamán, C.R., and J.P. Borkoski. 1994. Chemokines/intercrines and central regulation of feeding. Am. J. Physiol. 35:R1711–R1715.

Plata-Salamán, C.R., Y. Oomura, and Y. Kai. 1988. Tumor necrosis factor and interluekin-1β: suppression of food intake by direct action in the central nervous system. Brain Res. 448:106–114.

Plata-Salamán, C.R., J.R. Vasselli, and G. Sonti. 1997. Differentail responsiveness of obese (fa/fa) and lean (Fa/Fa) zucker rats to cytokine-induced anorexia. Obes. Res. 5:36–42. doi:10.1002/j.1550-8528.1997.tb00281.x.

Plaza, M.A., J. Fioramonti, and L. Bueno. 1997. Role of Central Interleukin-1β in Gastrointestinal Motor Disturbances Induced by Lipopolysaccharide in Sheep. Dig. Dis. Sci. 42:242–250.

Porter, M.H., B.J. Hrupka, G. Altreuther, M. Arnold, and W. Langhans. 2000. Inhibition of TNF-α production contributes to the attenuation of LPS-induced hypophagia by pentoxifylline. Am J Physiol Regul Integr Comp Physiol 279:R2113–R2120.

Pyörälä, S. 2008. Mastitis in Post-Partum Dairy Cows. Reprod. Domest. Anim. 43:252–259. doi:10.1111/j.1439-0531.2008.01170.x.

Qu, Y., A.N. Fadden, M.G. Traber, and G. Bobe. 2014. Potential risk indicators of retained placenta and other diseases in multiparous cows. J. Dairy Sci. 97:4151–4165. doi:10.3168/jds.2013-7154.

Robert, A., A.S. Olafsson, C. Lancaster, and W. Zhang. 1991. Interleukin-1 is cytoprotective, antisecretory, stimulates PGE2 synthesis by the stomach, and retards gastric emptying. Life Sci. 48:123–134.

Roche, J.R., D. Blache, J.K. Kay, D.R. Miller, A.J. Sheahan, and D.W. Miller. 2008. Neuroendocrine and physiological regulation of intake with particular reference to domesticated ruminant animals. Nutr. Res. Rev. 21:207–234. doi:10.1017/S0954422408138744.

Rodrigues, M.C., R.F. Cooke, R.S. Marques, B.I. Cappellozz, S.A. Arispe, D.H. Keisler, and

D.W. Bohnert. 2015. Effects of vaccination against respiratory pathogens on feed intake, metabolic, and inflammatory responses in beef heifers. J. Anim. Sci. 93:4443–4452. doi:10.2527/jas2015-9277.

Romanatto, T., M. Cesquini, M.E. Amaral, E. Roman, J.C. Moraes, M.A. Torsoni, A.P. Curz-Neto, and L.A. Velloso. 2007. TNF- a acts in the hypothalamus inhibiting food intake and increasing the respiratory quotient — Effects on leptin and insulin signaling pathways. Peptides 28:1050–1058. doi:10.1016/j.peptides.2007.03.006.

Sachot, C., S. Poole, and G.N. Luheshi. 2004. Circulating leptin mediates lipopolysaccharide-induced anorexia and fever in rats. J. Physiol. 561:263–272. doi:10.1113/jphysiol.2004.074351.

Sandberg, F.B., G.C. Emmans, and I. Kyriazakis. 2006. A model for predicting feed intake of growing animals during exposure to pathogens. J. Anim. Sci. 84:1552–1566. doi:10.2527/2006.8461552x.

Sandberg, F.B., G.C. Emmans, and I. Kyriazakis. 2007. The effects of pathogen challenges on the performance of naive and immune animals: the problem of prediction. Animal 1:67–86. doi:10.1017/S175173110765784X.

Sarraf, P., R.C. Frederich, E.M. Turner, G. Ma, N.T. Jaskowiak, D.J. Rivet, J.S. Flier, B.B. Lowell, D.L. Fraker, and H.R. Alexander. 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: Potential role in inflammatory anorexia. J. Exp. Med. 185:171–175. doi:10.1084/jem.185.1.171.

Schirmann, K., D.M. Weary, W. Heuwieser, N. Chapinal, R.L.A. Cerri, and M.A.G. von Keyserlingk. 2016. Short communication: Rumination and feeding behaviors differ between healthy and sick dairy cows during the transition period. J. Dairy Sci. 99:9917–9924. doi:10.3168/jds.2015-10548.

Scrimshaw, N.S. 1991. Effect of Infection on Nutrient Requirements. J. Parenter. Enter. Nutr. 15:589–600. doi:10.1177/0148607191015006589.

Segall, M.A., and L.S. Crnic. 1990. An Animal Model for the Behavioral Effects of Interferon. Behav. Neurosci. 104:612–618. doi:10.1037/0735-7044.104.4.612.

Shwartz, G., K.L. Hill, M.J. VanBaale, and L.H. Baumgard. 2009. Effects of flunixin meglumine on pyrexia and bioenergetic variables in postparturient dairy cows. J. Dairy Sci. 92:1963–1970. doi:10.3168/jds.2008-1581.

Soliman, M., S. Abdelhady, I. Fattouh, K. Ishioka, H. Kitamura, K. Kimura, and M. Saito. 2001. No Alteration in Serum Leptin Levels during Acute Endotoxemia in Sheep. J. Vet. Med. Sci. 63:1143–1145. doi:10.1292/jvms.63.1143.

Sonti, G., S.E. Ilyin, and C.R. Plata-Salamán. 1996. Anorexia induced by cytokine interactions at

pathophysiological concentrations. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 270:R1394–R1402. doi:10.1152/ajpregu.1996.270.6.r1394.

Sordillo, L., G.M. Pighetti, and M.R. Davis. 1995. Enhanced production of bovine tumor necrosis factor-α during the periparturient period. Vet. Immunol. Immunopathol. 49:263–270.

Sordillo, L.M., G.A. Contreras, and S.L. Aitken. 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Anim. Heal. Res. Rev. 10:53–63. doi:10.1017/S1466252309990016.

Spurlock, M.E., G.R. Frank, G.M. Willis, J.L. Kuske, and S.G. Cornelius. 1997. Effect of Dietary Energy Source and Immunological Challenge on Growth Performance and Immunological Variables in Growing Pigs 1. J. Anim. Sci. 75:720–726.

Steffen, B.T., S.J. Lees, and F.W. Booth. 2008. Anti-TNF treatment reduces rat skeletal muscle wasting in monocrotaline-induced cardiac cachexia. J. Appl. Physiol. 105:1950–1958. doi:10.1152/japplphysiol.90884.2008.

Sun, Y., Y. Yang, Z. Qin, J. Cai, X. Guo, Y. Tang, J. Wan, D.F. Su, and X. Liu. 2016. The acute-phase protein orosomucoid regulates food intake and energy homeostasis via leptin receptor signaling pathway. Diabetes 65:1630–1641. doi:10.2337/db15-1193.

Swiergiel, A.H., and A.J. Dunn. 1999. The Roles of IL-1, IL-6, and TNFα in the Feeding Responses to Endotoxin and Influenza Virus Infection in Mice. Brain. Behav. Immun. 13:252–265.

Swiergiel, A.H., G.N. Smagin, A.J. Dunn, A.H. Swiergiel, G.N. Smagin, and A.J. Dunn. 1997. Influenza Virus Infection of Mice Induces Anorexia: Comparison with Endotoxin and Interleukin-1 and the Effects of Indomethacin. Pharmacol. Biochem. Behav. 57:389–396.

Tachibana, T., T. Kodama, S. Yamane, R. Makino, S.I. Khan, and M.A. Cline. 2017. Possible role of central interleukins on the anorexigenic effect of lipopolysaccharide in chicks. Br. Poult. Sci. 58:305–311. doi:10.1080/00071668.2017.1280774.

Takiya, C.S., S.R. Montgomery, L.K. Mamedova, G. Kra, N. Nemes-Navon, Y. Levin, S.D. Fleming, B.J. Bradford, and M. Zachut. 2019. Proteomic analysis reveals greater abundance of complement and inflammatory proteins in subcutaneous adipose tissue from postpartum cows treated with sodium salicylate. J. Proteomics 204:103399. doi:10.1016/j.jprot.2019.103399.

Teeling, J.L., L.M. Felton, R.M.J. Deacon, C. Cunningham, J.N.P. Rawlins, and V.H. Perry. 2007. Sub-pyrogenic systemic in X ammation impacts on brain and behavior , independent of cytokines. Brain. Behav. Immun. 21:836–850. doi:10.1016/j.bbi.2007.01.012.

Threlkeld, S.W., J.L. Lynch, K.M. Lynch, G.B. Sadowska, W.A. Banks, and B.S. Stonestreet.

2010. Ovine proinflammatory cytokines cross the murine blood-brain barrier by a common saturable transport mechanism. Neuroimmunomodulation 17:405–410. doi:10.1159/000288265.

Torelli, G.F., M.M. Meguid, L.L. Moldawer, C.K. Edwards, H.J. Kim, J.L. Carter, A. Laviano, and F.R. Fanelli. 1999. Use of recombinant human soluble TNF receptor in anorectic tumor-bearing rats. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 277:850–855. doi:10.1152/ajpregu.1999.277.3.r850.

Tracey, K.J., H. Wei, K.R. Manogue, Y. Fong, D.G. Hesse, H.T. Nguyen, G.C. Kuo, B. Beutler, R.S. Cotran, A. Cerami, and S.F. Lowry. 1988. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. J. Exp. Med. 167:1211–1227.

Trevisi, E., N. Jahan, G. Bertoni, A. Ferrari, and A. Minuti. 2015. Pro-inflammatory cytokine profile in dairy cows: Consequences for new lactation. Ital. J. Anim. Sci. 14:285–292. doi:10.4081/ijas.2015.3862.

Trevisi, E., N. Jahan, G. Bertoni, A. Ferrari, and A. Minuti. 2016. Pro-Inflammatory Cytokine Profile in Dairy Cows: Consequences for New Lactation Pro-inflammatory cytokine profile in dairy cows: consequences for new lactation. Ital. J. Anim. Sci.. doi:10.4081/ijas.2015.3862.

Tsigos, C., D.A. Papanicolaou, C.S. Mitsiadis, I. Kyrou, and G.P. Chrousos. 1997. Dose effects of recombinant human interleukin-6 on pituitary hormone secretion and energy expenditure. Neuroendocrinology 66:54–62.

Tsuchiya, Y., T. Nozu, S. Kumei, M. Ohhira, and T. Okumura. 2012. IL-1 Receptor Antagonist Blocks the Lipopolysaccharide-Induced Inhibition of Gastric Motility in Freely Moving Conscious Rats. Dig Dis Sci. 57:2555–2561. doi:10.1007/s10620-012-2210-8.

Turrin, N.P., M.C. Flynn, and C.R. Plata-Salamán. 1998. Neuropeptide Y Counteracts Interferon-Alpha-Induced Anorexia. Neuroimmunomodulation 6:361–366.

Uehara, A., T. Okumura, K. Okamura, Y. Takasugi, and M. Namiki. 1990. Lipopolysaccharide-induced inhibition of gastric acid and pepsin secretion in rats. Eur. J. Pharmacol. 181:141–145. doi:10.1016/0014-2999(90)90256-6.

Uehara, A., C. Sekiya, Y. Takasugi, M. Namiki, and A. Arimura. 1989. Anorexia induced by interleukin involvement of corticotropin-releasing. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 257:R613–R617.

Vels, L., C.M. Røntved, M. Bjerring, and K.L. Ingvartsen. 2009. Cytokine and acute phase protein gene expression in repeated liver biopsies of dairy cows with a lipopolysaccharide-induced mastitis. J. Dairy Sci. 92:922–934. doi:10.3168/jds.2008-1209.

Waggoner, J.W., C.A. Löest, C.P. Mathis, D.M. Hallford, and M.K. Petersen. 2009a. Effects of

rumen-protected methionine supplementation and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. J. Anim. Sci. 87:681–692. doi:10.2527/jas.2008-1068.

Waggoner, J.W., C.A. Löest, J.L. Turner, C.P. Mathis, and D.M. Hallford. 2009b. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. J. Anim. Sci. 87:3656–3668. doi:10.2527/jas.2009-2011.

Waldron, M.R., T. Nishida, B.J. Nonnecke, and T.R. Overton. 2003a. Effect of lipopolysaccharide on indices of peripheral and hepatic metabolism in lactating cows. J. Dairy Sci. 86:3447–3459. doi:10.3168/jds.S0022-0302(03)73949-6.

Waldron, M.R., B.J. Nonnecke, T. Nishida, R.L. Horst, and T.R. Overton. 2003b. Effect of Lipopolysaccharide Infusion on Serum Macromineral and Vitamin D Concentrations in Dairy Cows 1. J. Dairy Sci 86:3440–3446. doi:10.3168/jds.S0022-0302(03)73948-4.

Wang, A., S.C. Huen, H.H. Luan, S. Yu, C. Zhang, J.D. Gallezot, C.J. Booth, and R. Medzhitov. 2016. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. Cell 166:1512-1525.e12. doi:10.1016/j.cell.2016.07.026.

Wang, Z., D. Liu, F. Wang, S. Liu, S. Zhao, E.A. Ling, and A. Hao. 2012. Saturated fatty acids activate microglia via Toll-like receptor 4/NF-κB signalling. Br. J. Nutr. 107:229–241. doi:10.1017/S0007114511002868.

Warren, E.J., B.N. Finck, S. Arkins, K.W. Kelley, R.W. Scamurra, M.P. Murtaugh, and R.W. Johnson. 1997. Coincidental changes in behavior and plasma cortisol in unrestrained pigs after intracerebroventricular injection of tumor necrosis factor-α. Endocrinology 138:2365–2371. doi:10.1210/endo.138.6.5180.

Williams, N.H., T.S. Stahly, and D.R. Zimmerman. 1997a. Effect of Level of Chronic Immune System Activation on the Growth and Dietary Lysine Needs of Pigs Fed from 6 to 112 kg. J. Anim. Sci. 75:2481–2496.

Williams, N.H., T.S. Stahly, and D.R. Zimmerman. 1997b. Effect of Chronic Immune System Activation on the Rate, Efficiency, and Composition of Growth and Lysine Needs of Pigs Fed from 6 to 27 kg. J. Anim. Sci 75:2463–2471.

Wing, E.J., and J.B. Young. 1980. Acute Starvation Protects Mice Against Listeria monocytogenes. Infect. Immun. 28:771–776.

Wong, S., and J. Pinkney. 2004. Role of Cytokines in Regulating Feeding Behaviour. Curr. Drug Targets 5:251–263.

Wostmann, B.S., C. Larkin, A. Moriarty, and E. Bruckner-Kardoss. 1983. Dietary Intake, Energy Metabolism, and Ecretory Losses of Adult Male Germfree Wistar Rats. Lab. Anim. Sci.

33:46–50.

Yamaguchi, M., T. Murakami, T. Tomimatsu, Y. Nishio, N. Misuda, T. Kanzaki, H. Kurachi, K. Hima, T. Aono, and Y. Murata. 1998. Autocrine Inhibition of Leptin Production by Tumor Necrosis Factor-α (TNF-α) through TNF-α Type-I Receptor in Vitro. Biochem. Biophys. Res. Commun. 244:30–34.

Yang, Z.-J., M. Koseki, M.M. Meguid, J.R. Gleason, and D. Debonis. 1994. Synergistic effect of rhTNF-α and rhIL-1α in inducing anorexia in rats. Am. J. Physiol. - Regul. Integr. Comp. Physiol. R1056–R1064.

Yeiser, E.E., K.E. Leslie, M.L. McGilliard, and C.S. Petersson-Wolfe. 2012. The effects of experimentally induced Escherichia coli mastitis and flunixin meglumine administration on activity measures, feed intake, and milk parameters. J. Dairy Sci. 95:4939–4949. doi:10.3168/jds.2011-5064.

Yuan, K., J.K. Farney, L.K. Mamedova, L.M. Sordillo, and B.J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8. doi:10.1371/journal.pone.0080316.

Yuan, K., L.G.D. Mendonça, L.E. Hulbert, L.K. Mamedova, M.B. Muckey, Y. Shen, C.C. Elrod, and B.J. Bradford. 2015. Yeast product supplementation modulated humoral and mucosal immunity and uterine inflammatory signals in transition dairy cows. J. Dairy Sci. 98:3236–3246. doi:10.3168/jds.2014-8469.

Zebeli, Q., K. Ghareeb, E. Humer, B.U. Metzler-Zebeli, and U. Besenfelder. 2015. Nutrition, rumen health and inflammation in the transition period and their role on overall health and fertility in dairy cows. Res. Vet. Sci. 103:126–136. doi:10.1016/j.rvsc.2015.09.020.

Zebeli, Q., S. Sivaraman, S.M. Dunn, and B.N. Ametaj. 2011. Intermittent parenteral administration of endotoxin triggers metabolic and immunological alterations typically associated with displaced abomasum and retained placenta in periparturient dairy cows. J. Dairy Sci. 94:4968–4983. doi:10.3168/jds.2011-4194.

Zhang, H.H., S. Kumar, A.H. Barnett, and M.C. Eggo. 2000. Tumour necrosis factor-α exerts dual effects on human adipose leptin synthesis and release. Mol. Cell. Endocrinol. 159:79–88.

Zhou, Y., and L. Rui. 2013. Leptin Signaling and Leptin Resistance. Front. Med. 7:207–222. doi:10.1007/s11684-013-0263-5.

Chapter 2 - Acute-phase protein alpha-1-acid glycoprotein is negatively associated with feed intake in postpartum dairy cows

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ABSTRACT

Alpha-1-acid glycoprotein (AGP) is an acute-phase protein that may suppress dry matter intake (DMI), potentially by acting on the leptin receptor in the hypothalamus. Our objective was to characterize plasma AGP concentration and associations with DMI during the transition period, and to determine the utility of AGP to identify or predict cows with low DMI. Plasma samples (n = 2,086) from 434 Holstein cows in 6 studies were analyzed on d -21, -13 ± 2, -3, 1, 3, 7 ± 1, 14 ± 1, and 21 ±1 relative to parturition. A commercially available ELISA kit specific for bovine AGP was validated, and two internal controls were analyzed on each plate with inter-plate variation of 15.0 and 17.3%, respectively. Bivariate analysis was used to assess the relationship between AGP and DMI. For significant associations, treatment(study) was added to the model and quadratic associations were included in the model, if significant. Plasma AGP concentration (± SEM) increased from 213 ± 37.3 μg/mL on d -3 to 445 ± 60.0 μg/mL on d 14. On d 3, AGP was associated negatively with DMI in a quadratic manner for wk 1 and wk 2 and linearly for wk 3. Day 7 AGP was associated negatively with DMI in a quadratic manner for wk 2 and linearly for wk 3. Similarly, d 14 AGP was negatively associated with DMI for wk 3 and wk 4. As d 3 AGP concentration increased over the interquartile range, there was a calculated 1.4 (8.5%), 0.5 (2.7%) and 0.4 (1.9%) kg/d reduction in predicted DMI during wk 1, 2, and 3, respectively. Using bivariate analysis, d 3 AGP explained 10.0% of the variation in DMI during wk 1. We explored the clinical utility of d 3 AGP to diagnose low DMI, defined as wk 1 DMI more than 1 standard deviation below the mean. Receiver operating characteristic analysis identified a threshold of 480.9 μg/mL providing 76% specificity and 48% sensitivity (AUC = 0.60). There were limited associations between AGP and blood biomarkers; however, AGP was associated with plasma haptoglobin concentration postpartum and incidence of displaced abomasum,

retained placenta and metritis. These results demonstrate a negative association between plasma AGP concentration and DMI in early postpartum dairy cows, although its diagnostic performance was marginal. Further investigation into whether AGP directly suppresses DMI in dairy cattle is warranted.

INTRODUCTION

Dairy cows transitioning to lactation experience systemic inflammation, with chronic sub-acute inflammation appearing to lead to long-term production losses (Bradford et al., 2015). Inflammation is the biological mechanism whereby the body copes with pathogenic or non-pathogenic stimuli to resolve an issue and return to homeostasis. Cytokine and acute phase protein production are hallmarks of inflammation, with cytokine production by immune cells markedly increasing following tissue insult, further leading to production of acute-phase proteins. These compounds, as indicators of inflammatory status, have consistently been linked to poor performance in dairy cows (Humblet et al., 2006; McCarthy et al., 2016), and there has been extensive evaluation of acute phase proteins as biomarkers of various diseases in ruminants (Ceciliani et al., 2012). Additionally, cytokines have been directly implicated in reducing feed intake through action on the hypothalamus (Wong and Pinkney, 2004), linking the immune response as an additional pathway for feed intake regulation beyond the traditional physical, endocrine and metabolic homeostatic factors eloquently outlined by Allen and Piantoni (2013).

Orosomucoid, or alpha-1-acid glycoprotein (**AGP**), is a positive acute-phase protein produced primarily in the liver (Hochepied et al., 2003). The role of AGP is not entirely clear, but it is a common lipocalin that also has immunomodulatory effects, working mostly in an anti-inflammatory manner (Ceciliani and Lecchi, 2019; Fournier et al., 2000). Two reports demonstrated compelling evidence that AGP works directly to reduce feed intake in rats after intracerebroventricular infusion (Bellinger and Mendel, 1990), intraperitoneal injection, or peripheral administration (Sun et al., 2016). More mechanistically, AGP was found to bind the hypothalamic leptin receptor in mice, which in turn activates the downstream JAK2-STAT3 pathway to relay satiety signals (Sun et al., 2016). The hypothalamus is an important structure in

the regulation of energy intake (Schwartz et al., 2000). However, very recent reports demonstrate a lack of effect of AGP on STAT3 signaling and feed intake in ruminants (Gregg et al., 2019; McGuckin et al., 2020).

Considering that plasma AGP concentration increases in postpartum dairy cows in a pattern similar to other acute phase proteins (Cairoli et al., 2006; Jafari et al., 2006) and peripartum cows often exhibit depressed feed intake, we hypothesized that plasma AGP concentration is negatively related to dry matter intake (**DMI**) during the transition period. The objective of this study was 1) to assess peripartum AGP concentration using a large sample size, 2) to determine the relationship between AGP and DMI in dairy cows during the transition period, 3) to assess the prognostic value of AGP relative to other biomarkers to predict cows at risk of low DMI, and 4) to access the prognostic value of AGP to predict postpartum disease.

MATERIALS AND METHODS

Animals and samples

Blood plasma samples (n = 2,086) from 434 Holstein cows were obtained from six previously-conducted studies conducted since 2014 at three institutions (Cornell University, University of Florida and Kansas State University; Leno et al., 2017a; b; Carpenter et al., 2018; Zenobi et al., 2018; Olagaray et al., 2019; Bollatti et al., 2020). All samples were obtained ± 21 d relative to parturition with specific sample days for each study outlined in Table 2.1. Individual daily feed intake data was available from all cows included in analysis. Detailed descriptions of study methodology, including analysis of other blood biomarkers, were previously published for each of these studies. Samples were analyzed in duplicate.

Sample Analysis

Initial attempts at quantifying AGP involved validating a previously reported ELISA procedure (Blees et al., 2017) modified to use a commercially-available purified bovine AGP (lot # 111M7401V; Sigma-Aldrich, St. Louis, MO) dissolved in distilled, deionized water for the standard curve to avoid non-specific binding. Lack of detectability of the purified bovine AGP prompted evaluation of commercial ELISA kits (MyBioSource, Inc., San Diego, CA; ICL, Inc., Portland, OR) to determine if the commercially purified AGP could be detected, and to potentially validate the kits for large-scale AGP quantification. The purified bovine AGP was not detectable on either kit, necessitating the use of commercial kit standards.

Samples for the complete analysis were analyzed using a bovine-specific ELISA kit (ICL Inc., Portland, OR) according to manufacturer specifications at a dilution of 1:20,000. The kit utilizes a lyophilized purified bovine AGP from serum as a standard curve. A robotic multichannel pipetting system was utilized to achieve the 4-step dilution (Integra Viaflo Assist, Integra Biosciences Corp., Hudson, NH) using the diluent buffer provided in the kit. Assay performance was assessed using spike and recovery and linearity of dilution techniques. Recovery of AGP was determined by spiking a known quantity of the standard into diluted sample (Table 2.2). A linearity of dilution experiment was conducted to test the behavior of the samples at various concentrations in the range of the standard curve by serially diluting plasma in the assay buffer (Table 2.3). According to the results in comparison with the linear portion of the standard curve, plasma samples were diluted 1:20,000 to achieve approximately 20 ng/mL final concentration. Two internal control samples were analyzed in duplicate on each plate, with intra-assay CV of 3.8 and 3.0% and inter-assay CV of 15.0 and 17.3%, respectively, for each sample.

Statistical Analysis

Plasma AGP mean concentration on each sampling date was obtained by using repeated measures in the Mixed Procedure of SAS (version 9.4; SAS Institute, Cary, NC), with the model including the random effect of study. Data were $\log_{10}$ transformed to achieve normality and reverse transformed to obtain means. Bivariate linear regression, and subsequently linear and quadratic regression, was used to assess the relationship between AGP and DMI in subsequent weeks using JMP (version 13.0, SAS Institute, Cary, NC). DMI was averaged over a three-day period following each blood sampling timepoint prepartum (d -21, -12 ± 3, and -3), except for blood sample timepoint d -21 for which DMI was averaged over d -15 to -13. For postpartum data, DMI was averaged over a one-week period for each week postpartum. Days used to calculate DMI averages for each week relative to parturition are outlined in Table 2.4. For significant bivariate associations, the following linear and quadratic model was utilized to further analyze the association between plasma AGP concentration and DMI:

$$Y_{ij} = \mu + A + A*A + T_i + e_{ij}$$

where μ is the overall treatment mean, A is the linear effect of plasma AGP concentration, A*A is the quadratic effect of plasma AGP concentration, T_i is the random effect of unique treatment within each study (i = 1 to 19), and e_{ij} is the residual error (j = 1 to 30). Analysis of the association between plasma AGP concentration and other blood biomarkers concentration was conducted with the same model. Quadratic effects were removed from the model when $P > 0.10$.

Residuals were analyzed for normality and data were natural log transformed when appropriate. Studentized residuals were evaluated, and outliers > 3.5 or < -3.5 were excluded from analysis. Significance was declared at $P < 0.05$, and tendencies when $P < 0.10$.

Nominal logistic modeling was used to determine the association between plasma AGP concentration on a given day and incidence of postpartum disease using the fixed effect of AGP and the random effect of study. From this information, we used receiver operating characteristic (ROC) analysis using JMP (Version 13.0; SAS Institute, Cary, NC) to assess the ability of AGP to predict disease incidence for diseases presenting sufficient sample size (sensitivity and specificity = 0.70; marginal error = 0.20; $\alpha = 0.05$; Hajian-Tilaki, 2014; Bujang and Adnan, 2016) based upon previously reported rates of disease and culling (Berge and Vertenten, 2014; McLaren et al., 2006; Smith et al., 2000).

We also carried out ROC analysis to determine the utility of d 3 AGP for detecting cows with low feed intake. Day 3 AGP concentration was utilized because the association between AGP and DMI was most significant early in the postpartum period. Low feed intake was defined as 1 standard deviation less than the mean wk 1 DMI. For all ROC analyses we determined the threshold AGP concentration offering the optimal balance between sensitivity and specificity, determined based on the concentration offering the greatest sum of calculated sensitivity + specificity.

A proportional hazards regression model was constructed using the fixed effects of AGP and study to determine the effects of AGP concentration on culling by 280 DIM. Furthermore, the concentration of AGP was classified as being above or below the threshold determined from ROC analysis to construct survival curves assessing the association of peripartum AGP with culling by 280 DIM. 280 d was chosen because, for two of the studies (Bollatti et al., 2020; Zenobi et al., 2018) representing half of the data, information was only available to 280 DIM, limiting the ability to evaluate the full lactation on all animals.

RESULTS

Prepartum AGP concentrations were relatively stable for d -21 (248 ± 35.0 µg/mL), d -13 (223 ± 30.2 µg/mL), d -3 (212 ± 30.2 µg/mL) and d 1 (273 ± 37.4 µg/mL; Figure 2.1). Plasma AGP concentration increased beginning on d 3 (377 ± 51.0 µg /mL) before numerically increasing to a peak on d 14 (445 ± 59.0 µg/mL) and declining numerically to d 21 (387 ± 52.2 µg/mL). Plasma concentrations of AGP on d 3 to d 21 were significantly greater than AGP on any day from d -21 to d 1 ($P < 0.001$). Results for associations between AGP and DMI are presented in Table 2.4. All associations between the variables were negative over the range of observed values (accounting for the quadratic nature of some relationships). Day -21 AGP was negatively related in a quadratic manner with wk -2 DMI ($P = 0.07$). On d 3, AGP was negatively associated with DMI on wk 1 and wk 2 in a quadratic fashion ($P = 0.01$; $P = 0.03$) and was negatively associated in a linear manner with wk 3 DMI ($P < 0.001$). AGP concentration on d 7 had a negative quadratic association with wk 2 DMI ($P < 0.01$), and was negatively associated with wk 3 DMI ($P < 0.001$). Day 14 AGP concentration was negatively associated with DMI in a linear manner for both wk 3 and wk 4 ($P < 0.001$). AGP concentration on d 21 was negatively associated with DMI in wk 4 ($P = 0.001$).

Using the intercepts and parameter estimates shown in Table 2.4, predicted DMI changes over the interquartile range of d 3 AGP were calculated. As d 3 AGP concentration increased over the interquartile range, there was a calculated 1.4 (8.5%), 0.5 (2.7%) and 0.4 (1.9%) kg/d reduction in predicted DMI during wk 1, 2, and 3, respectively (Figure 2.2A). Using bivariate analysis, d 3 AGP explained 10.0% of the variation in DMI during wk 1 (Figure 2.2B). Receiver operating characteristic analysis identified a threshold of 480.9 µg/mL providing 76% specificity and 48% sensitivity (Figure 2.3; AUC = 0.60) in detecting low feed intake.

Results for associations between AGP and other blood plasma biomarkers can be found in the supplementary file. Plasma AGP concentration on d 1 tended to be positively associated with plasma glucose concentration on d 7 ($P = 0.06$) and d 14 ($P = 0.07$). On d -3, plasma AGP was positively associated with d 21 free fatty acids (FFA; $P = 0.04$) and tended to be positively associated with d 14 FFA ($P = 0.08$), whereas d 7 AGP tended to be negatively associated with d 14 FFA ($P = 0.07$). Plasma AGP on d -13 was negatively associated with insulin concentration at the same time point ($P = 0.04$). The strongest associations were between AGP and beta-hydroxybutyrate (**BHB**) and haptoglobin. On d -13, AGP was negatively associated with BHB ($P = 0.02$), while d 7 AGP concentration was positively associated with BHB on d 7 ($P < 0.01$; Table 2.5). The concentrations of AGP and haptoglobin were positively associated on d 3, 7, and 14 ($P \leq 0.03$; Table 2.5), and d 7 AGP was also associated with d 14 haptoglobin ($P < 0.01$; Table 2.5).

There were strong associations between postpartum AGP concentration and transition cow diseases. The concentration of AGP was positively associated with postpartum incidence of retained placenta (**RP**) on d 3 to d 14 ($P < 0.001$) and d 21 ($P = 0.02$; Table 2.6). In a similar fashion, AGP was positively associated with incidence of metritis on d 3 to 21 ($P < 0.001$; Table 2.6). On d 7 ($P = 0.03$), d 14 ($P = 0.04$) and d 21 ($P < 0.001$; Table 2.6), AGP was positively associated with hyperketonemia. On d 21, AGP was positively associated with mastitis ($P = 0.03$). Furthermore, AGP was positively associated on d 1 ($P = 0.04$) with culling by 280 DIM. Overall, 19% of at-risk cows left the herd by 280 DIM, and proportional hazard analysis revealed that increased AGP concentration over the interquartile range (432 vs. 271 μg/mL) on d 1 was associated ($P = 0.02$) with a 15% decreased risk of culling through 280 DIM (0.85 hazard ratio, 95% CI: 0.82 to 0.95). Survival curves were constructed for cohorts using the optimal threshold

for d 1 AGP concentration (351 µg/mL) determined with ROC analysis, but there was no evidence of difference between cows above and below the threshold (P = 0.11 Wilcoxon test; data not shown).

DISCUSSION

To accomplish most of our objectives for this study, we first had to identify an accurate, high-throughput method for determination of AGP concentration in bovine plasma. To date, most of the quantification of circulating AGP has been conducted by radial immunodiffusion assays (Eckersall et al., 2007; Jafari et al., 2006), immunoturbidimetric assay (Bence et al., 2005; Mooney et al., 2006), gel electrophoresis methods (Cairoli et al., 2006; Ling et al., 1996), or by precipitating the protein and measuring absorbance (Lewis et al., 1989), none of which are suitable for analyzing large quantities of samples. More recent efforts have resulted in development of ELISA procedures to quantify AGP (Agra et al., 2017; Caperna et al., 2017), with only one study quantifying bovine plasma AGP (McGuckin et al., 2020). For this reason, we tested several ELISA kits and found the kit utilized in this study to be acceptable for linearity and recovery, and it most closely aligned with previously reported bovine plasma AGP concentrations using other techniques (Ceciliani et al., 2012; Horadagoda et al., 1999; Jafari et al., 2006).

Changes in peripartum AGP concentration

Our first objective was to assess AGP concentrations on a large sample size of peripartum dairy cows. The pattern of plasma AGP concentration shifts during the transition period in this study is similar to those reported for other positive acute-phase proteins, such as haptoglobin,

lipopolysaccharide binding protein, and serum amyloid A (Abuajamieh et al., 2016; Jafari et al., 2006; McCarthy et al., 2016). The concentration of AGP significantly increases postpartum by d 3, reaching an apparent peak at 14 DIM before numerically trending towards prepartum concentrations. This is similar to the pattern shown by McGuckin et al. (2020), where postpartum concentrations of AGP were significantly greater than prepartum concentrations, but lacked evidence of differences across time points in the first 3 weeks postpartum. In another study, plasma AGP peaked at 7 DIM and gradually declined to near prepartum concentrations by 21 DIM (Jafari et al., 2006). Bovine plasma AGP concentration is correlated with *AGP* mRNA abundance in the liver during the transition period, with an upregulation of hepatic *AGP* mRNA postpartum (McGuckin et al., 2020). In comparison with plasma haptoglobin concentrations, AGP declines at a much slower rate postpartum (Jafari et al., 2006; Sheldon et al., 2001). In a study conducted by Horadagoda et al. (1999), cattle with chronic or acute inflammation had serum AGP concentrations of 815 and 1,101 µg/mL, respectively, with a normal reference range of 200-450 µg/mL. Based upon the plasma AGP concentrations in the present study, most postpartum cows experienced elevated inflammatory tone, but perhaps not chronic inflammation. However, there is great variation in AGP concentration postpartum, so it is possible that a sizable minority of cows were experiencing chronic or even acute inflammation. We must also consider that the reference values in the study by Horadagoda et al. (1999) were determined by a single radial immunodiffusion assay compared with an ELISA in our study, so direct comparisons are tenuous.

Associations between AGP and DMI

One of the primary objectives of this study was to assess the relationship between AGP concentration and DMI. The negative associations between plasma AGP concentration and DMI were most significant postpartum, and AGP concentration on any given postpartum sampling day was negatively associated with DMI in subsequent weeks. This is consistent with previous work associating postpartum inflammatory markers with subsequent performance reduction in dairy cows (Huzzey et al., 2015; McCarthy et al., 2016). In pigs, serum AGP concentration is negatively correlated with daily feed intake and weight gain at 18 and 24 wk of age (Clapperton et al., 2005), and activation of the immune system causing an increase in AGP tended to decrease daily feed intake in another study (Williams et al., 1997a, 1997b). In fact, others have suggested AGP may serve as an independent predictor of production performance impairment in livestock (Caperna et al., 2013).

Administration of exogenous human AGP reduces voluntary feed intake in mice by acting on the hypothalamic leptin receptor and activating the JAK2/STAT3 pathway (Bellinger and Mendel, 1990; Sun et al., 2016), although very recent *in vitro* evidence suggests that bovine and human AGP do not elicit STAT3 signaling in cells expressing bovine leptin receptors (McGuckin et al., 2020). Another recent study administered purified bovine AGP both peripherally and centrally in sheep, with no effect on feeding rate or cumulative intake (Gregg et al., 2019). It is unclear why human AGP activates the leptin receptor in mice but bovine and human AGP do not activate the bovine leptin receptor. It is certainly possible that species-specific differences in AGP modes of action exist. Further, plasma leptin concentration declines by ~50% in the transition to lactation and remains low postpartum (Janovick et al., 2011; Liefers et al., 2003) as energy balance is declining in dairy cows at the same time. Ruminant feed intake

during periods of negative energy balance is generally refractory to leptin (Ehrhardt et al., 2016; Henry et al., 2001; Morrison et al., 2001); the same "leptin resistance" phenomenon may prevent AGP from having effects on downstream leptin receptor signaling in transition dairy cows.

While we cannot determine cause and effect relationships of AGP on DMI in the present study, it is intriguing that a negative association exists postpartum. Postpartum dairy cows experience elevated inflammation and there are many factors involved in an inflammatory response, including the release of cytokines, which have been implicated in modulating feed intake through hypothalamic action (Wong and Pinkney, 2004). To our knowledge, no other acute-phase proteins have been directly implicated in central control of feed intake, so it could be that a variety of molecules related to inflammation work centrally to affect intake during an inflammatory response.

To further evaluate the negative association between AGP and DMI, we calculated the predicted change in DMI for the d 3 AGP interquartile range (25^{th} to 75^{th} percentile) using parameter estimates from the best-fit equation for wk 1 DMI (Table 2.4). As d 3 AGP increased over the interquartile range, average daily DMI during wk 1 was calculated to decrease 8.5%, or 1.4 kg (Figure 2.2). For comparison, the reduction in DMI for retained placenta, metritis and milk fever was estimated to be 0.8, 5.1 and 14.7 kg/d, respectively (Bareille et al., 2003). While the reduction of DMI over the AGP interquartile range does not initially appear substantial compared with diseases that plague modern dairy operations, it is still concerning and warrants further investigation. Furthermore, weak forward associations suggest that AGP may serve as a leading indicator of suboptimal DMI rather than a trailing indicator like clinical disease diagnosis. For example, d 3 AGP was negatively associated with DMI beyond week 1 (Table 2.4), and AGP on d 7 and 14 was negatively associated with DMI for multiple weeks. One

limitation of this data is the relatively high variability in inter-plate CV (up to 17.3%) for AGP quantification, which may in part explain a portion of the 15.4% variation in DMI on d 3. A more precise measurement of AGP may have better explained any variation in DMI postpartum.

Prognostic ability of AGP to predict poor DMI

The fact that AGP may serve as a leading indicator of suboptimal postpartum DMI lead us to our third objective, which was to assess the prognostic value of AGP to predict cows at risk of low DMI using ROC analysis. The ROC analysis is a method for evaluation of the accuracy of a clinical diagnostic test, where the optimum combination of sensitivity (Se; true positive) and specificity (Sp; lack of false positive) of the test are maximized in ideal situations (Gardner and Greiner, 2006). As a continuous variable, DMI presents challenges for ROC analysis due to the difficulty in selecting a useful cutoff point (Gardner and Greiner, 2006). For determination of low DMI in this study, considering there has been no proposed threshold of acute DMI depression in transition cows, we used the arbitrary definition of wk 1 DMI that is 1 SD below the mean. In the current study, the area under the curve (AUC) of 0.6 (Figure 2.3) does not create confidence in the ability of AGP to predict acute depressed DMI. Constraining the definition of low DMI to 2 SD below the mean did not improve diagnostic performance (AUC = 0.55; data not shown). Therefore, while AGP is clearly negatively associated with DMI postpartum, it is a poor predictor of acute DMI depression.

Relationship between AGP and common biomarkers

Should AGP be a useful indicator of future DMI depression, it would ideally be independent of other blood biomarkers that are cheaply and easily measured today, such as glucose or BHB.

Out of 107 measured associations in this study between AGP and other blood biomarkers throughout the peripartum period (Appendix A), only 8 were significant. Considering the number of analyses for these comparisons, the 8 significant results may be a function of Type I error at the 95% confidence rate. The finding that AGP is poorly associated with other biomarkers at many timepoints suggests that it could serve as an independent biomarker for predicting postpartum health or performance.

Despite minimal associations between AGP and plasma biomarkers, there were several associations that may be biologically meaningful. The positive association between AGP and BHB on d 7 (Table 2.5) occurs at a time when both factors are normally increasing due to calving and the induction of negative energy balance. Others have demonstrated an association between acute-phase proteins and ketosis in postpartum cows (Abuajamieh et al., 2016) while more controlled studies have administered inflammatory components, such as tumor necrosis factor-α, and increased liver fat concentration (Bradford et al., 2009), BHB (Trevisi et al., 2009), incidence of ketosis (Yuan et al., 2013). In this light, the limited associations between AGP and BHB concentrations postpartum are biologically plausible.

Not surprisingly, plasma AGP concentration was positively associated with plasma haptoglobin concentration on d 3, d 7 and d 14 (Table 2.5). Postpartum AGP and haptoglobin have been shown in other studies to follow a similar pattern postpartum with a spike on d 7 before declining (Jafari et al., 2006), but the haptoglobin concentration declines to stable concentrations about one week earlier than does AGP (Sheldon et al., 2001). This may explain the lack of persistent future associations between AGP and haptoglobin in this study despite the strong associations on the same day, and may reflect the anti-inflammatory properties of AGP and its function to resolve inflammation (Ceciliani and Lecchi, 2019). Conversely, AGP and

haptoglobin have an extremely weak correlation in healthy pigs (Clapperton et al., 2007). Others have shown a tendency for a relationship between prepartum haptoglobin and postpartum disease incidence (Huzzey et al., 2011), and peripartum haptoglobin is negatively associated with milk production (Huzzey et al., 2015).

Relationship and prognostic ability of AGP for disease and culling

Our final objective was to assess the prognostic value of AGP to predict postpartum disease. In this study, postpartum AGP concentration was positively associated with incidence of RP and metritis, but for variables with sufficient sample size the diagnostic ability was marginal based upon the ROC AUC (Table 2.6). These diseases are common for postpartum dairy cows (Dubuc and Denis-Robichaud, 2017). Despite this strong positive association between AGP and postpartum disease, it is unlikely that AGP is a predictor of disease but rather AGP is increasing with other positive acute-phase proteins in response to the disease insult (Horadagoda et al., 1999; Sheldon et al., 2001). In normal uterine defense systems postpartum, bacterial invasion causes immune cell infiltration and inflammation which can persist with the pathogenesis of endometritis and metritis (Esposito et al., 2014). Unfortunately, lack of data prevented us from further analyzing onset of disease and its relationship with AGP concentration in transition cows. Incidence of disease for the dataset is outlined in Table 2.7.

We were surprised to observe AGP on d 1 to be associated with reduced culling through 280 DIM. While inflammation can be associated with negative production outcomes in chronic instances, it may be required as a part of the natural adaptive mechanisms for a successful transition to lactation (Bradford et al., 2015). Transgenic overexpression of AGP increased survivability from lethal *Klebsiella pneumonia* infection in mice, and administration of AGP in

normal animals prior to infection also had dramatic non-specific protective effects (Hochepied et al., 2000). Similarly, administration of AGP prior to a lethal dose of tumor necrosis factor-α prevented death (Libert et al., 1994). The cows in this study were generally not exposed to lethal quantities of harmful pathogens or cytokine storms, and their survivability to 280 days is much longer than the mouse studies. However, we could hypothesize that the elevated AGP concentrations as a result of the natural increase in inflammation postpartum had some protective effects upon subsequent pathogen exposure, considering postpartum cows have elevated susceptibility to infection. Nonetheless, despite this negative association, the diagnostic ability of d 1 AGP to predict culling as determined by ROC analysis was poor (AUC = 0.63; Table 2.6).

CONCLUSIONS

Plasma AGP concentration significantly increases by d 3 postpartum, with an apparent peak at 14 days in milk. AGP was negatively related with DMI postpartum, and DMI decreased approximately 8.5% as plasma AGP increased over the interquartile range. AGP could be used as a measure of inflammatory status in transition dairy cows, and an ELISA can serve as a reliable and repeatable method of quantification. We found AGP was not related to many other blood biomarkers except for haptoglobin, providing independence from other common biomarkers commonly used today. While there was a strong association between AGP and transition diseases, the disease may be causing the increase in AGP instead of AGP predicting disease. However, based upon ROC analysis, the diagnostic ability of AGP to predict sub-optimal DMI is poor. Further work should focus on *in vivo* AGP administration to determine impact on feeding behavior, and work to determine potential mechanisms of AGP action on the hypothalamus in ruminant models should be conducted.

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REFERENCES

Abuajamieh, M., S.K. Kvidera, M.V.S. Fernandez, A. Nayeri, N.C. Upah, E.A. Nolan, S.M. Lei, J.M. DeFrain, H.B. Green, K.M. Schoenberg, W.E. Trout, and L.H. Baumgard. 2016. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. Res. Vet. Sci. 109:81–85. doi:10.1016/j.rvsc.2016.09.015.

Agra, R.M., A. Varela-Román, R. González-Ferreiro, J.E. Viñuela, A. Castro-Pais, Á. Fernández-Trasancos, E. Díaz-Rodríguez, E. Álvarez, M.C. Carreira, F.F. Casanueva, J.R. González-Juanatey, and S. Eiras. 2017. Orosomucoid as prognosis factor associated with inflammation in acute or nutritional status in chronic heart failure. Int. J. Cardiol. 228:488–494. doi:10.1016/j.ijcard.2016.11.134.

Allen, M.S., and P. Piantoni. 2013. Metabolic Control of Feed Intake. Vet. Clin. North Am. Food Anim. Pract. 29:279–297. doi:10.1016/j.cvfa.2013.04.001.

Bareille, N., F. Beaudeau, S. Billon, A. Robert, and P. Faverdin. 2003. Effects of health disorders on feed intake and milk production in dairy cows. Livest. Prod. Sci. 83:53–62. doi:10.1016/S0301-6226(03)00040-X.

Bellinger, L.L., and V.E. Mendel. 1990. The Effects of Semi-and HPLC-Purified Human Satietin and Alpha-1-Glycoprotein on Ingestion and Body Weight. Brain Res. Bull. 25:941–947.

Bence, L.M., D.D. Addie, and P.D. Eckersall. 2005. An immunoturbidimetric assay for rapid quantitative measurement of feline alpha-1-acid glycoprotein in serum and peritoneal fluid. Vet. Clin. Pathol. 34:335–340. doi:10.1111/j.1939-165X.2005.tb00058.x.

Berge, A.C., and G. Vertenten. 2014. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. J. Dairy Sci. 97:2145–2154. doi:10.3168/jds.2013-7163.

Blees, T., C. Catozzi, C. Urh, S. Danicke, S. Haussler, H. Sauerwein, and F. Ceciliani. 2017. Development of an ELISA for the acute-phase protein alpha-1-acid glycoprotein (AGP) in cattle. Page 122 in Book of Abstracts of the 68th Annual Meeting of the European Federation of Animal Science, Tallinn, Estonia. European Federation of Animal Science, Rome, Italy.

Bollatti, J.M., M.G. Zenobi, N.A. Artusso, A.M. Lopez, C.D. Nelson, and J.E.P. Santos. 2020. Effects of rumen-protected choline on the inflammatory and metabolic status and health of dairy cows during the transition period. J. Dairy Sci. 103:4192–4205. doi:10.3168/jds.2019-17294.

Bradford, B., K. Yuan, J. Farney, L. Mamedova, and A. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650. doi:10.3168/jds.2015-9683.

Bradford, B.J., L.K. Mamedova, J.E. Minton, J.S. Drouillard, and B.J. Johnson. 2009. Daily Injection of Tumor Necrosis Factor-α Increases Hepatic Triglycerides and Alters Transcript Abundance of Metabolic Genes in Lactating Dairy Cattle. J. Nutr. 139:1451–1456. doi:10.3945/jn.109.108233.

Bujang, M.A., and T.H. Adnan. 2016. Requirements for Minimum Sample Size for Sensitivity and Specificity Analysis. J. Clin. Diagnostic Res. 10:YE01–YE06. doi:10.7860/JCDR/2016/18129.8744.

Cairoli, F., M. Battocchio, M.C. Veronesi, D. Brambilla, F. Conserva, I. Eberini, R. Wait, and E. Gianazza. 2006. Serum protein pattern during cow pregnancy: Acute-phase proteins increase in the peripartum period. Electrophoresis 27:1617–1625. doi:10.1002/elps.200500742.

Caperna, T.J., A.E. Shannon, L.A. Blomberg, M. Stoll, and T.G. Ramsay. 2013. Identification of alpha-1 acid glycoprotein (AGP) as a potential marker of impaired growth in the newborn piglet. Reprod. Fertil. Dev. 25:1126–1133. doi:10.1071/RD12103.

Caperna, T.J., A.E. Shannon, M. Stoll, S. Kahl, L.A. Blomberg, J.L. Vallet, and T.G. Ramsay. 2017. A sandwich ELISA for porcine alpha-1 acid glycoprotein (pAGP, ORM-1) and further demonstration of its use to evaluate growth potential in newborn pigs. Domest. Anim. Endocrinol. 60:75–82. doi:10.1016/j.domaniend.2017.04.001.

Carpenter, A., C. Ylioja, L. Mamedova, K. Olagaray, and B. Bradford. 2018. Effects of early postpartum sodium salicylate treatment on long-term milk, intake, and blood parameters of dairy cows. J. Dairy Sci. 101:1437–1447. doi:10.3168/jds.2017-13057.

Ceciliani, F., J.J. Ceron, P.D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. J. Proteomics 75:4207–4231. doi:10.1016/j.jprot.2012.04.004.

Ceciliani, F., and C. Lecchi. 2019. The Immune Functions of α 1 Acid Glycoprotein. Curr. Protein Pept. Sci. 20:505–524. doi:10.2174/1389203720666190405101138.

Clapperton, M., S.C. Bishop, N.D. Cameron, and E.J. Glass. 2005. Associations of acute phase protein levels with growth performance and with selection for growth performance in Large White pigs. Anim. Sci. 81:213–220.

Clapperton, M., S.C. Bishop, M. Piñeiro, F.M. Campbell, and E.J. Glass. 2007. The association between plasma levels of acute phase proteins, haptoglobin, alpha-1 acid glycoprotein (AGP), Pig-MAP, transthyretin and serum amyloid A (SAA) in Large White and Meishan pigs. Vet. Immunol. Immunopathol.. doi:10.1016/j.vetimm.2007.05.008.

Dubuc, J., and J. Denis-Robichaud. 2017. A dairy herd-level study of postpartum diseases and their association with reproductive performance and culling. J. Dairy Sci. 100:3068–3078. doi:10.3168/jds.2016-12144.

Eckersall, P.D., F.P. Lawson, L. Bence, M.M. Waterston, T.L. Lang, W. Donachie, and M.C. Fontaine. 2007. Acute phase protein response in an experimental model of ovine caseous lymphadenitis. BMC Vet. Res. 3. doi:10.1186/1746-6148-3-35.

Ehrhardt, R.A., A. Foskolos, S.L. Giesy, S.R. Wesolowski, C.S. Krumm, W.R. Butler, S.M. Quirk, M.R. Waldron, and Y.R. Boisclair. 2016. Increased plasma leptin attenuates ptive metabolism in early lactating dairy cows. J. Endocrinol. 229:145–157. doi:10.1530/JOE-16-0031.

Esposito, G., P.C. Irons, E.C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. Anim. Reprod. Sci. 144:60–71. doi:10.1016/j.anireprosci.2013.11.007.

Fournier, T., N. Medjoubi-N B, and D. Porquet. 2000. Alpha-1-acid glycoprotein. Biochim. Biophys. Acta 1482:157–171.

Gardner, I.A., and M. Greiner. 2006. Receiver-operating characteristic curves and likelihood ratios: Improvements over traditional methods for the evaluation and application of veterinary clinical pathology tests. Vet. Clin. Pathol. 35:8–17. doi:10.1111/j.1939-165X.2006.tb00082.x.

Gregg, B.A., P.A. Parker, K.M. Waller, L.G. Schneider, M. Garcia, B. Bradford, J.A. Daniel, and B.K. Whitlock. 2019. Effects of central and peripheral administration of an acute-phase protein, α-1-acid-glycoprotein, on feed intake and rectal temperature in sheep. J. Anim. Sci. 97:4783–4791. doi:10.1093/jas/skz336.

Hajian-Tilaki, K. 2014. Sample size estimation in diagnostic test studies of biomedical informatics. J. Biomed. Inform. 48:193–204. doi:10.1016/j.jbi.2014.02.013.

Henry, B.A., J.W. Goding, A.J. Tilbrook, F.R. Dunshea, and I.J. Clarke. 2001. Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. J. Endocrinol. 168:67–77. doi:10.1677/joe.0.1680067.

Hochepied, T., F.G. Berger, H. Baumann, and C. Libert. 2003. Alpha-1-Acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties.

Hochepied, T., W. Van Molle, F.G. Berger, H. Baumann, and C. Libert. 2000. Involvement of the acute phase protein α1-acid glycoprotein in nonspecific resistance to a lethal Gram-negative infection. J. Biol. Chem. 275:14903–14909. doi:10.1074/jbc.275.20.14903.

Horadagoda, N.U., K.M. G Knox, H.A. Gibbs, S.W. J Reid, A. Horadagoda, S.E. R Edwards, and P.D. Eckersall. 1999. Acute phase proteins in cattle: discrimination between acute and chronic inflammation. Vet. Rec. 144:437–441. doi:10.1136/vr.144.16.437.

Humblet, M.-F., H. Guyot, B. Boudry, F. Mbayahi, C. Hanzen, and J.-M. Godeau. 2006.

Relationship between haptoglobin, serum amyloid A, and clinical status in a survey of dairy herds during a 6-month period. Vet. Clin. Pathol. 35:188–193.

Huzzey, J.M., S. Mann, D. V. Nydam, R.J. Grant, and T.R. Overton. 2015. Associations of peripartum markers of stress and inflammation with milk yield and reproductive performance in Holstein dairy cows. Prev. Vet. Med. 120:291–297. doi:10.1016/j.prevetmed.2015.04.011.

Huzzey, J.M., D. V. Nydam, R.J. Grant, and T.R. Overton. 2011. Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94:5878–5889. doi:10.3168/jds.2010-3391.

Jafari, A., D.G. V Emmanuel, R.J. Christopherson, J.R. Thompson, G.K. Murdoch, J. Woodward, C.J. Field, and B.N. Ametaj. 2006. Parenteral administration of glutamine modulates acute phase response in postparturient dairy cows. J. Dairy Sci. 89:4660–4668. doi:10.3168/jds.S0022-0302(06)72516-4.

Janovick, N.A., Y.R. Boisclair, and J.K. Drackley. 2011. Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows 1. J. Dairy Sci. 94:1385–1400. doi:10.3168/jds.2010-3303.

Leno, B., S. LaCount, C. Ryan, D. Briggs, M. Crombie, and T. Overton. 2017a. The effect of source of supplemental dietary calcium and magnesium in the peripartum period, and level of dietary magnesium postpartum, on mineral status, performance, and energy metabolites in multiparous Holstein cows. J. Dairy Sci. 7183–7197. doi:10.3168/jds.2017-12773.

Leno, B., C. Ryan, T. Stokol, D. Kirk, K. Zanzalari, J. Chapman, and T. Overton. 2017b. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance of multiparous Holstein cows. J. Dairy Sci. 100:4604–4622. doi:10.3168/jds.2016-12221.

Lewis, E.J., J. Bishop, and C.H. Cashin. 1989. Automated Quantification of Rat Plasma Acute Phase Reactants in Experimental Inflammation. J. Pharmacol. Methods 21:183–194.

Libert, C., P. Brouckaert, and W. Fiers. 1994. Protection by α1-acid glycoprotein against tumor necrosis factor-induced lethality. J. Exp. Med. 180:1571–1575. doi:10.1084/jem.180.4.1571.

Liefers, S.C., R.F. Veerkamp, M.F.W. Te Pas, C. Delavaud, Y. Chilliard, and T. Van Der Lende. 2003. Leptin concentrations in relation to energy balance, milk yield, intake, live weight, and estrus in dairy cows. J. Dairy Sci. 86:799–807. doi:10.3168/jds.S0022-0302(03)73662-5.

Ling, P.R., J.H. Schwartz, M. Jeevanandam, J. Gauldie, B.R. Bistrian, P. Ling, J.H. Ra, and J.-A. Schwartz. 1996. Metabolic changes in rats during a continuos infusion of recombinant

interleukin-1. Am. J. Physiol. Endocrinol. Metab. 270:E305–E312.

McCarthy, M., T. Yasui, M. Felippe, and T. Overton. 2016. Associations between the degree of early lactation inflammation and performance, metabolism, and immune function in dairy cows. J. Dairy Sci 99:680–700. doi:10.3168/jds.2015-9694.

McGuckin, M.M., S.L. Giesy, A.N. Davis, M.A. Abyeta, E.A. Horst, S.S. Samii, Y. Zang, W.R. Butler, L.H. Baumgard, J.W. Mcfadden, and Y.R. Boisclair. 2020. The acute phase protein orosomucoid 1 is upregulated in early lactation but does not trigger appetite-suppressing STAT3 signaling via the leptin receptor. J. Dairy Sci. 103:4765–4776. doi:10.3168/jds.2019-18094.

McLaren, C.J., K.D. Lissemore, T.F. Duffield, K.E. Leslie, D.F. Kelton, and B. Grexton. 2006. The relationship between herd level disease incidence and a return over feed index in Ontario dairy herds. Can. Vet. J. 47:767–773.

Mooney, P., P. Hayes, and K. Smith. 2006. The putative use of alpha-1-acid glycoprotein as a non-invasive marker of fibrosis. Biomed. Chromatogr. 20:1351–1358. doi:10.1002/bmc.

Morrison, C.D., J.A. Daniel, B.J. Holmberg, J. Djiane, N. Raver, A. Gertler, and D.H. Keilser. 2001. Central infusion of leptin into well-fed and undernourished ewe lambs: Effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinol. 168:317–324. doi:10.1677/joe.0.1680317.

Olagaray, K., S. Sivinski, B. Saylor, L. Mamedova, J. Sauls-Hiesterman, I. Yoon, and B. Bradford. 2019. Effect of Saccharomyces cerevisiae fermentation product on feed intake parameters, lactation performance, and metabolism of transition dairy cattle. J. Dairy Sci. 102:8092–8107. doi:10.3168/jds.2019-16315.

Schwartz, M.W., S.C. Woods, D. Porte Jr, R.J. Seeley, and D.G. Baskin. 2000. Central nervous system control of food intake. Nature 404:661–671.

Sheldon, M., D.E. Noakes, A. Rycroft, and H. Dobson. 2001. Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet. Rec. 148:172–175.

Smith, J.W., L.O. Ely, and A.M. Chapa. 2000. Effect of region, herd size, and milk production on reasons cows leave the herd. J. Dairy Sci. 83:2980–2987. doi:10.3168/jds.S0022-0302(00)75198-8.

Sun, Y., Y. Yang, Z. Qin, J. Cai, X. Guo, Y. Tang, J. Wan, D.F. Su, and X. Liu. 2016. The acute-phase protein orosomucoid regulates food intake and energy homeostasis via leptin receptor signaling pathway. Diabetes 65:1630–1641. doi:10.2337/db15-1193.

Trevisi, E., M. Amadori, A.M. Bakudila, and G. Bertoni. 2009. Metabolic changes in dairy cows induced by oral, low-dose interferon-alpha treatment. J. Anim. Sci. 87:3020–3029. doi:10.2527/jas.2008-1178.

Williams, N.H., T.S. Stahly, and D.R. Zimmerman. 1997a. Effect of Level of Chronic Immune System Activation on the Growth and Dietary Lysine Needs of Pigs Fed from 6 to 112 kg. J. Anim. Sci. 75:2481–2496.

Williams, N.H., T.S. Stahly, and D.R. Zimmerman. 1997b. Effect of Chronic Immune System Activation on the Rate, Efficiency, and Composition of Growth and Lysine Needs of Pigs Fed from 6 to 27 kg. J. Anim. Sci 75:2463–2471.

Wong, S., and J. Pinkney. 2004. Role of Cytokines in Regulating Feeding Behaviour. Curr. Drug Targets 5:251–263.

Yuan, K., J.K. Farney, L.K. Mamedova, L.M. Sordillo, and B.J. Bradford. 2013. TNFα altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. PLoS One 8. doi:10.1371/journal.pone.0080316.

Zenobi, M., R. Gardinal, J. Zuniga, A. Dias, C. Nelson, J. Driver, B. Barton, J. Santos, and C. Staples. 2018. Effects of supplementation with ruminally protected choline on performance of multiparous Holstein cows did not depend upon prepartum caloric intake. J. Dairy Sci. 101:1088–1110. doi:10.3168/jds.2017-13327.

TABLES

Table 2.1 Experiments and number of plasma samples analyzed (n = 2,086) on day relative to parturition

	Day relative to parturition								
Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1	Treatments
Bollatti et al., 2020	0	90	0	0	90	94	96	94	Transition period rumen-protected choline
Carpenter et al., 2018	0	0	0	0	52	53	56	56	Postpartum sodium salicylate drench
Leno et al., 2017a	0	41	0	0	41	41	41	41	Ca/Mg source, postpartum dietary Mg conc.
Leno et al., 2017b	0	89	0	0	89	58	87	88	Prepartum DCAD level with low K diet
Olagaray et al., 2019	0	62	0	0	0	0	59	0	Transition period yeast fermentation product
Zenobi et al., 2018	91	85	44	87	90	90	92	92	Rumen-protected choline, prepartum dietary energy density
Total	91	367	44	87	362	336	431	371	

Table 2.2 Spike and recovery results of the ICL ELISA kit

	AGP concentration (ng/mL)			
Spike (ng/mL)	Expected	Observed	Recovery (%)	SD[1]
5	26.3	29.0	110.3	2.1
10	31.3	32.7	104.6	2.7
20	41.3	38.3	92.9	0.7

[1]Standard deviation of the observed concentrations, performed in triplicate

Table 2.3 Linearity of dilution results for AGP using the ICL ELISA kit

	AGP concentration (ng/mL)			
Dilution rate	Observed	Expected	% of Expected	SD[1]
1:5,000	88.5	-	-	11.5
1:10,000	36.4	44.2	82.2	0.1
1:20,000	20.9	18.2	115.2	1.2
1:40,000	10.7	10.5	102.5	0.7
1:80,000	5.1	5.4	95.4	0.4

[1]Standard deviation of the observed concentrations, performed in duplicate

Table 2.4. Association between plasma AGP concentration (natural log µg/mL) and DMI (kg/d). Significant linear associations were subsequently analyzed with quadratic and linear regression including random effect of treatment(study) in the model.

		P-value[1]						
Plasma AGP sample day	Week of DMI	Linear	Quadratic	Intercept	Parameter Estimate	R^2	N	RMSE
-21								
	-2[2]	0.09		-31.37	16.14	0.15	82	2.08
			0.07		-1.50			
-13 ± 2								
	-2	0.10		13.96	-0.53	0.33	348	3.43
	1[3]	0.22		18.87	-0.46	0.16	367	3.27
	3	0.46		21.90	-0.25	0.21	253	3.14
	4	0.80		22.02	-0.09	0.28	291	2.88
3 ± 1								
	1	0.02		-2.50	8.36	0.27	355	3.21
			0.01		-0.87			
	2[4]	0.07		3.25	6.61	0.24	318	3.08
			0.03		-0.67			
	3[5]	<0.001		26.81	-1.08	0.27	350	2.92
7 ± 1								
	2	0.02		6.54	5.86	0.32	330	2.91
			<0.01		-0.63			
	3	<0.001		21.53	-0.002	0.30	327	2.80
14 ± 1								
	3	<0.001		28.49	-1.31	0.25	423	3.06
	4[6]	<0.001		28.54	-1.15	0.31	358	2.76
21 ± 1								
	4	0.001		27.30	-0.97	0.30	363	2.77

[1]All data were transformed using natural log to achieve normality and data are presented in the transformed state.
[2]DMI averaged over 3 d following plasma sample date (d -13 ±2)

[3]DMI averaged over d 1 to 7
[4]DMI averaged over d 8 to 14
[5]DMI averaged over d 15 to 21
[6]DMI averaged over d 22 to 28

Table 2.5. Association between plasma AGP concentration (natural log µg/mL) and plasma BHB (µM) and haptoglobin (Hp) concentrations[1] for select sampling points postpartum.

		P-value[2]						
AGP plasma sample day	Variable sample day	Linear	Quadratic	Intercept	Parameter Estimate	R-square	N	RMSE
7 ± 1	BHB d 7	<0.01		54.1	113.3	0.09	322	425.8
3 ± 1	Hp d 3	<0.001		-1,439.5	272.1	0.24	50	233.1
7 ± 1	Hp d 7	<0.001		-1,875.8	406.6	0.32	143	518.0
7 ± 1	Hp d 14	<0.01		-532.8	149.6	0.28	140	318.3
14 ± 1	Hp d 14	0.03		3598.4	-1286.8	0.31	145	337.0
14 ± 1	Hp d 14		0.02		123.2			

[1]Data for haptoglobin from Carpenter et al. (2018) were reported as µg/mL and data from Bollatti et al. (2020) and Zenobi et al. (2018) were reported as optical density. Differences between concentration reporting methods are accounted for with the random effect of treatment(study) in our model.

[2]All data for AGP concentration were transformed using natural log to achieve normality and data are presented in the transformed state.

Table 2.6. Association between alpha-1-acid glycoprotein (AGP; natural log µg/mL) and incidence of postpartum disease and culling in transition dairy cows. All associations were positive unless otherwise denoted by superscript.

	AGP Sample Day (relative to parturition)							
Disease	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
Cows at risk, n	91	360	44	87	306	280	369	310
Hyperketonemia								
P-value								
AGP[1]	0.10	0.20	0.13	0.61	0.15	0.03	0.04	<0.001
Study	-	0.69	-	-	0.12	0.08	0.15	0.04
Cases, n[2]	20	91	10	18	78	69	91	77
AUC[3]	0.60	0.59	0.51	0.50	0.59	0.62	0.60	0.66
AGP threshold[4], µg/mL	246	258	211	246	531	165	464	275
Retained placenta								
P-value								
AGP	0.81[6]	0.16[6]	0.87[6]	0.72[6]	<0.001	<0.001	<0.001	0.02
Study	-	<0.01	-	-	0.46	0.69	<0.001	0.25
Cases, n	19[5]	33	5[5]	17[5]	37	30	38	40
AUC	-	0.70	-	-	0.74	0.77	0.83	0.69
AGP threshold[4], µg/mL	-	77	-	-	496	853	477	431
Metritis								
P-value								
AGP	0.63[6]	0.71[6]	0.96	0.81[6]	<0.001	<0.001	<0.001	<0.001
Study	-	0.19	-	-	0.08	0.01	0.07	0.13
Cases, n	19[5]	37	5[5]	18[5]	40	34	41	41
AUC	-	0.61	-	-	0.78	0.86	0.81	0.74
AGP threshold[4], µg/mL	-	330	-	-	401	643	365	548
Mastitis								
P-value								
AGP	0.95[6]	0.55	0.11	0.31	0.25	0.11	0.10	0.03
Study	-	<0.001	-	-	<0.01	<0.01	<0.001	0.03
Cases, n	23	51	10	24	49	45	49	49

AUC	0.54	0.74	0.63	0.53	0.67	0.74	0.74	0.7
AGP threshold[4], µg/mL	223	152	291	236	164	383	1,216	413
Culling by 280 DIM								
P-value								
AGP	0.10[6]	0.15[6]	0.84	0.04[6]	0.99[6]	0.71	0.52	0.98
Study	-	0.10	-	-	0.07	0.13	0.11	0.07
Culled, n	15	61	8	14	60	51	68	58
AUC	0.61	0.62	0.58	0.63	0.61	0.62	0.62	0.61
AGP threshold[4], µg/mL	183	50	291	350	419	410	182	357

[1]Data for AGP concentration were transformed using natural log to achieve normality and data are presented in the transformed state.
[2]Number of observations of animals in the data set for a particular sampling day that were diseased at some point during the study. period. Data was not available for onset of disease.
[3]Receiver operating characteristic area under the curve for AGP predicting postpartum disease
[4]Concentration offering the maximum difference between sensitivity and (1-specificity)
[5]Insufficient sample size for diagnostic testing using ROC analysis (Hajian-Tilaki, 2014)
[6]Negative association between plasma AGP and disease or culling

Table 2.7. Total incidence of postpartum disease and survival to 280 DIM by study in transition dairy cows.

	n	
Item	Total cases	Total animals[1]
Hyperketonemia	94	377
Retained placenta	41	377
Metritis[2, 3]	41	318
Mastitis	52	377
Culled by 280 DIM	69	371

[1]No disease incidence data were available from Carpenter et al. (2018).
[2]Data on metritis incidence were not available from Olagaray et al. (2019).
[3]Defined as fetid, watery discharge. Complete descriptions can be found in the respective publications (Leno et al., 2017a; b; Zenobi et al., 2018; Bollatti et al., 2020)

FIGURES

Figure 2.1. Box and whisker plots and outliers of plasma AGP concentration of peripartum dairy cows.

Refer to Table 2.1 for number of samples analyzed at each time point. Data were transformed using $\log_{10}$ to achieve normality and reverse-transformed data is shown below. Symbols above each day indicate difference from postpartum timepoints (* = different from d1 to d21 timepoints, $P < 0.001$; † = different from d 3 to d21 timepoints, $P < 0.001$). Data for each plot are represented in each box by the 25th percentile, median, and 75th percentile, while whiskers are data within 1.5 times the interquartile range. Black dots are outliers.

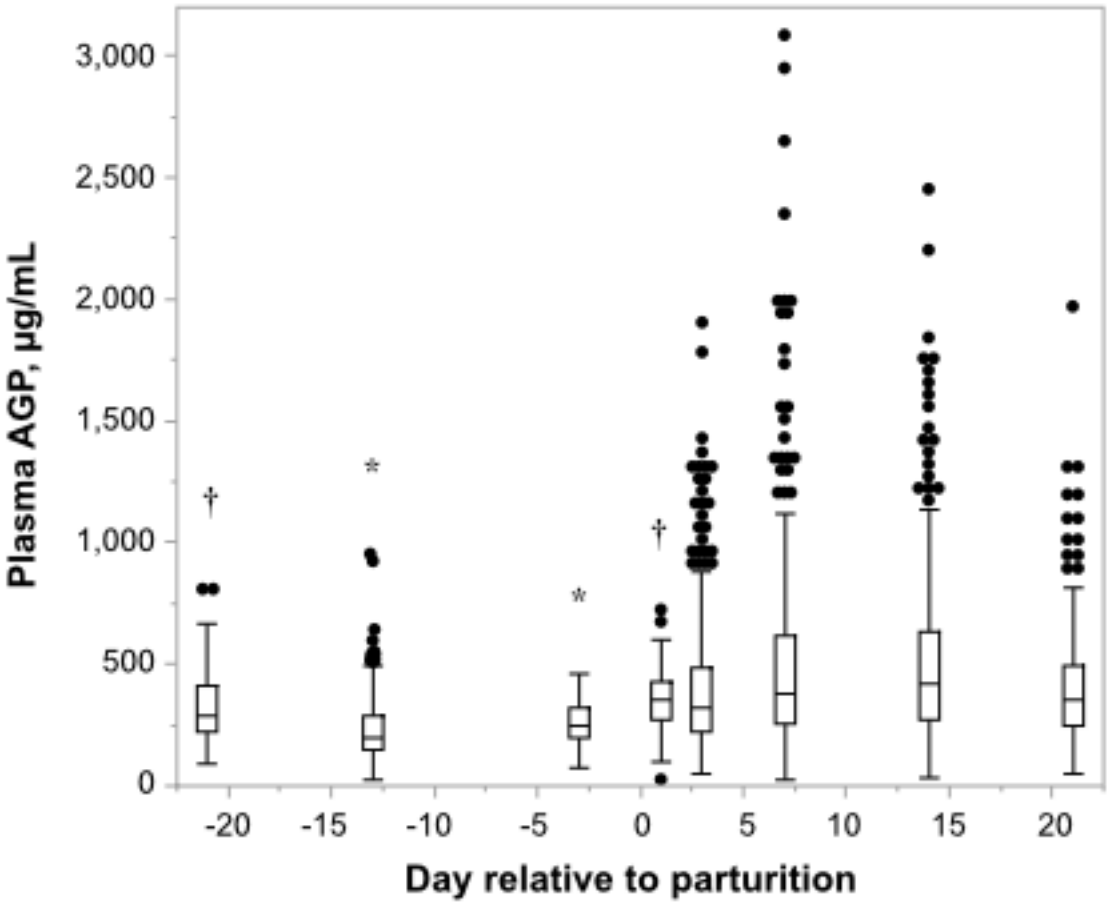
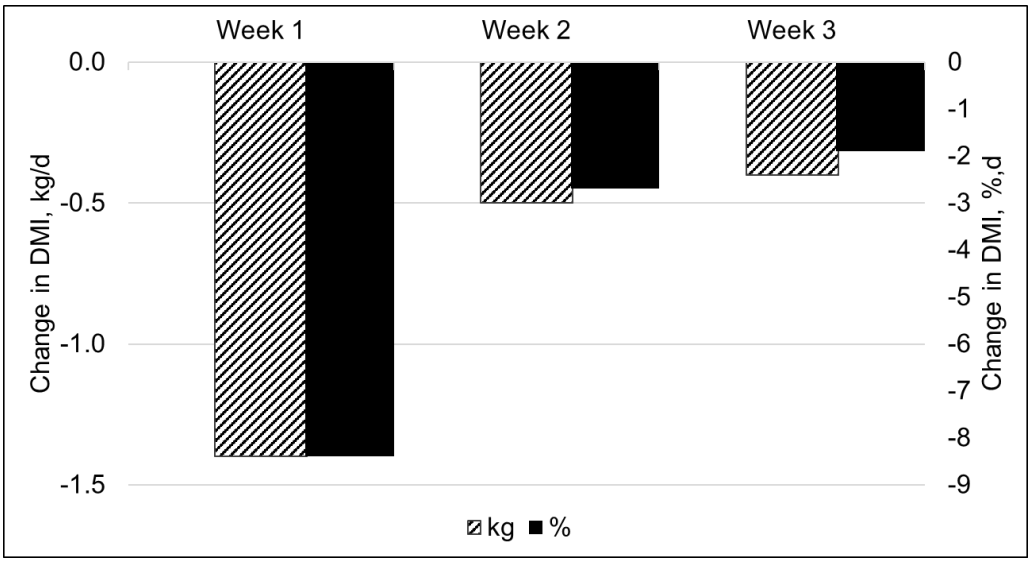


Figure 2.2. Plasma AGP concentration on d 3 postpartum is negatively associated with early lactation DMI.

The reduction in DMI calculated over the interquartile range of d 3 plasma AGP concentration (222 to 488 µg/mL; Panel A) and the bivariate fit of d 3 AGP (µg/mL) and wk 1 DMI (Panel B).

A)



B)

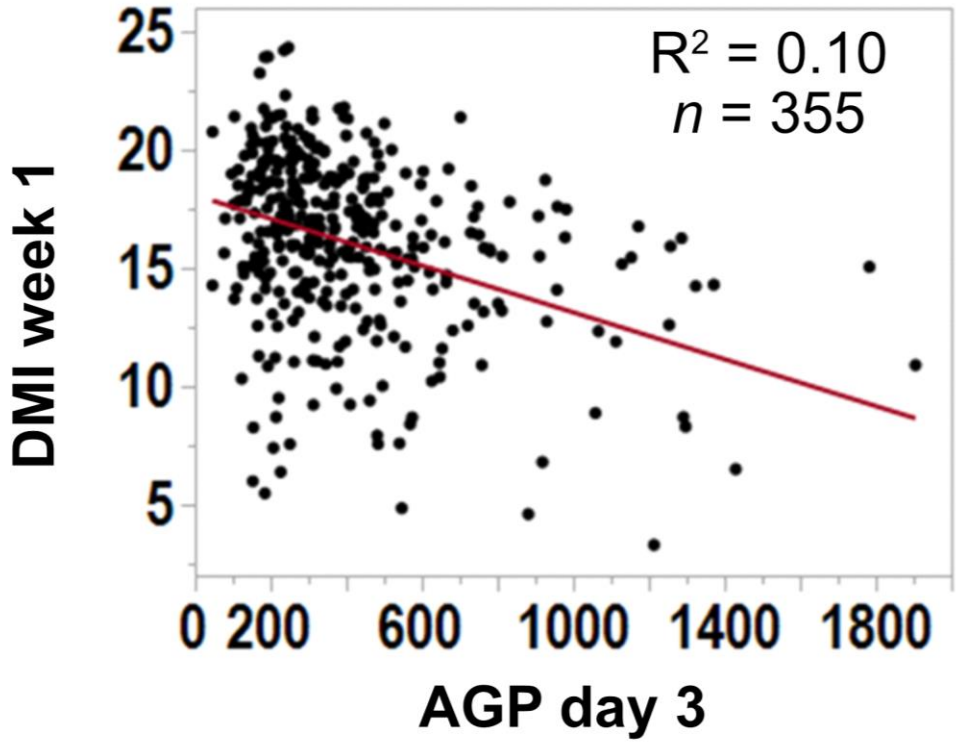
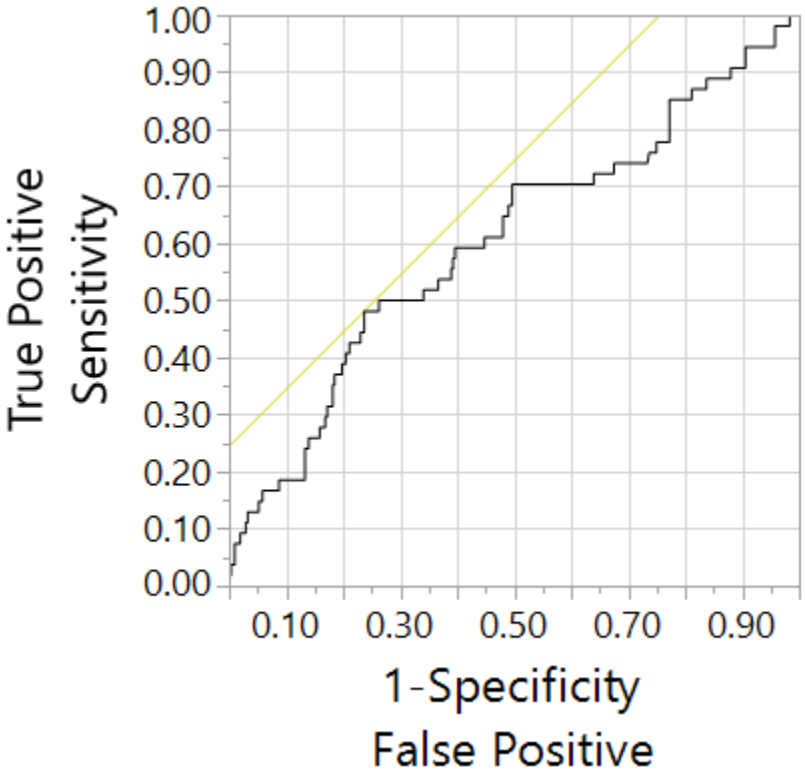


Figure 2.3. Receiver operating characteristics curve for plasma AGP concentration on d 3 predicting low DMI for wk 1.

Receiver operating characteristics curve (jagged line) that shows the critical threshold (intersection with the solid line) for plasma AGP concentration on d 3 (480.9 µg/mL) predicting low DMI for wk 1 (AUC = 0.60). The critical threshold AGP concentration offered the optimal balance between sensitivity and specificity, determined based on greatest sum of calculated sensitivity + specificity. Low DMI was defined as wk 1 DMI less than 1 SD below the mean (< 12.5 kg/d; n = 54).



Effects of a high-protein corn product compared with soy and canola protein sources on nutrient digestibility and production responses in mid-lactation dairy cows

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ABSTRACT

An experiment was conducted to assess the effects of a novel and proprietary high-protein corn product (56% crude protein; CP) relative to other common sources of protein on the lactation performance of dairy cows. Twenty-four Holstein cows (620 ± 47.7 kg of body weight, 111 ± 34 d in milk, 2.28 ± 0.46 lactations; mean ± SD) were randomly assigned to treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects. Cows were individually fed one of four diets with a different protein concentrate source during each 28-d period, including: soybean meal (SBM), high-protein corn product (HPCP), soybean meal with rumen-bypass soy protein (SBMBP), and canola meal with rumen-bypass soy protein (CANBP). Diets were formulated for equal concentrations of CP and balanced to meet predicted lysine and methionine requirements. The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), while SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. Data were analyzed using mixed models with the fixed effects of treatment, period, square, the interactions of treatment and period, treatment and square, and the random effect of cow. The CANBP diet increased dry matter intake compared with SBM and HPCP. Treatment affected milk yield, as SBMBP and CANBP increased yield compared with SBM, but HPCP decreased milk yield compared to all treatments. The HPCP diet reduced CP intake as a percent of total DMI and increased the CP content of orts indicative of selection against HPCP. The HPCP diet also decreased apparent total tract and CP digestibility, leading to less urine N excretion and greater fecal N output. The SBMBP and CANBP diets performed similarly in nearly every variable measured, except that SBMBP increased MUN. In conclusion, the HPCP diet reduced yield of milk and milk components likely because of reduced apparent total tract DM and CP digestibility.

INTRODUCTION

Corn grain is a useful feed ingredient in lactating dairy cow diets, serving as a readily available energy substrate; however, it is low in CP (approximately 7%). Through corn processing to produce value-added products - such as ethanol or corn sweeteners - the resulting co-products possess greater concentrations of protein, which have utility as protein sources for livestock (Loy and Lundy, 2019).

Corn wet-milling and bio-ethanol production are two common processes that generate high-protein corn byproducts (Loy and Lundy, 2019). Wet-milling removes the carbohydrate component through various mechanisms, resulting in the production of corn gluten feed (25% CP) and corn gluten meal (60% CP; Loy and Lundy, 2019). Gluten meal is a high-value product destined for feed ingredients in pet, poultry and swine diets, while corn gluten feed is primarily an ingredient for ruminant diets (Loy and Lundy, 2019). Bio-ethanol production most commonly utilizes starch in grains for fermentation. The fermentation process results in spent grains known as distiller's grains with solubles (DGS) which have a CP concentration of 27 to 31% CP (Belyea et al., 2004; Liu, 2011). Removal of fat from the DGS increases CP concentration marginally (34%; Wang et al., 2007; Morris et al., 2018), and removal of bran and germ components enhances the efficiency of ethanol production and increases the CP concentration to more than 40% (Corredor et al., 2006; Robinson et al., 2008). Distiller's grains have been fed to beef (Klopfenstein et al., 2008) and dairy (Kelzer et al., 2009; Schingoethe et al., 2009) cattle successfully as a protein source, and the inclusion has become more common as ethanol production grows.

More nascent technologies are being evaluated to further add value to DGS. One concept uses sieving and elutriation to separate particles based upon size and density, increasing protein

concentration up to 40% (Srinivasan et al., 2005). Distiller's grains are an attractive feedstock for second-generation, fiber-based ethanol production because of its fiber content and ability to streamline production processes (White et al., 2008; Kim et al., 2010; Mikulski and Kłosowski, 2018), producing a co-product of 34 to 50% CP (Kim et al., 2008; Lundy et al., 2015). Ability to cost-effectively integrate various technologies into existing production processes will ultimately determine their viability in the future, and the feed value of resulting co-products is critical.

A new, proprietary high-protein corn product with 56% CP may be a novel option for lactating dairy cow diets. With very limited data available on livestock feeding performance using this new technology, we chose to determine its utility for replacing commonly-fed protein sources in North America, such as soybean meal and canola meal, as has been done with other high-protein corn products (Nichols et al., 1998; Mulrooney et al., 2009; Christen et al., 2010). Care must be taken when feeding different protein sources, because not all protein sources have equal value due to differences in their AA profiles relative to the AA requirements of the animal. The two most limiting AA in the lactating dairy cow are typically considered to be lysine and methionine (NRC, 2001). Lysine concentration as a percentage of CP is greatest in soybean meal and least in corn products, while methionine concentration is greatest in canola meal and least in soybean meal (Maxin et al., 2013). When diets are formulated for similar CP concentration, the lack of methionine or lysine may inhibit performance depending on diet characteristics (Donkin et al., 1989; Nichols et al., 1998; Wang et al., 2010). Therefore, the objective of this experiment was to assess milk production and nitrogen digestibility responses of high-producing Holstein cows following replacement of common protein sources with a novel high-protein corn product, while maintaining consistent predicted supply of metabolizable

methionine and lysine across diets. We hypothesized that the novel high-protein corn product would perform similarly to other common protein sources fed in dairy diets.

MATERIALS AND METHODS

Animals, Design, and Diets

Twenty-four multiparous Holstein cows near peak lactation (111 ± 34 DIM, 2.3 ± 0.46 lactations, mean ± SD) from the Kansas State University Dairy Teaching and Research Center were randomly assigned to treatment sequence using a random number sequence generator (Microsoft Excel, Microsoft Corporation, Redmond, WA) in a replicated 4 x 4 Latin Square design balanced for carryover effects. Power analysis conducted prior to study initiation determined 24 animals were sufficient to detect a difference in mean milk yield of 2.7 kg/d (α = 0.05, β = 0.16; Kononoff and Hanford, 2006). Cows were housed in a tie-stall barn. All experimental procedures were conducted with approval from the Institutional Animal Care and Use Committee at Kansas State University. Treatment periods were 28 d, with 25 d for diet adaptation and 3 d for sample collection. Feeding of treatments began in October 2018 and continued through January 2019. Cows were fed twice daily at 0600 and 1900 h and milked 3 times daily at 0400, 1100 and 1800 h.

Cows were individually fed 1 of 4 diets *ad libitum* with a different protein concentrate source during each 28-d period, including: soybean meal (**SBM**), high-protein corn product (**HPCP; 56.1% CP**), soybean meal with bypass protein (**SBMBP**) and canola meal with bypass protein (**CANBP**). Soybean meal and canola meal were chosen as treatments for comparison because they are typical protein sources fed on North American farms and can be used as a standard benchmark for comparison with the novel HPCP. A base TMR was delivered to each

cow with treatment top-dressed and mixed by hand at each feeding. Diets were formulated (NDS Professional, RUM&N Sas, Reggio Emilia, Italy) to meet nutrient requirements of a 629-kg cow producing 52 kg/d milk with 3.6% fat and 3.1% protein. Diets were formulated for equal concentrations of CP and balanced to meet metabolizable lysine (79.5 g/d) and methionine (225 g/d) requirements. Amino acid composition of the HPCP (Official Method 982.30; AOACInternational, 2016) is shown in Table 3.1, while library values were used for all other common feed ingredients (NRC, 2001). The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), while SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. The HPCP RUP was estimated in commercial laboratories with a combination of 16 h incubation *in situ* (Calsamiglia and Stern, 1995) and *in vitro* (Ross et al., 2013), and intestinal digestibility was determined with the modifications of Ross et al. (2013). The diets were formulated to provide equal concentrations of forage NDF and starch. Nutrient composition and ingredients are shown in Table 3.2.

One cow was removed from the experiment during the second collection period due to hemorrhagic diarrhea and was subsequently replaced for the remaining two periods. Period 2 data were not retained from this cow. Another cow was dried off in one quarter following an udder injury during period 3. Data were analyzed both with and without her inclusion post-dry-off, and it was determined that this did not impact evidence of significance for any treatment, so data were included in the final analysis. This resulted in analysis of data from 95 of 96 total collection period observations, with only 23 of 24 observations for the HPCP due to the removal of one cow during the collection period.

Data and Sample Collection and Analysis

Feed offered and refused for each cow was recorded daily during the final 3 d of each treatment period. Water intake was recorded daily. Samples of the TMR, concentrate treatment mixes and individual feed ingredients were collected daily and composited over the collection period for analysis. Feed refusals were frozen and later composited over the collection period for each cow and dried in a 55°C forced-air oven. All feed samples were sent to Dairyland Laboratories, Inc. (Arcadia, WI) for nutrient analysis (Table 3.3) using wet chemistry. Dry matter was determined by drying at 60°C until moisture was less than 6%, and then 105°C for 3 h (Shreve et al., 2006). Neutral detergent fiber was determined using an amylase treated method (method 2002.04; AOACInternational, 2016). Indigestible NDF was determined in fermentation flasks according to Goering and Van Soest (1970) after 240-h fermentation (Raffrenato et al., 2018), and residual NDF determined as described above. Crude protein was determined by analysis for nitrogen with AOAC method 990.03 (2016) while ADICP was determined on grain treatments by collecting ADF residue on filter paper using (method 973.18; AOACInternational, 2016) and analyzing for nitrogen to determine crude protein. Crude fat was determined with the use of diethyl ether on a Foss Soxtec 2047 (Foss North America, Eden Prairie, MN; AOAC method 920.39) and grain treatments containing calcium salts of fatty acids were subjected to an additional acid hydrolysis step using the SoxCap 2047 (Foss North America). Ash concentration was determined using AOAC method 942.05 (2016). Orts samples were analyzed for 240-h indigestible NDF and CP with the same methods.

Milk samples were collected at every milking during the collection period. Samples were analyzed for fat, true protein, lactose, and non-fat solids and MUN using infrared spectroscopy (Bentley FTS; Bentley Instruments, Inc., Chaska, MN), and somatic cells were counted using a

flow cytometer (Bentley FCM; Bentley Instruments, Inc.) at MQT Labs (Kansas City, MO). 3.5% FCM was calculated as FCM = (milk kg × 0.432) + (fat kg × 16.216), and energy-corrected milk was calculated as ECM = (0.327 × milk kg) + (12.95 × fat kg) + (7.65 × protein kg).

Fecal grab samples were collected for determination of total tract digestibility every 9 h during the 3-d collection period to represent every 3 h over a 24-h period. Samples were frozen immediately and were later thawed and composited by cow on an equal volume basis and dried in a 55°C forced-air oven. Dried samples were later ground through a 6 mm screen for analysis by Dairyland Labs, Inc. (Arcadia, WI). Indigestible NDF was determined in fermentation flasks according to Goering and Van Soest (1970) after 240-h fermentation (Raffrenato et al., 2018), and residual NDF determined as outlined above. Crude protein was determined by analysis for nitrogen with AOAC method 990.03. Total tract digestibility was calculated using 240-h indigestible NDFom as an internal marker to estimate total fecal output, with digestibility determined by the ratio of nutrient concentration in the feces and diet consumed (Cochran and Galyean, 1994).

Urine samples were collected via vulva stimulation every 9 h during the 3-d collection period to represent every 3 h over a 24-h period. Urine was diluted 1:3 in 0.15 *N* H_2SO_4 to achieve pH < 2, and then frozen at -20°C. After each collection period, samples were thawed and composited by cow and period on an equal volume basis. Urine was analyzed in duplicate for creatinine using alkaline picrate (Cayman Chemical, Ann Arbor, MI) and total urine output was estimated assuming a constant creatinine excretion rate of 29 mg/kg BW (Valadares et al., 1999). Urine N concentration was determined in duplicate using a LECO TruMac N analyzer (LECO Corporation, St. Joseph, MO). Total daily urinary N excretion was calculated using the estimated volume of urine excretion from creatinine analysis.

Cows were weighed individually on the initial 2 days of the study and during the final 2 d of each collection period to determine body weight change. Body condition score was recorded blindly for each cow by two trained investigators blinded to treatment at the initiation of the study and at the end of each period to determine body condition change.

Statistical Analysis

During period 1, some individual milk weight data were missing due to an equipment recording error. To account for missing data in an unbiased manner, milk yield, component yield and composition data were analyzed by individual milking over the 3-d collection period. Data were analyzed using JMP (version 13.0, SAS Institute, Cary, NC) according to the following statistical models. The model for milk production data was:

$$Y_{ijkln} = \mu + T_i + P_j + S_k + T_i \times P_j + T_i \times S_k + M_l(P_j) + C_n + C_n \times P_j + e_{ijkln},$$

where μ is the overall treatment mean, T_i is the fixed effect of treatment (i = 1 to 4), P_j is the fixed effect of period (j = 1 to 4), S_k is the fixed effect of square (k = 1 to 6), Ti*Pj is the interaction of treatment and period, T_i*S_k is the interaction of treatment and square, $M_l(P_j)$ is the random effect of milking time nested within period (l = 1 to 9), C_n is the random effect of cow (n = 1 to 24), $C_n* P_j$ is the interaction of cow and period, and e_{ijkln} is the residual error.

The model for feed and water intake, feed efficiency, nitrogen balance and output, digestibility, body weight, and body condition score data was:

$$Y_{ijkn} = \mu + T_i + P_j + S_k + T_i*P_j + T_i*S_k + C_n + e_{ijkn}$$

where μ is the overall treatment mean, T_i is the fixed effect of treatment (i = 1 to 4), P_j is the fixed effect of period (j = 1 to 4), S_k is the fixed effect of square (k = 1 to 6), Ti*Pj is the

interaction of treatment and period, T_i*S_k is the interaction of treatment and square, C_n is the random effect of cow (n = 1 to 24), and e_{ijkln} is the residual error.

Best linear unbiased predictors for cow*period milk yields were utilized to determine daily means for FCM and milk N excretion to calculate feed efficiency and nitrogen balance. Denominator degrees of freedom were checked to verify the lack of pseudoreplication in all models.

Data were evaluated for normality, and data points with studentized residuals > 4 or < -4 were considered outliers and removed from analysis. Interactions for fixed effects with $P > 0.20$ were removed from the model. Differences between treatments were declared significant when $P < 0.05$ according to Tukey's HSD.

RESULTS

Dry matter intake

Treatment affected DMI, with CANBP increasing DMI compared with SBM and HPCP (Table 3.4; $P < 0.01$), and HPCP increased orts CP concentration compared with all other treatments (Table 3.4; $P < 0.001$). These two factors altered N intake, as both SBMBP and CANBP increased N intake compared with HPCP (Table 3.4; $P < 0.001$). HPCP reduced CP intake as a proportion of total DMI compared with all other treatments (Table 3.4; $P < 0.001$). The HPCP and SBMBP diets increased water consumption relative to CANBP (Table 3.4; $P < 0.001$).

Milk yield and components

Treatment affected milk yield (Table 3.5; $P < 0.001$), whereby cows fed SBM produced less milk than SBMBP and CANBP but more than HPCP. There was no evidence of differences in milk fat concentration (Table 3.5; $P = 0.86$) but diet affected milk fat yield (Table 3.5; $P < 0.001$). CANBP increased milk fat yield compared with SBM and HPCP, and SBMBP increased fat yield compared with HPCP. The SBM diet increased milk protein concentration compared with HPCP (Table 3.5; $P < 0.01$) and HPCP decreased protein yield compared with all other treatments (Table 3.5; $P < 0.01$). There was no evidence of treatment effects on milk lactose concentration (Table 3.5; $P = 0.59$), but HPCP decreased lactose yield ($P < 0.001$). The SBM and SBMBP diets increased MUN compared with CANBP (Table 3.5; $P < 0.01$), but HPCP decreased MUN. There was no evidence of a treatment effect on SCC (Table 3.5; $P = 0.41$). Feeding HPCP reduced milk nitrogen secretion compared with all other treatments (Table 3.5; $P < 0.001$)

Cows fed SBMBP and CANBP produced more FCM compared with SBM and HPCP (Table 3.5; $P < 0.001$). Treatment also affected ECM (Table 3.5; $P < 0.001$) whereby CANBP increased ECM yield compared with SBM and HPCP. Cows fed HPCP produced the least ECM. There was a tendency for a treatment effect on feed efficiency (FCM:DMI; Table 3.5; $P = 0.06$); SBMBP increased feed efficiency compared with HPCP ($P < 0.05$, Tukey's HSD).

Digestibility and N balance

CANBP increased urinary N concentration compared with HPCP (Table 3.6; $P < 0.01$). There was no evidence of a treatment effect on urine output (Table 3.6; $P = 0.15$); however, HPCP reduced urinary N excretion compared with SBM and CANBP (Table 3.6, $P = 0.02$).

When compared with SBMBP and CANBP, SBM reduced fecal N concentration and HPCP increased fecal N concentration (Table 3.6; $P < 0.001$). There was a significant F-test for fecal DM output ($P = 0.04$); however, the conservative Tukey's HSD did not identify any significant contrast among treatments. Cows fed HPCP excreted more N than all other treatments (Table 3.6; $P < 0.001$) and SBM decreased N excretion compared with CANBP.

The HPCP diet reduced total tract DM digestibility compared with all other treatments (Table 3.6; $P < 0.001$). Treatment also affected apparent total tract N digestibility, whereby CANBP reduced digestibility compared with SBM, but increased digestibility compared with HPCP (Table 3.6; $P < 0.001$). There was no evidence of a treatment effect on N balance (Table 3.6; $P = 0.65$), body weight change ($P < 0.89$) nor BCS change ($P < 0.19$).

Treatment nutrient profile

Post-hoc analysis of top-dress premixes was carried out to confirm that formulated nutrient profiles were achieved and to evaluate whether Maillard product formation may have contributed to HPCP responses. The results revealed a dramatically greater concentration of ADICP in the HPCP mix (7.9%) compared with all other treatments (1.0% or less; data not shown).

DISCUSSION

The reduction of milk production of cows fed the HPCP diet appears to result from two issues: first through the reduction of CP intake and secondly through the reduction in apparent total tract CP digestibility. The cows fed HPCP had greater orts CP concentration and lower CP intake as a percentage of total DMI compared with the other treatments, suggesting

they sorted against the HPCP. While most mature dairy cattle sort during lactation to obtain a more energy dense diet (Miller-Cushon and DeVries, 2017), instances arise where cattle sort against protein concentrate sources due to palatability (Lardy and Kerley, 1994). It may be that the production of HPCP imparted a flavor or odor prompting the cows to select against the HPCP, since corn coproducts are typically highly palatable (Klopfenstein et al., 2008).

The bypass protein was added to the SBMBP and CANBP diets in this study to assess the HPCP on an equal-RUP basis, which was not possible with the SBM diet alone. In the present study, the HPCP decreased total tract apparent N digestibility and urinary N excretion, and increased fecal N excretion compared with the other treatments. In other studies, increasing RUP decreased apparent CP digestibility in mid-lactation cows, and cows fed diets with greater RUP had reduced urinary N excretion and elevated fecal N excretion despite similar N intake (Brito and Broderick, 2007). This suggests that the ruminal digestibility of the HPCP in our study was perhaps less than anticipated, leading to the greater fecal N excretion and lesser urinary N excretion. The results from Brito and Broderick (2007) suggest that the lesser fecal N excretion of the SBM diet compared with all other treatments was likely due to the lesser RUP content of that diet. Additionally, Noftsger and St-Pierre (2003) reported a decrease in milk production from cows fed less digestible RUP sources at the same dietary CP concentration, which aligns with the lesser milk production of HPCP compared with the other treatments in this study.

One explanation for the reduction in digestibility of CP that led to reduced milk production for HPCP compared with other treatments may be the formation of Maillard products while drying. Excessive heating of feed products is known to increase acid-detergent insoluble crude protein (ADICP) concentrations in distiller's grains, which is positively correlated with

fecal N concentration (van Soest and Mason, 1991). Previous HPCP protein digestibility analysis provided by the suppliers using *in situ* (Ross et al., 2013) and *in vitro* (Krishnamoorthy et al., 1983) methods conducted in commercial laboratories reported 52.9% RUP. ADICP content was unknown. Nonetheless, post-hoc analysis of the protein source top-dresses in the present study clearly demonstrated elevated ADICP for the HPCP top-dress. The HPCP top dress was 7.9% ADICP, whereas the other top dress protein treatments were less than 1.0% ADICP (data not shown). This coincides with the high fecal N excretion for those cows and the dark color of the product (Figure 1) consistent with Maillard product formation (Urriola et al., 2013). Similarly, bench-top production of a high-protein corn product during the initial concept development of cellulosic ethanol fermentation using distillers' grains resulted in heat damage and decreased lysine and methionine concentration compared with protein from distillers' grains (Kim et al., 2008). Traditional heating and cooking of the grain before the dry-milling process for ethanol production resulted in similar RUP concentrations (55%) as a high-protein DGS from fractionation of corn prior to the fermentation process, but the high-protein DGS had greater total tract digestibility (Kelzer et al., 2010). In contrast, a high-protein corn product (39.1%) from cellulosic fermentation of distillers' grains decreased DM and OM digestibility compared with traditional distillers' grains (34.1% CP; Lundy et al., 2015). It is unclear if the reduction in protein availability for HPCP is due to the drying process for long-term storage or another upstream process, so further investigation into those processes are warranted before determining if HPCP in this study is a feasible protein supplement for lactating dairy cows.

Milk protein concentration in this study was greater for cows fed SBM vs. HPCP. Milk protein concentration of milk is not driven by dietary CP concentration (NRC, 2001; Olmos Colmenero and Broderick, 2006a), but rather milk protein content increases as metabolizable

essential AA supply increases, at least until requirements are met (NRC, 2001). In the present study, dietary CP concentration was the same across diets and all were formulated to meet lysine and methionine requirements using synthetic rumen-protected AA. The reduction in milk protein content of cows fed HPCP compared with SBM points to decreased protein quality in HPCP, especially considering the lower RUP concentration for SBM.

The supply of metabolizable protein to the cow is important for maximizing milk production, and the SBMBP and CANBP diets increased milk yield and fat-corrected milk yield compared with SBM. Similar results have been noted by others who have increased RUP content in the diet (Zanton et al., 2013), although the work of Zanton et al. (2013) did not influence DMI despite the increase in milk yield, in contrast with our study. Additionally, Broderick et al. (2009) fed greater RUP concentration, which increased fat-corrected milk through increased milk fat concentration despite no difference in milk yield or DMI. Maintaining the correct balance of rumen-degradable protein and RUP is important to maximize microbial protein yield to achieve optimum milk production (Santos et al., 1998), which may explain a lack of effect of increasing RUP at constant CP concentration on milk yield in other instances (Olmos Colmenero and Broderick, 2006b). The SBM diet was formulated to meet the Met and Lys requirements of the cow, but total MP supply was still only 95% of the requirement (data not shown), indicating that there may have been a lack of metabolizable protein to achieve the milk production of the SBMBP and CANBP diets where the cow's MP requirements were met.

Many studies have been conducted evaluating canola meal supplementation compared with soybean meal. Results have largely indicated similar performance (Sanchez and Claypool, 1983; Oba et al., 2010; Paula et al., 2018) or an increase in DMI and milk production in cows fed

canola meal (Broderick et al., 2015; Gidlund et al., 2015). In this study, SBMBP and CANBP resulted in similar DMI, milk production, FCM and milk component yields and nitrogen excretion. The only difference arose in MUN concentration, which was greater for SBMBP than CANBP, corresponding with the data of Maxin et al. (2013), Martineau et al. (2014), and Paula et al. (2018). The meta-analysis by Martineau and others (2014) noted that concentrations of blood AA, including essential and branched-chain AA, increase with canola meal supplementation. While we did not measure blood AA concentrations in this study, other work has demonstrated that the combination of methionine, lysine, and branched-chain AA infusion decreased MUN without altering N efficiency (Appuhamy et al., 2011), suggesting that the canola meal in our study may have been utilized more extensively for protein synthesis. This work, combined with previous evidence mentioned above, reinforces the importance and interchangeability of these protein sources in dairy diets as market conditions fluctuate.

Increasing diet complexity by incorporating feeds from different plant sources may provide a more complete amino acid profile for the animal to enhance production. Weiss (2019) fed an only corn-based diet or a diet with other forage and protein concentrate types, and noted reduced milk production despite methionine and lysine supplementation and a similar RUP concentration suggesting AA other than Met and Lys may have been limiting. In contrast, adding additional protein supplement types to the diet compared with a corn-based diet including distiller's grains as a protein source did not enhance performance in different study (Liu et al., 2000). In the present study, the SBMBP and CANBP had more variety of ingredients than the mostly corn-based HPCP. An additional limiting AA from a single feed source may have also contributed minorly to the reduced milk yield; however, the poor digestibility of the HPCP compared with the other treatments likely remains the primary cause.

The production process for the HPCP is proprietary; nonetheless, it is worthwhile to compare performance of this product with that of other high-protein corn products. In contrast to the present study, feeding a high-protein, corn-based DDGS resulting from fractionation prior to fermentation that was approximately 44% CP and 3.4% fat failed to elicit a response in DMI, milk yield, ECM yield (Kelzer et al., 2009; Christen et al., 2010) or FCM yield (Kelzer et al., 2009). In the case of Hubbard et al. (2009), a high-protein corn product increased milk and FCM yields, and a high-protein distillers' grain corn product decreased milk protein concentration in another case (Kelzer et al., 2009). Reduced-fat distillers grains maintained (Castillo-Lopez et al., 2014; Morris et al., 2018) or increased milk yield (Ramirez-Ramirez et al., 2016), but decreased 3.5% FCM, fat, and protein yields in a more recent study (Morris et al., 2018). Cows fed a diet with corn gluten meal as the primary protein source, compared with soybean meal, produced similar quantities of milk and FCM but produced less milk protein in early lactation (Wohlt et al., 1991). Beef cattle fed a high-protein corn product (39.1% CP) from secondary cellulosic ethanol fermentation of DGS had similar outcomes relative to a traditional DDGS (34.1% CP) for final body weight and hot carcass weight, but it decreased backfat thickness (Lundy et al., 2015). Chemical composition of various high-protein corn products fed to livestock is summarized in Table 3.7 for comparison purposes.

Investigations into the ruminal degradation parameters of high-protein corn products have demonstrated variable results. A high-protein distillers' grain product from corn fractionation increased the RUP content of the supplement compared with both soybean meal and canola meal (Maxin et al., 2013), but there was no evidence of differences in total-tract digestibility of DM, NDF, or CP in the study of Kelzer et al. (2009), in contrast to the present study. When evaluating ruminal fermentation profiles, the only effect of a high protein corn product was a

decrease of ruminal acetate concentration compared with soybean meal (Kelzer et al., 2009) or a decrease in isobutyrate concentration (Christen et al., 2010). Ruminal ammonia concentration was not effected in either instance, suggesting similar crude protein degradability (Kelzer et al., 2009; Christen et al., 2010). Corn gluten meal has lesser ruminal degradability compared with soybean meal (Koeln and Paterson, 1986; Annexstad et al., 1987). While ruminal fermentation profiles were not obtained in this study, the total tract digestibility of the HPCP was clearly decreased, as indicated by the increase in fecal N excretion and decrease in urine N excretion, and ruminal degradability was most likely decreased as well.

CONCLUSIONS

Cows fed the HPCP diet produced less milk and milk components. Cows fed HPCP consumed less CP and had lower apparent total tract and CP digestibility, suggesting the production process may have resulted in Maillard production formation, measured as ADICP. The SBMBP and CANBP diets performed similarly when balanced for methionine and lysine supply, supporting previous data demonstrating canola meal as a valuable substitute for soybean mean. Further efforts to improve protein quality from this HPCP are warranted.

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REFERENCES

Annexstad, R.J., M.D. Stern, D.E. Otterby, J.G. Linn, and W.P. Hansen. 1987. Extruded Soybeans and Corn Gluten Meal as Supplemental Protein Sources for Lactating Dairy Cattle. J. Dairy Sci. 70:814–822. doi:10.3168/jds.S0022-0302(87)80078-4.

AOACInternational. 2016. Official Methods of Analysis. 20th ed. AOAC International, Gaithersburg, MD.

Appuhamy, J.A.D.R.N., J.R. Knapp, O. Becvar, J. Escobar, and M.D. Hanigan. 2011. Effects of jugular-infused lysine, methionine, and branched-chain amino acids on milk protein synthesis in high-producing dairy cows. J. Dairy Sci. 94:1952–1960. doi:10.3168/jds.2010-3442.

Belyea, R.L., K.D. Rausch, and M.E. Tumbleson. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresour. Technol. 94:293–298. doi:10.1016/j.biortech.2004.01.001.

Brito, A.F., and G.A. Broderick. 2007. Effects of Different Protein Supplements on Milk Production and Nutrient Utilization in Lactating Dairy Cows. J. Dairy Sci. 90:1816–1827. doi:10.3168/jds.2006-558.

Broderick, G.A., A.P. Faciola, and L.E. Armentano. 2015. Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. J. Dairy Sci.. doi:10.3168/jds.2015-9563.

Broderick, G.A., M.J. Stevenson, and R.A. Patton. 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. J. Dairy Sci. 92:2719–2728. doi:10.3168/jds.2008-1277.

Calsamiglia, S., and M. Stern. 1995. A Three-Step In Vitro Procedure for Estimating Intestinal Digestion of Protein in Ruminants. J. Anim. Sci. 73:1459–1465.

Castillo-Lopez, E., H.A. Ramirez Ramirez, T.J. Klopfenstein, D. Hostetler, K. Karges, S.C. Fernando, and P.J. Kononoff. 2014. Ration formulations containing reduced-fat dried distillers grains with solubles and their effect on lactation performance, rumen fermentation, and intestinal flow of microbial nitrogen in Holstein cows. J. Dairy Sci. 97:1578–1593. doi:10.3168/jds.2013-6865.

Christen, K.A., D.J. Schingoethe, K.F. Kalscheur, A.R. Hippen, K.K. Karges, and M.L. Gibson. 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. J. Dairy Sci. 93:2095–2104. doi:10.3168/jds.2009-2687.

Cochran, R.C., and M.L. Galyean. 1994. Measurement of in vivo forage digestion by ruminants. Forage Qual. Eval. Util. 613–643.

Corredor, D.Y., S.R. Bean, T. Schober, and D. Wang. 2006. Effect of decorticating sorghum on ethanol production and composition of DDGS. Cereal Chem. 83:17–21. doi:10.1094/CC-83-0017.

Donkin, S.S., G.A. Varga, T.F. Sweeney, and L.D. Muller. 1989. Rumen-Protected Methionine and Lysine: Effects on Animal Performance, Milk Protein Yield, and Physiological Measures. J. Dairy Sci. 72:1484–1491. doi:10.3168/jds.S0022-0302(89)79258-4.

Gidlund, H., M. Hetta, S.J. Krizsan, S. Lemosquet, and P. Huhtanen. 2015. Effects of soybean meal or canola meal on milk production and methane emissions in lactating dairy cows fed grass silage-based diets. J. Dairy Sci. 98:8093–8106. doi:10.3168/jds.2015-9757.

Goering, H.K., and P.J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agriculture Handbook No. 379. Agricultural Research Service - USDA, Washington D.C.

Holter, J.B., H.H. Hayes, W.E. Urban, S. Ramsey, and H. Rideout. 1992. Response of Holstein Cows to Corn Gluten Meal Used to Increase Undegradable Protein in Early or Later Lactation. J. Dairy Sci. 75:1495–1506. doi:10.3168/jds.S0022-0302(92)77906-5.

Hubbard, K.J., P.J. Kononoff, A.M. Gehman, J.M. Kelzer, K. Karges, and M.L. Gibson. 2009. Short communication: The effect of feeding high protein distillers dried grains on milk production of Holstein cows. J. Dairy Sci. 92:2911–2914. doi:10.3168/jds.2008-1955.

Kelzer, J.M., P.J. Kononoff, A.M. Gehman, L.O. Tedeschi, K. Karges, and M.L. Gibson. 2009. Effects of feeding three types of corn-milling coproducts on milk production and ruminal fermentation of lactating Holstein cattle. J. Dairy Sci. 92:5120–5132. doi:10.3168/jds.2009-2208.

Kelzer, J.M., P.J. Kononoff, L.O. Tedeschi, T.C. Jenkins, K. Karges, and M.L. Gibson. 2010. Evaluation of protein fractionation and ruminal and intestinal digestibility of corn milling co-products. J. Dairy Sci. 93:2803–2815. doi:10.3168/jds.2009-2460.

Kim, Y., R. Hendrickson, N. Mosier, M. Ladisch, B. Bals, V. Balan, and B.E. Dale. 2008. Enzyme hydrolysis and ethanol fermentation of liquid hot water and AFEX pretreated distillers' grains at high-solids loadings. Bioresour. Technol. 99:5206–5215.

Kim, Y., R. Hendrickson, N.S. Mosier, M.R. Ladisch, B. Bals, V. Balan, B.E. Dale, B.S. Dien, and M.A. Cotta. 2010. Effect of compositional variability of distillers' grains on cellulosic ethanol production. Bioresour. Technol. 101:5385–5393. doi:10.1016/j.biortech.2010.02.054.

Klopfenstein, T.J., G.E. Erickson, and V.R. Bremer. 2008. BOARD-INVITED REVIEW: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci 86:1223–1231. doi:10.2527/jas.2007-0550.

Koeln, L.L., and J.A. Paterson. 1986. Nitrogen balance and amino acid disappearance from the small intestine in calves fed soybean meal-, toasted soybean meal- or corn gluten meal-supplemented diets.. J. Anim. Sci. 63:1258–1266. doi:10.2527/jas1986.6341258x.

Kononoff, P.J., and K.J. Hanford. 2006. Technical Note: Estimating Statistical Power of Mixed Models Used in Dairy Nutrition Experiments. J. Dairy Sci 89:3968–3971. doi:10.3168/jds.S0022-0302(06)72439-0.

Krishnamoorthy, U., C.J. Sniffen, M.D. Stern, and P.J. Van Soest. 1983. Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. Br. J. Nutr. 50:555–568. doi:10.1079/bjn19830127.

Lardy, G.P., and M.S. Kerley. 1994. Effect of Increasing the Dietary Level of Rapeseed Meal on Intake by Growing Beef Steers. J. Anim. Sci. 72:1936–1942.

Liu, C., D.J. Schingoethe, and G.A. Stegeman. 2000. Corn distillers grains versus a blend of protein supplements with or without ruminally protected amino acids for lactating cows. J. Dairy Sci. 83:2075–2084. doi:10.3168/jds.S0022-0302(00)75089-2.

Liu, K. 2011. Chemical composition of distillers grains, a review. J. Agric. Food Chem. 59:1508–1526. doi:10.1021/jf103512z.

Loy, D.D., and E.L. Lundy. 2019. Nutritional Properties and Feeding Value of Corn and Its Coproducts. Corn. 633–659. doi:10.1016/b978-0-12-811971-6.00023-1.

Lundy, E.L., D.D. Loy, and S.L. Hansen. 2015. Influence of distillers grains resulting from a cellulosic ethanol process utilizing corn kernel fiber on nutrient digestibility of lambs and steer feedlot performance. J. Anim. Sci. 93:2265–2274. doi:10.2527/jas.2014-8572.

Martineau, R., D. Ouellet, and H. Lapierre. 2014. The effect of feeding canola meal on concentrations of plasma amino acids. J. Dairy Sci. 97:1603–1610. doi:10.3168/jds.2013-7125.

Maxin, G., D.R. Ouellet, and H. Lapierre. 2013. Ruminal degradability of dry matter, crude protein, and amino acids in soybean meal, canola meal, corn, and wheat dried distillers grains. J. Dairy Sci. 96:5151–5160. doi:10.3168/jds.2012-6392.

Mikulski, D., and G. Kłosowski. 2018. Efficiency of dilute sulfuric acid pretreatment of distillery stillage in the production of cellulosic ethanol. Bioresour. Technol. 268:424–433. doi:10.1016/j.biortech.2018.08.005.

Miller-Cushon, E., and T. DeVries. 2017. Feed sorting in dairy cattle: Causes, consequences, and management. J. Dairy Sci. 100:4172–4183. doi:10.3168/jds.2016-11983.

Morris, D.L., S.H. Kim, P.J. Kononoff, and C. Lee. 2018. Continuous 11-week feeding of

reduced-fat distillers grains with and without monensin reduces lactation performance of dairy cows. J. Dairy Sci. 101:5971–5983. doi:10.3168/jds.2017-14170.

Mulrooney, C.N., D.J. Schingoethe, K.F. Kalscheur, and A.R. Hippen. 2009. Canola meal replacing distillers grains with solubles for lactating dairy cows. J. Dairy Sci. 92:5669–5676. doi:10.3168/jds.2009-2276.

Nichols, J.R., D.J. Schingoethe, H.A. Maiga, M.J. Brouk, and M.S. Piepenbrink. 1998. Evaluation of Corn Distillers Grains and Ruminally Protected Lysine and Methionine for Lactating Dairy Cows. J. Dairy Sci. 81:482–491. doi:10.3168/jds.S0022-0302(98)75600-0.

Noftsger, S., and N.R. St-Pierre. 2003. Supplementation of Methionine and Selection of Highly Digestible Rumen Undegradable Protein to Improve Nitrogen Efficiency for Milk Production. J. Dairy Sci. 86:958–969. doi:10.3168/jds.S0022-0302(03)73679-0.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. e. National Academy Press, Washington, DC.

Oba, M., G.B. Penner, T.D. Whyte, and K. Wierenga. 2010. Effects of feeding triticale dried distillers grains plus solubles as a nitrogen source on productivity of lactating dairy cows. J. Dairy Sci. 93:2044–2052. doi:10.3168/jds.2009-2454.

Olmos Colmenero, J.J., and G.A. Broderick. 2006a. Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows. J. Dairy Sci. 89:1704–1712. doi:10.3168/jds.S0022-0302(06)72238-X.

Olmos Colmenero, J.J., and G.A. Broderick. 2006b. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. J. Dairy Sci. 89:1635–1643. doi:10.3168/jds.S0022-0302(06)72230-5.

Paula, E.M., G.A. Broderick, M.A.C. Danes, N.E. Lobos, G.I. Zanton, and A.P. Faciola. 2018. Effects of replacing soybean meal with canola meal or treated canola meal on ruminal digestion, omasal nutrient flow, and performance in lactating dairy cows. J. Dairy Sci. 101:328–339. doi:10.3168/jds.2017-13392.

Raffrenato, E., D.A. Ross, and M.E. Van Amburgh. 2018. Development of an in vitro method to determine rumen undigested aNDFom for use in feed evaluation. J. Dairy Sci. 101:9888–9900. doi:10.3168/jds.2018-15101.

Ramirez-Ramirez, H.A., E. Castillo Lopez, C.J.R. Jenkins, N.D. Aluthge, C. Anderson, S.C. Fernando, K.J. Harvatine, and P.J. Kononoff. 2016. Reduced-fat dried distillers grains with solubles reduces the risk for milk fat depression and supports milk production and ruminal fermentation in dairy cows. J. Dairy Sci. 99:1912–1928. doi:10.3168/jds.2015-9712.

Robinson, P.H., K. Karges, and M.L. Gibson. 2008. Nutritional evaluation of four co-product feedstuffs from the motor fuel ethanol distillation industry in the Midwestern USA. Anim.

Feed Sci. Technol. 146:345–352. doi:10.1016/j.anifeedsci.2008.01.004.

Ross, D.A., M. Gutierrez-Botero, and M.E. Van Amburgh. 2013. Development of an in vitro intestinal digestibility assay for ruminant feeds. Pages 190–202 in Cornell Nutrition Conference. Cornell University, East Syracuse, NY.

Sanchez, J.M., and D.W. Claypool. 1983. Canola Meal as a Protein Supplement in Dairy Rations. J. Dairy Sci. 66:80–85. doi:10.3168/jds.S0022-0302(83)81756-1.

Santos, F.A.P., J.E.P. Santos, C.B. Theurer, and J.T. Huber. 1998. Effects of Rumen-Undegradable Protein on Dairy Cow Performance : A 12-Year Literature Review. J. Dairy Sci. 81:3182–3213. doi:10.3168/jds.S0022-0302(98)75884-9.

Schingoethe, D.J., K.F. Kalscheur, A.R. Hippen, and A.D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802–5813. doi:10.3168/jds.2009-2549.

Shreve, B., N. Thiex, and M. Wolf. 2006. NFTA Method 2.1.4 - Dry Matter by Oven Drying for 3 hr at 105C. National Forage Testing Association, Lincoln, NE.

van Soest, P.J., and V.C. Mason. 1991. The influence of the Maillard reaction upon the nutritive value of fibrous feeds. Anim. Feed Sci. Technol. 32:45–53.

Srinivasan, R., R.A. Moreau, K.D. Rausch, R.L. Belyea, M.E. Tumbleson, and V. Singh. 2005. Separation of fiber from distillers dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chem. 82:528–533. doi:10.1094/CC-82-0528.

Urriola, P.E., L.J. Johnston, H.H. Stein, and G.C. Shurson. 2013. Prediction of the concentration of standardized ileal digestible amino acids in distillers dried grains with solubles. J. Anim. Sci. 91:4389–4396. doi:10.2527/jas.2013-6362.

Valadares, R.F.D., G.A. Broderick, S.C. Valadares Filho, and M.K. Clayton. 1999. Effect of Replacing Alfalfa Silage with High Moisture Corn on Ruminal Protein Synthesis Estimated from Excretion of Total Purine Derivatives. J. Dairy Sci. 82:2686–2696. doi:10.3168/jds.S0022-0302(99)75525-6.

Wang, C., H.Y. Liu, Y.M. Wang, Z.Q. Yang, J.X. Liu, Y.M. Wu, T. Yan, and H.W. Ye. 2010. Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows. J. Dairy Sci. 93:3661–3670. doi:10.3168/jds.2009-2750.

Wang, L., C.L. Weller, V.L. Schlegel, T.P. Carr, and S.L. Cuppett. 2007. Comparison of supercritical CO2 and hexane extraction of lipids from sorghum distillers grains. Eur. J. Lipid Sci. Technol. 109:567–574. doi:10.1002/ejlt.200700018.

Weiss, W.P. 2019. Effects of feeding diets composed of corn silage and a corn milling product with and without supplemental lysine and methionine to dairy cows. J. Dairy Sci.

102:2075–2084. doi:10.3168/jds.2018-15535.

White, J.S., B.K. Yohannan, and G.M. Walker. 2008. Bioconversion of brewer's spent grains to bioethanol. FEMS Yeast Res. 8:1175–1184. doi:10.1111/j.1567-1364.2008.00390.x.

Wohlt, J.E., S.L. Chmiel, P.K. Zajac, L. Backer, D.B. Blethen, and J.L. Evans. 1991. Dry Matter Intake, Milk Yield and Composition, and Nitrogen Use in Holstein Cows Fed Soybean, Fish, or Corn Gluten Meals. J. Dairy Sci. 74:1609–1622. doi:10.3168/jds.S0022-0302(91)78323-9.

Zanton, G.I., A.J. Heinrichs, and C.M. Jones. 2013. Short communication: Effects of level of rumen-degradable protein and corn distillers grains in corn silage-based diets on milk production and ruminal fermentation in lactating dairy cows1. J. Dairy Sci. 96:4638–4642. doi:10.3168/jds.2012-6030.

TABLES

Table 0.1. Amino acid profile of a proprietary high-protein corn product fed to lactating Holstein cows

Item	HPCP[1]
Crude Protein, %	56.1
Amino acid, g/kg of DM	
Ala	31.7
Arg	12.9
Asp	27.6
Glu	67.5
His	9.1
Ile	17.7
Lys	8.7
Met	8.4
Phe	20.9
Pro	33.2
Ser	18.2
Thr	16.0
Tyr	18.3
Val	21.9

[1]HPCP = high-protein corn product

Table 0.2. Ingredients and nutrient composition of experimental diets fed to lactating Holstein cows supplemented with a protein source from either soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP) or canola meal with bypass protein (CANBP)

	Diet[1]			
Item, % of DM	SBM	HPCP	SBMBP	CANBP
Dietary ingredients				
Corn silage	34.5	34.5	34.5	34.5
Alfalfa hay	19.5	19.5	19.5	19.5
Soybean meal, 47.5% solvent extracted	10.7	…	3.9	…
High protein corn product[2]	…	9.4	…	…
Canola meal, 37% solvent extracted	…	…	…	8.9
Expeller soybean meal[3]	…	…	8.0	4.7
Ground corn grain	22.6	22.6	22.6	22.6
Soybean hulls	7.8	8.8	6.8	5.0
Ca salts of long chain fatty acids[4]	0.75	0.75	0.75	0.75
Lysine hydrochloride[5]	0.32	0.68	0.11	0.26
HMBi[6]	0.24	0.17	0.17	0.15
Micronutrient premix[7]	3.6	3.7	3.6	3.6
Diet nutrient composition				
Dry matter, % as-fed	52.8	52.6	52.9	52.9
Crude protein	16.8	16.8	16.8	16.8
aNDFom	30.2	30.3	30.0	30.3
Starch	22.5	22.7	22.6	22.7
Ether extract	4.3	4.9	4.5	4.7
Ash	8.2	8.1	8.2	8.3

[1]SBM = soybean meal; HPCP = high-protein corn product; SBMBP = soybean meal + bypass protein; CANBP = canola meal + bypass protein

[2]Proprietary high-protein corn product

[3]SoyPlus (Landus Cooperative, Ames, IA).

[4]Megalac-R (Arm & Hammer Animal Nutrition, Princeton, NJ).

[5]Lysine hydrochloride, lecithin and hydrogenated vegetable oil, AjiPro-L (Ajinimoto Animal Nutrition North America, Chicago, IL).

[6]Isopropyl ester of 2-hydroxy-4-methylthio butanoic acid, MetaSmart (Adisseo Inc., Antony, France).

[7]The premix consists of 30.5% limestone, 20.8% sodium bicarbonate, 30.5% Kruse lactation premix, 4.40% trace mineral salt, 4.42% white salt, 7.08% Magnesium oxide, 4.42% vitamin E premix, 0.69% Zinpro 4-plex, 0.35% Zinpro 120, and 0.19% Rumensin 90.

Table 0.3. Nutrient composition of major feed ingredients fed to lactating Holstein cows.

	Feed ingredient[1]						
Component, % of DM	Corn silage	Alfalfa hay	Grain mix	SBM[2]	HPCP[2]	SBMBP[2]	CANBP[2]
Dry matter, % as-fed	29.1	92.7	90.6	91.7	87.5	92.4	92.1
Crude protein	9.3	23.6	9.3	36.1	35.8	35.6	35.7
aNDFom	47.0	33.5	16.3	16.5	17.1	15.7	17.1
Starch	16.6	2.3	49.5	12.8	13.6	13.5	13.9
Ether extract[3]	4.2	2.2	3.9	7.7	10.9	8.9	9.7
Ash	5.9	10.1	10.5	6.8	6.1	6.5	7.4

[1]SBM = soybean meal topdress; HPCP = high-protein corn product topdress; SBMBP = soybean meal + bypass protein topdress; CANBP = canola meal + bypass protein topdress

[2]Protein topdress treatments included 16.4% dry ground corn, 4.3% calcium salts of long-chain fatty acids, soy hulls, AjiPro-L, and MetaSmart

[3]Protein top-dress treatments were subjected to an additional acid hydrolysis step due to inclusion of calcium salts of fatty acids

Table 0.4. Dry matter and water intake of lactating Holstein cows supplemented with a protein source consisting or soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP), or canola meal with bypass protein (CANBP)

	Diet[1]					
Item	SBM	HPCP	SBMBP	CANBP	SEM	*P*-value
DMI, kg/d	27.6[b]	27.3[b]	28.1[ab]	28.9[a]	0.46	<0.01
Orts CP, %	15.5[b]	18.8[a]	14.6[b]	15.0[b]	0.37	<0.001
N intake, g/d	753[ab]	722[b]	762[a]	783[a]	12.7	<0.001
CP intake, % of Total DMI	17.0[a]	16.5[b]	17.0[a]	16.9[a]	0.04	<0.001
Water intake, L/d	108.5[ab]	110.4[a]	111.9[a]	102.8[b]	3.0	<0.001

[1]SBM = soybean meal; HPCP = high-protein corn product; SBMBP = soybean meal + bypass protein; CANBP = canola meal + bypass protein

[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$)

Table 0.5. Production of milk, milk components, and milk nitrogen excretion of lactating Holstein cows supplemented with a protein source consisting of soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP) or canola meal with bypass protein (CANBP)

	Diet[1]					
Item	SBM	HPCP	SBMBP	CANBP	SEM	*P*-value
Milk, kg/d	39.9[b]	37.4[c]	42.0[a]	42.4[a]	1.79	<0.001
Fat, %	3.86	3.91	3.88	3.87	0.10	0.86
Protein, %	3.08[a]	3.00[b]	3.03[ab]	3.04[ab]	0.04	<0.01
Lactose, %	4.81	4.84	4.84	4.84	0.03	0.59
MUN, mg/dL	11.7[a]	10.0[c]	11.6[a]	10.8[b]	0.34	<0.01
SCC[2], 1,000 cells/mL	41.7	39.8	33.1	38.0	1.23	0.28
Fat, kg/d	1.50[bc]	1.44[c]	1.58[ab]	1.61[a]	0.05	<0.001
Protein, kg/d	1.21[a]	1.12[b]	1.27[a]	1.28[a]	0.05	<0.001
Lactose, kg/d	1.92[b]	1.80[c]	2.04[a]	2.06[a]	0.08	<0.001
Solids non-fat, kg/d	3.41[b]	3.17[c]	3.58[ab]	3.62[a]	0.15	<0.001
Fat-corrected milk, kg/d	41.5[b]	39.4[b]	43.8[a]	44.4[a]	1.50	<0.001
Energy-corrected milk, kg/d	41.7[b]	39.3[c]	43.9[ab]	44.4[a]	1.54	<0.001
Feed efficiency, kg FCM/kg DMI	1.52[ab]	1.49[b]	1.56[a]	1.52[ab]	0.03	0.06
Milk nitrogen secretion, g/d	198[a]	180[b]	204[a]	207[a]	8.2	<0.001

[1]SBM = soybean meal; HPCP = high-protein corn product; SBMBP = soybean meal + bypass protein; CANBP = canola meal + bypass protein

[2]Data were $\log_{10}$-transformed for analysis and means were reverse-transformed

[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$)

Table 0.6. Urine and feces nitrogen excretion, whole-body nitrogen balance, apparent total tract DM and CP digestibility, and body weight and body condition score change of lactating Holstein cows supplemented with protein source consisting of soybean meal (SBM), a high-protein corn product (HPCP), soybean meal with bypass protein (SBMBP), or canola meal with bypass protein (CANBP)

	Diet[1]					
Item	SBM	HPCP	SBMBP	CANBP	SEM	*P*-value
Urine N, %	0.70[ab]	0.63[b]	0.69[ab]	0.76[a]	0.03	<0.01
Urine output, L/d[2]	49.4	40.9	45.0	44.9	2.56	0.15
Urine N excretion, g/d	348[a]	255[b]	308[ab]	338[a]	23.9	0.02
Fecal N, % DM	2.7[c]	3.1[a]	2.8[b]	2.8[b]	0.02	<0.001
Fecal output, kg DM/d[3]	9.8[a]	10.2[a]	9.8[a]	10.4[a]	0.22	0.04
Fecal N excretion, g/d	264[c]	313[a]	270[bc]	287[b]	6.8	<0.001
N balance[4], g/d	-56	-29	-22	-53	25.1	0.65
Apparent total tract DM digestibility[5], %	64.7[a]	62.8[b]	65.2[a]	64.2[a]	0.34	<0.001
Apparent total tract N digestibility[6], %	65.1[a]	56.5[c]	64.6[ab]	63.4[b]	0.47	<0.001
Body weight change, kg/28 d	17.9	14.4	17.0	17.2	3.11	0.89
BCS change, units/28 d	0.03	0.01	0.07	0.11	0.04	0.19

[1]SBM = soybean meal; HPCP = high-protein corn product; SBMBP = soybean meal + bypass protein; CANBP = canola meal + bypass protein

[2]Estimated assuming a constant creatinine excretion rate of 29 mg/kg BW (Valadares et al., 1999)

[3]Estimated using 240-h indigestible NDF as an internal marker (Cochran and Galyean, 1994)

[4]Nitrogen balance = nitrogen intake – (milk nitrogen + urine nitrogen + fecal nitrogen)

[5]Apparent total tract digestibility = 100 – 100 x (g fecal DM ÷ g DM consumed); (Cochran and Galyean, 1994)

[6]Apparent total tract N digestibility = 100 – 100 x (g fecal N ÷ g N consumed); (Cochran and Galyean, 1994)

[abc]Means with different superscripts within a row are significantly different by Tukey's HSD ($P < 0.05$)

Table 0.7. Composition of high-protein corn products reported in the literature for comparison to the current novel and proprietary product utilized in this experiment.

	Nutrient analysis (% of DM)					
Source	CP	ADICP	NDF	ADF	Starch	EE
HPCP (this study)	56.1	-	4.0	3.3	2.6	1.6
Corn gluten meal						
Wohlt et al., 1991	66.6	-	-	-	-	0.7
Wohlt et al., 1991	51.8	-	-	-	-	1.8
Holter et al., 1992	66.8	-	14.5	8.3	-	1.2
High-protein distillers' grains						
Hubbard et al., 2009	46.1	-	26.4	15.6	-	4.6
Kelzer et al., 2009	46.1	4.1	26.4	15.6	9.1	4.6
Reduced fat distillers' grains						
Morris et al., 2018	34.4	-	27.5	12.3	-	7.4
Ramirez-Ramirez et al., 2016	31.5	-	37.7	11.0	6.7	6.6
Secondary fermentation of distillers' grains						
Lundy et al., 2015	39.1	-	32.7	15.2	1.6	7.3

FIGURES

Figure 0.1. Proprietary high-protein corn product fed to lactating Holstein cows as the sole protein concentrate source in the present study demonstrating a dark color indicative of Maillard product formation.

Chapter 3 - Effects of pre-cutting round alfalfa hay bales on forage quality and processing time

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ABSTRACT

Round hay balers with knives that cut the hay as it enters the baling chamber reduce the particle size upon baling and eliminating the need for a tub-grinder. The objective of this study was to evaluate the effects of a round hay baler with knives on forage quality of alfalfa hay at baling and after storage, and the effects of processing method on nutrient composition and particle size distribution. Alfalfa hay was baled (560 M Megawide HC^2, John Deere, Moline, IL) with knives every 10.2 cm (CUT; theoretical length of cut) or without knives (NORM). At baling and after 6 mo storage uncovered, bales were weighed, measured and 10 core samples obtained for nutrient analysis. Cores were separated into outer 15 cm and inner 15 to 46-cm segments to determine depth of spoilage. After storage, particle size was reduced to approximately 10 cm using a mixer wagon for CUT (CUT-MIX) or a tub grinder for NORM (NORM-GRIND). Grinding time, grinding shrink and post-processing factors were analyzed as a completely randomized design. Bale dimensions, weight, density, and post-processing factors were analyzed as a split-plot design, while pre-processing nutrient analysis was analyzed as a split-split-plot design with treatment as the whole plot, storage period as the sub-plot, and core depth as the sub-sub-plot. Compared with NORM, CUT increased bale weight and density (P<0.001). Core depth interacted with storage timepoint whereby ADF concentration increased more for outer than inner cores from baling to the end of storage (P<0.01), with similar effects for lignin (P<0.001) and 240-h uNDF (P=0.01). Compared with NORM, CUT increased concentrations of aNDFom, ADF and lignin (P<0.01), and decreased RFQ (P=0.02). The CUT-MIX treatment increased time to reduce particle size (P<0.001), but decreased processing shrink by 6.1% (P<0.001) compared with NORM-GRIND. Additionally, when compared with NORM-GRIND, CUT-MIX increased fiber content and decreased fiber digestibility, which may have been due to sampling

error from longer particle size. In summary, CUT produced larger, more dense bales and increased fiber content slightly, and CUT-MIX decreased processing shrink but increased fiber content with more longer particles after processing, which could be advantageous for physically effective fiber in ruminant diets. Further work should continue to evaluate leaf loss during baling and options for processing and incorporating pre-cut hay into diets.

Key words: density, shrink, round bale, particle size, grinding

Abbreviations: CUT, bales from cut treatment; NORM, bales from normal treatment; CUT-MIX, CUT bales combined with processing in a mixer wagon; NORM-GRIND, NORM bales combined with processing in a tub-grinder; DM, dry matter; ADF, acid detergent fiber; NDF, neutral detergent fiber; NDFom, neutral detergent fiber of organic matter; NDFD, neutral detergent fiber digestibility; uNDF, undigested neutral detergent fiber; RFV, relative forage value; RFQ, relative forage quality; TTNDFD, total tract neutral detergent fiber digestibility; CP, crude protein; IVDMD, *in vitro* dry matter digestibility; ADICP, acid-detergent insoluble crude protein

INTRODUCTION

The use of round hay bales in livestock feeding in North America is common, either through animals eating directly from the bale or caretakers incorporating hay into mixed diets delivered to the animals. If hay from round bales is to be incorporated into diets and delivered to animals, it must be disassembled to facilitate ease of mixing and to reduce particle size (Heinrichs et al., 1999) for stimulation of feed intake in high forage diets limited by gut fill (Jaster and Murphy, 1983; Kononoff and Heinrichs, 2003). A common method for round bale disassembly is the use of tub grinders which rapidly break down the bale and reduces hay particle size. While this method is relatively quick and effective, there are several drawbacks. First, a large amount of shrink occurs as high-value components of the feed drift away as small fragments into the environment, especially on windy days. Secondly, the ground hay typically is stored outdoors and exposed to moisture and wind for extended periods of time until it can be fed, with estimated losses incurred up to 20% (Kertz, 1998). Finally, the use of a tub-grinder requires specialized equipment, either purchased or contracted, creating an additional expense per ton of feed offered to the animal. The energy requirements of grinding also increase energy costs to reduce particle size.

Round hay balers with vertical knives reduce the particle size of hay before entering the baling chamber, which potentially eliminates the need for tub grinders to break apart round bales before incorporation into animal diets (Shinners, 2003). Hay produced in this manner is widely referred to as precut or presliced hay and provides unique feed management possibilities to protect forage losses during preparation for mixed ration delivery. While precut hay baler models have been available from various equipment manufacturers for over two decades, little peer-reviewed data is available on forage produced in this manner. The data that is available

focuses on animal feeding behavior and production performance (Hoffman et al., 2006; Loya-Olguin et al., 2008; Müller, 2009), forage quality and fermentation of wrapped precut hay bales (Borreani and Tabacco, 2006; Müller, 2009), and bale characteristics and power requirements for bale production (Tremblay et al., 1997).

There are a number of factors related to hay harvest and storage that contribute to hay quality. Harvest of alfalfa hay requires special consideration for retaining the leaf portion of the plant to prevent nutrient loss at multiple stages. Dry matter loss can be influenced by mower conditioner type and settings (Greenlees et al., 1999; Koegel et al., 1985), raking (Shearer et al., 1992), moisture content (Buckmaster, 1993) and baler type (Koegel et al., 1985). Furthermore, hay storage is estimated to be the greatest source of loss on the farm (Buckmaster et al., 1990), and storing bales under cover prevents DM loss (R L Belyea et al., 1985). Increasing density of wet hay at baling increases DM loss (Coblentz et al., 1996), but greater density in appropriately dried hay prevents moisture penetration and outer layer spoilage of hay stored outside (Russell et al., 1990). Precutting bales may alter several haying factors, such as leaf loss at baling or bale density, and therefore it may have effects on forage quality.

In hay production an estimated DM loss of 25% may occur between harvest and feeding (Buckmaster et al., 1990), so maximizing nutrient retention from harvest to feed delivery is critical ensure the nutritional requirements of the animal are met cost effectively. Considering the nutrient loss that occurs from harvest through feeding of round hay bales and the possibility that precutting bales may eliminate the need for tub-grinding, the objectives of this study were to 1) assess the effect of precutting alfalfa hay on forage quality at baling and after storage, and 2) determine the effects of post-storage processing method determined by bale production system on forage quality. We hypothesized that precutting alfalfa hay would have minimal impacts on

forage quality at baling and through storage, and that precut bales would retain more nutrients during processing with a mixer wagon due to less leaf loss to the environment compared with grinding bales.

MATERIALS AND METHODS

Experimental design and bale measurements

This trial was conducted on four farms in South Central Nebraska in the summer and winter of 2019 as a split-split-plot design. A John Deere 560 M Megawide HC^2 (John Deere Corporation, Moline, IL) was used to produce round alfalfa bales of either first or second cutting, with two farms for each cutting. Bales were produced using vertical knives spaced 10.2 cm apart between the pickup and the baling chamber (CUT) or with the knives retracted to produce a normal bale (NORM) and each treatment was applied to alternate bales as the baler moved through the field. Knives were not present on the outer 15 cm of the baler to preserve bale stability. There were 6 bales of each treatment produced on each farm for a total of 48 bales. Time to bale and wrap each bale was recorded. Before bales were moved, 4 diameter measurements were recorded for each end of the bale and 4 length measurements were recorded on each side. The diameter and length measurements were averaged, respectively, to calculate bale volume. Bales were weighed individually and stored uncovered in rows for 5 to 6 months. Bale measurements and weights were recorded again after storage.

Forage sampling and nutrient analysis

Core samples were obtained from each bale at baling and after storage. Ten core samples were obtained randomly from the sides of each bale before and after storage using a 46-cm hay

probe (Nasco, Ft. Atkinson, WI). Core samples were split into sections representing the outer 15 cm of the core, and the inner 15 to 46 cm of the core. Core depth sections from 2 bales of the same treatment at each farm were composited into one sample for analysis. All samples were ground through a 1 mm screen and analyzed for chemical composition using near infrared spectroscopy (NIR; Rock River Laboratory, Inc., Watertown, WI), which was calibrated using historical samples also analyzed with wet-chemistry methods in the same laboratory. The total tract neutral detergent fiber digestibility (TTNDFD) was calculated using standardized *in vitro* neutral detergent fiber (NDF) digestion measures (Goeser et al., 2009) for 24, 30 and 48 h, 240-h undigested NDF (uNDF) according to Lopes et al. (2015). Relative feed value (RFV) was calculated according to the equation: RFV = (digestible dry matter * dry matter intake)/1.29. Relative forage quality (RFQ) was calculated according to the equation: (dry matter intake, % of body weight)*(total digestible nutrients, % of body weight)/1.23.

Bale processing

After post-storage core samples and measurements were obtained, bales were subjected to different particle size reduction strategies based upon baling treatment. The NORM bales were ground individually in a tub-grinder with a 10 to 15 cm screen and ground hay was deposited directly into a twin-screw mixer wagon (NORM-GRIND; XLRation 2580, Helm Welding Limited, Lucknow, Ontario). Time to grind the entire bale was recorded. About 10% of each bale would not feed through the grinder, so the residual was subsequently dumped and added to the mixer wagon. Weight of the hay recovered in the mixer wagon was recorded on the wagon scales. The CUT bales were individually placed in the mixer wagon and mixed until the long particles were approximately the same length as the longest particles for NORM-GRIND

(approximately 10 cm; CUT-MIX). Time to reduce the particle size to the desired length in the mixer wagon was recorded and the weight was recorded on the wagon scales. The net wrap was left on each bale during processing.

After particle size reduction, the wagon was used to deposit each bale in a windrow approximately 15 m in length. Next, 2 sets of 12 subsamples were obtained from each windrow using a small hand scoop (Weiss et al., 2016) for nutrient analysis and determination of particle size distribution. The subsamples were taken alternately from the top, middle, and bottom of each windrow to account for variation of density among different particle sizes. For each set of subsamples, nutrient composition was determined using NIR and particle size distribution was determined twice using a Penn State Particle Size Separator with 3 sieves (19, 8 and 4-mm) and a bottom pan (Nasco, Ft. Atkinson, WI; Heinrichs, 2007). Contents of each sieve were dried in a 55° forced-air oven to determine dry matter content for each sieve.

Statistical analysis

Data were analyzed using JMP Pro (version 14.0, SAS Institute, Cary, NC) according to the following statistical models. Significant differences were declared at $P<0.05$ and tendencies were declared at $P<0.10$. The data for core sample nutrient analysis was analyzed as a split-split-plot design. The model for nutrient composition of bale core samples prior to processing was:

$$Y_{ijklm} = \mu + T_i + S_j + C_k + (T*S)_{ij} + (T*C)_{ik} + (S*C)_{jk} + (T*S*C)_{ijk} + F_l + B_m(F_l) + (B*T)_{mi}(F_l) + (B*S)_{mj}(F*T)_{li} + e_{ijklm}$$

where T_i is the fixed effect of treatment (i = 1 to 2), S_j is the fixed effect of point of time in storage (j = 1 to 2), C_k is the fixed effect of core depth (k = 1 to 2), T_i*S_j is the interaction of treatment and point of time in storage, $(T*C)_{ik}$ is the interaction of treatment and core depth,

$(S*C)_{jk}$ is the interaction of point of time in storage and core depth, $(T*S*C)_{ijk}$ is the three-way interaction of treatment, point of time in storage, and core depth, F_l is the random effect of farm ($l = 1$ to 4), $B_m(F_l)$ is the random effect of bale composite nested within farm ($m = 1$ to 24), $(B*T)_{mi}(F_l)$ is the random effect of the interaction of bale composite and treatment nested within farm, $(B*S)_{mj}(F*T)_{li}$ is the random effect of the interaction of bale composite and point of time in storage nested within treatment and farm, and e_{ijklm} is the residual error.

The data for bale dimensions, weight and density were analyzed as a split-plot design according to the model:

$$Y_{ijlm} = \mu + T_i + S_j + (T*S)_{ij} + F_l + B_m(F_l) + (B*T)_{mi}(F_l) + e_{ijlm}$$

where T_i is the fixed effect of treatment ($i = 1$ to 2), S_j is the fixed effect of point of time in storage ($j = 1$ to 2), $(T*S)_{ij}$ is the interaction of treatment and point of time in storage, F_l is the random effect of farm ($l = 1$ to 4), $B_m(F_l)$ is the random effect of bale composite nested within farm ($m = 1$ to 24), $(B*T)_{mi}(F_l)$ is the random effect of the interaction of bale composite and treatment nested within farm, and e_{ijlm} is the residual error.

Factors related to baling time and rate, and shrink for bale and processing were analyzed as a completely randomized design. The model for baling time and rate, and bale and processing shrink was:

$$Y_{ilm} = \mu + T_i + F_l + B_m(F_l) + e_{ilm}$$

where T_i is the fixed effect of treatment ($i = 1$ to 2), F_l is the random effect of farm ($l = 1$ to 4), $B_m(F_l)$ is the random effect of bale nested within farm ($m = 1$ to 48), and e_{ijklm} is the residual error.

Data for chemical composition and particle size analysis of bale and processing treatment combinations were analyzed as a completely randomized design according to the following model:

$$Y_{ilm} = \mu + T_i + F_l + B_m(T*F)_{il} + e_{ilm}$$

where T_i is the fixed effect of treatment (i = 1 to 2), F_l is the random effect of farm (l = 1 to 4), $B_m(T*F)_{il}$ is the random effect of bale nested within the interaction of farm and treatment (m = 1 to 48), and e_{ilm} is the residual error.

RESULTS

Baling time, rate, weight and dimensions

The CUT bales increased baling time per bale by 6 sec compared with NORM ($P = 0.01$; Table 4.1), but there was no evidence of difference for baling rate ($P = 0.74$). Accordingly, CUT increased bale weight 13% ($P < 0.001$; Table 4.1) and bale length by 2 cm ($P < 0.001$) compared with NORM, but there was no evidence of difference between treatment for bale diameter ($P = 0.74$; Table 4.1). The increase in CUT bale length increased bale volume by 0.06 m^3 ($P = 0.01$; Table 4.1); however, CUT increased bale density 10% compared with NORM ($P < 0.001$).

All bales lost a mean of 7.7% of dry matter (DM), or 44 kg, over the storage period ($P < 0.001$; Table 4.1), leading to a reduction diameter, volume and density ($P < 0.001$); however, there was no evidence of interaction between treatment and storage for any of these variables ($P > 0.14$). CUT had greater DM shrink compared with NORM ($P = 0.02$; Table 4.1), but there was no evidence of difference for percentage of shrink between treatment ($P = 0.16$).

Chemical composition of bales before processing

There tended to be a core and storage interaction for bale DM % whereby inner core segments increased more in DM% from baling to processing compared with outer core segments (P=0.06; Table 4.2). For all bales, inner core segments increased DM % (P=0.01; Table 4.2) and bale dry matter content after storage was less than at baling (P=0.02). There was no evidence of treatment, core or storage effects on crude protein (CP) or ash content ($P \geq 0.10$); however, there was a tendency for an interaction of core and storage for ash content (P=0.08; Table 4.2) with ash content decreasing from baling to processing for inner cores (11.1 vs 10.7 ± 0.38%) but not in outer cores.

Treatments had distinct impacts on fiber content. There was an interaction of core and storage for acid detergent fiber (ADF; P<0.01; Table 4.2) content whereby inner cores increased 1.1% in ADF content from harvest to processing (36.9 vs 38.0 ± 0.48%) but outer cores increased 2.7% (37.1 vs 39.7 ± 0.48%). There was also an interaction for core and storage for lignin (P<0.001; Table 4.2) whereby outer cores increased 0.7% over the storage period. For both ADF and lignin, content was increase by CUT compared with NORM (P<0.01; Table 4.2), outer cores compared with inner cores (P<0.001), and at the end of storage compared with at baling (P<0.001). The CUT increased NDF and ash-free NDF (NDFom; P<0.01; Table 4.2) content compared with NORM, and both NDF and NDFom were higher at the end of storage than at baling (P<0.001; Table 4.2). Furthermore, outer cores tended to have greater NDF content compared with inner cores (P=0.06; Table 4.2).

There was in interaction for acid-detergent insoluble crude protein (ADICP) as a percentage of total DM, whereby ADICP increased more for outer cores than inner cores during the storage period (0.18 vs 0.06 ± 0.04%; P<0.001; Table 4.2). There was a similar interaction

for ADICP as percent of CP (P<0.001; Table 4.2). Additionally, treatment and storage period interacted for ADICP as a percent of DM (P=0.02; Table 4.2) and of CP (P=0.05). As a percent of total DM, ADICP increased more for CUT than NORM over the storage period (0.08 vs 0.15 ± 0.04%); however, these differences were not significant according to a Tukey's HSD comparison (P > 0.05). Similarly, as a percent of CP, ADICP increased more for CUT than NORM over the storage period (0.77 vs 0.45 ± 0.21%). The ADICP content as a percent of DM and CP increased over the storage period (P<0.001) and for outer cores (P<0.001). The CUT bales tended to increase ADICP as a percent of CP (P=0.08; Table 4.2).

There was an interaction of core and storage for 240 h NDF digestibility (NDFD; P=0.001; Table 4.2), whereby inner cores did not change in digestibility, but outer cores decreased in digestibility 2.2% from baling to the end of storage (52.2 vs 50.0 ± 0.52%). The CUT tended to decrease NDF digestibility at 30, 48 (P=0.08; Table 4.2) and 120 h (P=0.06) compared with NORM. The concentration of 30, 120, and 240 h uNDF was greater at the end of storage than at baling (P<0.001 Table 4.2). The CUT increased 30, 120 and 240 h uNDF content (P=0.01; Table 4.2) compared with NORM. Outer cores had greater 240 h uNDF content (P=0.03; Table 4.2) and tended to increase 30 h uNDF content (P=0.08). There was an interaction between storage and core for TTNDFD (P<0.001; Table 4.2), whereby there was no change for inner cores over the storage period, but outer cores decreased in TTNDFD over the storage period (29.1 vs 33.5 ± 0.94%). Overall, storage and outer cores decreased TTNDFD (P<0.001; Table 4.2), and CUT tended to decrease TTNDFD compared with NORM (P=0.08)

The relative forage value (RFV, P<0.001; Table 4.2) and relative forage quality (RFQ, P=0.001) was decreased after storage, and CUT decreased both RFV (P<0.01; Table 4.2) and

RFQ (P=0.02) compared with NORM. Outer cores had lower RFV compared with inner cores (P=0.01; Table 4.2).

Chemical composition and particle size after processing, and processing efficiency

The CUT-MIX tended to increase windrow DM % (91.5 vs. 91.1 ± 0.36%; P=0.06; Table 4.3) and decrease windrow CP % (19.1 vs 19.6 ± 1.26%; P=0.06) compared with NORM-GRIND. The CUT-MIX also increased windrow ADF, NDF, ADFom and lignin content by 1.2, 1.6, 1.8, and 0.5%, respectively (P<0.001; Table 4.3) compared with NORM-GRIND, but there was no evidence of difference in ash % (P=0.93). Further, compared with NORM-GRIND, CUT-MIX increased ADICP as a percent of DM (1.2 vs 1.1 ± 0.05%; P<0.01; Table 4.3) and as a percent of CP (6.3 vs 5.9 ± 0.39%; P<0.001). Treatment affected NDFD whereby CUT-MIX decreased NDFD at 30 h (39.7 vs 41.1 ± 0.96%; P<0.001; Table 4.3), 48 h (44.6 vs. 47.0 ± 0.62%; P<0.001), 120 h (46.9 vs. 48.7 ± 0.98%; P=0.001), and 240 h (49.0 vs. 50.8 ± 1.30%; P<0.01) compared with NORM-GRIND. Accordingly, CUT-MIX increased uNDF by 1.6, 1.7, and 1.6% for 30, 120, and 240 h, respectively (P<0.001; Table 4.3) compared with NORM-GRIND, and decreased TTNDFD (28.7 vs. 29.7 ±0.94%, P=0.03). The CUT-MIX also decreased RFV and RFQ by 5 and 10 points, respectively (P<0.001; Table 4.3) compared with NORM-GRIND.

Treatment also affected particle size distribution of the processed hay bales. Compared with NORM-GRIND, CUT-MIX increased the percent of total DM remaining on screen 1 (35.1 vs. 13.7 ± 1.58%; P<0.001; Table 4.3) and screen 2 (20.3 vs. 19.6 ± 1.14%; P=0.04). Accordingly, CUT-MIX decreased the percent of total DM remaining on screen 3 (13.3 vs 16.6 ±

0.65%; $P<0.001$; Table 4.3) and the bottom pan (31.2 vs. 49.9 ± 1.82%; $P<0.001$) compared with NORM-GRIND.

The CUT-MIX increased time to reduce particle size by 7.4 min ($P < 0.001$; Table 4.3), which reduced the processing rate by 130 kg DM/min ($P < 0.001$). Despite this, CUT-MIX had 73% less DM loss during processing ($P < 0.001$; Table 4.3), which reduced the loss as a % of total DM (1.6 vs. 8.1 ± 0.95%; $P < 0.001$).

DISCUSSION

Bale weight and dimensions

In the present study, CUT increased weight and density compared with NORM, which is supported by another study that created baleage with a precutting system (Borreani and Tabacco, 2006). Other precutting round balers have failed to alter alfalfa bale weight (Jones et al., 2010b) or timothy/bluegrass bale mass and density across a range of DM from 36 to 82% (Tremblay et al., 1997). The ability to produce heavier bales equates to less time spent wrapping bales considering round balers must stop during this final step, and this should result in less time required for baling large fields. Furthermore, creating fewer bales of greater density saves space in the storage area, and heavier bales have less handling and storage costs per ton of DM (Harrigan et al., 1994). There was no evidence of difference for baling rate in our study, but baling time was increased for CUT compared with NORM because of the greater bale mass. Tremblay et al. (1997) reported a numerical reduction in material flow and field capacity for pre-cut bales compared with normal bales, but they unfortunately did not statistically analyze this difference.

We did not expect differences in bale dimensions at the time of baling since the baler was set to produce the same size of bale for each treatment; however, the added length for CUT may be due to the greater internal density of the bale creating pressure on the outer portions of the bale. The baler does not have blades on the outer 15 cm to help maintain bale integrity during moving, so the internal pressure may have forced the less dense ends out slightly. This minimal length increase was enough to significantly increase the volume for CUT compared with NORM bales but CUT bales were still more dense when combined with the greater overall weight.

All bales lost DM weight, diameter, volume and density from the beginning to end of storage, but there was no interaction with treatment. Loss of DM is common with round hay bales stored on the ground (Huhnke, 1990, 1988) and exposed to the elements (Coblentz, 2009); however some have observed no loss of DM in uncovered bales during dry years (Taylor et al., 1994). While CUT bales had greater DM shrink over the storage period, this was a factor of heavier bales because shrink as a percentage of initial bale weight was not different between treatments (Table 4.1). It is common for hay bales to lose from 5 to 20% of total dry matter over the storage period depending on storage conditions (Russell and Buxton, 1985; Montgomery et al., 1986; Coblentz, 2009), so the 7% DM shrink in this study aligns with previous data.

Bale quality before processing

An increase in DM content for inner core segments from beginning to end of storage compared with outer core segments may have been a factor of being stored outside where moisture penetration could occur on the outer layer. Bale DM decreases in outer bale segments that are exposed to the elements (Coblentz, 2009; Russell et al., 1990), but bale DM is dynamic depending on moisture at baling. Bale DM increases after baling as a normal process when

baled at greater moisture content (Taylor et al., 1994); however, bale DM may also decrease over storage if hay is baled at low DM and stored outside (Taylor et al., 1994). The fact that bales in this study were wetter at the end of storage may reflect exposure to the elements considering the very low moisture content of the hay at baling, and moisture can vary over the storage period depending on conditions (Russell and Buxton, 1985). Additionally, bales stored directly on the ground compared with being elevated on pallets have greater moisture content in the surface layers (Coblentz, 2009), so it is possible that our bales absorbed moisture from the ground. We did not obtain samples from the bottom of the bale, so we cannot confirm if this is the mechanism of the observed change.

There was no evidence of treatment effects on CP concentration for any of the main effects. This aligns with other reports for pre-cut alfalfa baleage (Borreani and Tabacco, 2006) and pre-cut dry alfalfa hay in square bales (Hoffman et al., 2006), while others have demonstrated a decrease in CP in grass hay harvested as baleage (Müller, 2009) or an increase in CP for pre-cut bales (Jones et al., 2010a). Additionally, other authors have noted no change in CP concentration in conventional bales of dry alfalfa hay during storage, or even an increase in CP and available CP (Huhnke, 1988; Russell and Buxton, 1985; Taylor et al., 1994). The alfalfa hay in those studies had a similar CP content to that reported in this study, about 18%. More intuitively, other studies have documented a decline in nitrogen content over storage for the weathered layer (Collins et al., 1987). Hay stored outside has greater ADIN as a percentage of total N in outer layers, which may indicate nutrient leaching if stored outdoors (Rotz and Muck, 1994; Russell et al., 1990). This aligns with the increased ADICP as a percent of CP in outer layers over storage in the present study. The ADICP content may also increase over time (Coblentz et al., 1996), especially in wetter bales (Coblentz et al., 2000; Russell and Buxton,

1985), with ADICP being a factor of heat formation within the bale (Coblentz et al., 2010). Higher density round bales have more internal heating (Buckmaster et al., 1989; Russell et al., 1990) from reduced relative surface area to dissipate heat from post-harvest respiration (Shinners et al., 1996); however, in this study the low DM of the bales at baling is not likely a contributor to the increased ADICP.

Furthermore, content of NDF, ADF and lignin increased over the storage period for the bales in the present study. It is common to observe an increase in ADF, NDF (Coblentz et al., 1996; Huhnke, 1990; Taylor et al., 1994) and lignin (Rotz and Abrams, 1988) over the storage period. It has been postulated that increases in fiber content during the storage period are not a factor of changes of the quantity of fiber components, but rather the reduction of soluble carbohydrates that are lost during microbial respiration during the initial storage period, or from soluble carbohydrate leaching in the outer layers (Rotz and Muck, 1994). The interaction between core depth and storage in the present study indicated that ADF and lignin increased more for outer segments of the bale over the storage period than inner segments. This is supported by other studies where bales stored outside experienced greater NDF, ADF and lignin content in the outer layers of the bale (Coblentz, 2009; Russell et al., 1990; Taylor et al., 1994) where exposure to moisture could leach soluble components of the plant. Along with these storage and core effects, CUT increased NDF, ADF and lignin content compared with NORM. A 1.5% increase in NDF has been previously demonstrated for pre-cut alfalfa square bales (Hoffman et al., 2006) while Müller (2009) did not observe a difference in fiber components at baling of wet forages. It is difficult to determine if the increase in fiber content for CUT is a factor of the baling process or the storage conditions imparted by more dense bales. Theoretically, the reduced particle size of the forage may have allowed more leaching of soluble

nutrients. Further, additional agitation of the forage by the knives in the baler may increase leaf loss, and while manipulation of hay prior to baling has been shown to alter CP concentration due to leaf loss, it does not necessarily affect ADF or NDF content (Shearer et al., 1992). Baling hay at moisture content above 20% can increase fiber content through increased microbial respiration depleting soluble carbohydrates during the initial storage phase (Rotz and Muck, 1994), but low bale moisture of less than 10% at the time of baling in this study is not indicative of much post-baling heating (Buckmaster et al., 1989; Coblentz et al., 2000, 1996) common of microbial respiration of soluble carbohydrate. We could also speculate that the agitation of the cutting processed reduced the quantity of leaves that were attached to the stem, which potentially allowed them to fall through the bale matrix as the core sampling device was moving through the bale, thus increasing the fiber content.

In the present study, CUT tended to decrease NDF digestibility at 30, 48 and 120 h compared with NORM, and outer cores decreased more in 240 h NDF digestibility over the storage period (Table 4.2). Much of the digestibility data in the literature for hay bales over the storage period and for different segments of the bale has been conducted using *in vitro* or *in situ* techniques and only evaluate total DM disappearance, compared with using NIR in the present study to evaluate NDF digestibility. *In vitro* DM digestibility (IVDMD) for bales stored outside decreases when stored outside (Huhnke, 1988; Rotz and Abrams, 1988), particularly for the outer portions of the bale (Coblentz, 2009; Huhnke, 1988; Russell et al., 1990), which is similar to our results at 240 h for NDF digestibility of outer cores. Furthermore, NDF digestibility is influenced greatly by stage of maturity of the alfalfa plant at harvest (Palmonari et al., 2014), and leaf ADF and NDF digestibility is greater than that of the stems (Collins, 1988) suggesting that

leaf loss may have occurred for CUT since this bales within farm were harvested from the same field and bales were produced with alternating treatments.

Bale processing

Processing the two bale treatments with different methods was conducted to compare a typical processing method of NORM bales in the Great Plains region with a potential application with the CUT bales to reduce machinery needs. As such, the processing methods combined with the baling treatments are considered a separate treatment from the bales before processing. Additionally, there is a paucity of data available evaluating DM losses and forage quality changes during particle size reduction, so this study begins to fill a void in that area.

The increased time for processing CUT-MIX compared with NORM-GRIND is likely due to the efficiency and aggressive nature of the tub-grinding method compared with mixer wagons. In comparing the ability of a mixer wagon to break apart pre-cut bales, an extension publication demonstrated that pre-cut alfalfa and cornstalk bales broke apart more rapidly in a mixer wagon than conventional bales (Jones et al., 2010b), but the bales were only broken down in a mixer wagon and were not subject to full particle size reduction for feeding. In the present study, reduction of the particle size for CUT-MIX was determined to be achieved by visual appraisal to be similar to the NORM-GRIND for the longest particle size. Particle size distribution of these forages will be discussed below.

The CUT-MIX bales had less shrink during the particle size reduction, presumably due to less dust emitted compared with NORM-GRIND. Dry matter shrink is evident in grinding of hay, and while some effort has been placed by the medical community on determining the health effects of chopping and grinding hay particulates in agricultural environments (Jones et al.,

1995), this is the first data we are aware of being published quantifying the loss of hay during grinding or mixing.

Most data available on the change in chemical composition of forages due to grinding or chopping are for very small particle size. In general, chemical composition of forages in studies where treatments consist of a long and short particle size derived from the same source is largely assumed to be the same (R. L. Belyea et al., 1985; Kononoff and Heinrichs, 2003; Leonardi and Armentano, 2003). However, a few studies reporting chemical composition of chopped alfalfa show variable numerical differences in CP and NDF (Belyea et al., 1989; Rogers et al., 1985; Shaver et al., 1986) or no evidence of difference in chopped sorghum (Osafo et al., 1997). This demonstrates either the potential effects of chopping or grinding on forage quality, or a difficulty in obtaining accurate samples depending on particle size.

In the present study, CUT-MIX consistently increased fiber components and reduced digestibility compared with NORM-GRIND, while also reducing CP content. We were surprised by the increase in fiber after processing for CUT-MIX because of the lesser DM shrink during processing. Admittedly, the CUT bales prior to processing had greater fiber content, but the magnitude of the difference was amplified when the processing treatments were applied leading us to believe that difficulty in sampling may have been realized due to the differences in particle size distribution between treatments. The CUT-MIX had greater long particles compared with the NORM-GRIND. Sampling loose forages and total mixed rations is notoriously difficult because of the variability in particle size and the ability of small particles to fall through the forage matrix during sampling (Weiss et al., 2016). Therefore, sampling is the greatest contributor to nutrient composition variability within a farm (St-Pierre and Weiss, 2015), and others have purposely avoided sampling long alfalfa hay because of the difficulty involved in

obtaining accurate results (Heinrichs et al., 1999). In our case, the NORM-GRIND windrows had greater flowability with the smaller particle size that likely allowed us to more minimally disturb the matrix while sampling.

The mixing process for CUT-MIX was stopped when the apparent particle size reached similar length as the longest particles on the NORM-GRIND. Indeed, mixing for a longer period of time may have reduced the particle size of the hay more, but the amount of reduction is incrementally less after approximately 8 to 15 minutes of mixing (Heinrichs et al., 1999). Mixing a bale to achieve reduced particle size for greater than 15 minutes is not likely realistic in an on-farm scenario. The overall distribution of the NORM-GRIND particle size is similar to the distribution of 2,815 legume forage samples analyzed at a commercial laboratory in the eastern U.S. (Heinrichs et al., 1999).

One advantage of the longer particle size for CUT-MIX is the ability of that hay to provide physically effective fiber. Alfalfa hay is typically one of the most costly forage sources in a ruminant diet. Grinding forages through a tub grinder extensively reduces particle size, so using the CUT-MIX as a method to reduce particle size while still maintaining physically effective fiber in diets that lack other long fiber sources may be of benefit (Zebeli et al., 2012).

The design of the experiment that combines the baling and processing methods into one treatment for the particle size reduction process limits our interpretation of the impact of the two processing methods on forage quality and particle size, especially considering there was a significant difference for chemical composition before processing. It is unclear if there would have been a difference for forage quality and particle size between the CUT and NORM bales if they had both been processed in the mixer wagon, and perhaps a factorial design for the processing portion of this study may have helped isolate the effects of the baling and processing

methods. To attempt to elucidate any differences that may have existed due to processing method, we tested the interaction of treatment and chemical composition for newly formed bales to predict post-processing chemical composition. There was no evidence of interaction (Appendix B), indicating a lack of processing method influence on the final chemical composition, but these results should be interpreted with caution.

CONCLUSION

Producing round alfalfa hay bales using a pre-cutting mechanism increased bale mass and density. The CUT treatment significantly increased the content of NDF, ADF and lignin compared with NORM, but the magnitude of differences were small. There were minimal interactions between treatment and core depth or storage time. During processing, CUT-MIX decreased DM shrink compared with NORM-GRIND, but it increased fiber content and reduced digestibility of the hay while altering the particle size distribution. The increase in fiber content after processing may have been influenced by sampling error due to different particle size distribution between treatments. Further work should be conducted to determine the cause of the increased fiber content for CUT bales, and additional options for incorporating CUT bales into total mixed rations should be evaluated.

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REFERENCES

Belyea, R. L., Marin, P.J., Sedgwick, H.T., 1985. Utilization of Chopped and Long Alfalfa by Dairy Heifers. J. Dairy Sci. 68, 1297–1301. https://doi.org/10.3168/jds.S0022-0302(85)80960-7

Belyea, R L, Martz, F., Bell, S., 1985. Storage and Feeding Losses of Large Round Bales. J. Dairy Sci. 3371–3375. https://doi.org/10.3168/jds.S0022-0302(85)81250-9

Belyea, R.L., Martz, F.A., Mbagaya, G.A., 1989. Effect of Particle Size of Alfalfa Hay on Intake, Digestibility, Milk Yield, and Ruminal Cell Wall of Dairy Cattle. J. Dairy Sci. 72, 958–963. https://doi.org/10.3168/jds.S0022-0302(89)79189-X

Borreani, G., Tabacco, E., 2006. The Effect of a Baler Chopping System on Fermentation and Losses of Wrapped Big Bales of Alfalfa. Agron. J. 98, 1–7. https://doi.org/10.2134/agronj2004.0134

Buckmaster, D.R., 1993. Alfalfa Raking Losses as Measured on Artificial Stubble. Trans. Am. Soc. Agric. Eng. 36, 645–651.

Buckmaster, D.R., Rotz, C., Black, J., 1990. Value of alfalfa losses on dairy farms. Trans. Am. Soc. Agric. Eng. 33, 351–360.

Buckmaster, D.R., Rotz, C.A., Martens, D.R., 1989. A Model of Alfalfa Hay Storage. Trans. Am. Soc. Agric. Eng. 32, 30–36.

Coblentz, W.K., 2009. Effects of Wrapping Method and Soil Contact on Hay Stored in Large Round Bales in Central Wisconsin. Appl. Eng. Agric. 25, 835–850. https://doi.org/10.1002/mrdd

Coblentz, W.K., Fritz, J.O., Bolsen, K.K., Cochran, R.C., 1996. Quality Changes in Alfalfa Hay during Storage in Bales. J. Dairy Sci. 79, 873–885. https://doi.org/10.3168/jds.S0022-0302(96)76436-6

Coblentz, W.K., Hoffman, P.C., Martin, N.P., 2010. Effects of spontaneous heating on forage protein fractions and in situ disappearance kinetics of crude protein for alfalfa-orchardgrass hays packaged in large round bales. J. Dairy Sci. 93, 1148–1169. https://doi.org/10.3168/jds.2009-2701

Coblentz, W.K., Turner, J.E., Scarbrough, D.A., Lesmeister, K.E., Johnson, Z.B., Kellogg, D.W., Coffey, K.P., Mcbeth, L.J., Weyers, J.S., 2000. Storage Characteristics and Nutritive Value Changes in Bermudagrass Hay as Affected by Moisture Content and Density of Rectangular Bales. Crop Sci. 40, 1375–1383. https://doi.org/10.2135/cropsci2000.4051375x

Collins, M., 1988. Composition and Fibre Digestion in Morphological Components of an Alfalfa-Timothy Sward. Anim. Feed Sci. Technol. 19, 135–143.

Collins, M., Paulson, W.H., Finner, M.F., Jorgensen, N.A., Keuler, C.R., 1987. Moisture and Storage Effects on Dry Matter and Quality Losses of Alfalfa in Round Bales. Trans. ASAE 30, 913–917.

Goeser, J.P., Hoffman, P.C., Combs, D.K., 2009. Modification of a rumen fluid priming

technique for measuring in vitro neutral detergent fiber digestibility. J. Dairy Sci. 92, 3842–3848. https://doi.org/10.3168/jds.2008-1745

Greenlees, W., Hanna, H., Shinners, K., Marley, S.J., Bailey, T., 1999. A Comparison of Four Mower Conditioners on Drying Rate and Leaf Loss in Alfalfa and Grass. Appl. Eng. Agric. 16, 15–21.

Harrigan, T.M., Rotz, C.A., Black, J.R., 1994. Comparison of large round bale storage and feeding systems on dairy farms. Appl. Eng. Agric. 10, 479–491.

Heinrichs, A.J., 1996. Evaluating particle size of forages and TMRs using the Penn State Particle Size Separator, Coop. Ext. Bull. DAS 96-20, College of Agricultural Sciences, Pennsylvania State University, University Park.

Heinrichs, A.J., Buckmaster, D.R., Lammers, B.P., 1999. Processing, mixing, and particle size reduction of forages for dairy cattle. J. Anim. Sci. 77, 180–186. https://doi.org/10.2527/1999.771180x

Hoffman, P.C., Simson, C.R., Shinners, K.J., 2006. Evaluation of Hay Feeding Strategies on Feed Sorting Behavior of Dairy Heifers Fed Mock Lactation Diets. Prof. Anim. Sci. 22, 71–79. https://doi.org/10.15232/S1080-7446(15)31063-9

Huhnke, R.L., 1990. Round bale bermudagrass hay storage losses. Appl. Eng. Agric. 6, 396–400.

Huhnke, R.L., 1988. Large round bale alfalfa hay storage. Trans. Am. Soc. Agric. Eng. 4, 316–318.

Jaster, E.H., Murphy, M.R., 1983. Effects of Varying Particle Size of Forage on Digestion and Chewing Behavior of Dairy Heifers. J. Dairy Sci. 66, 802–810. https://doi.org/10.3168/jds.S0022-0302(83)81860-8

Jones, S.Q., DeRouchey, J.M., Waggoner, J.W., Breiner, R.M., Marston, T.T., Kraus, T.J., 2010a. Round bale alfalfa processing method affects heifer growth but does not influence wastage or eating preference, in: Kansas Agricultural Experiment Station Research Reports. pp. 43–46. https://doi.org/https://doi.org/10.4148/2378-5977.2883

Jones, S.Q., Marston, T.T., Kraus, R.M., Derouchey, J.M., Waggoner, J.W., Breiner, R.M., 2010b. Precutting round alfalfa and cornstalk bales decreases time and fuel required for bale breakup in a vertical mixer, in: Kansas Agricultural Experiment Station Research Reports. pp. 11–14. https://doi.org/https://doi.org/ 10.4148/2378-5977.2882 This

Jones, W.G., Dennis, J.W., May, J.J., Whitmer, M.P., Siegel, P.D., Sorenson, W.G., Schwegler-Berry, D., Kullman, G.J., 1995. Dust Control During Bedding Chopping. App. Occup. Environ. Hyg. 10, 467–475.

Kertz, A.F., 1998. Variability in Delivery of Nutrients to Lactating Dairy Cows. J. Dairy Sci. 81, 3075–3084. https://doi.org/10.3168/jds.S0022-0302(98)75872-2

Koegel, R.G., Straub, R.J., Walgenbach, R.P., 1985. Quantification of Mechanical Losses in Forage Harvesting. Trans. Am. Soc. Agric. Eng. 28, 1047–1051. https://doi.org/10.13031/2013.32385

Kononoff, P.J., Heinrichs, A.J., 2003. The Effect of Reducing Alfalfa Haylage Particle Size on Cows in Early Lactation. J. Dairy Sci. 86, 1445–1457. https://doi.org/10.3168/jds.S0022-0302(03)73728-X

Leonardi, C., Armentano, L.E., 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86, 557–564. https://doi.org/10.3168/jds.S0022-0302(03)73634-0

Lopes, F., Cook, D.E., Combs, D.K., 2015. Validation of an in vitro model for predicting rumen and total-tract fiber digestibility in dairy cows fed corn silages with different in vitro neutral detergent fiber digestibilities at 2 levels of dry matter intake. J. Dairy Sci. 98, 574–585. https://doi.org/10.3168/jds.2014-8661

Loya-Olguin, F., Avendaño-Reyes, L., Encinias, A.M., Walker, D.A., Elam, N.A., Soto-Navarro, S.A., 2008. Influence of slice baling on feeding value of alfalfa hay in receiving and finishing diets for feedlot cattle. J. Anim. Sci. 86, 2749–2755. https://doi.org/10.2527/jas.2007-0637

Montgomery, M.J., Tineo, A., Al, M.E.T., 1986. Effect of Moisture Content at Baling on Nutritive Value of Alfalfa Orchardgrass Hay in Conventional and Large Round Bales. J. Dairy Sci. 69, 1847–1853. https://doi.org/10.3168/jds.S0022-0302(86)80610-5

Müller, C.E., 2009. Long-stemmed vs. cut haylage in bales — Effects on fermentation, aerobic storage stability, equine eating behaviour and characteristics of equine faeces. Anim. Feed Sci. Technol. 152, 307–321. https://doi.org/10.1016/j.anifeedsci.2009.04.016

Osafo, E.L.K., Owen, E., Said, A.N., Gill, M., Sherington, J., 1997. Effects of amount offered and chopping on intake and selection of sorghum stover by Ethiopian sheep and cattle. Anim. Sci. 65, 55–62.

Palmonari, A., Fustini, M., Canestrari, G., Grilli, E., Formigoni, A., 2014. Influence of maturity on alfalfa hay nutritional fractions and indigestible fiber content. J. Dairy Sci. 97, 7729–7734. https://doi.org/10.3168/jds.2014-8123

Rogers, J.A., Muller, L.D., Snyder, T.J., Maddox, T.L., 1985. Milk Production, Nutrient Digestion, and Rate of Digesta Passage in Dairy Cows Fed Long or Chopped Alfalfa Hay Supplemented with Sodium Bicarbonate. J. Dairy Sci. 68, 868–880. https://doi.org/10.3168/jds.S0022-0302(85)80904-8

Rotz, C.A., Abrams, S.M., 1988. Losses and Quality Changes During Alfalfa Hay Harvest and Storage. Trans. Am. Soc. Agric. Eng. 31, 350–355. https://doi.org/10.13031/2013.30713

Rotz, C.A., Muck, R.E., 1994. Changes in Forage Quality During Harvest and Storage, in: National Conference on Forage Quality, Evaluation, and Utilization. Lincoln, NE, pp. 828–868. https://doi.org/10.2134/1994.foragequality.c20

Russell, J.R., Buxton, D.R., 1985. Storage of large round bales of hay harvested at different moisture concentrations and treated with sodium diacetate and/or covered with plastic. Anim. Feed Sci. Technol. 13, 69–81.

Russell, J.R., Yoder, S.J., Marley, S.J., 1990. The Effects of Bale Density, Type of Binding and Storage Surface on the Chemical Composition, Nutrient Recovery and Digestibility of

Large Round Hay Bales. Anim. Feed Sci. Technol. 29, 131–145.

Shaver, R.D., Nytes, A.J., Satter, L.D., Jorgensen, N.A., 1986. Influence of Amount of Feed Intake and Forage Physical Form on Digestion and Passage of Prebloom Alfalfa Hay in Dairy Cows. J. Dairy Sci. 69, 1545–1559. https://doi.org/10.3168/jds.S0022-0302(86)80571-9

Shearer, S.A., Turner, G.M., Collins, M., Peterson, W.O., 1992. Effect of swath and windrow manipulation on alfalfa drying and quality. Appl. Eng. Agric. 8, 303–307.

Shinners, K.J., 2003. Engineering Principles of Silage Harvesting Equipment, in: Buxton, D.R., Muck, R.E., Harrison, J.H. (Eds.), Silage Science and Technology. American Society of Agronomy, Madison, WI, USA, pp. 361–403.

Shinners, K.J., Straub, R.J., Huhnke, R.L., Undersander, D.J., 1996. Harvest and storage losses associated with mid-size rectangular bales. Appl. Eng. Agric. 12, 167–173.

St-Pierre, N.R., Weiss, W.P., 2015. Partitioning variation in nutrient composition data of common feeds and mixed diets on commercial dairy farms. J. Dairy Sci. 98, 5004–5015. https://doi.org/10.3168/jds.2015-9431

Taylor, R.K., Blasi, D.A., Shroyer, J.P., 1994. Storage losses in net-wrapped, large round bales of alfalfa. Appl. Eng. Agric. 10, 317–320. https://doi.org/10.4148/2378-5977.2099

Tremblay, D., Savoie, P., LePhat, Q., 1997. Power requirements and bale characteristics for a fixed and a variable chamber baler. Can. Agric. Eng. 39, 73–76.

Weiss, B., Zhang, P., Goeser, J., St-Pierre, N., 2016. TMR Sampling: Valuable Exercise or a Random Number Generator?, in: Tri-State Dairy Nutrition Confererence. Ft. Wayne, IN, pp. 137–147.

Zebeli, Q., Aschenbach, J.R., Tafaj, M., Boguhn, J., Ametaj, B.N., Drochner, W., 2012. Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle. J. Dairy Sci. 95, 1041–1056. https://doi.org/10.3168/jds.2011-4421

TABLES

Table 3.1. Effects of a pre-cutting hay baler on round alfalfa bale baling efficiency, bale weight, density, dimensions and shrink over a 5 to 6-month storage period.

	Treatment		Storage			*P*-value		
Item	Cut	Normal	Begin	End	SEM	Trt	Storage	Trt*Storage
Baling time, seconds	60	54	-	-	3.7	0.01	-	-
Baling rate, kg DM/min	617	611	-	-	53.8	0.74	-	-
Bale characteristics								
Weight, kg DM	581	513	569	525	19.8	<0.001	<0.001	0.14
Length, cm	160	158	159	159	0.2	<0.001	0.81	0.34
Diameter, cm	165	165	166	163	4.3	0.74	<0.001	0.59
Volume, m^3	3.43	3.37	3.45	3.34	0.18	0.01	<0.001	0.82
Density, kg DM/m^3	170	154	165	159	5.9	<0.001	<0.001	0.10
Storage shrink, kg DM	49	38	-	-	11.9	0.02	-	-
Storage shrink, %	7.9	7.0	-	-	1.82	0.16	-	-

Table 3.2. Effect of a pre-cutting baler, core depth, and storage timepoint on alfalfa bale chemical composition, quality and digestibility[1].

	Treatment		Core Depth		Storage			*P*-value[2]				
Item[1]	Cut	Normal	Inner	Outer	Begin	End	SEM	Trt	Core	Storage	Core* Storage	Trt* Storage
Bale DM%	89.5	89.5	89.8	89.3	90.5	88.6	1.36	0.96	0.01	0.02	0.06	0.46
Crude protein, %	18.8	19.0	19.0	18.8	18.9	18.9	1.07	0.31	0.10	0.36	0.98	0.70
ADF, %	38.2	37.6	37.4	38.4	37.0	38.8	0.48	<0.01	<0.001	<0.001	<0.01	0.41
NDF, %	45.4	44.8	44.9	45.4	44.4	45.9	0.81	<0.01	0.06	<0.001	0.64	0.64
NDFom, %	41.9	41.3	41.4	41.7	40.7	42.4	0.95	<0.01	0.25	<0.001	0.71	0.61
Lignin, %	8.7	8.5	8.5	8.7	8.4	8.9	0.10	<0.01	<0.001	<0.001	<0.001	0.47
Ash, %	11.1	10.8	10.9	11.1	11.1	10.9	0.38	0.32	0.18	0.39	0.08	0.77
ADICP, % DM	0.8	0.8	0.8	0.9	0.8	0.9	0.04	0.36	<0.001	<0.001	<0.001	0.02
ADICP, % CP	4.5	4.4	4.3	4.6	4.1	4.8	0.21	0.08	<0.001	<0.001	<0.001	0.05
NDFD-30hr, % NDF	41.1	41.6	41.4	41.3	41.4	41.3	0.70	0.08	0.53	0.41	0.69	0.47
NDFD-48hr, % NDF	47.7	48.5	48.1	48.1	48.3	47.9	0.57	0.08	0.98	0.50	0.43	0.77
NDFD-120hr, % NDF	49.2	50.0	49.8	49.4	49.9	49.3	0.50	0.06	0.30	0.26	0.20	0.98
NDFD-240hr, % NDF	51.2	51.5	51.6	51.1	50.7	52.0	0.80	0.10	0.10	0.11	0.001	0.89
uNDF-30hr, % DM	26.7	26.2	26.3	26.6	26.0	27.0	0.47	0.01	0.08	<0.001	0.60	0.48
uNDF-120hr, % DM	23.1	22.4	22.6	23.0	22.2	23.3	0.51	<0.01	0.10	<0.001	0.28	0.87
uNDF-240hr, % DM	22.2	21.7	21.7	22.2	21.3	22.6	0.52	0.01	0.03	<0.001	0.01	0.76
TTNDFD, % NDF	31.7	32.3	32.8	31.3	33.5	30.6	0.94	0.08	<0.001	<0.001	<0.001	0.49
RFV	121	124	124	121	126	119	3.0	<0.01	0.01	<0.001	0.18	0.57
RFQ	112	117	115	114	118	111	3.2	0.02	0.27	0.001	0.55	0.70

[1]Determined by near-infrared spectroscopy (Rock River Laboratory, Inc., Watertown, WI)

[2]Interactions for treatment*core and the three-way interaction of main effects all lacked significance and are not reported in this table.

Table 3.3. Effect of the combination of baling and processing methods (CUT-MIX vs. NORM-GRIND) on the chemical composition and particle size distribution of alfalfa hay.

	Treatment			
Item	CUT-MIX	NORM-GRIND	SEM	*P*-value
DM, %	91.5	91.1	0.36	0.06
CP, %	19.1	19.6	1.26	0.06
ADF, %	41.5	40.3	0.57	<0.001
NDF, %	48.6	47.0	0.96	<0.001
NDFom, %	45.1	43.3	1.05	<0.001
Lignin, %	9.9	9.4	0.14	<0.001
Ash, %	11.1	11.0	0.38	0.93
ADICP, % DM	1.2	1.1	0.05	<0.01
ADICP, % CP	6.3	5.9	0.39	<0.001
NDFD-30hr, % NDF	39.7	41.1	0.92	<0.001
NDFD-48hr, % NDF	44.6	47.0	0.62	<0.001
NDFD-120hr, % NDF	46.9	48.7	0.98	0.001
NDFD-240hr, % NDF	49.0	50.8	1.30	<0.01
uNDF-30hr, % DM	29.3	27.7	0.49	<0.001
uNDF-120hr, % DM	25.8	24.1	0.93	<0.001
uNDF-240hr, % DM	24.8	23.2	1.08	<0.001
TTNDFD, % NDF	28.7	29.7	0.94	0.03
RFV	109	114	3.0	<0.001
RFQ	95	105	3.3	<0.001
Processing time, min/bale	11.0	3.6	1.53	<0.001
Processing rate, kg DM/min	55	185	35.9	<0.001
Processing shrink, kg DM[3]	9.1	33.2	2.83	<0.001
Processing shrink, % DM[3]	1.6	8.1	0.95	<0.001
Particle Size[2]				
Screen 1, % total DM	35.1	13.7	1.58	<0.001
Screen 2, % total DM	20.3	19.6	1.14	0.04
Screen 3, % total DM	13.3	16.6	0.65	<0.001
Pan, % total DM	31.2	49.9	1.82	<0.001

[1]Determined by near-infrared spectroscopy (Rock River Laboratory, Inc., Watertown, WI)

[2]Screen sizes were 19 mm, 8 mm, and 4 mm, respectively.

[3]Does not include data from one farm due to issues with operating the tub-grinder.

Chapter 4 - Association of horizontal silo pad type, elevation and core depth with indicators of silo ramp hygiene, forage quality, and digestibility

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ABSTRACT

Horizontal silo piles without walls are constructed using equipment to adequately pack the forage for air exclusion. During packing, the equipment uses a ramp of forage to access the top of the pile, potentially introducing soil into the forage when the base of the silo is made of soil. Soil contains microorganisms which may cause malfermentation and pose health risks to livestock. The objective of this study was to assess the association of horizontal silo pad type, elevation, and core depth with factors related to silage hygiene and nutrient quality. We hypothesized that ash and mineral content, microbiological profile, and fermentation profile in silos with soil pads would be indicative of soil contamination, and that measures of potential contamination would be lesser at higher elevations. Eleven horizontal silos on 7 farms were sampled in a split-split-plot design, with silo pad type as the whole plot, elevation on the ramp as the sub-plot, and core depth as the sub-sub-plot. Concrete pads (CONC) increased silage pH, and soil pads (SOIL) increased P concentration at outer (OUTER) but not inner (INNER) core depths. Further, Fe was greater at lower (LOW) vs. medium (MED) elevations. On SOIL, OUTER had lesser 240-h NDF and 7-h starch digestibilities. The OUTER segments also had increased pH and decreased density. Further OUTER increased NDF, ADF, lignin, ash, and minerals, but decreased CP and starch. Additionally, OUTER decreased NDF and starch digestibility, and increased undigestible NDF. There were minimal effects of elevation on silage chemical composition, digestibility or microbiological profile. Overall, changes in Fe and P may be indicative of some soil contamination. Furthermore, the decreased quality of forage in the outer layers of the silo reinforce the importance of an anaerobic environment for adequate preservation of silage.

INTRODUCTION

Achieving high-quality silage on beef and dairy operations requires careful attention to oxygen exclusion to accomplish excellent fermentation (Muck, 1988). Inadequate fermentation or allowing silage to spoil during the feed-out phase permits secondary aerobic fermentation known to increase dry matter loss and reduce dry matter intake and performance in livestock, and it may also contribute to animal health issues (Borreani et al., 2018). Specifically, a lack of pH reduction by lactic acid bacteria at the onset of fermentation or in spoiled sections may allow growth of *Clostridium* spp, known to have deleterious effects (Queiroz et al., 2018; Zheng et al., 2020).

Clostridial fermentation in silages produces butyric acid (> 1%; Kung Jr. et al., 2018), ammonia (Zheng et al., 2020), and biogenic amines (Queiroz et al., 2018). Some biogenic amines produced during this process may be involved in short-term dry matter intake reductions (Scherer et al., 2015), and silages with greater butyric acid concentration typical of clostridial fermentation have reduced digestibility (Mills and Kung, 2002). Others have suggested that the elevated butyric acid concentration in silage from clostridial fermentation can potentiate hyperketonemia in at-risk dairy cows, a concept which is confounded with reduced dry matter intake for butyric silages (Driehuis et al., 2018).

Clostridium tyrobutyricum is one of the most studied clostridial species in silage due to its implication as a causative agent in late-blowing of cheese (Klijn et al., 1995). While there are a vast number of *Clostridium* spp. associated with silages (Borreani et al., 2019), most are non-pathogenic. *Clostridium perfringens* strains are sometimes found in silage (Fohler et al., 2016) and are associated with enteritis and enterotoxemia in ruminants (Niilo, 1987; Songer, 1996; Savic et al., 2012). Despite *C. perfringens* and its toxins being found in some hemorrhagic

bowel cases in dairy cows (Dennison et al., 2005; Ceci et al., 2006), implicating it as a causative agent in the disease is difficult because of its presence in healthy animals.

Similar *to Clostridium spp.,* coliform bacteria have potential health implications in cattle and humans. Coliforms can be easily be isolated in feeds and feedbunks on farms (Lynn et al., 1998; Sanderson et al., 2005; Hutchison et al., 2006) but their presence on feed in those cases may be due to contamination directly from the animals and environment. *Escherichia coli*, a common coliform bacteria, are largely commensal in cattle, but certain strains are causative of mastitis (Kremer et al., 1990), and fecal coliforms can be found in a majority of bulk tanks (Kessel et al., 2004) posing a potential human health risk. *Escherichia coli* does not survive proper acidification of silage, which may be a challenge with certain forage sources based on the forage's buffering capacity (Bach et al., 2002; Ogunade et al., 2016, 2017). While coliforms are not common to soil (Turco et al., 1994), their presence in soil for a short period after manure or contaminated water application (Fenlon et al., 2000; Islam et al., 2005) and proximity of silage piles to animal housing or contaminated equipment could potentially introduce them to drive-over silage piles.

Clostridia are ubiquitous, being found in soil, forage, manure, feed, and milk (Julien et al., 2008; Borreani et al., 2019) and their universal presence is aided by the fact they form spores during less-than-ideal growing conditions, allowing them to re-emerge when the conditions improve. Some evidence suggests that prevalence of clostridia in silage may be influenced by the application of animal manure and the timing of its application (Rammer and Lingvall, 1997; Rammer et al., 1997; Coblentz et al., 2014). While some authors have suggested that soil contamination may be the primary cause of *Clostridium spp.* contamination in silage (Dunière et al., 2013; Kung Jr. et al., 2018; Queiroz et al., 2018) due to their ubiquitous nature in soil, a

paucity of work has been conducted to quantify soil contamination of silage. One group monitored radiocaesium and titanium in fresh forages, soil, and silage over time, presenting evidence that soil contamination did occur between harvest and ensiling (Rafferty et al., 1994). One case study identified an extreme case of sand contamination in a silage pile stored on soil as a causative factor for bovine mortality from rumen sand impaction, thus highlighting the possibility of soil contamination during packing by inexperienced individuals (Erickson and Hendrick, 2011). Ash and iron content has been utilized as a measure of soil contamination of silages after flooding events (Kung et al., 2015), and of hay during normal harvest conditions utilizing different harvesting methods (Digman et al., 2013; Neu et al., 2017).

Around 90% of U.S. dairy farms feed corn silage (USDA, 2016), and many large farms utilize drive-over silage piles of some type to store large quantities of forage. Drive-over silage piles may have a base of soil, or a harder base such as concrete or asphalt. Therefore, considering the ubiquitous nature of clostridia bacteria in soil and coliforms in livestock facilities, the objective of this study was to assess the association of silage pad type, elevation, and core depth with silage hygiene and nutritional characteristics. We hypothesized that ash and mineral content would be greater on silage piles stored on soil compared with concrete, and that those piles would have greater counts of clostridia and coliform bacteria. We also hypothesized that the ash and mineral content would be greater at lower elevations where tires first interact with the ramp.

MATERIALS AND METHODS

Experimental design

This observational trial was conducted from November 2019 to March 2020 on 7 farms in north central and western Kansas using forages grown in 2019. All silages had fermented for at least 60 days. The trial design was a randomized block design with a split-split-plot treatment structure. In total, 11 drive-over corn, sorghum, and triticale silage piles were sampled, 3 of which were on concrete pads (CONC) with the remainder on soil pads (SOIL). Detailed explanation of the silage piles sampled are provided in Table 5.1. All silage piles were covered with plastic.

Each pile was sampled at 18 locations on the silage ramp where tractors accessed the pile for packing, with cores obtained at an elevation of approximately 0.6 m (LOW), 1.2 m (MED) and at the peak (PEAK; approximately 6 m). Specifically, 6 cores were obtained across the ramp at each elevation. Cores of 45.5 to 56 cm in length were obtained with a coring device 5.6-cm in width. Cores were separated into two segments, 1) 15 cm of the core representing the outer layers of the silage pile (OUTER), and 2) the remainder of the core (15 to 56 cm) representing the inner portions of the pile (INNER). Plastic caught in the corer during piercing the plastic shield of the silo was removed to ensure it did not influence laboratory results. Samples were immediately placed on ice.

After samples were collected, three samples from the same median plane, elevation and core depth were composited for a total of 6 samples for each ramp at each core depth. One half of the silage composite was evaluated for chemical composition by near-infrared spectroscopy (Rock River Laboratory, Inc., Watertown, WI). The remaining half of the composite sample was sent overnight to ARM and HAMMER Animal Nutrition (Waukesha, WI) for microbial and

fermentation acid profile. Eight samples sent for microbiological and fermentation acid analysis were exposed to water during the shipping process, and therefore were not analyzed.

Forage chemical composition

Forage chemical composition was determined at Rock River Laboratory, Inc. (Watertown, WI). Samples were dried and ground through a 1-mm screen before being cooled for analysis using near-infrared spectroscopy (Foss Analytics, Hillerød, Denmark), which was calibrated using historical samples also analyzed with wet-chemistry methods in the same laboratory. The total tract neutral detergent fiber digestibility (TTNDFD) was calculated using standardized *in vitro* neutral detergent fiber (NDF) digestion measures (Goeser et al., 2009) for 24, 30 and 48 h, and 240 h undigested NDF (uNDF) according to Lopes et al. (2015). The MILK2006 variables were determined according to Shaver (2007).

Fermentation acid analysis

Feed samples were first diluted 1:10 in 5 m*M* H_2SO_4 followed by a 15-minute incubation period at room temperature. Samples were homogenized for 1 min at 300 rpm. Aliquots were centrifuged at 22,000 x g for 7 min. The supernatant was filter sterilized. Lactate, acetate, butyrate, propionate and valerate were detected using high performance liquid chromatography (Shimadzu RID-20A, Kyoto, Japan) with a ROA-Organic acid H+ (8%) column (Phenomenex, Torrance, CA) at a flow rate of 0.5 ml/min, an ambient column temperature (approximately 19°C), and an injection volume of 10 µL. All samples were analyzed in duplicate.

Microbiological analysis

For microbiological analysis, samples were suspended in sterile peptone and homogenized using mastication for 1 min. at 300 rpm. Samples for clostridia quantification were diluted 10^{-1} to 10^{-3} prior to plating, and samples for yeast and mold quantification were diluted 10^{-3} to 10^{-5}. All samples were plated in duplicate for each method using the pour plate method. For culture of clostridia, samples were plated on tryptose sulfite cycloserine agar base (Thermo Fisher Scientific, Waltham, MA) with D-cycloserine (400 mg/L) and incubated anaerobically for 24 h at 37°C. Plates were heat-shocked prior to enumeration, with black colonies with iron precipitation identified as *Clostridia spp.* *Escherichia coli* and coliforms were cultured with colorimetric CHROMagar™ ECC (CHROMagar, Springfield, NJ) for 24 h aerobically at 37°C. The *E. coli* colonies were blue and the coliforms were identified as purple and blue colonies, and enumerated accordingly. Yeasts and molds were cultured for 5 d at room temperature (approximately 23°C) with potato dextrose agar (Beckton, Dickinson and Company, Franklin Lakes, NJ) and 0.16% tartaric acid. Yeasts were identified and enumerated as small, smooth, white morphological colonies, with mold identified and enumerated as multi-filamentous organisms.

Statistical analysis

The study was conducted using a split-split-plot design, with silo pad type as the whole plot, elevation as the sub-plot, and core depth as the sub-sub-plot. A general linear mixed model was utilized for analysis of continuous variables using the GLIMMIX procedure SAS (version 9.4, SAS Institute, Cary, NC) according to the following statistical model:

$Y_{ijklm} = \mu + Farm_i + Pad_j + (Farm*Pad)_{ij} + Elevation_k + (Pad*Elevation)_{jk} +$

$(Farm*Pad*Elevation)_{ijk} + Triad_l(Farm*Pad*Elevation)_{ijk} + Depth_m + (Pad*Depth)_{im} +$

$(Elevation*Depth)_{km} + (Pad*Elevation*Depth)_{ikm} + Forage_n + e_{ijklmn}$

Where Y_{ijklm} = the response variable; μ = overall intercept; $Farm_i$ = random blocking effect of farm (i = 1 to 7), Farm_i~NIID(0, σ^2_f) , Pad_j = fixed effect of pad type (j = 1 to 2); $Farm_i*Pad_j$ = random effect of the silage pile, represented as the cross product of farm and pad type, assumed as $(Farm*Pad)_{ij}$ ~NIID(0, σ^2_pile); $Elevation_k$ = fixed effect of elevation (k = 1 to 3); $(Pad*Elevation)_{ik}$ = fixed effect of the interaction of pad and elevation; $(Farm*Pad*Elevation)_{ijk}$ = random effect of the pair of core triad, represented as cross product of farm, pad and elevation, assumed Farm*Pad*Elevation ~NIID(0, _pair); $Triad_l(Farm*Pad*Elevation)_{ijk}$ = random effect of a core triad (l = 1 to 2) nested within pair of core triads, assumed NIID(0, σ^2_cyl); $Depth_m$ = fixed effect of core depth (m = 1 to 2); $(Pad*Depth)_{im}$ = fixed effect of the interaction of pad type and core depth; $(Elevation*Depth_{)km}$ = fixed effect of the interaction of elevation and core depth; $(Pad*Elevation*Depth_{)jkm}$ = fixed effect of the interaction of pad type, elevation and core depth; $Forage_n$ = fixed effect of forage type (n = 1 to 4), and e_{ijklm} = left-over residual for the sample composite, represented by the combination of depth and triad, assumed NIID(0, σ^2_e). All random effects are assumed to be normal and unrelated.

During model fitting, random effects, which were estimated using restricted maximum likelihood, were dropped from the model if variance components converged to zero. Model assumptions were evaluated using externally studentized residuals. After accounting for heterogeneous variances, any outlying residuals remaining that were greater than 3.4 were excluded from analysis based on a conservative Bonferroni adjusted test on studentized

residuals, which suggested they were not representative of the population of interest. Relevant pairwise comparisons were conducted using a Tukey-Kramer or Bonferroni adjustment to avoid inflation of the Type I error rate due to multiple comparisons. Significant differences were declared at $P \leq 0.05$ and marginal significance was declared at $P \leq 0.10$.

Modeling of microbiological data used a 2-step approach. The first step analyzed the detection of the microbe of interest with a generalized linear mixed model assuming a binomial distribution of the response, the probability of which was connected to the linear predictor using a logit link function. For the second step of the analysis, concentration of the variable of interest for those samples above the detection limit was fitted using a general linear mixed model. Adjustments were conducted for denominator degrees of freedom in the variables for which multiple samples were missing or below the detection limit.

RESULTS

Silage chemical composition, pH, and density

There was marginal evidence of an interaction between elevation and core depth on density ($P = 0.08$; Table 5.2), whereby density was greater for INNER vs OUTER cores at MED (279 vs 236 ± 42.8 kg/m^3; Table 5.9), but not at any other elevation ($P > 0.31$). There was no evidence of difference for any main effects or interactions on DM % ($P > 0.28$; Table 5.2), but CONC was associated with an increase pH 0.2 units compared with SOIL ($P = 0.02$), and OUTER was associated with a decrease in pH 0.1 units compared with INNER ($P = 0.01$).

Core was associated with measures of the chemical composition of the silage, whereby OUTER had CP Content compared with INNER (10.1 vs. 9.9 ± 0.32%; $P = 0.01$; Table 5.2), ADF (33.3 vs. 32.5± 0.75%; $P = 0.05$), lignin (5.4 vs. 5.0 ± 0.51%; $P < 0.001$), and had

marginally increased aNDF (47.4 vs. 46.5 ± 1.07%; $P = 0.08$). There was no evidence of difference for ADF ($P > 0.12$; Table 5.2) or starch ($P > 0.71$) content.

There were several effects for ash and mineral content. Compared with INNER, OUTER had increased of ash content (12.3 vs. 10.9 ± 3.38%; $P < 0.001$; Table 5.2), K (2.3 vs. 2.2 ± 0.15%; $P = 0.001$), Ca (0.40 vs. 0.37 ± 0.11%; $P = 0.01$), Mg (0.28 vs. 0.26 ± 0.03%; $P < 0.001$), and Fe (496 vs. 394 ± 240.2 ppm; $P = 0.01$). Further, elevation affected Fe concentration ($P = 0.02$; Table 5.2) whereby LOW was greater than MED (518 vs 381 ± 240.2 ppm; $P = 0.01$), but there was no evidence of difference between PEAK and the other elevations ($P > 0.23$). For P, there were interactions between pad and elevation ($P = 0.01$; Table 5.2) and pad and core depth ($P = 0.03$). For the interaction between pad type and elevation, there was greater P content at PEAK vs. LOW in CONC (0.26 vs. 0.23 ± 0.03%; Table 5.8). For the interaction between pad and core depth, P was greater for OUTER vs. INNER cores on SOIL (0.27 vs 0.24 ± 0.02%; $P < 0.01$; Table 5.7).

Core depth tended to have decreased ammonia N for INNER vs OUTER (0.17 vs 0.18 ± 0.01%; $P = 0.06$; Table 5.2), but there was no evidence of difference for ammonia N as a % of CP ($P > 0.23$). Further, OUTER had increased ADICP as a % of CP by 0.8% ($P < 0.01$; Table 5.2), but there was no evidence of difference for ADICP as a % of DM ($P > 0.19$).

Butyric acid was detectable in only 7 of 124 samples analyzed, and thus was not statistically analyzed. Lactic acid was undetectable in 15 of 124 samples. Core depth affected lactic acid, as INNER had increased lactic acid vs. OUTER (3.34 vs. 2.05 ± 0.57%; $P < 0.001$; Table 5.2). There was marginal evidence for an interaction between pad type and core depth for acetic acid ($P = 0.07$); however, differences were only numerical after adjustment for multiple comparisons using a Bonferroni adjustment. Specifically, INNER was numerically greater than

OUTER for CONC (1.68 vs. 1.23 ± 0.63%; $P = 0.17$), but INNER was numerically lesser than OUTER for SOIL (1.94 vs. 20.7 ± 0.36; $P = 0.71$).

Silage digestibility

Core depth was the primary factor for significant differences on NDF digestibility variables. Compared with INNER, OUTER had reduced 30, 48, and 120 h NDF digestibility 2.6% ($P = 0.001$; Table 5.3), 2.0%, and 2.2% ($P < 0.01$), respectively. Pad and core depth interacted for 120-h NDF digestibility ($P = 0.02$; Table 5.3) and 240-h NDF digestibility ($P = 0.04$), whereby INNER had greater digestibility than OUTER for SOIL ($P < 0.01$; Table 5.7). Undigestible NDF behaved in a reasonably inverse manner at 30, 120 and 240 h, in which OUTER had increased uNDF compared with INNER by 1.8% ($P < 0.001$; Table 5.3), 1.1%, and 1.2% ($P = 0.01$), respectively. There was only a tendency for OUTER to have decreased TTNDFD compared with INNER (34.7 vs. 35.8 ± 1.63%; $P = 0.06$; Table 5.3).

For starch digestibility, OUTER had decreased 0 h starch digestibility by 4.4% ($P = 0.01$; Table 3). For 7 h starch digestibility, pad and core depth interacted ($P < 0.001$), whereby digestibility was greater for INNER vs OUTER on SOIL (68.9 vs. 64.8 ± 2.13%; Table 5.7).

Core depth significantly altered MILK2006 variables at 30 and 48 h. Specifically, at 30 h, OUTER had reduced ($P < 0.001$; Table 5.3) NE_L (1.18 vs 1.23 ± 0.01 Mcal/kg), TDN (49.6 vs. 51.2 ± 1.10%), and milk/mt (1,045 vs. 1,110 ± 80.0 kg/mt) compared with INNER. Furthermore, pad type tended to affect Milk2006 TDN, where SOIL had increased TDN compared with CONC by 4.2% ($P = 0.08$; Table 5.3). For 48 h, OUTER had reduced ($P < 0.001$; Table 5.3) NE_L (1.20 vs 1.25 ± 0.02 Mcal/kg), TDN (51.6 vs. 52.9 ± 1.07%), and milk/mt (1,140 vs. 1,192 ± 108.1 kg/mt) compared with INNER. Elevation marginally affected Milk2006 NE_L ($P = 0.09$) as MED was numerically greater than LOW and PEAK (1.24 vs. 1.22

± 0.02%), but pairwise comparisons were not significant after a Tukey-Kramer adjustment for multiple comparisons ($P > 0.10$).

Microbiological profile

The frequency of detection of microbiological species is outlined in Table 6. Due to low detection of *C. perfringens*, *E. coli*, total coliforms, and mold, these variables were not statistically analyzed due to the complex nature of the factorial design which makes it difficult to make an accurate inference. There was no evidence of difference for any treatment factor on the probability of detecting *Clostridia spp*. or yeast ($P > 0.42$; Table 5.5). Analysis of probability of detecting *Clostridia spp.* where not statistically relevant, noting excessive confidence intervals, and therefore data are not shown. When samples that were above the detection threshold were analyzed for quantification of CFU, there was an interaction of elevation and core depth for yeast ($P = 0.05$; Table 5.4). The CFU of yeast were greater for OUTER vs INNER at MED (5.5 vs. 4.1 ± 0.57 $\log_{10}$ CFU; $P = 0.01$; Table 5.9), but not at the other elevations ($P > 0.23$). There was no evidence of difference for any treatment factor for the quantification of *Clostridia spp.* CFU ($P \geq 0.10$; Table 5.4).

DISCUSSION

Contrary to our hypothesis, there was minimal evidence of significant effects of silage pad type on ash, Fe, or microbiological factors. The interactions involving pad type for P are intriguing, but inconclusive. For SOIL, P was greater in OUTER vs. INNER core depths. This could indicate some soil contamination for SOIL, but we would anticipate similar differences for ash and other mineral content. Coincidentally, the 120-h and 240-h NDF, and 7-h starch

digestibility, were both reduced on OUTER for SOIL. A direct mechanism for why this occurred on SOIL but not CONC is elusive based upon our data, especially considering the lesser pH of SOIL.

The lack of overall effects for pad type on ash and minerals may be representative of care taken by drivers of packing equipment not to incorporate soil into the pile, or variability of deterioration in the outer layers of the silo. The degradation in the outer layer of the silo concentrated the ash component, and variability in degradation between silos may have been greater than any introduction of ash from soil making it difficult to detect differences. The ability to detect significant differences in ash in the outer layer of these piles may have been easier in fresh silage that had not yet been covered.

Most of the significant effects identified in this experiment were for core depth, which is supported by multiple studies reporting greater deterioration of silage in outer layers (McLaughlin et al., 1978; Dickerson et al., 1991; Bolsen et al., 1993; Borreani and Tabacco, 2014). The outer layers of a silo are more exposed to oxygen, which promotes aerobic degradation of feed (Borreani et al., 2018). Further, because of this aerobic degradation, anaerobic microbes that produce lactic acid do not thrive, which results in greater pH and allows less desirable microorganism to thrive, such as Clostridia or Enterobacter species (Pahlow et al., 2003). In our study, lactic acid was greater in INNER vs. OUTER, which is consistent with other studies (Okatsu et al., 2019). However, Okatsu et al. (2019) reported a reduction in acetic acid in outer layers, which was not the case in this study. Butyric acid was only detectable in 7 of the 124 samples analyzed, which is indicative of high-quality fermentation and lack of clostridial fermentation in the silage pile. Of the 7 samples in which butyric acid was detected, 6 were in the outer core depths. The difference in lactic acid at different core depths in this study likely

drove the concurrent difference in pH between core depths. Others have reported similar changes in pH from inner to outer core segments depending on various management factors (Borreani and Tabacco, 2010, 2014; Okatsu et al., 2019). The pH at different silage depths is altered with the type of plastic cover and silage pile size, both of which are factors that alter oxygen presence at different locations in the pile and the resulting fermentation (Borreani and Tabacco, 2014).

In this study, only yeast and clostridia were detectable in sufficient samples to warrant accurate statistical analysis in a complex split-split-plot treatment structure, and there was greater concentration of yeast in the outer layer (Table 5.4), but there was no evidence of an increase in the probability of finding either type of microorganism (Table 5.5). Multiple studies report an increase in yeasts and molds on the outer layers of horizontal silage piles (Borreani and Tabacco, 2010, 2014; Okatsu et al., 2019), but results have been mixed for *Clostridium spp.* (Borreani and Tabacco, 2010; Okatsu et al., 2019). Concurrent with the increase in these microbes in the outer layer, there was a reduction of lactic acid and an increase in pH in the outer layer in our study which likely aided the growth of those species, which has been demonstrated by others (Borreani and Tabacco, 2010, 2014; Okatsu et al., 2019).

The aerobic degradation in the outer layers leads to a reduction in the nutritive quality of the forage (Table 5.2). In this study, there was an increase in NDFom, ADF, lignin, and a decrease in CP and starch. The digestibility of the fiber and starch components also decreased in the outer layers, resulting in greater undigestible NDF and lesser total tract NDF digestibility. Feeding deteriorated silage has a marked ability to decrease apparent digestibility (Whitlock et al., 2000). There are fewer studies that report chemical composition of the silage in the outer layers, but our results align with a study where they measured changes in chemical composition based on distance from the wall of the silo (Borreani and Tabacco, 2014). Overall, this leads to a loss of

DM (Borreani et al., 2007; Borreani and Tabacco, 2014) and an increase in in inorganic substances as ash (Borreani and Tabacco, 2014). Our data also demonstrate an increase in ash in the outer layers where degradation was present. Specifically, K, P, Mg and Fe increased in those outer layers, although caution must be taken in interpretation of these minerals because NIR is not a wholly reliable quantification method for minerals (NRC, 2001).

Other measures of degradation of feeds and forages in this study, such as the increase in ADICP and ammonia N, further demonstrate the decay of the outer layers of the forage. McGuffey and Owens (1979) reported an increase in ADICP in outer layers, but not in ADICP as a percent of total CP. Other authors have not detected a change in ADICP based on sample depth (Borreani and Tabacco, 2014; Okatsu et al., 2019). The ammonia N in this study was marginally greater for outer layers, which is opposite of the work of McGuffey and Owens (1979). In other studies, ammonia N was not effected by silage depth (Borreani and Tabacco, 2014; Okatsu et al., 2019).

From an economic perspective, the level of deterioration in the outer layers of the silage will inhibit production and profitability if fed to livestock. The production per ton of feed as estimated by Milk2006 was 52 to 65 kg/mt less for OUTER than INNER. Assuming a 60-m long silo with a 20-m base with, 4-m top width, and density of 260 kg/m^3, a total of 51.5 mt is represented in the outer 15 cm of the pile (Holmes and Muck, 2008). This would equate to an estimated loss of approximately 3.4 mt of milk production.

There were minimal effects of elevation in this study. We hypothesized that there may be more evidence of soil contamination and ash at lower elevations because of the proximity to soil to be introduced by tractor tires during packing. The greater Fe at LOW vs. MED may indicate some soil contamination at the beginning of the ramp, but based on a lack of interaction with pad

type we would have to assume that contamination could occur equally either pad type. The decrease in density for OUTER vs. INNER in MED likely contributed to the greater yeast CFU for the same factors, but it is unclear why this did not also occur at LOW and PEAK.

To our knowledge, this is the first data evaluating silage properties at different elevations of a horizontal silo ramp. Typically, a greater proportion of silage in the silo ramp must be discarded due to deterioration. Since there were no effects of elevation in this study, any apparent increase in deteriorated silage at the lower end of a ramp would be because of a relatively larger proportion of the deteriorated layer compared with higher elevations. Further, elevation did not affect density in the present study which is contrary to other published data. Typically, silage in horizontal piles has greater density at lower elevations (Savoie and D'Amours, 2008) because of the added compaction from the increased mass from upper layers. However, in this study the samples were obtained from the ramp where the only mode of compaction was from packing equipment, and we did not sample at points deep enough in the pile to detect a difference.

There are several limitations to this study which make data analysis and interpretation difficult. Specifically, the unbalanced design by pad and forage type introduce variability in the data that limit our ability to detect small differences. Balancing the number of concrete and soil pads would be helpful for making accurate inference about the results. Increasing the number of silos sampled would also add confidence about the reproducibility of these results. Additionally, variability in chemical composition and fermentative characteristics between forage types introduce additional variability. Ideally, this study would have utilized silos with the same forage type; however, there is value in testing different forages. The ability to reduce pH more rapidly during ensiling in corn and sorghum forages prevents the growth of unfavorable

microbes compared with forages that have greater buffering capacity, such as alfalfa (Buxton and O'Kiely, 2003). Perhaps in future studies it would be more advantageous to focus on forages like alfalfa or triticale where soil contamination could more easily facilitate growth of *Clostridia* or *Enterobacter spp.* Finally, as mentioned earlier, the variability in degradation of the outer layers of the silage creates variability for detection of ash and iron concentration, making it difficult to draw inference as to whether equipment added soil to the outer layers during the final packing process. Collecting fresh forages samples during the packing process and immediately after may packing may help to enhance the ability to detect soil contamination. Despite these sources of variation, extreme care was taken with statistical analysis to meet all necessary assumptions for analysis, such as homogeneity of residual variances.

CONCLUSION

There were minor indications that soil contamination may have occurred based on mineral content of Fe at LOW, and for P in OUTER for SOIL. Further, SOIL reduced NDF and 7-h starch digestibility for OUTER on SOIL. The most numerous effects noted in this study were for core depth, where OUTER exhibited decreased density, nutritive quality, and nutrient digestibility. Future work should evaluate single forage types with high buffering capacity that may be more prone to malfermentation from soil bacterial contamination, such as alfalfa or winter forage crops. Further, efforts to secure samples prior to fermentation and degradation of outer layers may enhance the ability to detect subtle differences in ash and mineral content as indicators of soil contamination.

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REFERENCES

Bach, S.J., T.A. McAllister, J. Baah, L.J. Yanke, D.M. Veira, V.P.J. Gannon, and R.A. Holley. 2002. Persistence of Escherichia coli O157:H7 in barley silage: Effect of a bacterial inoculant. J. Appl. Microbiol. 93:288–294. doi:10.1046/j.1365-2672.2002.01695.x.

Bolsen, K.K., J.T. Dickerson, B.E. Brent, R.N. Sonon, B.S. Dalke, C. Lin, and J.E. Boyer. 1993. Rate and Extent of Top Spoilage Losses in Horizontal Silos. J. Dairy Sci. 76:2940–2962. doi:10.3168/jds.S0022-0302(93)77634-1.

Borreani, G., F. Ferrero, D. Nucera, M. Casale, S. Piano, and E. Tabacco. 2019. Dairy farm management practices and the risk of contamination of tank milk from Clostridium spp . and Paenibacillus spp . spores in silage , total mixed ration , dairy cow feces , and raw milk. J. Dairy Sci. 102:8273–8289. doi:10.3168/jds.2019-16462.

Borreani, G., and E. Tabacco. 2010. The relationship of silage temperature with the microbiological status of the face of corn silage bunkers. J. Dairy Sci. 93:2620–2629. doi:10.3168/jds.2009-2919.

Borreani, G., and E. Tabacco. 2014. Improving corn silage quality in the top layer of farm bunker silos through the use of a next-generation barrier film with high impermeability to oxygen. J. Dairy Sci. 97:2415–2426. doi:10.3168/jds.2013-7632.

Borreani, G., E. Tabacco, and L. Cavallarin. 2007. A new oxygen barrier film reduces aerobic deterioration in farm-scale corn silage. J. Dairy Sci. 90:4701–4706. doi:10.3168/jds.2007-0310.

Borreani, G., E. Tabacco, R.J. Schmidt, B.J. Holmes, and R.E. Muck. 2018. Silage review : Factors affecting dry matter and quality losses in silages. J. Dairy Sci. 101:3952–3979. doi:10.3168/jds.2017-13837.

Buxton, D.R., and P. O'Kiely. 2003. Preharvest Plant Factors Affecting Ensiling.

Ceci, L., P. Paradies, M. Sasanelli, D. De Caprariis, F. Guarda, M.T. Capucchio, and G. Carelli. 2006. Haemorrhagic Bowel Syndrome in Dairy Cattle : Possible Role of Clostridium perfringens Type A in the Disease Complex. J. Vet. Med. A. 53:518–523.

Coblentz, W.K., R.E. Muck, M.A. Borchardt, S.K. Spencer, W.E. Jokela, and M.G. Bertram. 2014. Effects of dairy slurry on silage fermentation characteristics and nutritive value of alfalfa. J. Dairy Sci. 97:7197–7211. doi:10.3168/jds.2014-8582.

Dennison, A.C., D.C. Van Metre, P.S. Morley, R.J. Callan, and E.C. Plampin. 2005. Comparison of the odds of isolation , genotypes , and in vivo production of major toxins by Clostridium perfringens obtained from the gastrointestinal tract of dairy cows with hemorrhagic bowel syndrome or left-displaced abomasum. J. Am. Vet. Med. Assoc. 227:132–138.

Dickerson, J.T., G. Ashbell, L. Pfaff, K.K. Bolsen, B.E. Brent, J.E. Bradford, and R.L. Smith. 1991. Top spoilage losses in horizontal silos in western Kansas. Pages 71–74 in Cattlemen's Dairy, Progress Report. Kansas State University, Manhattan, KS.

Digman, M.F., K.J. Shinners, and M.E. Boettcher. 2013. Crop Mergers: Management of Soil

Contamination and Leaf Loss in Alfalfa. Appl. Eng. Agric. 29:179–185.

Driehuis, F., J.M. Wilkinson, Y. Jiang, I. Ogunade, and A.T. Adesogan. 2018. Silage review : Animal and human health risks from silage. J. Dairy Sci. 101:4093–4110. doi:10.3168/jds.2017-13836.

Dunière, L., J. Sindou, F. Chaucheyras-Durand, I. Chevallier, and D. Thevenot-Sergentet. 2013. Silage processing and strategies to prevent persistence of undesirable microorganisms. Anim. Feed Sci. Technol. 182:1–15. doi:10.1016/j.anifeedsci.2013.04.006.

Erickson, N., and S. Hendrick. 2011. Case report: Sand impactions in a Saskatchewan beef cow-calf herd. Can. Vet. J. 52:74–76.

Fenlon, D.R., A. Vinten, and I. Svoboda. 2000. The fate of Escherichia coli and E . coli 0157 in cattle slurry after application to land. J. Appl. Microbiol. Symp. Suppl. 88:149S-156S.

Fohler, S., G. Klein, M. Hoedemaker, T. Scheu, C. Seyboldt, A. Campe, K.C. Jensen, and A. Abdulmawjood. 2016. Diversity of Clostridium perfringens toxin- genotypes from dairy farms. BMC Microbiol. 1–7. doi:10.1186/s12866-016-0812-6.

Goeser, J.P., P.C. Hoffman, and D.K. Combs. 2009. Modification of a rumen fluid priming technique for measuring in vitro neutral detergent fiber digestibility. J. Dairy Sci. 92:3842–3848. doi:10.3168/jds.2008-1745.

Holmes, B.J., and R.E. Muck. 2008. Bunker Silo Silage Density Calculator. Madison, WI, USA.

Hutchison, M.L., D.J.I. Thomas, L.D. Walters, and S.M. Avery. 2006. Shiga toxin-producing Escherichia coli, faecal coliforms and coliphage in animal feeds. Lett. Appl. Microbiol. 43:205–210. doi:10.1111/j.1472-765X.2006.01929.x.

Islam, M., M.P. Doyle, S.C. Phatak, P. Millner, and X. Jiang. 2005. Survival of Escherichia coli O157:H7 in soil and on carrots and onions grown in fields treated with contaminated manure composts or irrigation water. Food Microbiol. 22:63–70. doi:10.1016/j.fm.2004.04.007.

Julien, M., P. Dion, C. Lafrenie, H. Antoun, and P. Drouin. 2008. Sources of Clostridia in Raw Milk on Farms. Appl. Environ. Microbiol. 74:6348–6357. doi:10.1128/AEM.00913-08.

Kessel, J.S. Van, J.S. Karns, L. Gorski, B.J. Mccluskey, and M.L. Perdue. 2004. Prevalence of Salmonellae , Listeria monocytogenes , and Fecal Coliforms in Bulk Tank Milk on US Dairies. J. Dairy Sci. 87:2822–2830. doi:10.3168/jds.S0022-0302(04)73410-4.

Klijn, N., F.F.J. Nieuwenhof, J.A.N.D. Hoolwerf, C.B.V.A.N.D.E.R. Waals, and A.H. Weerkamp. 1995. Identification of Clostridium tyrobutyricum as the Causative Agent of Late Blowing in Cheese by Species-Specific PCR Amplification. Appl. Environ. Microbiol. 61:2919–2924.

Kremer, W.D.J., E.N. Noordhuizen-Stassen, and J.A.C.M. Lohuis. 1990. Host defence and bovine coliform mastitis Host defence and bovine coliform mastitis. Vet. Q. 12:103–113. doi:10.1080/01652176.1990.9694252.

Kung Jr., L., R.D. Shaver, R.J. Grant, and R.J. Schmidt. 2018. Silage review : Interpretation of chemical , microbial , and organoleptic components of silage. J. Dairy Sci. 101:4020–4033.

doi:10.3168/jds.2017-13909.

Kung, L., J.M. Lim, D.J. Hudson, J.M. Smith, and R.D. Joerger. 2015. Chemical composition and nutritive value of corn silage harvested in the northeastern United States after Tropical Storm Irene. J. Dairy Sci. 98:2055–2062. doi:10.3168/jds.2014-8621.

Lopes, F., D.E. Cook, and D.K. Combs. 2015. Validation of an in vitro model for predicting rumen and total-tract fiber digestibility in dairy cows fed corn silages with different in vitro neutral detergent fiber digestibilities at 2 levels of dry matter intake. J. Dairy Sci. 98:574–585. doi:10.3168/jds.2014-8661.

Lynn, T. V, D.D. Hancock, T.E. Besser, J.H. Harrison, D.H. Rice, N.T. Stewart, and L.L. Rowan. 1998. The Occurrence and Replication of Escherichia coli in Cattle Feeds. J. Dairy Sci. 81:1102–1108. doi:10.3168/jds.S0022-0302(98)75672-3.

McGuffey, R.K., and M.J. Owens. 1979. Effect of covering and dry matter at ensiling on preservation of alfalfa in bunker silos. 1Journal Anim. Sci. 49:298–305.

McLaughlin, N.B., D.B. Wilson, and D.M. Bowden. 1978. Effect of a plastic cover on dry matter loss from a horizontal silo. Can. J. Agric. Eng. 20:1–4.

Mills, J.A., and L. Kung. 2002. The Effect of Delayed Ensiling and Application of a Propionic Acid-Based Additive on the Fermentation of Barley Silage. J. Dairy Sci. 85:1969–1975. doi:10.3168/jds.S0022-0302(02)74273-2.

Muck, R.E. 1988. Factors Influencing Silage Quality and Their Implications for Management. J. Dairy Sci. 71:2992–3002. doi:10.3168/jds.S0022-0302(88)79897-5.

Neu, A.E., C.C. Sheaffer, D.J. Undersander, M.H. Hall, D.M. Kniffen, M.S. Wells, D.N. Catalano, and K.L. Martinson. 2017. Hay rake-type effect on ash and forage nutritive values of alfalfa hay. Agron. J. 109:2163–2171. doi:10.2134/agronj2017.03.0185.

Niilo, L. 1987. Toxigenic Characteristics of Clostridium perfringens Type C in Enterotoxemia of Domestic Animals. Can. J. Vet. Res. 51:224–228.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. e. National Academy Press, Washington, DC.

Ogunade, I.M., Y. Jiang, D.H. Kim, A.A.P. Cervantes, K.G. Arriola, D. Vyas, Z.G. Weinberg, K.C. Jeong, and A.T. Adesogan. 2017. Fate of Escherichia coli O157:H7 and bacterial diversity in corn silage contaminated with the pathogen and treated with chemical or microbial additives. J. Dairy Sci. 100:1780–1794. doi:10.3168/jds.2016-11745.

Ogunade, I.M., D.H. Kim, Y. Jiang, Z.G. Weinberg, K.C. Jeong, and A.T. Adesogan. 2016. Control of Escherichia coli O157:H7 in contaminated alfalfa silage: Effects of silage additives. J. Dairy Sci. 99:4427–4436. doi:10.3168/jds.2015-10766.

Okatsu, Y., N. Swanepoel, E.A. Maga, and P.H. Robinson. 2019. Impacts of some factors that effect spoilage of silage at the periphery of the exposed face of corn silage piles. Anim. Feed Sci. Technol. 247:234–247. doi:10.1016/j.anifeedsci.2018.11.018.

Pahlow, G., R.E. Muck, F. Driehuis, S.J.W.H. Oude Elferink, and S.F. Poelstra. 2003.

Microbiology of Ensiling.

Queiroz, O.C.M., I.M. Ogunade, Z. Weinberg, and A.T. Adesogan. 2018. Silage review : Foodborne pathogens in silage and their mitigation by silage additives. J. Dairy Sci. 101:4132–4142. doi:10.3168/jds.2017-13901.

Rafferty, B., D.E. Dawson, and P.A. Colgan. 1994. Soil and radiocaesium contamination of winter fodders. Sci. Total Environ. 153:69–76.

Rammer, C., and P. Lingvall. 1997. Influence of Farmyard Manure on the Quality of Grass Silage. J Sci Food Agric 133:133–140.

Rammer, C., P. Lingvall, and E. Salomon. 1997. Ensiling of manured crops - Does repeated spreading of slurry increase the hygienic risk?. J. Sci. Food Agric. 73:329–336. doi:10.1002/(SICI)1097-0010(199703)73:3<329::AID-JSFA734>3.0.CO;2-Q.

Sanderson, M.W., J.M. Sargeant, D.G. Renter, D.D. Griffin, and R.A. Smith. 2005. Factors Associated with the Presence of Coliforms in the Feed and Water of Feedlot Cattle. Appl. Environ. Microbiol. 71:6026–6032. doi:10.1128/AEM.71.10.6026.

Savic, B., R. Prodanovic, V. Ivetic, O. Radanovic, and J. Bojkovski. 2012. Enteritis associated with Clostridium perfringens type A in 9-month-old calves. Can. Vet. J. 174–176.

Savoie, P., and L. D'Amours. 2008. Density profile of herbage silage in bunker silos. Can. Biosyst. Eng. 50:57–65.

Scherer, R., K. Gerlach, and K. Südekum. 2015. Biogenic amines and gamma-amino butyric acid in silages : Formation , occurrence and influence on dry matter intake and ruminant production. Anim. Feed Sci. Technol. 210:1–16. doi:10.1016/j.anifeedsci.2015.10.001.

Shaver, R.D. 2007. Evaluating corn silage quality for dairy cattle. Pages 1–11 in Univ. of Wisconsin, Madison Extension.

Songer, J.G. 1996. Clostridial Enteric Diseases of Domestic Animals. Clin. Microbiol. Rev. 9:216–234.

Turco, R.F., A.C. Kennedy, and M.D. Jawson. 1994. Microbial indicators of soil quality.

USDA. 2016. Dairy 2014: Dairy Cattle Management Practices in the United States, 2014. Fort Collins, CO.

Whitlock, L.A., M.K. Siefers, R. V. Pope, and B.E. Brent. 2000. Effect of level of surface-spoiled silage on teh nutritive value of corn silage-based rations. Kansas Agric. Exp. Stn. Res. Reports 36–38. doi:10.4148/2378-5977.3195.

Zheng, M., D. Niu, D. Jiang, R. Li, L. Meng, and C. Xu. 2020. Metagenome analyses reveal the role of Clostridium perfringens in alfalfa silage anaerobic deterioration. FEMS Microbiol. Lett. 367:fnaa052. doi:10.1093/femsle/fnaa052.

TABLES

Table 4.1. Description of horizontal silo forage type and pad type by farm.

Item	Pad Type	Forage
Farm 1		
Silo 1	Soil	Corn
Silo 2	Soil	Corn
Farm 2		
Silo 1	Soil	Sorghum
Farm 3		
Silo 1	Soil	Triticale
Silo 2	Concrete	Sorghum
Farm 4		
Silo 1	Concrete	Sorghum
Farm 5		
Silo 1	Soil	Triticale
Silo 2	Concrete	Corn
Farm 6		
Silo 1	Soil	Corn/Sorghum
Farm 7		
Silo 1	Soil	Corn
Silo 2	Soil	Corn

Table 4.2. Association of pad type, elevation, and core depth with the chemical composition (as determined by NIR) and density of silage samples obtained from horizontal silo ramps.

	Pad Type		Elevation			Core Depth			*P*-value[2]					
Item[1]	Conc	Soil	Low	Med	Peak	Inner	Outer	SEM	Pad	Elev.	Core	Pad* Elev	Pad* Core	Elev* Core
DM, %	38.1	34.5	36.4	36.5	36.0	36.0	36.6	7.47	0.55	0.89	0.55	0.99	0.28	0.63
pH	5.0	4.8	4.8	4.8	4.9	4.8	4.9	0.10	0.02	0.45	0.01	0.74	0.45	0.43
CP, %	9.6	10.4	10.0	9.9	10.1	9.9	10.1	0.32	0.16	0.56	0.01	0.27	0.21	0.11
aNDF, %	47.5	46.4	46.8	46.9	47.1	46.5	47.4	1.07	0.62	0.92	0.08	0.95	0.88	0.90
aNDFom, %	43.8	43.0	43.3	43.5	43.5	43.0	43.8	3.44	0.91	0.92	0.12	0.98	0.47	0.86
ADF, %	33.3	32.5	32.9	32.8	33.0	32.5	33.3	0.75	0.56	0.88	0.05	0.45	0.78	0.99
Lignin, %	5.2	5.2	5.2	5.1	5.3	5.0	5.4	0.51	0.87	0.76	<0.001	0.24	0.37	0.39
Starch, %	13.3	13.8	13.3	13.6	13.6	13.6	13.5	1.31	0.86	0.91	0.92	0.77	0.78	0.71
Ash, %	12.6	10.7	11.8	11.1	11.9	10.9	12.3	3.38	0.48	0.12	<0.001	0.23	0.35	0.33
K, %	2.4	2.1	2.3	2.2	2.3	2.2	2.3	0.15	0.45	0.64	0.001	0.51	0.41	0.56
P, %[4,5]	-	-	-	-	-	-	-	0.02	0.42	0.14	0.04	0.01	0.03	0.13
Ca, %	0.45	0.32	0.40	0.37	0.38	0.37	0.40	0.11	0.15	0.27	0.01	0.77	0.36	0.85
Mg, %	0.27	0.27	0.27	0.27	0.27	0.26	0.28	0.03	0.98	0.54	<0.001	0.98	0.48	0.55
Fe, ppm	418	468	518[a]	381[b]	438[ab]	394	496	240.2	0.78	0.02	0.01	0.29	0.37	0.23
NH_3 N, %	0.17	0.17	0.18	0.18	0.17	0.17	0.18	0.01	0.69	0.42	0.06	0.65	0.71	0.17
NH_3 N, % CP	10.2	8.9	9.6	9.9	9.3	9.5	9.6	1.39	0.23	0.61	0.93	0.94	0.33	0.28
ADICP, %	0.85	0.91	0.90	0.87	0.88	0.86	0.90	0.07	0.64	0.88	0.19	0.58	0.86	0.99
ADICP, % CP	8.5	8.7	8.8	8.5	8.5	8.2	9.0	0.70	0.85	0.40	<0.01	0.70	0.48	0.88
Lactic acid, %	2.03	3.37	2.73	2.79	2.57	3.34	2.05	0.57	0.40	0.81	<0.001	0.75	0.50	0.86
Acetic acid, %[4]	-	-	1.80	1.75	1.63	-	-	0.40	0.49	0.68	0.30	0.82	0.07	0.82
Density, kg DM/m[3,6]	243	272	-	-	-	-	-	51.1	0.48	0.74	0.05	0.90	0.11	0.08

[1]Percentage values are represented as % of DM unless otherwise noted

[2]There was no evidence of difference for any 3-way interaction ($P > 0.27$)

[4]Means for the interaction of pad and core depth are reported in Table 7
[5]Means for the interaction of pad and elevation are reported in Table 8
[6]Means for the interaction of elevation and core depth are reported in Table 9
[a,b]Means for elevation with differing superscripts are different ($P < 0.05$)

Table 4.3. Association of pad type, elevation, and core depth with the digestibility (as determined by NIR) of silage samples obtained from horizontal silo ramps.

	Pad		Elevation			Core Depth			*P*-value[1,2,3]			
Item	Conc	Soil	Low	Med	Peak	Inner	Outer	SEM	Pad	Elev	Core	Pad* Core
NDF Digestibility												
30 h, % NDF	41.8	44.3	43.3	43.3	42.5	44.3	41.7	2.89	0.31	0.55	0.001	0.27
48 h, % NDF	56.6	57.8	57.4	57.9	56.3	58.2	56.2	4.77	0.74	0.36	<0.01	0.38
120 h, % NDF	-	-	60.1	60.9	59.1	-	-	4.99	0.79	0.35	<0.01	0.02
240 h, % NDF	-	-	61.8	63.2	60.9	-	-	6.05	0.90	0.19	<0.01	0.04
Undigestible NDF												
30 h, % DM	27.6	25.7	26.5	26.4	27.1	25.8	27.6	1.37	0.12	0.43	<0.001	0.40
120 h, % DM	20.5	20.2	20.3	20.0	20.9	19.8	20.9	1.17	0.90	0.25	0.01	0.19
240 h, % DM	19.3	19.1	19.1	18.8	19.7	18.6	19.8	1.17	0.94	0.21	0.01	0.28
TTNDFD, % NDF[4]	36.1	34.4	35.5	35.8	34.4	35.8	34.7	1.63	0.60	0.46	0.06	0.86
Starch Digestibility												
0 h, %	32.5	27.8	29.6	29.1	31.9	32.4	28.0	1.93	0.35	0.46	0.01	0.81
7 h, %[5]	-	-	65.3	65.8	67.9	-	-	1.91	0.67	0.18	0.19	<0.001
Milk2006 30 hr												
kg milk/mt	1,070	1,085	1,072	1,099	1,061	1,110	1,045	80.0	0.74	0.16	<0.001	0.89
TDN, %	48.2	52.6	50.2	50.9	50.0	51.2	49.6	1.10	0.08	0.20	<0.001	0.88
NE_L, Mcal/kg	1.21	1.20	1.20	1.23	1.20	1.23	1.18	0.01	0.75	0.12	<0.001	0.34
Milk2006 48 hr												
kg milk/mt	1,182	1,151	1,156	1,186	1,157	1,192	1,140	108.1	0.68	0.16	<0.001	0.71
TDN, %	50.4	54.1	52.0	52.8	51.9	52.9	51.6	1.07	0.11	0.19	<0.001	0.81
NE_L, Mcal/kg	1.23	1.22	1.22	1.24	1.21	1.25	1.20	0.02	0.68	0.09	<0.001	0.31

[1]There was no evidence of difference for any 3-way interaction ($P > 0.17$).

[2]There was no evidence of difference for any 2-way interaction between pad type and elevation ($P > 0.13$)

[3]There was no evidence of difference for any 2-way interaction between elevation and core depth ($P > 0.14$)

[4]Total tract NDF digestibility
[5]Means for the interaction of pad type and core depth are reported in Table 7

Table 4.4. Association of pad type, elevation, and core depth with the quantification of *Clostridium spp.* and yeast from silage samples obtained on horizontal silo ramps.

	Pad		Elevation			Core Depth			*P*-value[2]					
Item	Conc	Soil	Low	Med	Peak	Inner	Outer	SEM	Pad	Elev.	Core	Pad* Elev	Pad* Core	Elev* Core
log_{10} CFU/g[1]														
Clostridia	2.4	2.5	2.5	2.3	2.7	2.4	2.6	0.36	0.92	0.23	0.12	0.10	0.11	0.87
Yeast[3]	5.3	5.0	-	-	-	-	-	0.54	0.72	0.47	<0.01	0.40	0.64	0.05

[1]Data for quantification of CFU was determined only using data for samples greater than the minimum detectable limit as outlined in Table 5.

[2]There was no evidence of difference for any 3-way interaction ($P > 0.38$).

[3]Means for the interaction between elevation and core depth are reported in Table 5.9

Table 4.5. Association of pad type, elevation, and core depth with the probability of detection of *Clostridium spp.* and yeast in silage samples obtained from horizontal silo ramps.

	Microbe type
	Yeast
Item	Probability (95% CI)
Pad Type	
Conc	0.95 (0.06, 1.00)
Soil	0.76 (0.16, 0.98)
Elevation	
Low	0.91 (0.44, 0.99)
Med	0.89 (0.38, 0.99)
High	0.86, 0.34, 0.99)
Core Depth	
Inner	0.87 (0.43, 0.98)
Outer	0.90 (0.49, 0.99)
P-value	
Pad	0.42
Elevation	0.87
Core Depth	0.69
Pad*Elev.	0.54
Pad*Core	0.69
Elev.*Core	0.46
Pad*Elev.*Core	0.53

Table 4.6. Empirical prevalence of microbiological species silage samples obtained from the ramp of horizontal silos.

Item	Total detected, n[1]
Clostridia *spp.*	89/124 (0.71)
C. perfringens	44/124 (0.35)
E. coli	29/124 (0.23)
Total coliforms	54/124 (0.45)
Yeast	84/124 (0.68)
Mold	46/124 (0.37)

[1]Proportion of samples in which a given microbe type was detected, and percent of total in the parenthesis.

Table 4.7. Esimated means for variables with marginal or significant interactions of pad type and core depth.

	Conc		Soil			*P*-value
Item	Inner	Outer	Inner	Outer	SEM	Pad*Core
Phosphorus, %[1]	0.24	0.24	0.25[a]	0.27[b]	0.02	0.03
Acetic acid, %	1.68	1.23	1.94	2.07	0.49	0.07
NDFd 120 h, % NDF[1]	59.8	59.2	62.4[a]	58.7[b]	5.01	0.02
NDFd 240 h, % NDF[1]	62.1	61.3	64.5[a]	59.9[b]	3.52	0.04
Starch Dig, 7 h, %[1]	64.8	66.6	68.9[a]	64.8[b]	2.13	<0.001

[1]Superscripts that differ within a pad type are different ($P < 0.05$) based on a Bonferroni adjustment for multiple comparisons

Table 4.8. Estimated means for variables with significant interactions of pad type and elevation.

	Conc			Soil				*P*-value
Item	Low	Med	Peak	Low	Med	Peak	SEM	Pad*Elev
Phosphorus, %[1]	0.23[a]	0.24[ab]	0.26[b]	0.26	0.26	0.26	0.03	0.01

[1]Superscripts within a pad type that differ are different ($P < 0.05$) based on a Bonferroni adjustment for multiple comparisons

Table 4.9. Estimated means for variables with a marginal or significant interaction of elevation and core depth.

	Low		Med		Peak			*P*-value
Item	Inner	Outer	Inner	Outer	Inner	Outer	SEM	Elev*Core
Density, kg/m^{3}, [1]	276	249	279[a]	235[b]	247	258	42.8	0.08
Yeast, $\log_{10}$ CFU/g[1]	5.2	5.2	4.1[a]	5.5[b]	5.1	5.8	0.57	0.05

[1]Superscripts within an elevation that differ are different ($P < 0.05$) based on a Bonferroni adjustment for multiple comparisons

Appendix A - Chapter 2 Supplementary Tables

Table A.1. Linear associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and glucose (mg/dL) in transition Holstein cows.[1]

		Plasma glucose sample day							
Plasma AGP sample day	Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
-21	Transformed	-	No	No	-	-	No	No	No
	$AGP^2 < 0.1$	-	No	No	-	-	No	No	No
	AGP^2 included	-	No	No	-	-	No	No	No
	P	-	0.37	0.97	-	-	0.63	0.19	0.22
	r^2	-	0.14	0.30	-	-	0.01	0.03	0.00
	r	-	0.37	0.55	-	-	0.10	0.17	0.03
	n	-	44	36	-	-	85	90	89
	RMSE	-	5.38	3.63	-	-	6.70	5.96	6.02
	Slope	-	Negative	Negative	-	-	Negative	Negative	Negative
-13 ± 2	Transformed	-	No	No	-	-	No	No	No
	$AGP^2 < 0.1$	-	No	No	-	-	No	No	No
	AGP^2 included	-	No	No	-	-	No	No	No
	P	-	0.12	0.59	-	-	0.16	0.56	0.75
	r^2	-	0.72	0.24	-	-	0.32	0.27	0.39
	r	-	0.85	0.49	-	-	0.57	0.52	0.62
	n	-	191	36	-	-	217	311	231
	RMSE	-	6.92	3.80	-	-	7.77	8.75	6.91
	Slope	-	Negative	Positive	-	-	Positive	Negative	Negative
-3	Transformed	-	-	Yes	-	-	Yes	Yes	Yes
	$AGP^2 < 0.1$	-	-	No	-	-	No	No	No
	AGP^2 included	-	-	No	-	-	No	No	No
	P	-	-	0.88	-	-	0.103	0.15	0.43

	r^2	-	-	0.23	-	-	-0.27	0.08	-0.01
	r	-	-	0.48	-	-	0.52	0.28	0.10
	n	-	-	37	-	-	40	44	43
	RMSE	-	-	3.79	-	-	5.48	6.60	6.24
	Slope	-	-	Positive	-	-	Negative	Negative	Negative
1	Transformed	-	-	-	-	-	Yes	Yes	Yes
	$AGP^2 < 0.1$	-	-	-	-	-	Yes	Yes	No
	AGP^2 included	-	-	-	-	-	Yes	Yes	No
	P	-	-	-	-	-	0.01	0.07	0.1
	r^2	-	-	-	-	-	0.02	0.06	-0.03
	r	-	-	-	-	-	0.124097	0.244949	0.173205
	n	-	-	-	-	-	85	87	85
	RMSE	-	-	-	-	-	7.26	5.57	5.63
	Slope	-	-	-	-	-	Negative	Negative	Negative
3 ± 1	Transformed	-	-	-	-	-	No	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	0.72	0.24	0.93
	r^2	-	-	-	-	-	0.36	0.4	0.4
	r	-	-	-	-	-	0.60	0.63	0.63
	n	-	-	-	-	-	271	308	286
	RMSE	-	-	-	-	-	7.92	7.57	7.88
	Slope	-	-	-	-	-	Positive	Positive	Negative
7 ± 1	Transformed	-	-	-	-	-	No	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	0.58	0.55	1
	r^2	-	-	-	-	-	0.36	0.38	0.36
	r	-	-	-	-	-	0.60	0.62	0.60

	n	-	-	-	-	-	282	285	270
	RMSE	-	-	-	-	-	7.95	7.86	8.22
	Slope	-	-	-	-	-	Positive	Positive	Negative
14 ± 1	Transformed	-	-	-	-	-	-	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	No	No
	AGP^2 included	-	-	-	-	-	-	No	No
	P	-	-	-	-	-	-	0.85	0.81
	r^2	-	-	-	-	-	-	0.3	0.37
	r	-	-	-	-	-	-	0.55	0.61
	n	-	-	-	-	-	-	380	296
	RMSE	-	-	-	-	-	-	8.71	8.19
	Slope	-	-	-	-	-	-	Negative	Positive
21 ± 1	Transformed	-	-	-	-	-	-	-	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	-	No
	AGP^2 included	-	-	-	-	-	-	-	No
	P	-	-	-	-	-	-	-	0.32
	r^2	-	-	-	-	-	-	-	0.37
	r	-	-	-	-	-	-	-	0.61
	n	-	-	-	-	-	-	-	300
	RMSE	-	-	-	-	-	-	-	8.14
	Slope	-	-	-	-	-	-	-	Positive

[1]Model included the fixed effect of AGP and the random effect of treatment nested within study.

Table A.2. Quadratic associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and glucose (mg/dL) in transition Holstein cows.[1]

Plasma AGP sample day	Item	Plasma glucose sample day							
		-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
1	Transformed	-	-	-	-	-	Yes	Yes	-
	$AGP^2 < 0.1$	-	-	-	-	-	Yes	Yes	-
	AGP^2 included	-	-	-	-	-	Yes	Yes	-
	P	-	-	-	-	-	0.01	0.07	-
	r^2	-	-	-	-	-	0.01	0.06	-
	r	-	-	-	-	-	0.10	0.24	-
	n	-	-	-	-	-	85	87	-
	RMSE	-	-	-	-	-	7.26	5.57	-
	Slope	-	-	-	-	-	Positive	Positive	-

[1]Model included the fixed effect of linear and quadratic AGP and the random effect of treatment nested within study.

Table A.3. Linear associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and free fatty acid (µE/L) in transition Holstein cows.[1]

		Plasma free fatty acid sample day							
Plasma AGP sample day	Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
-21	Transformed	-	Yes	Yes	-	-	No	No	No
	$AGP^2 < 0.1$	-	No	No	-	-	No	No	No
	AGP^2 included	-	No	No	-	-	No	No	No
	P	-	0.87	0.79	-	-	0.48	0.73	0.88
	r^2	-	-0.01	-0.09	-	-	-0.29	0.06	0.04
	r	-	0.10	0.30	-	-	0.54	0.24	0.20
	n	-	43	37	-	-	85	90	90
	RMSE	-	97.22	172.40	-	-	219.59	199.05	205.13
	Slope	-	Positive	Positive	-	-	Positive	Positive	Positive
-13 ± 2	Transformed	-	Yes	Yes	-	No	No	No	Yes
	$AGP^2 < 0.1$	-	No	No	-	No	No	No	No
	AGP^2 included	-	No	No	-	No	No	No	No
	P	-	0.04	0.78	-	0.49	0.29	0.82	0.5
	r^2	-	0.27	-0.08	-	0.05	0.01	0.33	0.1
	r	-	0.52	-	-	0.22	0.10	0.57	0.32
	n	-	230	37	-	120	254	352	263
	RMSE	-	53.03	173.06	-	322.03	265.50	224.94	204.98
	Slope	-	Positive	Negative	-	Negative	Negative	Negative	Negative
-3	Transformed	-	-	No	-	-	No	Yes	Yes
	$AGP^2 < 0.1$	-	-	No	-	-	No	No	No
	AGP^2 included	-	-	No	-	-	No	No	No
	P	-	-	0.49	-	-	0.92	0.08	0.04
	r^2	-	-	0.002	-	-	-0.32	-0.02	0.05
	r	-	-	0.04	-	-	0.57	0.14	0.22

	n	-	-	38	-	-	40	44	44
	RMSE	-	-	167.33	-	-	238.81	207.97	217.61
	Slope	-	-	Positive	-	-	Negative	Positive	Positive
1	Transformed	-	-	-	-	-	Yes	Yes	Yes
	$AGP^2 < 0.1$	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	0.53	0.89	0.42
	r^2	-	-	-	-	-	-0.39	0.1	0.08
	r	-	-	-	-	-	0.62	0.32	0.28
	n	-	-	-	-	-	84	87	87
	RMSE	-	-	-	-	-	219.59	186.22	188.52
	Slope	-	-	-	-	-	Negative	Positive	Positive
3 ± 1	Transformed	-	-	-	-	No	No	No	No
	$AGP^2 < 0.1$	-	-	-	-	No	No	No	No
	AGP^2 included	-	-	-	-	No	No	No	No
	P	-	-	-	-	0.81	0.91	0.1	0.5
	r^2	-	-	-	-	0.05	0.03	0.11	0.11
	r	-	-	-	-	0.22	0.17	0.33	0.33
	n	-	-	-	-	169	312	349	320
	RMSE	-	-	-	-	282.44	253.88	229.83	196.95
	Slope	-	-	-	-	Positive	Positive	Negative	Negative
7 ± 1	Transformed	-	-	-	-	-	Yes	No	Yes
	$AGP^2 < 0.1$	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	0.67	0.07	0.64
	r^2	-	-	-	-	-	0.03	0.08	0.1
	r	-	-	-	-	-	0.17	0.28	0.32
	n	-	-	-	-	-	323	325	306
	RMSE	-	-	-	-	-	252.39	231.48	197.08

	Slope	-	-	-	-	-	Positive	Negative	Negative
14 ± 1	Transformed	-	-	-	-	-	-	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	No	No
	AGP^2 included	-	-	-	-	-	-	No	No
	P	-	-	-	-	-	-	0.61	0.91
	r^2	-	-	-	-	-	-	0.3	0.08
	r	-	-	-	-	-	-	0.55	0.28
	n	-	-	-	-	-	-	420	329
	RMSE	-	-	-	-	-	-	215.28	194.57
	Slope	-	-	-	-	-	-	Negative	Positive
21 ± 1	Transformed	-	-	-	-	-	-	-	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	-	No
	AGP^2 included	-	-	-	-	-	-	-	No
	P	-	-	-	-	-	-	-	0.43
	r^2	-	-	-	-	-	-	-	0.09
	r	-	-	-	-	-	-	-	0.3
	n	-	-	-	-	-	-	-	333
	RMSE	-	-	-	-	-	-	-	196.22
	Slope	-	-	-	-	-	-	-	Positive

[1]Model included the fixed effect of AGP and the random effect of treatment nested within study.

Table A.4. Linear associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and beta-hydroxybutyrate (µM) in transition Holstein cows.[1]

		Plasma beta-hydroxybutyrate sample day							
Plasma AGP sample day	Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
-21	Transformed	-	No	-	-	-	Yes	Yes	No
	$AGP^2 < 0.1$	-	No	-	-	-	No	No	No
	AGP^2 included	-	No	-	-	-	No	No	No
	P	-	0.06	-	-	-	0.3	0.14	0.81
	r^2	-	-0.22	-	-	-	0.11	0.05	0.05
	r	-	0.47	-	-	-	0.33	0.22	0.22
	n	-	44	-	-	-	84	88	87
	RMSE	-	64.05	-	-	-	507.26	581.11	360.05
	Slope	-	Negative	-	-	-	Positive	Positive	Positive
-13 ± 2	Transformed	-	No	-	-	No	No	No	No
	$AGP^2 < 0.1$	-	Yes	-	-	No	No	No	No
	AGP^2 included	-	Yes	-	-	No	No	No	No
	P	-	0.38	-	-	0.99	0.36	0.61	0.64
	r^2	-	0.65	-	-	0.11	0.05	0.18	0.15
	r	-	0.81	-	-	0.33	0.22	0.42	0.39
	n	-	234	-	-	119	252	345	264
	RMSE	-	98.60	-	-	309.71	404.75	409.03	456.32
	Slope	-	Negative	-	-	Negative	Negative	Positive	Negative
-3	Transformed	-	-	Yes	-	-	No	Yes	Yes
	$AGP^2 < 0.1$	-	-	No	-	-	No	No	No
	AGP^2 included	-	-	No	-	-	No	No	No
	P	-	-	0.24	-	-	0.88	0.38	0.41
	r^2	-	-	-0.25	-	-	0.14	0.09	0.14
	r	-	-	0.50	-	-	0.37	0.30	0.37

	n	-	-	36	-	-	39	42	41
	RMSE	-	-	53.04	-	-	269.51	346.13	323.62
	Slope	-	-	Positive	-	-	Positive	Positive	Positive
1	Transformed	-	-	-	-	-	Yes	Yes	Yes
	$AGP^2 < 0.1$	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	0.56	0.95	0.84
	r^2	-	-	-	-	-	0.03	0.04	0.05
	r	-	-	-	-	-	0.17	0.20	0.22
	n	-	-	-	-	-	80	85	85
	RMSE	-	-	-	-	-	307.94	305.62	360.14
	Slope	-	-	-	-	-	Positive	Positive	Positive
3 ± 1	Transformed	-	-	-	-	No	Yes	No	No
	$AGP^2 < 0.1$	-	-	-	-	No	No	No	No
	AGP^2 included	-	-	-	-	No	No	No	No
	P	-	-	-	-	0.49	0.29	0.58	0.93
	r^2	-	-	-	-	0.15	0.08	0.14	0.13
	r	-	-	-	-	0.39	0.28	0.37	0.36
	n	-	-	-	-	167	314	337	316
	RMSE	-	-	-	-	266.99	593.55	320.26	390.99
	Slope	-	-	-	-	Positive	Positive	Negative	Negative
7 ± 1	Transformed	-	-	-	-	-	Yes	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	<0.01	0.94	0.29
	r^2	-	-	-	-	-	0.09	0.13	0.09
	r	-	-	-	-	-	0.30	0.36	0.30
	n	-	-	-	-	-	322	311	297
	RMSE	-	-	-	-	-	425.65	270.60	357.35

	Slope	-	-	-	-	-	Positive	Positive	Negative
14 ± 1	Transformed	-	-	-	-	-	-	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	No	No
	AGP^2 included	-	-	-	-	-	-	No	No
	P	-	-	-	-	-	-	0.84	88
	r^2	-	-	-	-	-	-	0.21	0.09
	r	-	-	-	-	-	-	0.46	0.30
	n	-	-	-	-	-	-	404	322
	RMSE	-	-	-	-	-	-	291.58	346.54
	Slope	-	-	-	-	-	-	Positive	Negative
21 ± 1	Transformed	-	-	-	-	-	-	-	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	-	No
	AGP^2 included	-	-	-	-	-	-	-	No
	P	-	-	-	-	-	-	-	0.14
	r^2	-	-	-	-	-	-	-	0.11
	r	-	-	-	-	-	-	-	0.33
	n	-	-	-	-	-	-	-	326
	RMSE	-	-	-	-	-	-	-	343.60
	Slope	-	-	-	-	-	-	-	Positive

[1]Model included the fixed effect of AGP and the random effect of treatment nested within study.

Table A.5. Quadratic associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and beta-hydroxybutyrate (µM) in transition Holstein cows.[1]

Plasma AGP sample day	Item	Plasma beta-hydroxybutyrate sample day							
		-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
-13 ± 2	Transformed	-	No	-	-	-	-	-	-
	$AGP^2 < 0.1$	-	Yes	-	-	-	-	-	-
	AGP^2 included	-	Yes	-	-	-	-	-	-
	P	-	0.02	-	-	-	-	-	-
	r^2	-	0.66	-	-	-	-	-	-
	r	-	0.81	-	-	-	-	-	-
	n	-	233	-	-	-	-	-	-
	RMSE	-	97.42	-	-	-	-	-	-
	Slope	-	Negative	-	-	-	-	-	-

[1]Model included the fixed effect of linear and quadratic AGP and the random effect of treatment nested within study.

Table A.6. Linear associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and insulin (ng/mL) in transition Holstein cows.[1]

		Plasma insulin sample day							
Plasma AGP sample day	Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
-13 ± 2	Transformed	-	Yes	-	-	-	-	Yes	-
	$AGP^2 < 0.1$	-	No	-	-	-	-	No	-
	AGP^2 included	-	No	-	-	-	-	No	-
	P	-	0.04	-	-	-	-	0.69	-
	r^2	-	0.17	-	-	-	-	-0.03	-
	r	-	0.41	-	-	-	-	0.17	-
	n	-	61	-	-	-	-	56	-
	RMSE	-	0.06	-	-	-	-	0.03	-
	Slope	-	Negative	-	-	-	-	Positive	-
-3	Transformed	-	-	-	-	No	Yes	Yes	No
	$AGP^2 < 0.1$	-	-	-	-	No	No	No	No
	AGP^2 included	-	-	-	-	No	No	No	No
	P	-	-	-	-	0.7	0.46	0.36	0.25
	r^2	-	-	-	-	0.04	-0.12	-0.06	-0.01
	r	-	-	-	-	0.20	0.35	0.24	0.10
	n	-	-	-	-	51	48	50	51
	RMSE	-	-	-	-	0.08	0.08	0.09	0.15
	Slope	-	-	-	-	Negative	Negative	Negative	Negative
3 ± 1	Transformed	-	-	-	-	No	Yes	Yes	No
	$AGP^2 < 0.1$	-	-	-	-	No	No	No	No
	AGP^2 included	-	-	-	-	No	No	No	No
	P	-	-	-	-	0.7	0.46	0.36	0.25
	r^2	-	-	-	-	0.04	-0.12	-0.06	-0.01
	r	-	-	-	-	0.20	0.35	0.24	0.10

	n	-	-	-	-	51	48	50	51
	RMSE	-	-	-	-	0.08	0.08	0.09	0.15
	Slope	-	-	-	-	Negative	Negative	Negative	Negative
7 ± 1	Transformed	-	-	-	-	No	No	No	Yes
	$AGP^2 < 0.1$	-	-	-	-	No	No	No	No
	AGP^2 included	-	-	-	-	No	No	No	No
	P	-	-	-	-	0.6	0.26	0.95	0.95
	r^2	-	-	-	-	0.03	-0.09	-0.42	-0.79
	r	-	-	-	-	0.17	0.30	0.65	0.89
	n	-	-	-	-	48	52	52	51
	RMSE	-	-	-	-	0.08	0.08	0.10	0.23
	Slope	-	-	-	-	Negative	Negative	Negative	Positive
14 ± 1	Transformed	-	-	-	-	-	-	No	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	No	No
	AGP^2 included	-	-	-	-	-	-	No	No
	P	-	-	-	-	-	-	0.36	0.64
	r^2	-	-	-	-	-	-	0.27	-0.24
	r	-	-	-	-	-	-	0.52	0.49
	n	-	-	-	-	-	-	111	54
	RMSE	-	-	-	-	-	-	0.06	0.13
	Slope	-	-	-	-	-	-	Positive	Positive
21 ± 1	Transformed	-	-	-	-	-	-	-	No
	$AGP^2 < 0.1$	-	-	-	-	-	-	-	No
	AGP^2 included	-	-	-	-	-	-	-	No
	P	-	-	-	-	-	-	-	0.27
	r^2	-	-	-	-	-	-	-	-0.42
	r	-	-	-	-	-	-	-	0.65
	n	-	-	-	-	-	-	-	53
	RMSE	-	-	-	-	-	-	-	0.11

Slope	-	-	-	-	-	-	Positive

[1]Model included the fixed effect of AGP and the random effect of treatment nested within study.

Table A.7. Linear associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and haptoglobin[1] in transition Holstein cows.[2]

		Plasma haptoglobin sample day							
Plasma AGP sample day	Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
-13 ± 2	Transformed	-	-	-	-	-	Yes	Yes	Yes
	AGP^2 < 0.1	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	0.64	0.43	0.23
	r^2	-	-	-	-	-	-0.07	0.07	0.16
	r	-	-	-	-	-	0.26	0.26	0.40
	n	-	-	-	-	-	82	85	81
	RMSE	-	-	-	-	-	586.38	392.22	330.01
	Slope	-	-	-	-	-	Negative	Negative	Negative
3 ± 1	Transformed	-	-	-	-	Yes	No	No	Yes
	AGP^2 < 0.1	-	-	-	-	No	No	No	No
	AGP^2 included	-	-	-	-	No	No	No	No
	P	-	-	-	-	<0.001	0.33	0.55	0.36
	r^2	-	-	-	-	0.24	0.16	0.28	0.24
	r	-	-	-	-	0.49	0.40	0.53	0.49
	n	-	-	-	-	50	130	130	129
	RMSE	-	-	-	-	233.09	506.54	209.17	247.37
	Slope	-	-	-	-	Positive	Positive	Positive	Positive
7 ± 1	Transformed	-	-	-	-	-	Yes	Yes	Yes
	AGP^2 < 0.1	-	-	-	-	-	No	No	No
	AGP^2 included	-	-	-	-	-	No	No	No
	P	-	-	-	-	-	<.001	<0.01	0.86
	r^2	-	-	-	-	-	0.32	0.28	0.25
	r	-	-	-	-	-	0.57	0.53	0.50

	n	-	-	-	-	-	143	140	134
	RMSE	-	-	-	-	-	518.98	318.25	224.55
	Slope	-	-	-	-	-	Positive	Positive	Positive
14 ± 1	Transformed	-	-	-	-	-	-	Yes	Yes
	$AGP^2 < 0.1$	-	-	-	-	-	-	Yes	No
	AGP^2 included	-	-	-	-	-	-	Yes	No
	P	-	-	-	-	-	-	0.03	0.71
	r^2	-	-	-	-	-	-	0.31	0.22
	r	-	-	-	-	-	-	0.56	0.47
	n	-	-	-	-	-	-	145	135
	RMSE	-	-	-	-	-	-	337.03	204.15
	Slope	-	-	-	-	-	-	Positive	Positive
21 ± 1	Transformed	-	-	-	-	-	-	-	Yes
	$AGP^2 < 0.1$	-	-	-	-	-	-	-	No
	AGP^2 included	-	-	-	-	-	-	-	No
	P	-	-	-	-	-	-	-	0.22
	r^2	-	-	-	-	-	-	-	0.25
	r	-	-	-	-	-	-	-	0.50
	n	-	-	-	-	-	-	-	137
	RMSE	-	-	-	-	-	-	-	195.57
	Slope	-	-	-	-	-	-	-	Positive

[1]Data for haptoglobin from Carpenter et al. (2018) were reported as µg/mL and data from Bollatti et al. (2020) and Zenobi et al. (2018) were reported as optical density. Differences between concentration reporting methods is accounted for with the random effect of treatment(study) in our model.

[2]Model included the fixed effect of AGP and the random effect of treatment nested within study.

Table A.8. Quadratic associations between plasma alpha-1-acid glycoprotein (AGP) concentration (µg/mL) and haptoglobin[1] in transition Holstein cows.[2]

		Plasma glucose sample day							
Plasma AGP sample day	Item	-21	-13 ± 2	-3	1	3 ± 1	7 ± 1	14 ± 1	21 ± 1
1	Transformed	-	-	-	-	-	-	Yes	-
	$AGP^2 < 0.1$	-	-	-	-	-	-	Yes	-
	AGP^2 included	-	-	-	-	-	-	Yes	-
	P	-	-	-	-	-	-	0.02	-
	r^2	-	-	-	-	-	-	0.31	-
	r	-	-	-	-	-	-	0.56	-
	n	-	-	-	-	-	-	145	-
	RMSE	-	-	-	-	-	-	337.03	-
	Slope	-	-	-	-	-	-	Positive	-

[1]Data for haptoglobin from Carpenter et al. (2018) were reported as µg/mL and data from Bollatti et al. (2020) and Zenobi et al. (2018) were reported as optical density. Differences between concentration reporting methods is accounted for with the random effect of treatment(study) in our model.

[2]Model included the fixed effect of linear and quadratic AGP and the random effect of treatment nested within study.

Appendix B - Chapter 4 Supplementary Table

Table B. 1. Association of chemical composition for bales immediately after baling and after particle size reduction.

	P-value					
Item	Trt	Intercept	Parameter Estimate	R^2	N	RMSE
DM, %	0.27	82.48	0.19	0.27	24	0.82
CP, %	0.49	-1.99	1.13	0.93	24	0.06
ADF, %	0.03	23.83	0.55	0.38	24	1.17
NDF, %	0.03	5.46	0.54	0.76	24	1.10
NDFom, %	0.01	9.11	0.70	0.77	24	1.17
Lignin, %	0.86	9.93	-0.04	0.33	24	0.36
Ash, %	0.52	5.21	-0.04	0.86	24	0.31
ADICP, %	0.01	0.43	0.03	0.66	24	0.06
ADICP, % CP	0.03	0.61	1.31	0.67	24	0.45
NDFD-30hr, g/kg	0.03	0.12	-0.60	0.60	24	1.28
NDFD-48hr, g/kg	0.01	26.40	-1.10	0.43	24	1.69
NDFD-120hr, g/kg	0.05	58.20	-0.96	0.17	24	2.21
NDFD-240hr, g/kg	0.09	69.31	-0.93	0.21	24	2.58
uNDF-30hr, g/kg	<0.01	10.4	0.67	0.66	24	0.87
uNDF-120hr, g/kg	0.02	2.48	1.01	0.28	24	1.83
uNDF-240hr, g/kg	0.11	14.42	0.74	0.18	24	2.15
TTNDFD	0.97	29.04	0.01	0.07	24	1.96

[1]Model included the fixed effects of chemical composition at the time of baling and treatment.

The interaction of beginning chemical composition and treatment were dropped from the model for all variables ($P > 0.05$).