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CORN SILAGE GROWING DIETS AND RUP
DIGESTIBILITY OF CORN SILAGE, THE
UTILIZATION OF AGGRESSIVE IMPLANT
PROTOCOLS, AND THE EFFECT OF
INOCULATE ON CORN SILAGE AND HIGH
MOISTURE CORN

Colton R. Oney

University of Nebraska - Lincoln, colton.oney@unl.edu

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THE EFFECTS OF SUPPLEMENTAL RUP IN CORN SILAGE GROWING DIETS
AND RUP DIGESTIBILITY OF CORN SILAGE, THE UTILIZATION OF
AGGRESSIVE IMPLANT PROTOCOLS, AND THE EFFECT OF INOCULATE ON
CORN SILAGE AND HIGH MOISTURE CORN

BY

Colton Ray Oney

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THE EFFECTS OF SUPPLEMENTAL RUP IN CORN SILAGE GROWING DIETS
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Colton Ray Oney, M.S.

University of Nebraska, 2017

Advisor: Andrea K. Watson

Three experiments evaluated the effect of implant strategies on feedlot performance and carcass characteristics of crossbred steers and Holstein steers. Final BW, ADG, and G:F were not different between implant strategies in Exp. 1 and 2. The utilization of more aggressive implant strategies has minimal impact on both feedlot and carcass performance of cross breed steers.

Four experiments evaluated the effects of supplemental RUP in corn silage growing diets and RUP content and RUP digestibility of corn silage. Exp. 1, supplemented five concentrations of RUP to growing calves consuming a corn silage diet. As supplemental RUP increased ADG and ending BW increased. In Exp. 2, *in vitro* methods were used to determine RUP of 37 and 43% DM corn silage by correcting for microbial contamination using purines. As a % of CP, RUP averaged 22.7 and 23.9 for the 37 and 42% corn silages. In Exp. 3, *in situ* methods were used to determine RUP content and RUP digestibility of 37 and 42% DM corn silage. RUP digestibility without rinsing in neutral detergent fiber solution averaged 37.5 and RUP digestibility of corn silage rinsed in neutral detergent fiber solution averaged 32.3. In Exp. 4, DRC was

reconstituted to determine protein values of grain in corn silage. As DM of corn decreased % RUP decreased and % DMD increased. Corn silage has low RUP and increased moisture further decreases RUP of corn silage.

Two experiments evaluated the effects of inoculants on nutrient losses and aerobic stability of corn silage and HMC. In Exp. 1, corn silage was inoculated at either 0, 200,000, or 400,000 CFU/g and HMC was inoculated at either 0, 300,000 or 600,000 CFU/g then ensiled for 30 or 90 days. In Exp. 2, HMC was inoculated at either 0 or 600,000 CFU/g and ensiled for either 90 or 120 days. In both experiments pH increased linearly for both corn silage and HMC as level of inoculant increased. As inoculant level increased, the amount of lactic acid decreased and the amount of acetic acid increased.

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Introduction

In order to continue supporting the rapidly growing world population, the beef industry must continue to progress in efficiency and output. In the interest of supporting this ever-growing population the industry is continuing to develop and implement new technologies to improve efficiency and overall output of cattle while also staying profitable.

Exogenous compounds administered to the body have been studied, produced, and in use since the 1950s. They are administered to increase efficiency in the conversion of energy consumed into body mass. There are two major growth promoting agents approved for use in the cattle industry; β -adrenergic agonists and hormonal implants. Hormonal implants are used to enhance growth rate, feed efficiency, and lean tissue accretion (Hilscher et al. 2016). Implants are available for suckling calves, grazing cattle, and finishing cattle in both steers and heifers. Implants can be composed of a single compound or a combination of active ingredients. Currently, there are 30 anabolic implants available for use in beef cattle, which producers can implement into their own implant program allowing them to have a program that fits their marketing goals as well as gives them the highest return on investment (Nichols, 2009; Duckett and Pratt, 2014).

Along with hormonal implants, diet manipulation provides another opportunity to optimize cattle efficiency and performance. Manipulating the diet allows producers to provide cattle with the optimal level of each ingredient in order to meet nutritional requirements without feeding excess nutrients. One way to improve efficiencies is by producing high-quality forages and increasing their proportion in the diet. Corn silage is a major forage and energy source in both the North American dairy and beef industries.

With increased costs of grain and forage in recent years corn silage has once again become popular as a forage source (Johnson et al., 1999). Corn silage has rather low rumen undegradable protein (RUP) levels and therefore needs to be supplemented with an RUP source in order to meet the metabolizable protein (MP) requirements of growing cattle allowing for optimal performance and efficiency. Forage inoculates are another technology that can improve efficiency, and reduce cost by better preserving the silage. Inoculates reduce economic and nutritional losses of silage by stimulating a more efficient fermentation and inhibiting aerobic spoilage by inhibiting the metabolism of acid intolerant microbes.

Therefore, the objectives of these studies were: 1) determine the effects of aggressive implant programs on feedlot performance and carcass traits of weaned calf-fed steers; 2) determine rumen undegradable protein levels in corn silage and the effects of moisture content and length of ensiling period on the digestibility of protein in corn silage; 3) determine the effect of inoculation on nutrient losses of corn silage and high moisture corn.

CHAPTER 1. Review of Literature

Growth Promoting Anabolic Steroid Implants

Anabolic steroids are a group of natural and synthetic compounds including estrogens (female hormone), androgens (male hormone) and progestins approved for animal production as growth promoting agents to increase growth rate and feed efficiency (Meyer, 2001). Naturally occurring steroids include estradiol-17beta (E17), testosterone, and progesterone. There are three synthetic compounds used in beef cattle production to enhance growth and efficiency: zeranol, trenbolone acetate (TBA), and megestrol acetate (MGA). Currently, 90% of all feedlot cattle in the U.S. receive some type of implant (Johnson et al., 2013). There are numerous types of implants approved by the FDA for use in the beef cattle industry from suckling calves to finishing cattle; however, two-thirds of the implants marketed in the U.S. in 2012 were made up of a combination of E17 and TBA. Due to E17/TBA implants being the primary implant used, this review will focus on these compounds. Anabolic steroid implants are only approved for implantation in the back middle of the ear. Once in the ear, the pellets slowly dissolve into the bloodstream and are carried to all tissues in the body.

Mode of Action

The endocrine system is the body's mechanism for regulating physiological functions and controlling growth, and steroid hormones act upon the endocrine system. Therefore, the use of anabolic steroids has a significant effect on muscle growth (Trenkle, 1997; Meyer, 2001). The combination of the two hormones TBA and E17 are more effective than either TBA or E17 alone in stimulating growth in

feedlot steers (Pampusch et al., 2008). Compared to non-implanted cattle, cattle implanted with TBA and estradiol-17beta have shown increased circulating IGF-I and skeletal muscle IGF-I-mRNA abundance. The combined TBA and E17 implant results in approximately a 10% increase in carcass protein compared to non-implanted steers (Johnson et al., 2013).

Growth Hormone, insulin, insulin-like growth factors, glucocorticoids, thyroxine and β_2 – agonists are some of the hormones that affect protein metabolism. Johnson et al. (2013) discussed how steroid growth implants have been shown to induce postnatal skeletal muscle hypertrophy (increase in size of existing muscle fibers), through increased circulatory concentrations of the important growth factor for skeletal muscle, insulin like growth factor-I (IGF-I). IGF-I is an important stimulator of skeletal muscle growth and differentiation. Indirectly, E17 causes a stimulation of the anterior pituitary which in turn increases the secretion of growth hormone which leads to the production of IGF-I (Meyer, 2001). The increase in IGF-I helps stimulate skeletal muscle protein synthesis while reducing skeletal muscle protein degradation (Johnson et al., 2013). Hypertrophy increases the level of protein accretion in existing muscle tissue over a short period of time when increased circulatory levels of IGF-I and IGF-I-mRNA are present (Johnson and Chung, 2007).

Although not as well understood, Meyer (2001) suggested that TBA acts similar to other androgens; however, it shows strong binding with androgen, progestin, and glucocorticoid receptors. Trenbolone acetate has been documented to suppress tyrosine amino transferase, which is a key enzyme in amino acid degradation. This allows for the assumption that TBA behaves as an antiglucocorticoid when binding to glucocorticoid

receptors. Therefore, the strong growth-promoting effects of TBA can be attributed to both the anabolic activity as an androgen and the anticatabolic activity as an antigluccorticoid (Meyer, 2001). When comparing a combination of E17/TBA, E17 alone, and TBA alone, Pampusch et al. (2008) reported that *in vivo* the level of TBA was not high enough to measure a direct response to increased protein synthesis. When compared to E17 alone, and TBA alone the combination of E17/TBA was reported to have had a greater impact on IGF-I serum and IGF-I-mRNA levels and that TBA stimulated the muscle growth pathways allowing a synergistic effect with E17. While Kamanga-Sollo et al. (2001) reported that TBA is dose dependent and is directly involved in influencing protein synthesis and protein degradation, *in vitro* studies reported that other competitive inhibitors disrupt TBA from stimulating synthesis and degradation. E17 and TBA can be used in a synergistic combination to produce additive anabolic effects in implanted cattle due to the independent mechanisms by which estrogens and androgens illicit physiological effects (Trenkle, 1997).

Combining E17 and TBA in an implant has not only been shown to boost muscle protein through hypertrophy but also increases muscle protein through hyperplasia by increasing cells formed from satellite cells (Johnson and Chung, 2007; Pampusch et al., 2008; Chung et al., 2012). When E17 and TBA are combined there is an increase in the amount of satellite cells by 50%. Demonstrating that these hormones were causing the proliferation of satellite cells and the new cells were fusing with the muscle fibers, allows for increased amounts of DNA in the muscle which is then available to synthesis more protein. Anabolic steroid implants in the form of E17/TBA increase the number of satellite cells, circulating IGF-I concentrations, and production of IGF-I in the muscle

tissue. However, the complete mechanism of the intracellular pathways and receptors influenced by these hormones are not yet established or understood. Therefore, the complete effects of implanting are not totally understood (Dayton and White, 2014).

Implanting Strategies

Understanding the duration of implant effectiveness and knowing how long cattle will be on feed is important for cattle feeders. Understanding this allows feeders to match the proper implants and implant strategies to their cattle and allows for maximum return on investment (Brand, 1997). Based on cattle breed, sex, marketing conditions, body condition and approximate days on feed (DOF), cattle producers have developed specific implant strategies for different combinations of implants. Strategies are tailored to animal type and marketing opportunities by increasing or decreasing the dose of hormone administered (Johnson et al., 2013).

Increasing implant hormone dosage of single implants or using multiple implants to increase hormone dosage increases body weight (BW) when comparing animals at the same body composition (Guiroy et al., 2002). Implanted cattle tend to have less marbling and leaner carcasses when harvested on same DOF as non-implanted cattle, due to the increased deposition of muscle rather than fat (Johnson et al., 2013). Implants reduce the physiological age of cattle causing the animal's growth curve to shift to that of a younger animal, allowing for more efficient production of lean tissue. Due to this shift in the growth curve, when harvested on equal DOF, cattle that have received an implant tend to have a lower percentage of body fat compared to non-implanted cattle (Reinhardt, 2007). Therefore, implanted cattle should be fed for longer periods of time to achieve similar amounts of marbling as non-implanted cattle (Johnson et al., 2013).

Mader (1997) discussed that animal BW, age, and producer production goals are important factors to consider when determining the correct implant dose in order to maintain optimum hormone levels. Continental cattle are leaner and can benefit from an implant approach that begins with a low dose initial implant and then reimplant with a high dose implant in order to prevent a reduction in quality grade. Whereas, British breeds who tend to mature earlier can benefit from a longer growing period and can better utilize a more aggressive higher dose implant regimen (Johnson et al., 2013). Reinhardt (2007) and Johnson et al. (2013) both discussed how, before choosing an implant strategy, considerations must be made with the goals of the producers in mind. Targeting efficiency and lean meat yield or targeting quality along with efficiency must also be considered.

While there is no withdrawal period for implants, in order to receive the optimal response it is necessary to leave the implant in the animal until most of the compound has paid out (ranging from 50 to 200 days). Applying single dose implants on top of each other when the prior implant is still paying out is possible; however, utilizing a combination implant achieves the same purpose, but is much more controlled. Implant strategies are usually based on estimated DOF for cattle to reach the desired harvest weight (Johnson et al., 2013). Bruns et al., (2005) discussed that the effects of aggressively implanting during the early growth phases of cattle could cause considerable reduction in quality grade. However, using mild implants during the rapid growth phase and aggressive implants once growth has slowed down has been seen to provide substantial growth enhancement with minimal impact on the ability to deposit marbling. Weather can also play a role in the implant strategy decision. For example, producers in

the Southern US who face extremely high temperatures during the summer may choose to implant with a single long-lasting implant during the spring to avoid processing in the heat. Likewise, producers in the Midwestern US might choose a longer-acting implant to avoid implanting during the colder months of winter (Johnson et al., 2013).

Payout

In order for growth promotions to occur, the dissolving implant pellets must release a minimum amount of micro-gram/d of anabolic steroids. The rate of release from the implant controls the length (or payout) of the growth promoting phase (Reinhardt, 2007). Brandt (1997) and Reinhardt (2007) discussed how the blood serum hormone levels peak in the first few days after implantation and then the rate of release slowly decreases with additional DOF following first-order kinetics, making the rate of release biphasic. There is a common consensus that the functional life (period of time for which the implant releases hormone) is between 60 – 120 days (Heitzman et al., 1981; Hendricks et al., 1982; Blasi et al., 2000; Kraft et al., 2000). While it is likely that a theoretical level of serum or plasma concentration of hormone exists below which growth promotion will not occur, these levels have not been completely established and it might be that these thresholds differ for various classes of cattle and different rates of gain (Brandt 1997; Preston 1999).

Both the carrier and the hormone mixture in the implant affect the release rates of hormones from the implant into the animal. Disintegration of the pellet or dissolving of the carrier matrix allows for hormone release and exposure to the body. Due to its ability to form pellets and its harmlessness to tissue, lactose was initially used as a carrier in compressed pellet implants. Because of its high solubility, utilizing lactose as a carrier

led to rapid release of hormone and a shorter functional life. Therefore, because of its lower solubility, cholesterol is now used as a carrier and allows for a slower release rate of hormone from the pellet (Hunter, 2010). Heitzman and Harwood (1977) discussed how combining estradiol and trenbolone into a compressed pellet implant results in a slower and more sustained release of estradiol than from pellets containing only estradiol. In a study by Heitzman et al., (1981) when trenbolone was combined with estradiol in a single implant and compared to an implant containing only estradiol, plasma concentrations of estradiol were elevated for 91 days for the combination implant compared to only 28 days for the pellet containing only estradiol.

With demand for increased gain efficiency and lean meat yield, utilization of greater-dose implants has been increasing. Some implants like Revalor-XS (200 mg of TBA and 40 mg of E17; Merck Animal Health, De Soto, KS) have unique payouts due to the polymer coating on a fraction of the pellets included in the implant. Upon implanting, the pellets without the coating begin releasing TBA and E17, while the coated pellets are theoretically not supposed to start paying out until around 80 d after the initial administration of the implant. This delay in release of TBA and E17 prevent the need to reimplant cattle multiple times, due to the extended payout period of around 200 d (FDA, 2007). Parr et al., (2011) conducted four experiments comparing release patterns of anabolic implants and the effects on growth in finishing steers. They concluded that greater doses of TBA + E17 improves steer performance when steers are fed for longer periods of time. However, when comparing equal doses of TBA and E17 the delayed release of TBA and E17 associated with Revalor-X improved steer performance and quality grade when compared to the non-delayed release implants. It's important to note

that these effects could have been associated with the duration of feeding period and timing of reimplanting rather than implant payout. Because many cattle require more than 120 days to reach slaughter weight and the majority of implants only last 60 – 120 days, depending on dose, reimplanting is becoming an increasingly important management tool. Therefore it is increasingly common for cattle to be implanted with two consecutive combination implants containing both TBA and E17 which allows for hormone to be present and available the entire feeding period. When compared to nonimplanted cattle, Duckett and Pratt (2014) observed a 20% increase in ADG and a 13.5% improvement in BW gain efficiency in cattle implanted with two consecutive implants. When considering greater-dose implants and more aggressive implant protocols, data are limited on the use of these implant combinations in cattle fed for greater than 170 days. However, Hilscher et al., (2016) conducted three experiments evaluating initial implant strategies for finishing cattle utilizing high-dose implants. They concluded that implant protocols utilizing a greater-dose initial implant followed by a high-dose terminal implant yielded results not different from implant protocols that utilized a lesser-dose initial implant followed by a greater-dose terminal implant. This suggests that the payout for a lesser-dose implant lasts around 80 days and when followed by a higher-dose terminal implant, sufficient hormone is available to continue to advance the deposition of lean muscle tissue while allowing cattle to remain on feed long enough to deposit sufficient fat.

The past fifty years of safe use of growth promotants in beef cattle production has allowed for massive increases in beef cattle efficiency. Ultimately, the best available resource for producers to increase animal and financial efficiency in growing and

finishing beef cattle is anabolic implants (Nichols et al., 2002). The resulting increase in performance provides economic benefit to producers and also reflects on the competitiveness of beef as a dietary source of protein compared to other available sources.

Corn Silage Rumen Undegradable Protein

In 2013 U.S. corn silage production was estimated at 117.9 million tons and was the fourth highest level of production on record. It is estimated that in 2013 6.3 million acres were harvested as corn silage with an average per-acre yield of 18.8 tons (USDA, 2014). Due to record high corn prices in recent years, there has been a renewed interest in feeding corn silage to beef cattle. While average daily gain (ADG) and feed to gain efficiency (F:G) are not as favorable, feeding greater inclusions of corn silage offers an economical alternative to high priced corn. Feeding corn silage allows cattle feeders to take advantage of the entire corn plant at a time of maximum quality and tonnage, while also securing a substantial quantity of roughage inventory. Corn silage averages 6.5 to 8.5 % crude protein (CP), with most of it being rumen degradable protein (RDP) which is utilized for microbial protein synthesis. Very little protein from corn silage escapes the rumen (RUP) and previous estimates of RUP from corn silage are likely incorrect. Because corn silage consists of both forage and grain it is difficult to measure bypass protein. It is also key to distinguish between bypass protein and microbial crude protein (MCP), consequently making calculating correct values for bypass protein very challenging. Inaccurate estimates of RUP could result in not meeting metabolizable protein (MP) requirements (NRC, 2000). Therefore this section of the review will give a brief review of protein utilization in ruminants, and focus on how corn silage digestion

and levels of RUP have been measured in the past, and the supplemental RUP required to meet metabolizable protein (MP) requirements for growing cattle fed corn silage diets.

Ruminant Protein and Amino Acids

Adequate protein and amino acids are essential for proper development, growth and production efficiency. Cattle are pre-gastric fermenters, meaning that the microbes contained in the rumen have the first opportunity at the nutrients being supplied. Protein that is able to be used for nutritional needs is supplied by microbial and dietary sources (Wilkerson et al., 1993). Protein that is degraded by the microbes in the rumen is called rumen degradable protein (RDP) while protein that is not degraded by the rumen microbes, or bypasses the rumen, is called rumen undegradable protein (RUP). Microbial protein synthesis, feed protein that is resistant to rumen degradation (RUP) and amino acids that are able to bypass the rumen are what supplies the small intestine with absorbable amino acids (Chalupa, 1974). Microorganisms in the rumen are able to convert low quality nitrogen sources to microbial protein, which is a high quality protein. In non-ruminants, microbes are excreted and lost, but in ruminants they are flushed to the small intestine and are absorbed to be utilized as energy for the animal. The amount and quality of amino acids reaching the small intestine depends on the feed type, and the amount of intake. Variation of ruminal degradation of proteins among different feeds is brought about by two main factors. The first is solubility of protein in rumen liquid, and the second is the length of time protein is retained in the rumen. Proteins that have a higher degree of solubility in the rumen will be degraded to a greater extent and therefore will supply less RUP to the small intestine. This is also true for the amount of time retained in the rumen, longer periods of retention allow for more degradation of the

protein and smaller amounts being able to withstand degradation (Chalupa, 1974). Variation in protein source accounts for degree of degradation and total amino acids that flow to the duodenum and are available for absorption in the small intestine. Though microbial protein synthesis is able to produce some usable protein for the animal, it cannot supply sufficient amounts of amino acids to meet the high amino acid requirements of growing cattle (Polan et al., 1991). Therefore the influx of material from the rumen to the small intestine, consisting of microbial cells, fermentation end products, and undegraded feed materials, possibly does not supply the animal with an optimal amount of nutrients. Deficiencies in limiting amino acids prevent cattle from growing and performing at their optimal levels. Thus, providing opportunities to improve animal performance by providing a supplemental source of RUP (Chalupa, 1974).

There are currently three procedures that exist by which the quantity and quality of amino acids that are available to the ruminant might be altered. The first is maximizing net production of microbial protein. Improvement of net production of microbial protein has been studied in many labs and a haNDSul of ruminal and dietary factors have been identified that have the ability to influence the net production of microbial protein (Merchen and Titgemeyer 1992). The primary method is defaunation, or removal of protozoa. Defaunation has the ability to decrease nitrogen recycling in the rumen, therefore improving total bacterial protein synthesis. Although increased ruminal production of microbial protein results, there is limited opportunity for increasing microbial protein through defaunation enough to contribute significantly (Rowe et al., 1985; Meyer et al., 1986). The second strategy, and most feasible right now, is altering the amino acid supply with supplemental protein sources. Any protein that is high in

RUP will help to improve performance when total absorbable amino acids limit production. However, in situations when specific essential amino acids limit production, certain protein sources may be more advantageous to the animal in supplying these specific amino acids. Furthermore, feeding proteins that are high in RUP when limiting amino acids are deficient in the supplemental protein fed may be of little use. It is important to keep two things in mind when supplementing a protein source. First, be sure to account for maintaining microbial protein synthesis by including a RDP source such as urea, and secondly make sure that the protein you supplement is not degradable by the rumen, yet available for absorption in the small intestine (Merchen and Titgemeyer 1992). The third strategy to increase amino acids available to the ruminant is by feeding ruminally protected amino acids. Though Titgemeyer et al., (1988) showed that feeding rumen protected amino acids increased the availability and changed the essential amino acid profiles entering the small intestine of beef cattle, results from growing studies haven't proved the effectiveness of this strategy in growing cattle (Merchen and Titgemeyer 1992).

Limiting Amino Acids in Growing Cattle

Growing cattle have high nutritional needs. They must have energy to maintain day to day activities (respiration, digestion, movement, circulation, etc.) and also sufficient energy for growth (energy deposited as muscle and/or fat). As we continue to push cattle further than we ever have before with increased growth rate and efficiency of conversion of feed to gain it is becoming increasingly challenging to meet specific amino acid requirements of growing cattle (Richardson and Hatfield, 1978). Today growing cattle are putting on protein at increasingly quick rates. Because of this it is essential to

keep a constant supply of readily metabolizable protein available to the cattle. Shortage of essential amino acids delivered to the small intestine is possibly a result of insufficient quantity and/or quality of amino acids being produced. When amino acid requirement is high, as it is in growing cattle, microbial protein produced in the rumen from RDP may not be sufficient to meet the essential amino acid requirements of the animal (Merchen and Titgemeyer, 1992). Essential amino acids include: Phenylalanine, Valine, Threonine, Tryptophan, Isoleucine, Methionine, Histidine, Arginine, Lysine, and Leucine.

By observing the responses of abomasally infused amino acids, Richardson and Hatfield (1978) demonstrated that in growing steers, methionine, lysine and threonine, are the first three limiting amino acids, respectively. Growth rate cannot be increased until limiting amino acids are provided in sufficient amounts. Amino acids entering the small intestine normally have little resemblance, either quantitatively or qualitatively, to dietary amino acids due to alterations that occur in the rumen. This causes formulating diets to meet amino acid requirement to be a troublesome task. To formulate diets for amino acid requirements you must have reliable estimates of three quantities: 1) microbial amino acids reaching the small intestine, 2) dietary amino acids entering and being absorbed from the small intestine, and 3) amino acid requirements for maintenance and production (Merchen and Titgemeyer, 1992).

Although there are three key limiting amino acids in growing cattle, it is important to keep in mind that the value of other amino acids cannot be overlooked because they could quickly become limiting. Because the amount and type of essential amino acid supply at the duodenum from microbial protein synthesis is inconsistent, supplementing whole proteins usually evoke an improved response rather than supplementing with a

single amino acid. Merchen and Titgemeyer (1992) showed that when methionine alone was supplemented, a nitrogen retention of roughly 17% was observed, while responses to supplementation of casein (a whole protein) had increased nitrogen retentions of 33, 63, and 108% (at 100, 200, and 300 g/d of casein). This suggests that despite methionine being the first limiting amino acid, additional amino acids might be co-limiting and administering a wide range of essential amino acids amplifies response by the animal (Merchen and Titgemeyer 1992). In connection with Merchen and Titgemeyer (1992), Ali et al., (2009) found that by increasing the level of RUP in the diet the flow of amino acids into the small intestine for absorption was increased; therefore, increasing overall growth and performance of the animal. They also observed that when supplementing protected amino acids to low protein diets, increased performance was obtained.

Methods of Estimating Protein of Corn Silage

Forages are rapidly degraded by rumen microorganisms and are a good source of rumen degradable protein (RDP) and a fairly poor source of RUP. Lab techniques designed to measure bypass protein levels are specific to either forages or concentrates. Because silage is assumed to be 50% forage and 50% concentrate, measuring concentrations of bypass protein can be difficult due to the microbial attachment to forage particles. Haugen et al., (2006) validated the use of neutral detergent insoluble nitrogen to account for microbial attachment when measuring RUP; however, it has not been utilized for non-forage fiber sources and it is possible that a portion of the proteins may remain in the residue but were soluble in the neutral detergent solution, resulting in lower estimates of degradable RUP (Klopfenstein et al., 2001). Because corn silage is low in RUP it is necessary to have accurate measures of protein degradability in order to

predict animal performance and to correctly supplement to meet MP requirements. To accurately measure RUP content of corn silage it is critical to measure the amount of microbial crude protein (MCP) entering the small intestine (Klopfenstein et al., 2001). There are three main methods of estimating ruminal protein degradation: *in vivo*, *in vitro*, and *in situ*. *In vivo* procedures require considerable investment of resources and have many problems related to inaccuracies in: determination of flow due to marker, sampling, cannulation effects, differences between protein of feed microbial and endogenous origin and is therefore rarely used in determining rumen protein degradation (Broderick, 1994). Being more cost and time efficient than *in vivo*, *in situ* and *in vitro* procedures are used in order to estimate *in vivo* values. *In vitro* and *in situ* procedures are typically carried out utilizing rumen inoculum obtained from a ruminally cannulated animal on a 30% concentrate diet (Vanzant, 1998). However, when utilizing *in situ* and *in vitro* methods it is important to consider how dietary components can influence the ruminal microbial population and potentially affect the rate or extent of digestion (Varvikko and Lindberg, 1985).

Therefore, when performing an *in vitro* study it is important to utilize a good microbial marker. Utilizing a reliable microbial marker allows for a good estimation of microbial protein flow to the small intestine (Obispo and Dehority, 1999). Ideal microbial markers should 1) not be present in the feed; 2) not be absorbed; 3) be biologically stable; 4) occur in a similar percentage between the various types of microbes; 5) have a fairly simple assay procedure; 6) be a constant percentage of the microbial cell in all stages of growth; and 7) flow at a similar rate in all forms (i.e., free and bound; Dehority, 1995).

Based on critical analyses by Broderick and Merchen, (1992) and Stern et al., (1994), under practical conditions the total purine method has been suggested as one of the best procedures to quantify microbial protein yields. However, when using purine concentration to estimate microbial protein it is important to consider the quality of the bacterial standard used to establish the purine:protein ratio. Obispo and Dehority (1999), measured purine and protein concentrations in 10 pure cultures of the most prevalent species of ruminal bacteria and found a mean purine:protein ratio of 0.0883. They then compared this ratio to an overall mean ratio of 7 mixed bacterial samples isolated from ruminal contents and found a ratio of 0.0306. Therefore, they concluded that using the ratio obtained from the mixed bacterial cultures would overestimate the microbial protein content in the duodenum by approximately threefold. Thus, when utilizing total purine method to adjust for microbial crude protein it is critical to consider the variation amongst bacterial species in rumen sample used for the standard. Also when considering corn silage, it is important to consider the 50:50 ratio of forage to concentrate and that some microbes will be attached to feed particles (fibreolytic bacteria) and others will be free floating (amylolytic bacteria). In differential centrifugation free floating bacteria are harvested and they may have purine to N ratio different than microbes that are attached to feed particles (Klopfenstein et al., 2001).

In situ techniques reduce the need for ruminal stimulation by studying digestion within the rumen itself and offer a relatively inexpensive and quick means to quantify the ruminally degraded protein of feeds. However, *in situ* techniques are plagued by large variation, both within and among laboratories. Like *in vitro* when using *in situ* methods you must correct for microbial contamination. One of the main sources of variability

with *in situ* seems to be the lack of standardization of rinsing techniques and the failure to correct for microbial contamination (Vanzant et al., 1998). Whittet et al., (2003) observed no differences in washing by hand or washing by machine but found with increased rinsing washout can occur. Therefore, when utilizing *in situ* methods it is important to consider washout and microbial contamination.

Considerations for Corn Silage

The (1996) Beef NRC uses a common value of 80%, for the RUP digestibility of all feeds. However when considering work from Benton et al., (2005) and Buckner et al., (2013) this common value of 80% for multiple feed sources is a poor estimate of RUP digestibility. Benton et al., (2005) found a % RUP digestibility value of dry rolled corn (DRC) to be 95, wet distillers grains (WDGS) to be 89, wet corn gluten feed (WCGF) at 81, soybean meal (SBM) at 98 and SoyPass at 97. The 1996 NRC value of 80% in every case except for WCGF underestimates the % of RUP digestibility for concentrates. Buckner et al., (2013) conducted four experiments to evaluate RUP content and % RUP digestibility of different forages and found that meadow grass had a RUP digestibility of 38, range grass was 38.5, and brome grass was 45 while corn residue (leaf and husk) was 25.5%. Buckner's data shows that the common 80% value used by the NRC overestimates the % RUP digestibility of forages. These data suggest that concentrates have a higher % RUP digestibility while forages have a much lower % RUP digestibility when compared to the constant 80% value used by the 1996 NRC. Because corn silage is 50% concentrate and 50% forage, determining RUP values and % RUP digestibility has been difficult. Not only do we have to take into account both the forage and the concentrate portions of silage but it has also been shown that moisture content of silage at

harvest and the amount of time silage has been stored can impact protein degradability. Benton et al., (2005) harvested two different moisture levels (24 or 30%) of high moisture corn (HMC) and then reconstituted DRC to different moisture levels (28 or 35%) and then ensiled. He found that as moisture level increased and length of ensiling increased for both the HMC and reconstituted DRC, dry matter digestibility (DMD) and rumen degradable protein (RDP) increased ($P < 0.01$). This is important because when corn silage is harvested the kernel is typically between 60 – 70% DM; however, the grain in the silage will absorb the moisture from the forage and become similar to very wet HMC. When considering Benton's work it is important to account for the moisture effect on the grain in corn silage when attempting to quantify rumen bypass protein values.

The NRC of Dairy Cattle (2001) outlines the % digestible RUP of corn silage to be 70%. However, work by Kononoff et al., (2007) observed average % RUP digestibility values for corn silage to be $19.9 \pm 3.76\%$ suggesting that the 70% digestible RUP value used by the NRC overestimates % digestible RUP of corn silage. However it is important to note that Kononoff et al., (2007) used neutral detergent solution to correct for microbial contamination of the residue. Although the use of neutral detergent insoluble nitrogen to measure RUP has been validated (Haugen et al., 2006) it has not been utilized for non-forage fiber sources and it is possible that a portion of the proteins may have remained in the residue but were soluble in the neutral detergent solution, resulting in lower estimates of degradable RUP (Klopfenstein et al., 2001). Kononoff et al., (2007) concluded that values for degradable RUP of forages published by the NRC (2001) are overestimated. Further research is needed to more accurately describe the degradable RUP of corn silage.

Corn silage in Growing Programs

As previously mentioned corn silage is assumed to be 50% forage and 50% concentrate and with recent high corn prices has been utilized in least-cost growing programs. Corn silage growing diets allow for relatively cheap cost of gain but require a protein supplement in order to meet protein requirements (Guyer, 1978; Felix et al. 2014). The majority of both the forage and concentrate portions of silage are high in RDP, hence the need for a supplemental RUP source. While it is well known growing cattle require absorption of true metabolizable protein to meet their protein needs, data on supplemental protein levels included in corn silage growing diets is scarce. Felix et al. (2014) supplemented either urea (100% RDP), SBM (53% CP, 70% RDP) or DDGS (30% CP, 32% RDP; NRC. 2016) at 21% of diet DM to 79% corn silage diet (DM basis). Cattle supplemented with DDGS and SBM had greater ADG, DMI, G:F, and final BW compared to cattle supplemented with urea. While urea is a good RDP source it supplies no RUP making growing cattle with increased amino acid requirements deficient in bypass protein. Both DDGS and SBM have a greater % RUP and therefore allowed cattle that were supplemented with DDGS and SBM to have a larger supply of bypass protein and in turn more amino acids available to be absorbed in the small intestine. Hilscher et al. (2016) conducted a study similar to Felix et al. (2014) by supplementing increasing levels of RUP to growing cattle consuming an 88% corn silage diet (DM basis). Hilscher et al. (2006) used a combination of SoyPass (50% CP; 75% RUP as % of CP) and Emphyreal (Cargill, Blair. Neb; 75% CP; 65% RUP as % of CP) as the RUP source. The author reported that as supplemental RUP levels increased from 0 to 10% (diet DM) ADG and ending BW linearly increased.

With increased fluctuation in corn price, in some situations harvesting corn silage can provide an economic alternative to high corn prices. While ADG and G:F are not as favorable for finishing cattle, with a supplemental source of bypass protein growing cattle have the potential to gain well. Further research is needed in order to determine the ideal levels of supplemental protein in corn silage growing diets.

Corn Silage and High Moisture Corn Inoculants

Inoculants are biological products with a source of live, viable bacteria, combined with enzymes. Similar to live yeasts used to inoculate alcohol for fermentation, bacteria are applied to inoculate freshly harvested forages and sometimes high moisture corn. The enzymes are present to generate sugars for the inoculant bacteria to use for growth and fermentation (Lallemand Animal Nutrition, 2016). Continually growing industry and market dynamics apply constant pressure for beef producers to become more efficient and stay profitable. One way that efficiencies can be improved is by producing high-quality forages by ensiling and storing for long periods of time. The use of biological inoculants is one tool to assist in storing and properly fermenting stored feeds. This section of the review will cover the use of biological inoculants, specifically *Lactobacillus buchneri*, in both corn silage and high moisture corn.

Silage Fermentation and Effect of Inoculant

Silage is defined as an acidic, fermented, stored product from an agricultural crop (Wilkinson et al., 2003). Recovering maximum amounts of dry-matter and nutrients in order to retain maximum quantity of high quality materials to feed is the main reasoning behind ensiling crops. Indigenous lactic acid bacteria on the plant anaerobically

converting soluble sugars from the plant into organic acids is the key process behind ensiling crops (Schmidt, 2016). Pahlow et al. (2003) reported that silage retains the largest amount of nutrients from the original material when compared to other methods of forage preservation. However, even with excellent management strategies nutrient losses and changes in quality occur. Specifically, losses during storage and feedout from aerobic spoilage can be devastating to the quality of silage (Woolford, 1990). Microorganisms naturally colonize forage crops, some being beneficial while others are detrimental to the ensiling process. Muck (2008) reported that inoculants can improve the ensiling process by: preventing clostridial fermentation, enhancing aerobic stability, and making good fermentation better. Therefore, inoculating fresh forage prior to ensiling with fast-growing and effective lactic acid bacteria is recommended.

The ensiling process has four main phases. Phase 1 is considered the aerobic phase. In phase 1 the oxygen within the silage pile is reduced due to respiration of plant particles and aerobic and facultative aerobic microorganisms such as yeasts and enterobacteria. Because pH is still within the normal range of fresh forage (6.5-6) during this phase, proteases and carbohydrases are both still active. Phase 2, early fermentation, begins 2-3 days after the forage has been put up. There is a spike in temperature (usually no more than 20°F above the ambient temperature at harvest) due to the production of acetic acid, lactic acid and some ethanol predominately by lactic acid bacteria (LAB) which can grow in oxygen and help to create anaerobic conditions. Phase 2 is responsible for the initial drive to lower pH. Phase 3, lactic acid fermentation, begins when all the oxygen has been removed and it is a completely anaerobic environment about 3-4 days after ensiling and lasts between 2 and 3 weeks. Homolactic LAB begin

fermenting and produce lactic acid driving the pH of the forage down further (3.5-4.5). Phase 4, the stabilization phase, is when all bacterial activities stop, and the pH remains constant around 4.0. As long as no air penetrates the silage pile relatively little activity occurs until feedout begins (Charley, 2016; Kononoff, 2015).

Homofermentative lactic acid bacteria such as *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Lactobacillus plantarum* are able to convert one molecule of glucose directly to two molecules of lactic acid and are regarded as ‘traditional inoculants’ (Schmidt, 2016). The ability to convert one molecule of glucose into two molecules of lactic acid decreases pH and results in better DM and energy recovery in silages and improved animal performance in some cases (Muck and Kung, 1997). The main roles of homofermentative inoculants are to ensure rapid and efficient fermentation of water-soluble carbohydrates into lactic acid, rapidly decrease pH, and improve silage conservation with minimal fermentation losses (Weinberg et al., 1993). However, in many studies these homofermentative inoculants have not increased aerobic stability but rather decreased it due to enhancing the growth of spoilage yeasts (Moon et al., 1980). *Lactobacillus buchneri* is considered to be a non-traditional inoculant because it is a heterofermentative lactic acid bacterium while most inoculants are homofermentative. Oude Elferink et al. (2001) proposed a metabolic pathway for the use of lactic acid by *Lactobacillus buchneri* and reported that *Lactobacillus buchneri* degrades two molecules of lactic acid to form 1 molecule of acetic acid and 1 molecule of 1,2-propanediol, in addition to CO₂ and traces of ethanol, yielding 1 molecule of ATP. Kleinschmit and Kung (2006) also reported that the increased improvements in aerobic stability of silages have been credited to the increased concentrations of acetic acid produced by

Lactobacillus buchneri which were able to inhibit the growth of yeasts when they were exposed to air, which is the leading cause of silage spoilage. Moon (1983) reported that acetic acid has good antifungal properties which are strengthened with low pH; therefore, increases in acetic acid in the silage environment which contains lactic acid should effectively inhibit the growth of yeasts. *Lactobacillus buchneri* has recently been combined with homofermentative bacteria in an attempt to improve both aerobic stability and fermentation of silage (Kung et al., 2003). Driehuis et al. (2001) reported that silage treated with *Lactobacillus buchneri* alone or in combination with *Pediococcus pentosaceus* and *Lactobacillus plantarum* (homofermentative bacteria) decreased DM loss, improved aerobic stability, and reduced yeasts and mold counts compared to untreated silage. Arriola et al., (2011) compared non-inoculated silage to silage inoculated with *Lactobacillus buchneri* and silage inoculated with a combination of *Lactobacillus buchneri* and *Pediococcus pentosaceus* and reported that both treatments had a greater lactate concentration and lower pH compared to the control; however, only the *Lactobacillus buchneri* by itself reduced DM losses relative to the control treatment.

Effect of Inoculate on High-Moisture Corn

Harvesting and ensiling corn as high-moisture corn (HMC) offers an alternative to dry grain for the feeding of cattle. HMC has been shown to have 104% the energy value of dry-rolled corn (DRC; Vander Pol et al., 2006) and when combined with distillers grains has a significantly better feeding efficiency (Corrigan et al., 2007). Similar to silage, the primary preservation technique for HMC is ensiling. The ensiling process of HMC is similar to that of silage in that the predominant factor in the ensiling process is the reduction of pH from the production of organic acids from soluble sugars due to

bacterial fermentation (Rutherford, 2014). Also like silage, HMC is prone to aerobic deterioration or spoilage when it is exposed to air (Kung et al., 2007). Several factors can lead to the spoilage of HMC, restricted fermentation due to low amounts of fermentable sugar contents and its relatively low moisture both play a role in spoilage. High starch content of corn also plays a role because yeasts can use starch as an energy source and yeasts in aerobic conditions assimilate lactic acid which can initiate spoilage (Woolford, 1990). Hoffman and Ocker (1997) explained how spoilage of HMC is a concern due to the net loss in nutritive value affecting animal performance. Additives containing propionic acid and ammonia have been added to HMC as antifungal compounds with variable success (Britt and Huber, 1975; Sebastian et al., 1996). However, an alternative to applying chemicals to HMC in order to improve aerobic stability and prevent spoilage is the addition of a bacterial inoculate in order to alter the fermentation process and improve stability (Kung et al., 2007). Phillip and Fellner (1992) reported improved aerobic stability of HMC with the addition of a homolactic acid bacteria, however Wardynski et al. (1993) showed that this method is not always effective and attributed the poor results to lactic acid and decreased pH not being sufficient in inhibiting the growth of yeasts.

Lactobacillus buchneri, as explained in the silage fermentation section, is a heterofermentative bacteria that has the ability to produce acetic acid via a novel pathway that converts lactic acid to acetic acid, ethanol, and 1,2-propanediol (Oude Elferink et al., 2001). Even though *Lactobacillus buchneri* is found naturally on most plant species, Hesser et al. (1967) found that it only represented 3% of the lactobacilli isolates found naturally on HMC. There has been a substantial amount of research conducted by

Kleinschmit and Kung (2006) that has reported that inoculation of silage with *Lactobacillus buchneri* improves the aerobic stability of silages. However, little research has been conducted with the addition of *Lactobacillus buchneri* to HMC (Kendall et al., 2002; Taylor and Kung, 2002; Kung et al., 2007). Taylor and Kung (2002) compared HMC either with or without a *Lactobacillus buchneri* inoculant and reported that the aerobic stability of HMC was significantly improved for HMC that received an inoculant. They attributed this to the accumulation of acetic acid via the anaerobic degradation of lactic acid and the inhibition of yeasts by acetic acid. In the aerobic stability test when compared to untreated HMC, Taylor and Kung (2002) reported a six-fold increase in aerobic stability for HMC treated with *Lactobacillus buchneri*. Kung et al. (2007) followed up the previous study by once again comparing the effects of inoculating *Lactobacillus buchneri* on silage fermentation and aerobic stability. They observed that treating both ground and whole HMC with *Lactobacillus buchneri* increased the concentrations of acetic and propionic acids, decreased fungal populations and improved the aerobic stability. The authors concluded that the addition of *Lactobacillus buchneri* has the ability to improve the aerobic stability of HMC by increasing the proportion of acetic acid, in turn inhibiting the growth and reproduction of yeasts that cause spoilage.

Conclusion

Body weight gain and efficiency of gain are the driving forces of the overall profitability of cattle feeding operations. In the interest of supporting the ever-growing human population the beef cattle industry is continuing to develop and implement new technologies to improve efficiency and overall output of cattle while also continuing to stay profitable.

Anabolic growth promoting implants have been used in the industry since the 1950s. Today there are many implant options available to cattle producers and they all have the ability to improve growth rate, feed efficiency, and HCW. The specific implant regime chosen is dependent on the end goals of the producer for their specific cattle and can vary from a single implant protocol up to three to four implant protocols allowing for an implant regime that fits the marketing goals as well as allowing for the greatest return on investment possible (Nichols et al.,2002).

With increased corn grain prices in recent years, there has been a resurgence in the interest of feeding corn silage amongst cattle feeders. Although finishing cattle ADG and feed conversions of diets with high inclusions of corn silage are slightly diminished it is still a great economical alternative to high priced corn. While corn silage has sufficient levels of RDP, bypass protein levels are insufficient and unable to meet metabolizable protein requirements of growing cattle alone. Therefore, corn silage needs to be supplemented with a bypass protein source. Hilscher et al. (2016) demonstrated that with a supplemental protein source (0 to 10% of diet DM) growing cattle on an 88% corn silage diet showed linear increases in both ADG and ending BW as supplemental RUP increased in the diet. With cattle continuing to increase linearly as supplemental RUP increased Hilscher et al (2016) concluded that the supplemental protein levels used may be insufficient to meet metabolizable protein requirements. Further research is needed with increased levels of protein supplementation to quantify the amount of RUP needed to reach metabolizable protein requirements of growing cattle.

Inoculants, biological products with a source of live, viable bacteria, combined with enzymes, have become an increasingly common tool for silage and HMC

management. Both silage and HMC are susceptible to aerobic deterioration or spoilage when exposed to air. Spoilage reduces DM recovery and increases nutrient losses of the stored feed resulting in reduced feed conversions of cattle and reduced return on profit. Inoculants, specifically *Lactobacillus buchneri*, have been shown to significantly decrease DM losses and aerobic spoilage of both corn silage and HMC (Kleinschmit and Kung 2006; Kung et al., 2007). Therefore the use of inoculants is a recommended management tool for cattle producers utilizing silage and HMC.

Therefore, the objectives of these studies were: 1) determine the effects of aggressive implant programs on feedlot performance and carcass traits of weaned calf-fed steers; 2) determine Rumen Undegradable Protein levels in corn silage and the effects of moisture content and length of ensiling period on the digestibility of grain in corn silage; 3) determine the effect of inoculation on nutrient losses of corn silage and high moisture corn.

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Chapter II. Impact of utilizing increasingly aggressive implant protocols on feedlot performance and carcass characteristics of calf-fed steers ¹

C. R. Oney *, M. N. Streeter †, W.T. Nichols †, B.D. Dicke #, R. J. Cooper ‡, D.J. Jordon ‡, T. L. Scott ‡, †, E. Larson •, A. K. Watson *², G. E. Erickson *²

*Department of Animal Science, University of Nebraska, Lincoln 68583; †Merck Animal Health, Desoto, KS 66018; ‡Cattlemen's Nutrition Services, LLC, Lincoln, NE 68506; #Dicke Consulting, LLC, Lincoln, NE 68516 •Larson Nutrition Services, Fowler, CO 81039

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²Corresponding author: gerickson4@unl.edu

Abstract

Three experiments were conducted to evaluate the effects of aggressive implant strategies on feedlot performance and carcass characteristics of crossbred steers (Exp. 1 and Exp. 2) and Holstein steers (Exp. 3). In Exp. 1, steers ($n = 1,350$; 282 kg initial BW, $SD = 8$) were fed for an average of 215 d. Treatments were 1) Revalor-IS at initial processing with a terminal Revalor-200 on d 133; 2) Revalor-IS at initial processing followed 67 d later by Revalor-200 with a terminal Revalor-200 on d 133; Revalor-XS at initial processing with a terminal Revalor-200 on d 133. Final BW, DMI, ADG and G:F were not different ($P \geq 0.16$) among implant strategies. Dressing percent, HCW and LM area did not differ ($P \geq 0.21$). In Exp. 2, steers ($n = 1,513$; 265 kg initial BW, $SD = 18$) were fed for an average of 208 d. Treatments were 1) Revalor-G at initial processing followed 50 days later by a Revalor-IS with a terminal Revalor-200 on day 140; 2) Ralgro at initial processing followed 50 days later by a Revalor-XS; 3) Revalor-XS at initial processing followed by a terminal Revalor-200 on day 140. Implanting treatments did not impact DMI, final BW or ADG ($P \geq 0.12$). Dressing percent, HCW and LM area were not different ($P \geq 0.19$) between treatments. In Exp. 3, Holstein steers ($n = 1,832$; 144 kg initial BW, $SD = 11$) were fed for an average of 360 d. Treatments were: 1) Ralgro at initial processing followed 120 days later by a Revalor-IS with a terminal Revalor-S at 240 days; 2) Ralgro at initial processing followed by a terminal Revalor-XS on day 120; 3) Ralgro at initial processing followed 60 days later by a Revalor-IS with a terminal Revalor-XS on day 160; 4) Revalor-XS at initial processing followed by a terminal Revalor-XS on day 160. Final BW and ADG were not different among treatments ($P \geq 0.81$); however, both DMI and G:F were different ($P < 0.01$). There

were no differences in HCW ($P = 0.92$); however, LM area, marbling score, and calculated YG were all different ($P \leq 0.03$). The utilization of increasingly aggressive implant protocols has little effect on both feedlot and carcass performance of beef steers; however, small effects were observed in Holstein steers.

Key Words: carcass characteristics, feedlot, implant, performance

Introduction

Implants are used to improve growth rate and feed efficiency (Meyer, 2001).

Johnson et al. (2013) reported that 90% of all feedlot cattle in the U.S. receive some type of growth-promoting implant. The majority of beef cattle are fed more than 160 days and Holsteins are fed for nearly a year. Many implants only last 60 to 120 d, depending on the dose, before they are no longer active. Therefore, re-implanting is an important management tool used to further improve animal performance (Preston, 1999).

Understanding the duration of implant effectiveness and knowing how long cattle will be on feed is important for cattle feeders. Understanding this allows feeders to match the proper implants and implant strategies to their cattle and allows for maximum return on investment (Brandt, 1997). When compared to nonimplanted cattle, Duckett and Pratt (2014) observed a 20% increase in ADG and a 13.5% improvement in gain efficiency in cattle implanted with two consecutive implants.

With an increased demand for efficiency, the use of more aggressive implant protocols has become increasingly common. When considering greater-dose implants and more aggressive implant protocols, data are limited on the use of these implant combinations in cattle fed for greater than 170 days. However, Hilscher et al., (2016) conducted three experiments evaluating initial implant strategies for finishing cattle utilizing high-dose implants. They concluded that implant protocols utilizing a greater-dose initial implant followed by a high-dose terminal implant did not differ from implant protocols that utilized a lesser-dose initial implant followed by a greater-dose terminal implant. Therefore, the objectives of these experiments were to compare feedlot and

carcass performance of long-fed steers receiving different aggressive initial implant strategies in commercial feedyard pens.

Material and Methods

The following experiments were conducted in collaboration with Merck Animal Health (De Soto, KS), Cattlemen's Nutrition Service, LLC. (Lincoln, NE), and the University of Nebraska-Lincoln. Research was conducted at commercial facilities and followed the guidelines stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Exp. 1

Animals and Treatments. Crossbred steer calves (n = 1,350; 282 kg initial BW) sourced from ranches and sale barns located in Nebraska, Iowa, Utah, South Dakota, Idaho, and California were fed at a commercial feedyard in central Nebraska. Days on feed across all blocks averaged 215 d (204-232 d). Treatments were 1) Revalor-IS (80 mg of trenbolone acetate (TBA) + 16 mg of estradiol (E) Merck Animal Health) at initial processing with a terminal Revalor-200 (200 mg of TBA + 20 mg of E; Merck Animal Health; **RevIS**) on d 133 (120-140 d); 2) Revalor-IS at initial processing followed 67 d (60-70 d) later by Revalor-200 with a terminal Revalor-200 on d 133 (**RevIS/200**); 3) Revalor-XS (200 mg of TBA + 40 mg of E; Merck Animal Health) at initial processing with a terminal Revalor-200 on d 133 (**RevXS**).

Steers were allotted randomly to pen within arrival block (n = 6) prior to initial processing. Steers were sorted by gate sorting every 2 steers into one of three pens prior

to processing. Implant treatments were assigned randomly to pen (n = 18) within a block. After steers were sorted into their respective pens, each pen was group weighed on a platform scale before processing to establish pen initial BW. At initial processing, steers were individually weighed, given an individual feedlot identification tag, received an infectious bovine rhinotracheitis virus, bovine virus diarrhea (type 1 and 2) combination vaccine (Vista 3, Merck Animal Health), treated for internal parasites with an oral dose of Safe-Guard (Merck Animal Health), external parasites with an injection of Cydectin (Boehringer Ingelheim St. Joseph, MO), and implanted as specified by treatment assignment. At reimplant (averaged 67 d), all pens within a block were brought to the processing facility, pen weighed and cattle on the RevIS/200 treatment were reimplanted with Revalor-200. On d 133, all cattle again were pen weighed and reimplanted with a terminal Revalor-200.

Cattle were housed in 18 open lots with earthen mounds and had ad libitum access to clean water and diets. A step-up period consisting of three adaption diets was used to adapt cattle to the finishing ration by increasing the amount of steam flaked corn and reducing the amount of alfalfa hay in each period. The finishing ration was identical across treatments and averaged 58.2% steam flaked corn, 17.5% WDG (range 9-25%), mixed hay 5.1% (range 4-7%), corn silage 4.7% (range 3-7%), liquid supplement 4.9% (range 4.1-5.2%), micro 0.04%, and 1.86% fat (range 0-2.7%), all on a DM basis. All ration changes that occurred during the feeding period were the same for all cattle on trial. Steers were fed twice daily at approximately 0700 h and 1300 h in concrete fence-line feedbunks, and feedbunks were visually evaluated each morning and managed to allow trace amounts of feed to remain in the bunk before feed delivery. Diet samples

were obtained monthly from feedbunks and composited for nutrient analysis (Servi-Tech laboratories; Hastings, NE).

Carcass Evaluation. Final live BW was determined at shipping by weighing steers on a platform scale and applying a 4% shrink to adjust for gut fill. After weighing, steers were immediately loaded on trucks and transported 201 km to JBS (Grand Island, NE). Carcass data were collected by personnel from West Texas A&M University (Canyon, TX). Individual HCW, 12th rib fat thickness, LM area, KPH, marbling scores, USDA quality grade, and USDA YG were collected for each pen. Yield grade was also calculated using the YG equation, (Boggs and Merkel, 1993) where $YG = 2.50 + (0.98425 \times 12^{\text{th}} \text{ rib fat depth, cm}) + (0.2 \times \% \text{ KPH}) + (0.00837 \times \text{HCW, kg}) - (0.0496 \times \text{LM area, cm}^2)$. Dressing percent was calculated by dividing the HCW by final shrunk BW. Carcass adjusted performance was calculated by dividing the HCW by dressing percent and using the carcass adjusted value as the final BW.

Exp. 2

Animals and Treatments. Crossbred steer calves (n = 1,513; 265 kg initial BW) sourced from a ranch in Oregon were fed at a commercial feedyard in Idaho. Across all blocks, DOF averaged 208 d (197-222 d). Treatments were 1) Revalor-G (40 mg of TBA and 8 mg of E; Merck Animal Health) at initial processing followed 50 days later by a Revalor-IS with a terminal Revalor-200 on day 140 (**Rev-G/IS/200**); 2) Ralgro (36 mg of Zeranol; Merck Animal Health) at initial processing followed 50 days later by a Revalor-XS (**Ral/Rev-XS**); 3) Revalor-XS at initial processing followed by a terminal Revalor-200 on day 140 (**Rev-XS/200**).

All steers were processed within two days of study initiation, split into two sort groups determined by target days to finish, and then were sequentially assigned to block (n = 5). With blocks, steers were assigned to a treatment group using a random number generator and then replicates were assigned to an alley and a pen (n = 15). During initial processing, steers were individually weighed, given an individual feedlot identification tag, received an infectious bovine rhinotracheitis virus, bovine viral diarrhea (type 1 and 2), parainfluenza3 viruse, and bovine respiratory syncytial virus combination vaccine (Bovi-shield Gold 5; Zoetis Inc., Florham Park, NJ), treated for gastrointestinal roundworms, lungworms, eyeworms, grubs, sucking lice and mange mites (Dectomax; Zoetis Inc., Florham Park, NJ) and Panacur for further dewormer (Merck Animal Health), and received the implant associated with treatment assignment. Pen weights were collected after initial processing to establish pen initial BW. Steers were housed in 15 outdoor pens and had ad libitum access to clean water and their respective diets. Cattle were stepped up to feed by decreasing the amount of alfalfa hay and corn silage in the diet while increasing flaked corn. The finishing ration was consistent across treatments and contained on average 5.83% alfalfa hay, 2.5% alfalfa haylage, 6.67% CCDS, 3.39% DDG, 48.35% flaked corn, 4% fry waste, 23.33% potatoes, 1.26% tallow and 4.33% liquid supplement. All feed analysis was performed by Servi-Tech Laboratories in Hastings, NE.

Carcass Evaluation. Steers were pen weighed prior to shipping and a 4% shrink was applied to adjust for gut fill in order to determine final BW. After weighing, steers were loaded on trucks and transported to Hyrum, UT to be processed at a JBS plant.

Carcass data were collected by a USDA certified camera and individual carcass measurements were collected the same as Exp. 1.

Exp. 3

Animals and Treatments. Holstein steer calves (n = 1,832; 144 kg initial BW) sourced from “calf ranches” in Idaho and Washington were fed at a commercial feedyard in Colorado. Days on feed across all treatments averaged 360 d (335 – 386 d). Treatments were: 1) Ralgro at initial processing followed 120 days later by a Revalor-IS with a terminal Revalor-S (120 mg of TBA + 24 mg of E; Merck Animal Health; **Ral/Rev-IS/S**) on d 240; 2) Ralgro at initial processing followed by a terminal Revalor-XS on day 120 (**Ral/Rev-XS**); 3) Ralgro at initial processing followed 60 days later by a Revalor-IS with a terminal Revalor-XS on day 160 (**Ral/Rev-IS/XS**); 4) Revalor-XS at initial processing followed by a terminal Revalor-XS on day 160 (**Rev-XS/XS**).

Steers were blocked (n = 6) by origin, each block consisted of two loads of calves. Each load of calves was sorted into 1 of 4 pens in groups of 5 head at a time. Once all calves were allotted within a block, each group was assigned a lot number, weighed on a platform scale, and randomly assigned to a pen. Pens were then randomly assigned to a treatment. Immediately after weighing, calves were processed and placed into their home pen.

During initial processing, steers were given an individual feedlot identification tag, and received an infectious bovine rhinotracheitis virus, bovine virus diarrhea virus (type 1 and 2) bovine parainfluenza3 virus, and bovine respiratory syncytial virus combination vaccine (TiterVac 10; Diamond Animal Health, Inc., Des Moines, IA), a

clostridium vaccine (Vision 7+H; Merck Animal Health), mycoplasma vaccine (Mycoplasma Bactrin; Zoetis Inc., Florham Park, NJ), bovine rhinotracheitis – parainfluenza3 vaccine (Nasalgen IP; Merck Animal Health), and received Multimin injectable (Multimin USA, Inc.). They were also treated with Safe-guard (Merck Animal Health) to remove and control lungworms, stomach worms, brown stomach worms, barberple worms, intestinal worms, hookworms, thread-necked intestinal worms, bankrupt worms, and nodular worms, and received an injectable Ivomec Plus (Merial Limited, Iselin, NJ) for further parasite control. All steers also received 4 cc of Draxxin (Zoetis Inc., Florham Park, NJ) to prevent and treat all four major BRD pathogens (*Mannheimia hamolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*) and then were administered an implant associated with their specific treatment.

Steers were housed in 24 outdoor pens at a stocking density of 74-80 animals per pen and had ad libitum access to water and feed. Cattle were fed twice daily, with 40% of the daily ration delivered in the morning and the remaining 60% delivered in the afternoon. All ration changes were performed by block so that all four treatments received the same ration at all times. The finishing diets were the same for each treatment but varied slightly across time; however, the ration averaged 76.81% steam flaked corn, 4.75% finisher suspension, 2.8% yellow grease, 8.42% alfalfa hay with the remainder of the diet consisting of either wheat midd pellets or corn syrup. Ration samples were analyzed by SDK Laboratories (Hutchinson, KS) and Servi-Tech Laboratories (Dodge City, KS).

Carcass Evaluation. Steers were pen weighed at approximately 0600, prior to first feeding, on the morning of shipping cattle to the processing plant. This weight was

shrunk 4% to adjust for gut fill. Cattle were then returned to their pens and fed their first feeding. At 1500, prior to second feeding, cattle were shipped 172 miles to the packing plant (Tyson, Holcomb, KS) and were processed at the start of the following days shift. Carcass data were collected by personnel from West Texas A&M University (Canyon, TX). Individual carcass measurements were the same as described in Exp. 1.

Deads Out Calculations

All data was analyzed with deads out. Deads out initial BW data was calculated by subtracting individual weight of dead steers or removals from the total initial pen weight, and divided by the number of animals slaughtered. Final live BW was calculated using the total weight of cattle at shipping (shrunk 4%) divided by the total number of cattle shipped excluding deads. Deads-out ADG was determined by dividing the total weight gain (average final weight – average starting weight) by days on feed. Deads out DMI was determined by dividing total feed delivered to the pen by the total number of animal days.

Statistical Analysis.

For all 3 experiments live performance and carcass data were analyzed as a randomized complete bock design using the Glimmix procedure of SAS (9.2, SAS Institute, Inc., Cary, NC). Steers were blocked by source and arrival time and pens were assigned randomly to a treatment within arrival blocks. Pen served as the experimental unit and the model included the fixed effect of treatment, while block served as a fixed effect. The LSMEANS option of SAS was used to calculate treatment averages and using the Glimmix procedure of SAS quality grade and YG were analyzed using

multinomial code to determine the distribution of quality grade and YG in each treatment. An alpha level less than or equal to 0.05 was used to determine statistical significance of outcomes.

Results and Discussion

Exp. 1 – Performance

There was no difference observed in DMI ($P = 0.19$) between the three implant strategies (Rev-IS, Rev-IS/200, and Rev-XS) over the entire feeding period (Table 1). Using carcass-adjusted performance, no differences in final BW or ADG were observed ($P \geq 0.38$). Therefore, G:F also was unaffected by implant strategy ($P = 0.16$). Observing no differences in performance between cattle implanted with either: Revalor-IS at initial processing with a terminal Revalor-200 on d 133; Revalor-IS at initial processing followed 67 d later by Revalor-200 with a terminal Revalor-200 on d 133; or Revalor-XS at initial processing with a terminal Revalor-200 on d 133 is consistent with data presented by both Nichols et al. (2014) and Hilscher et al. (2016). Nichols et al. (2014) compared cattle implanted with either a Revalor-IS on d 1 followed by a Revalor-S on day d 80 to cattle implanted with only a Revalor-XS. Hilscher et al. (2016) observed no differences in final BW, DMI, ADG, or G:F when comparing the effects of increased implant dose combinations on growth performance and carcass characteristics of steer calves fed for 216 d.

When considering interim performance, there was no differences in DMI, ADG, or G:F ($P \geq 0.16$) in the first 67 days on feed. This is what we would expect due to the payout of the initial implant. When considering day 67-133, DMI was not different

across all three treatments ($P = 0.62$); however, ADG was greatest for the Rev-IS/200 treatment ($P < 0.01$) compared to the Rev-XS treatment which was greater than the Rev-IS treatment. The increased ADG in the Rev-IS/200 treatment is from the re-implant received on day 67. While not statistically different, from day 133-215 ADG was numerically greater ($P = 0.11$) for the Rev-IS treatment compared to the Rev-IS/200 and Rev-XS treatments which were not different from each other. Interim data suggests that cattle implanted more aggressively early gained faster through the first 2/3 of the trial but by conclusion of the study had lost the gain and feed efficiency advantage.

Carcass characteristics, including ($P = 0.59$) HCW and dressing percent ($P = 0.93$) were not different between the three implant strategies (Table 1). Folmer et al. (2009) also observed no differences in HCW and dressing percent for steers implanted with either Revalor-IS or Synovex-S as an initial implant with a Revalor-S as a common terminal implant. Dressing percent averaged 63.92% between the three treatments and is consistent with findings from Hilscher et al. (2016). There were also no differences in LM area ($P = 0.21$) or the distributions of USDA quality ($P = 0.90$) and yield ($P = 0.23$) grades between implant treatments. No difference in LM area is consistent with findings from Nichols et al. (2014); however, contrary to this study Nichols observed a greater proportion of low choice and a lower proportion of select for cattle implanted with a Revalor-XS initially compared to cattle implanted with Revalor-IS initially and Revalor-S on d 80. Nichols et al. (2014) observed no differences in the proportion of USDA yield grades which is consistent with the current study.

Steers implanted with a high dose initial implant followed by a common terminal implant of Revalor-200 on d 133 had similar feedlot and carcass performance as steers

implanted with two high dose initial implants followed by a common terminal Revalor-200. These data suggest the use of more aggressive initial implant strategies have minimal impact on both feedlot and carcass performance and characteristics.

Exp. 2 – Performance

Steers from the three different implanting strategies did not differ ($P \geq 0.31$) in live- or carcass-adjusted final BW; however, cattle that received an initial implant of Ralgro had numerically greater live- and carcass-adjusted final BW (Table 3). Dry matter intake was similar ($P = 0.12$) across all three implant treatments. Both live- and carcass-adjusted ADG were also not different ($P \geq 0.78$) between treatments. While not statistically different ($P \geq 0.12$) steers that received the Rev-G/IS/200 treatment had numerically greater G:F. Both Parr et al. (2011) and Hilscher et al. (2016) also observed no differences in ADG and G:F when comparing initial implant programs. When considering a study comparing multiple implants with similar overall concentrations of TBA and E conducted by Samber et al. (1996), results were similar to the current study in that there were no differences observed in final BW, DMI, ADG, and G:F between treatments.

In Exp. 2, no differences were observed in HCW, dressing percent or LM area ($P \geq 0.19$). While not statistically different, steers implanted with the Ral/Rev-XS treatment had a tendency ($P = 0.07$) to have a numerically greater marbling score. When comparing Rev-XS with Rev-IS/S, Nichols et al. (2014) reported no differences in HCW or marbling score. Correspondingly, Hilscher et al. (2016) reported no differences in HCW, dressing percent and marbling score when comparing increased implant dose combinations on steers fed for 216 d.

In Exp. 2, USDA quality grade distributions were different ($P < 0.01$) among implant treatments. There were no differences in the proportions of prime, low choice, and standard quality grades. However, when considering upper 2/3 choice the Ral/Rev-XS treatment had a greater proportion of upper 2/3 choice compared to the Rev-G/IS/200 and Rev-XS/200 treatments. There was also a difference in the proportion of select quality grades. The Ral/Rev-XS treatment had a lower proportion of select compared to the Rev-G/IS/200 and the Rev-XS/200 treatments. Both Parr et al. (2011) and Nichols et al. (2014) reported differences in the percentages of choice and select carcasses with cattle who received a Rev-XS implant having a greater proportion of carcasses grading choice and less carcasses grading select compared to cattle implanted with Rev-IS/S. Contrary to the current study, Hilscher et al. (2016) reported no difference in quality grade distribution between implant treatments. There was also a difference in the proportion of USDA YG ($P < 0.01$) across treatments. There was no differences in the proportion of yield grades 1, 4 and 5; however the Ral/Rev-XS treatment had a lower proportion of YG 2 and a higher proportion of YG 3 compared to the Rev-G/IS/200 and Rev-XS/200 treatments. Contrary to the current study, Hilscher et al. (2016) reported no differences in the distribution of YG among treatments.

Exp. 3 – Performance

While previous studies have compared non-implanted Holstein cattle to single, double and triple implant programs (Scheffler et al., 2003; Apple et al., 1991), there is little information available on comparing different combinations of multiple aggressive implants on long fed Holstein steers in order to maximize production efficiency.

There were no differences in live or carcass-adjusted final BW or ADG ($P \geq 0.81$) between the four treatments (Table 4). Intake was greatest ($P < 0.01$) for the Rev-XS/XS and Ral/Rev-IS/XS treatments compared to the Ral/Rev-IS/S and Ral/Rev-XS treatments. Carcass-adjusted G:F was greatest ($P = 0.01$) for the Ral/Rev-IS/S and Ral/Rev-XS treatments compared to the Ral/Rev-IS/XS and Rev-XS/XS treatments. When comparing Holsteins implanted with either 2 or 3 Component TE-S (120 mg of TBA and 24 mg of E; Elanco; Greenfield, IN) implants to non-implanted Holsteins, Scheffler et al. (2003) reported decreased intake and improved G:F. Contrary to the current study, both Samber et al. (1996) and Nichols et al. (2014) reported no differences in DMI and G:F when comparing implant strategies in beef cattle.

Additionally, there was no difference in HCW ($P = 0.92$). However, both dressing percent ($P = 0.05$) and LM area ($P = 0.01$) were significantly different among treatments. The Rev-XS/XS treatment had the greatest dressing percent at 62.09% and was significantly greater than the Ral/Rev-XS and the Ral/Rev-IS/XS treatments (61.43 and 61.32%, respectively) but not different from the Ral/Rev-IS/S treatment (61.72%). The LM area was greatest for the Rev-XS/XS and Ral/Rev-IS/S treatments, while the Ral/Rev-XS and Ral/Rev-IS/XS treatments had a significantly smaller LM area. Marbling score ($P < 0.01$) and calculated YG ($P = 0.03$) were also significantly different among treatments. The Rev-XS/XS had a marbling score that was significantly less than the other three treatments which were not significantly different from each other. Calculated YG was greatest for the Ral/Rev-IS/XS treatment compared to the Ral/Rev-IS/S and Rev-XS/XS treatments which were not different from the Ral/Rev-XS treatment. Hilscher et al. (2016) reported cattle treated with a Rev-XS implant followed

by a Rev-200 had a greater LM area and decreased YG compared to cattle implanted with either 2 Rev-200 implants or a Rev-IS followed by a Rev-200.

The distribution of USDA YG was not different ($P = 0.54$) across all treatments; however, the distribution of USDA quality grade was significantly different ($P < 0.01$) between treatments. Cattle given the Ral/Rev-XS treatment had the greatest percent of prime compared to the other three treatments which were not different from each other. The Ral/Rev-XS once again had the highest percent of upper 2/3 choice but was not different from the Ral/Rev-IS/XS treatment which was greater than the Rev-XS/XS treatment but not different from the Ral/Rev-IS/S treatment. When considering low choice quality grades the Ral/Rev-IS/S and Ral/Rev-IS/XS treatments were not different and had a greater percent of low choice than the Ral/Rev-XS and Rev-XS/XS treatments. The Rev-XS/XS treatment had the highest percent of select quality grades compared to the other three treatments which were not different from each other. There was no differences among treatments in the percent of standard quality grades. Contrary to the current study, when comparing aggressive implant strategies Samber et al. (1996) reported a decrease in calculated YG as implant dosage increased. Similar to Samber et al. (1996), Hilscher et al. (2016) also reported a decrease in calculated YG for cattle implanted with a Rev-XS and Rev-200 compared to cattle implanted with either two Rev-200 implants or a Rev-IS and Rev-200 implant combination. Similar to the current study, Hilscher et al. (2016) also reported an increase in the proportion of carcasses grading select when treated with a Rev-XS treatment.

In Exp. 1, utilizing aggressive implant protocols (Rev-IS/200 and Rev-XS) did not affect feedlot performance or carcass characteristics of cattle when compared to a less

aggressive implant protocol (Rev-IS). In Exp. 2, there was no differences in feedlot performance; however, cattle implanted with Ralgro initially had a greater proportion of upper 2/3 choice and in turn a lower proportion of select QG scores compared to the treatments implanted initially with Revalor-G or Revalor-XS. In Exp. 3, Holsteins implanted with Ralgro initially had greater G:F compared to Holsteins implanted with Revalor-XS initially and also had a greater marbling score compared to Holsteins implanted with Revalor-XS initially. Holsteins implanted with Revalor-XS initially had a lower proportion of upper 2/3 choice and a greater proportion of standard QG scores compared to Holsteins implanted with Ralgro initially.

Implication

The current studies would suggest that the utilization of more aggressive implant strategies has minimal impact on both feedlot and carcass performance of cross breed steers. However; when Ralgro is used as an initial implant QG could be increased. It appears that Holsteins implanted with Ralgro initially have better feed conversions and higher QG scores compared to Holsteins implanted with Revalor-XS initially.

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Table 1. Effects of three initial implant programs on growth performance and carcass characteristics of calf-fed steers fed 215 d (Exp. 1)

Item	<i>Treatments¹</i>			SEM	<i>P</i> - <i>value</i>
	Rev-IS	Rev-IS/200	Rev-XS		
Steers, n	451	449	450	-	-
Pens, n	6	6	6	-	-
Initial BW, kg ²	284	282	283	1.4	0.47
DMI, kg/d ³	10.4	10.3	10.3	0.05	0.19
<i>Live Performance</i>					
Final BW, kg	662	662	664	2.7	0.91
ADG, kg	1.76	1.77	1.78	0.008	0.95
G:F	0.170	0.172	0.172	0.0001	0.55
<i>Carcass-adjusted Performance</i>					
Final BW, kg	661	663	663	3.6	0.60
ADG, kg	1.76	1.78	1.77	0.018	0.38
G:F	0.170	0.173	0.172	0.0003	0.16
<i>Carcass Characteristics</i>					
HCW, kg	425	426	426	2.31	0.59
Dressing percent, %	64.17	63.34	64.24	3.10	0.93
LM area, cm ²	89.7	89.4	88.8	0.48	0.21
<i>USDA Quality Grade, %⁴</i>					
Prime	0.9	0.8	0.8		
Upper 2/3 Choice	69.8	68.7	67.3		
Low Choice	27.2	28.2	29.6		
Select	1.3	1.3	1.4		
Standard	0.9	1.0	1.4		
<i>USDA YG, %⁵</i>					
1	1.4	2.0	1.5		
2	14.9	19.5	15.3		
3	60.6	60.9	60.7		
4	22.1	16.9	21.5		
5	1.1	0.8	1.1		

¹Rev-IS = Revalor-IS (Merck) at initial processing followed by a terminal with a terminal Revalor-200 (Merck) on d 133; Rev-IS/200 = Revalor-IS at initial processing a Revalor-200 at reimplant on d 67 with a terminal Revalor-200 on d 133; Rev-XS = Revalor-XS (Merck) at initial processing followed by a terminal Revalor-200 on d 133.

²Initial BW: total pen weight of cattle with no shrink divided by total number of steers.

³Dry matter intake: calculated from total kilograms delivered to the pen divided by the total number of head days.

⁴The numbers represent by treatment the proportion of carcasses within each quality grade category. The distribution of quality grade was not significant ($P = 0.90$).

⁵The numbers represent by treatment the proportion of carcasses within each YG category. The distribution of YG was not significant ($P = 0.23$).

Table 2. Effects of three initial implant programs on interim growth performance of calf-fed steers

Item	<i>Treatments</i> ¹			SEM	<i>P</i> -value
	Rev-IS	Rev-IS/200	Rev-XS		
<i>D 1-67</i>					
Initial BW, kg	284	282	283	1.4	0.47
D 67 BW, kg	418	413	419	1.2	0.06
DMI, kg/d	9.9	9.8	9.9	0.03	0.16
ADG, kg/d	2.01	1.97	2.01	0.021	0.49
G:F	0.203	0.201	0.202	0.002	0.87
<i>D 67-133</i>					
D 133 BW, kg	517 ^b	528 ^a	527 ^a	0.7	<0.01
DMI, kg/d	10.2	10.2	10.3	0.111	0.62
ADG, kg/d	1.55 ^c	1.82 ^a	1.71 ^b	0.050	<0.01
G:F	0.152 ^b	0.179 ^a	0.166 ^{ab}	0.004	0.02
<i>D 1-133</i>					
D 133 BW, kg	517 ^b	528 ^a	527 ^a	0.7	<0.01
DMI, kg/d	10.0	9.9	10.0	0.12	0.76
ADG, kg/d	1.78 ^b	1.90 ^a	1.86 ^{ab}	0.026	0.03
G:F	0.177 ^b	0.191 ^a	0.185 ^{ab}	0.003	0.05
<i>D 133-215</i>					
D 215 BW, lb	662	662	664	2.7	0.91
DMI, lb/d	11.1	11.0	11.1	0.12	0.78
ADG, lb/d	1.60	1.47	1.47	0.043	0.11
G:F	0.146	0.133	0.132	0.005	0.21

¹Rev-IS = Revalor-IS (Merck) at initial processing followed by a terminal with a terminal Revalor-200 (Merck) on d 133; Rev-IS/200 = Revalor-IS at initial processing a Revalor-200 at reimplant on d 67 with a terminal Revalor-200 on d 133; Rev-XS = Revalor-XS (Merck) at initial processing followed by a terminal Revalor-200 on d 133.

²Initial BW: total pen weight of cattle with no shrink divided by total number of steers.

³Dry matter intake: calculated from total kilograms delivered to the pen divided by the total number of head days.

Table 3. Effects of three initial implant strategies on growth performance and carcass characteristics of steer calves fed 208 d (Exp. 2)

Item	<i>Treatments¹</i>			SEM	<i>P</i> - <i>value</i>
	Rev- G/IS/200	Ral/Rev-XS	Rev- XS/200		
Steers, n	506	504	503	-	-
Pens, n	5	5	5	-	-
Initial BW, kg ²	264	267	265	1.3	0.23
DMI, kg/d ³	8.87	9.24	9.00	0.110	0.12
<i>Live Performance</i>					
Final BW, kg	610	619	612	3.7	0.31
ADG, kg	1.66	1.68	1.66	0.017	0.78
G:F	0.188	0.182	0.185	0.0001	0.07
<i>Carcass-adjusted Performance</i>					
Final BW, kg	611	617	613	3.7	0.48
ADG, kg	1.67	1.68	1.67	0.019	0.97
G:F	0.188	0.182	0.186	< 0.01	0.12
<i>Carcass Characteristics</i>					
HCW, kg	390	394	392	2.4	0.48
Dressing percent, %	63.93	63.76	64.02	0.20	0.68
LM area, cm ²	90.2	88.8	90.3	0.58	0.19
Marbling Score ⁴	456	476	460	5.4	0.07
Calculated YG	3.02	3.20	2.98	0.065	0.09
<i>USDA Quality Grade, %⁵</i>					
Prime	0.79	1.40	0.63		
Upper 2/3 Choice	19.50 ^b	27.39 ^a	20.28 ^b		
Low Choice	57.89	56.05	60.16		
Select	21.64 ^a	14.97 ^b	18.73 ^a		
Standard	0.19	0.19	0.21		
<i>USDA YG, %⁶</i>					
1	9.54	5.65	9.30		
2	42.11 ^a	34.19 ^b	42.49 ^a		
3	35.88 ^b	48.17 ^a	40.25 ^b		
4	11.65	10.35	7.34		
5	0.81	1.63	0.62		

¹ Rev-G/IS/200 = Revalor-G at processing, a Revalor-IS at reimplant on d 50 with a terminal Revalor-200 on d 140; Ralgro/Rev-XS = Ralgro at processing and Revalor-XS at reimplant on d 50; Rev-XS/200 = Revalor-XS at processing and a terminal Revalor-XS on d 140

²Initial BW: total pen weight of cattle with no shrink divided by total number of steers.

³Dry matter intake: calculated from total kilograms delivered to the pen divided by total number of head days.

⁴Marbling score: 400 = small⁰⁰, 500 = modest⁰⁰

⁵The numbers represent by treatment the proportion of carcasses within each quality grade category. The distribution of quality grade was different ($P < 0.01$).

⁶The numbers represent by treatment the proportion of carcasses within each YG category. The distribution of YG was different ($P < 0.01$).

^{a,b,c} Means within a row with different superscripts differ ($P < 0.05$).

Table 4. Effects of several different implant strategies on growth performance and carcass characteristics of calf-fed Holstein steers (Exp. 3)

Item	<i>Treatments¹</i>				SEM	<i>P-value</i>
	Ral/Rev-IS/S	Ral/Rev-XS	Ral/Rev-IS/XS	Rev-XS/XS		
Steers, n	457	458	459	458	-	-
Pens, n	6	6	6	6	-	-
Initial BW, kg ²	145	145	144	144	0.5	0.26
DMI, kg/d ³	7.92 ^b	7.92 ^b	8.10 ^a	8.18 ^a	0.047	<0.01
<i>Live Performance</i>						
Final BW, kg	642	643	644	640	3.3	0.84
ADG, kg	1.38	1.38	1.39	1.38	0.006	0.81
G:F	0.175 ^a	0.175 ^a	0.172 ^b	0.169 ^c	0.000	<0.01
<i>Carcass-adjusted Performance</i>						
Final BW, kg	643	641	641	645	4.7	0.91
ADG, kg	1.38	1.38	1.38	1.39	0.007	0.90
G:F	0.174 ^a	0.174 ^a	0.171 ^b	0.170 ^b	0.000	0.01
<i>Carcass Characteristics</i>						
HCW, kg	398	395	395	397	2.9	0.92
Dressing percent, %	61.72 ^{ab}	61.43 ^b	61.32 ^b	62.09 ^a	0.177	0.05
LM area, cm ²	83.5 ^a	80.3 ^b	80.3 ^b	83.8 ^a	0.45	0.01
Marbling Score ⁴	457 ^a	468 ^a	457 ^a	433 ^b	4.3	<0.01
Calculated YG	2.84 ^b	2.98 ^{ab}	3.04 ^a	2.83 ^b	0.048	0.03
<i>USDA Quality Grade, %⁵</i>						
Prime	1.38 ^b	3.29 ^a	0.91 ^b	0.95 ^b		
Upper 2/3 Choice	16.61 ^b	21.63 ^a	18.72 ^{ab}	11.62 ^c		
Low Choice	65.96 ^a	58.07 ^b	64.60 ^a	57.76 ^b		
Select	14.88 ^b	16.06 ^b	14.83 ^b	28.97 ^a		
Standard	1.18	0.95	0.94	0.71		

USDA YG,
%⁶

1	12.60	8.57	7.64	11.57
2	47.24	43.10	40.95	49.62
3	31.86	39.75	40.80	34.54
4	8.07	8.11	10.40	4.27
5	0.23	0.47	0.23	0.00

¹Ral/Rev-IS/S = Ralgro at processing, Revalor-IS at reimplant on d 120 with a terminal Revalor-S on d 240; Ral/Rev-XS = Ralgro at processing and Revalor-XS at reimplant on d 120; Ral/Rev-IS/XS = Ralgro at processing, Revalor-IS at reimplant on d 60 with a terminal Revalor-XS on day 160; Rev-XS/XS = Revalor-XS at processing and Revalor-XS at reimplant on d 160, Merck Animal Health, De Soto, KS.

²Initial BW: total pen weight of cattle with no shrink divided by total number of starting Holstein steers.

³Dry matter intake: calculated from total kilograms delivered to the pen divided by total number of head days.

⁴Marbling score: 400 = small⁰⁰, 500 = modest⁰⁰

⁵The numbers represent by treatment the proportion of carcasses within each quality grade category. The distribution of quality grade was different ($P < 0.01$).

⁶The numbers represent by treatment the proportion of carcasses within each YG category. The distribution of YG was not different ($P = 0.54$).

^{a,b,c} Means within a row with different superscripts differ ($P < 0.05$).

Chapter III. Corn silage rumen undegradable protein levels and extent of digestion and the effect of supplementing increasing levels of rumen undegradable protein on growing performance in calves fed a silage-based diet

C. R. Oney*, J. K. Gramkow*, F. H. Hilscher*, R. G. Bondurant*, A. K. Watson*¹, G. E. Erickson*¹, J. C. MacDonald*, T. J. Klopfenstein*

*Department of Animal Science, University of Nebraska, Lincoln 68583

¹Corresponding authors: gerickson4@unl.edu and awatson3@unl.edu

Abstract

The amount of RUP in corn silage and the extent to which it is digested in the small intestine are uncertain. Four studies were conducted to determine % RUP digestibility in corn silage, and the effect of supplementing increasing levels of RUP on growing performance in calves fed a silage-based diet. Exp. 1 utilized 60 steers ($n = 290$ kg initial BW, $SD = 18$) in an 84-d growing study evaluating the effects of supplementing 0, 3.25, 6.5, 9.75 and 13% (as a % of diet DM) RUP on performance. All steers were individually fed using the Calan gate system. Five levels of supplementation were evaluated with 12 steers per level of supplement. There were no differences in DMI ($P = 0.33$) among treatments for period 1 (d 1-37). However, ADG ($P < 0.01$) and G:F ($P < 0.01$) both increased linearly as RUP supplement inclusion increased from day 1-37. There were no differences in DMI ($P = 0.16$), ADG ($P = 0.11$) or G:F ($P = 0.32$) for period 2 (d 38-84). For the overall growing period (d 1-84), as supplemental RUP inclusion increased from 0 to 13%, a linear increase was observed in ending BW ($P < 0.01$). With no difference in DMI ($P = 0.19$) between the five treatments, averaging 7.67 kg/d, and a linear increase in ADG ($P < 0.01$), G:F linearly increased ($P < 0.01$) from 0.148 to 0.174 as RUP inclusion increased. The MP balance increased from -115 to +130 g/d as RUP inclusion increased from 0 to 13%. In Exp. 2, four feeds (37% and 42% DM corn silage, soybean meal (SBM), and Soypass (enzymatically browned SBM; 50% CP; 75% RUP) were utilized in an *in vitro* setting with 100-mL bottles incubated for 16 or 24 hours. Purine analysis was done to correct for microbial N when calculating RUP content. Exp. 3 utilized *in situ* methods with two ruminally cannulated steers and one duodenally cannulated steer to compare RUP content and RUP digestibility of two corn

silages (37% and 42% DM) along with Empyreal (75% CP; 65% RUP) and Soypass. Samples were ruminally incubated for 20 or 30 h and ½ of the samples were then duodenally incubated to determine RUP digestibility. In Exp. 4, dry rolled corn (14% DM) was reconstituted to 75, 70, 65, and 50% DM and ensiled in mini silos (2265 cm³) for either 30, 90, 180, or 270 days. After ensiling, samples were ruminally incubated for 20 or 30 hours to determine RUP content. In Exp. 2, RUP as a % of CP was greatest for Soypass ($P = 0.02$) with no differences between the two corn silages, averaging 23.2% RUP as a % of CP, similar to SBM. As a % of CP, RUP was greatest for Soypass ($P < 0.01$), intermediate for both corn silages averaging 14.7%, 1.1 SEM, and least for Empyreal due to washout from bags. In Exp. 4, as moisture content of the corn grain increased, RUP as a % of DM decreased linearly ($P < 0.01$). The RUP content and RUP digestibility of corn silage is low and growing steers respond to RUP supplementation.

Key Words: corn silage, crude protein, rumen undegradable protein

Introduction

In order to formulate a growing ration to meet protein requirements two pieces of information are needed, protein supplied by the diet and protein required by the calf. To measure the protein supplied by corn silage we need to break down CP content into rumen degradable protein (RDP) and rumen undegradable protein (RUP).

The 1996 NRC uses a common value of 80% for digestibility of rumen undegradable protein (RUP) for all feeds. Benton et al. (2005a) suggests that this common value of 80% underestimates the RUP digestibility of concentrate feeds. Furthermore, data presented by Buckner et al. (2013) suggests that RUP digestibility of forages is overestimated by the common 80% value used by the 1996 NRC. It is important to determine a value for both RUP as a % of CP and how much of the RUP is digestible.

Because lab techniques that are designed to measure RUP values of feedstuffs are specific to either forages or concentrates (Klopfenstein et al. 2001; Haugen et al. 2006) and corn silage is a blend of both, quantifying RUP of corn silage is difficult. Haugen et al. (2006) validated the use of neutral detergent insoluble nitrogen to remove for microbial contamination when measuring RUP; however, it has not been utilized for non-forage fiber sources and it is possible that a portion of the protein may remain in the residue but are soluble in the neutral detergent solution, resulting in lower estimates of degradable RUP (Klopfenstein et al., 2001). Furthermore, moisture content and ensiling

time continually impact protein degradability. At harvest, forage is wetter than the grain and during storage the grain absorbs the moisture from the forage becoming very wet high moisture corn (HMC; Benton et al., 2005b). As the grain absorbs moisture, the protein has a greater degree of rumen degradability.

Without adequate amounts of supplemental RUP, dietary protein will be limiting and will not meet metabolizable protein requirements, negatively impacting cattle performance. Therefore, the objectives of these experiments were to determine the effects of supplemental RUP in corn silage growing diets and also to determine the RUP content and RUP digestibility of corn silage.

Materials and Methods

Exp. 1 – Supplementing RUP to growing cattle on a corn silage based diet

An 83 day growing study was conducted at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE using 60 crossbred steers (BW = 290; SD = 18 kg). All steers were individually fed using the Calan gate system. Five days prior to trial initiation, steers were limit fed a diet of 50% alfalfa and 50% Sweet Bran (Cargill corn milling, Blair, NE) at 2% of BW to reduce gut fill variation (Watson et al., 2013). Steers were weighed in the morning before being fed on 3 consecutive days and the average was used as initial BW (Stock et al., 1983). Based on initial BW steers were assigned randomly to 1 of 5 treatments. Diets consisted of 85% corn silage with the remaining 15% fed as supplement (DM basis). The supplement included protein sources, urea, minerals, vitamins A-D-E, and a finely-ground corn carrier that was replaced with the RUP sources (Table 1). The RUP supplement consisted of 52% SoyPass (50% CP;

75% RUP as % of CP) and 34.7% Emphyreal (Cargill; 75% CP; 65% RUP as % of CP). SoyPass is an enzymatically browned soybean meal and Emphyreal is a concentrated corn gluten meal. Five levels of supplement were evaluated with 12 steers per level. Supplement levels consisted of 0, 3.25, 6.5, 9.75, and 13% SoyPass + Emphyreal (RUP sources as a % of diet DM).

All steers were implanted with Ralgro on day 0 and fed ad-libitum once daily at 0800. Feed refusals were collected weekly, weighed, and then dried in a 60°C forced air oven for 48 hours to calculate an accurate DMI for individual steers. Interim weights were taken at 0700 on day 36 and 37 and shrunk 4% to account for gut fill. At the conclusion of the study, steers were once again limit fed a diet of 50% alfalfa hay and 50% Sweet Bran (Cargill) at 2% of BW to reduce variation due to gut fill. Weights were collected on 3 consecutive days and averaged to calculate an ending BW.

Data were analyzed using the mixed procedure of SAS as a randomized block design. Linear and quadratic contrasts were developed to determine the effect of RUP inclusion. Significance was declared at $P \leq 0.05$.

Exp. 2 – Corn silage in vitro analysis

Corn silage was harvested at the ENREC near Mead, NE at 37 or 42% DM to mimic traditional corn silage harvest or a delayed harvest. Harvest began when the field was at approximately $\frac{3}{4}$ milkline for the 37% DM corn silage (9/4/2014), and then delayed two weeks coinciding with black layer formation for the 42% DM corn silage (9/16/2014). After harvesting, silages were stored in sealed silo bags (Ag-Bag, St.

Nazianz, WI). After 28 days, 10 kg was brought to the University of Nebraska-Lincoln metabolism area, freeze dried and stored in dry storage.

In June of 2015 the 37 and 42% DM corn silages, soybean meal (SBM), and SoyPass were analyzed for RUP content using an *in vitro* technique. Empty, 100 mL bottles were labeled and weighed empty to obtain a reference weight. Samples were ground through a 1-mm screen and then 0.5 g of each feed sample was weighed into a 100 mL bottle with 3 replicates (bottles) per sample. Three bottles used as blanks to get an initial Purine:Nitrogen ratio. After grinding, initial CP analysis was conducted on all samples. Whole rumen contents were collected from two fistulated steers on a 30% concentrate diet, prior to feeding. Rumen contents were squeezed through four layers of cheese cloth into a pre-warmed thermos. Rumen fluid was poured into a 1000 mL separatory funnel and placed in a 39°C water bath until the particulate matter rose to the top. The lower portion of the rumen fluid was removed and mixed in a 1:1 ratio with McDougall's buffer with 1 g urea/L (McDougall, 1948). Then, 50 mL of the rumen fluid / McDougall's buffer mixture was added to each bottle, bottles were flushed with CO₂ and a stopper with a gas release mechanism was placed in the top of the bottle. Bottles were lightly swirled to mix the feed sample into the solution and then were incubated in a water bath at 39°C for either 16 or 24 hours. During incubation, bottles were lightly swirled to mix contents every 8 hours. After incubation, bottles were frozen, and freeze dried. Bottles were then weighed after they were dried.

After the samples were freeze dried, CP was measured again and a modified purine assay was conducted to correct for microbial N when calculating RUP content. Next, 0.5 g of sample from each bottle was weighed in duplicates into 50 ml screw cap

tubes. Then 2.5 ml HClO₄ (2M perchloric acid) was added to each tube and then the sample was vortexed. Tubes were then incubated in a 90-95°C water bath for 30 minutes. After incubation tubes were vortexed and then put back in the water for 30 more minutes. After incubation 17.5 mL of 28.5 mM H₆NPO₄ was added and then vortexed and incubated for 15 minutes. After incubation samples were filtered through Whatman #1 filter paper into 60 x 125 mm disposable glass culture tubes. Tubes were then capped and refrigerated for 12 hours. After refrigeration 0.25 ml of filtrate was transferred into a 16 x 125 mm tube along with 0.25 ml of 0.4 M AgNO₃ and 4.5 ml of 0.2 M H₆NPO₄. Tubes were then centrifuged for 10 minutes at approximately 1,000 x g and supernatant liquid was drawn off without disturbing the pellet. The pellet was washed with 4.5 mL of washing solution and 250 µL AgNO₃ and vortexed. This step was repeated twice. Next 5 mL of 0.5 N HCL was added to the pellet and vortexed until thoroughly mixed. Tubes were then covered with foil and incubated in a 90-95°C water bath for 30 minutes. After incubation tubes were centrifuged and allowed to cool for 10 minutes. After cooling, 200 µL of standards, samples, and 0.5 N HCL (blanks) were pipetted into a microtiter plate in duplicates and spec at 260 nm (Zinn and Owens. 1986; Aharoni and Tagari. 1991). To determine the amount of microbial N the following equations were used:

$$\frac{\frac{\mu g}{mL} (from\ spec) * 400}{Sample\ weight * 1000} = \frac{mg}{g} Purines$$

$$\frac{\left(Purines \left(\frac{mg}{g} \right) \div Purine: Nitrogen\ ratio \right)}{10} = \% microbial\ N$$

It is important to note that there is variation in the Purine:Nitrogen ratio and it is influenced by the type and amount of microbes present in the rumen when whole rumen

contents are obtained (Obispo and Dehority, 1999). For this analysis the blank bottles were used to calculate the purine:nitrogen ratio and that ratio was applied to all the bottles in that time period.

The % microbial N calculated for the blank bottles was then averaged and subtracted from the total N found in the individual feed sample bottles to adjust for N from the rumen fluid and calculate total N remaining from the feed after *in vitro* incubation. As a % of CP, RUP was then calculated by taking the residual N divided by the initial N and RUP as a % of DM was calculated by taking the residual N times 6.25 and then divided by the initial sample weight. Data were analyzed as a 2 × 4 factorial using the MIXED procedure of SAS and bottle was the experimental unit. Steer was added as a random effect and the class statement consisted of incubation time and feed sample.

Exp. 3 – Corn silage in situ analysis

In Exp. 3, the same corn silages from Exp. 2 (37 and 42% DM) along with SBM and SoyPass were evaluated using an *in situ* technique. Small Ankom *in situ* bags (5 cm × 10 cm, 50 µm pore size) were labeled and weighed. Feed samples were ground through a 2-mm screen and 1.25 g of sample was added to bags, with 16 replicate bags per sample. After bags were sealed, bags were ruminally incubated for either 20 or 30 hours in one of two ruminally fistulated steers on a 30% concentrate diet. Half of the 20 hour bags and half of the 30 hour bags were incubated in each steer. After the designated incubation time, bags were removed and rinsed in a washing machine. Rinsing consisted of five rinses of a one minute agitation and a two minute spin (Whittet et al., 2003). After rinsing half of the bags were frozen for duodenal incubation. The remaining bags

were divided in half again with half refluxed in NDS solution using an ANKOM Fiber Analyzer to remove microbial contamination from residue. Bags were dried in a 60°C forced-air oven for 24 hours and weighed to determine DM disappearance.

Four bags of each feed sample from both time points (20 and 30 hours; 32 bags total) were duodenally incubated. Of the bags that were to be duodenally incubated, half of them (two bags of each feed) were washed in NDS solution using an ANKOM Fiber Analyzer to remove microbial contamination from bag residue and the other half was not. The bags were incubated in a duodenally fistulated steer consuming a concentrate diet. Bags were inserted into the cannula every 7 minutes (8 bags/day) and were retrieved from fecal matter on average 12 hours after being placed in the cannula. Once all bags were retrieved, bags were rinsed and placed in a 60°C forced-air oven for 24 hours to dry. Bags were allowed to air equilibrate for 12 hours before being weighed. After all bags (ruminally and duodenally incubated) were weighed, bags were cut open and N was analyzed on the remaining feed residue to calculate remaining CP. The following equations were then used to determine DMD, RUP % of CP, RUP % of DM, and % RUP digestibility:

$$DMD, \% = 1 - \frac{[(\text{residue} + \text{in situ bag}) - \text{in situ bag}]}{(\text{Sample wt.})(DM)}$$

$$RUP, \% \text{ of } CP = 100 \times \frac{(\text{residue Wt.})(\text{Residue, \% CP})}{(\text{Sample Wt.})(\text{Sample, \% CP})}$$

$$RUP, \% \text{ of } DM = (RUP, \% \text{ of } CP) \left(\frac{\% CP}{\% DM} \right)$$

RUP digestibility, %

$$= \frac{CP \text{ after rumen incubation, mg} - CP \text{ after duodenal incubation, mg}}{CP \text{ after rumen incubation, mg}}$$

Data were then analyzed as a 2×4 factorial using the mixed procedure of SAS, with the experimental units consisting of 4 *in situ* bags. The factors included two incubations times and four different feeds. Steer was added as a random effect for the rumen incubated bags and the class statement included incubation time and feed sample. It is important to note that only one steer was used for duodenal incubation, therefore it was not added as a random effect.

Exp. 4 – RUP content of reconstituted dry rolled corn

Dry rolled corn (DRC) was sampled from the feed mill located at the ENREC near Mead, NE and brought to the University of Nebraska-Lincoln metabolism area. Using a Leland Food Mixer, Model L-1000, different proportions of water and corn were mixed to reconstitute DRC to 50, 65, 70, and 75% DM. To reach 50% DM 19.99 kg of water and 25.36 kg of corn were added to the mixer, mixed thoroughly and then placed into a plastic tote overnight to allow the corn more time to absorb the water. The same procedure was repeated for the other DM points by varying the amount of water added at each moisture point. For the 35% DM corn 9.53 kg of water was added and 7.04 kg and 4.29 kg of water was added to the 30 and 25% DM corn, respectively. It is important to note that we attempted to reconstitute corn to 40% DM by adding 31.33 kg of water; however, this was too much water and the corn was not able to absorb it all. Once corn was reconstituted to its designated DM, wet corn was packed into mini PVC silos (0.002m^3 , 0.08ft^3) using a packing density 720 kg DM/m^3 (45 lbs DM/ft^3), which is

representative of the HMC packing density. Silos were sealed with lids equipped with a gas release valve and stored for 30, 90, 180 or 270 days. There were three silos at each moisture level for every ensiling time (48 total).

On the designated day, silos were weighed, emptied and sub-sampled for DM and CP. Within 1 hour of being opened, corn was weighed into Ankom rumen *in situ* bags (10 cm × 20 cm, 50 µm pore size). In order to get the same DM content in each bag, different as-is amounts of HMC were added to the bags based on the DM at which the corn was ensiled at. For the 75% DM corn, 3.04 g of sample was added to each bag. Using the 70% DM corn 3.28 g was added, 3.56 g was added for the 65% DM corn and 4.57 g was added for the 50% DM corn. This was equal to 2.3 g of DM for each sample. There were 4 *in situ* bags per steer (2) for each incubation time (2), therefore 16 bags / silo were made. Bags were ruminally incubated for 20 or 30 hours in ruminally fistulaed cattle consuming a 30% concentrate diet. After the designated incubation time, bags were removed and rinsed in a washing machine. Rinsing consisted of five rinses of a one minute agitation and a two minute spin. After rinsing, bags were dried in a 60°C forced-air oven for 24 hours. Bags were allowed to air equilibrate for 12 hours and then they were weighed and CP analysis was conducted on the bag residue. Rumen % DMD and % RUP was calculated using the following equations:

$$DMD, \% = 1 - \frac{[(\text{residue} + \text{in situ bag}) - \text{in situ bag}]}{(\text{Sample wt.})(DM)}$$

$$RUP, \% \text{ of CP} = 100 \times \frac{(\text{residue Wt.})(\text{Residue, \% CP})}{(\text{Sample Wt.})(\text{Sample, \% CP})}$$

There were two incubation times, four ensiling times and four different corn DMs; therefore, data were analyzed as a $2 \times 4 \times 4$ factorial using the mixed procedure of SAS. Experimental unit consisted of 4 *in situ* bags and the class statement included incubation time, ensiling time, and corn DM. There were linear interactions for ensiling time and corn DM; therefore, simple effects within corn DM across days is presented.

Results and Discussion

Exp. 1 - Supplementing RUP to growing cattle on a corn silage based diet

No differences in DMI ($P \geq 0.16$) were observed among treatments for either the first (day 1-37) or the second (day 38-83) feeding periods nor was there a difference in DMI for the total feeding period (day 1-83; Table 2). During the first feeding period, ADG ($P < 0.01$) and G:F ($P < 0.01$) both linearly increased as RUP inclusion increased in the diet. Using the NRC model, MP balance (supply minus requirement of MP) for period one increased from -200 to +65 g/d as RUP inclusion increased from 0 to 13%. At 9.75% RUP inclusion, MP requirements were met (MP balance of +2 g/d). There were no differences in DMI, ADG, or G:F for the second feeding period ($P \geq 0.11$; day 38-83). As RUP supplementation increased, G:F improved 30% in the first period. The improvement in feed efficiency in the first 37 days may be due to increased amino acid requirements of younger, lighter calves.

For the overall growing period (day 1-83), as supplemental RUP inclusion increased from 0 to 13%, a linear increase was observed in ending BW ($P = 0.01$). With no difference in DMI ($P = 0.54$) among the 5 treatments, averaging 7.76 kg/d, and a linear increase in ADG ($P < 0.01$), G:F linearly improved ($P < 0.01$) as RUP inclusion

increased from 0 to 13%. This is consistent with data presented by Hilscher et al., (2016) who reported a linear increase in both ADG and G:F as supplemental RUP was increased in a corn silage growing diet from 0 to 10% (DM basis). Felix et al., (2014) also reported increased ADG, final BW and G:F in corn silage growing diets supplemented with either dried distillers grains with solubles (DDGS) or soybean meal (SBM) compared to diets supplemented with only urea.

For the overall feeding period, the MP balance increased from -115 to +130 g/d as RUP inclusion increased from 0 to 13%. In period 1, cattle had greater requirement and the MP balance increased from -140 to -7 g/d as supplemental RUP increased from 0 to 13%. As cattle became more mature their requirements decreased and the MP balance was met with a lower level of supplement. Protein from BCP is 7.9% lysine but because BCP is only 80% true protein, this 7.9% needs to be divided by 0.8 to get an accurate amounts of lysine from BCP. Based on modeling we found that in cattle receiving the 13% supplement lysine was not deficient. Therefore, lysine is not the 1st limiting nutrient but rather total MP. Another explanation for additional improvement in ADG and G:F as supplemental RUP increased from 9.75 to 13% of diet DM is that excess MP provided from supplemental RUP can also be used as energy once MP requirements are met.

Exp. 2 – Corn silage in vitro

As a % of CP, RUP averaged 22.7 and 23.9 for the 37 and 42% corn silage, respectively (Table 3). SoyPass averaged 47.1 % RUP as a % of CP. SoyPass was included as a standard to compare corn silage too because the RUP of SoyPass is well established (75% RUP as a % of CP). Because our value of SoyPass is lower than it

should be, the values presented for corn silage in Exp. 2 could be underestimated. As a % of DM, RUP was 2.2 and 1.2 for the 37 and 42% corn silages, respectively, and 22.7 for SoyPass. The purine:nitrogen ratio is variable and is influenced by the type and amount of microbes present in the rumen during sampling. The current study could be either underestimating or overestimating RUP content due to variation in the purine:nitrogen ratio and the effect of using the same ratio for all feed samples. Because the RUP value of SoyPass was so low in this study, it is likely we underestimated RUP of corn silage. There has been minimal research conducted on RUP values of corn silage utilizing *in vitro* methods; therefore, more research is needed in order to confirm the values presented in the current study.

Exp. 3 – Corn silage in situ

The DM digestibility of the two corn silages was not affected by the DM of the silage or amount of time incubated in the rumen ($P \geq 0.19$; Table 4). Contrary to the current study, data presented by Andrae et al., (2001) suggests that silage harvested at black layer (42% DM in the current study) is less digestible than corn silage harvested at half milk line (37% in the current study). As a % of DM, RUP with and without NDS were not different between the two corn silages and was not affected by time incubated in the rumen ($P \geq 0.12$). The RUP as a % of DM was greater for the silage that was not incubated in NDS. As a % of CP, RUP with NDS incubation had a tendency ($P = 0.07$) to be less for the 42% CS and also had a tendency ($P = 0.07$) to be less for corn silage incubated for 30 hours compared to corn silage incubated for 20 hours. For bags that were not refluxed in NDS solution there were no differences in RUP digestibility due to type of corn silage or hours incubated ($P \geq 0.46$). The 37% corn silage without NDS

averaged 39.9% RUP digestibility and the 42% corn silage averaged 35.1% RUP digestibility without NDS. Bags that were corrected for microbial contamination by refluxing in NDS solution also had no differences in RUP digestibility ($P \geq 0.56$) due to type of corn silage or hours incubated; averaging 32.3% RUP digestibility for both silages.

Microbial contamination is a potential source of error when measuring RUP in feeds. The reason some bags were washed in NDS and some were not is because it is unclear which procedure should be used with corn silage. Refluxing bags in NDS corrects for microbial attachment to forage particles by removing microbial N (Mass et al., 1999). While refluxing in NDS has been validated when using forages (Haugen et al., 2006) it has not been utilized for non-forage fiber sources and it is possible that a portion of the protein may have remained in the grain residue but was soluble in the NDS, resulting in lower estimates of degradable RUP (Klopfenstein et al., 2001). Kononoff et al., (2007) refluxed corn silage in NDS after duodenal incubation to correct for microbial N and reported average RUP digestibility values for corn silage to be 19.9 ± 3.76 as a % of RUP. In the current study, values for % RUP digestibility in bags that were not refluxed in NDS were greater (averaged 37.5%) than the bags that were refluxed in NDS (averaged 32%). The bags that received the NDS treatment are corrected for microbial N but a portion of the concentrate protein could have been degraded by the NDS; therefore, we could be underestimating RUP and overestimating RUP digestibility when refluxing corn silage in NDS. The bags that did not receive the NDS treatment are not corrected for microbial N and are potentially underestimating RUP digestibility.

Exp. 4 – Reconstituted dry rolled corn

There was a linear interaction of corn DM and days ensiled ($P < 0.01$) for RUP (% of CP). For the driest corn (75% DM), there was no difference in RUP content across all four ensiling times ($P \geq 0.23$). There was also no difference in RUP content of the 70% DM corn across all four ensiling times ($P \geq 0.74$); however, the 70% DM corn had less RUP than the 75% corn with the lowest RUP content at 90 days (Figure 1). The 65% DM corn showed a quadratic ($P < 0.01$) decrease in RUP as ensiling time increased and had a lower RUP than both the 75 and 70% DM corn. The wettest corn (50% DM) showed a linear decrease ($P < 0.01$) in RUP as ensiling time increased, and in each time point had the lowest % RUP out of all treatments. These data agree with data presented by Benton et al. (2005b) who reported that as moisture content of reconstituted DRC and ensiling time increased, RUP decreased.

A linear interaction ($P < 0.01$) for corn DM and ensiling time was also observed for dry matter digestibility as a % of CP. The DMD of the 75% DM corn linearly increased ($P = 0.01$) as ensiling time increased from 30 to 270 days (Figure 2). There was no difference in dry matter digestibility for the 70% DM corn across all ensiling times ($P \geq 0.13$); however, dry matter digestibility was greater in all ensiling periods for the 70% DM compared to the 75% DM. There was no difference in dry matter digestibility for the 65% DM corn across ensiling times ($P \geq 0.21$); however, the 65% DM corn had a greater dry matter digestibility than both the 70 and 75% DM corn. The wettest corn (50% DM) showed a quadratic increase ($P = 0.01$) in dry matter digestibility as ensiling time increased from 30 to 270 days with the greatest dry matter digestibility at 90 days. The 50% DM corn also had the greatest dry matter digestibility at each ensiling time compared to the drier corn samples. These data also agree with data presented by

Benton et al. (2005b) who reported that dry matter digestibility increased as DM of corn decreased and ensiling time increased.

There was no interaction of rumen incubation time and DM ($P = 0.08$) on RUP as a % of CP (Figure 3). For each DM, as corn was incubated longer in the rumen, RUP decreased ($P < 0.01$). This was expected because leaving the sample in the rumen longer gives rumen microbes longer to break down the feed. As DM of corn decreased from 75 to 50% DM, RUP linearly decreased ($P < 0.01$). The dry matter digestibility was greatest for samples incubated for 30 hours compared to 20 hours for all moisture levels, which was expected (Figure 4). The dry matter digestibility linearly increased ($P < 0.01$) as DM of corn decreased from 75 to 50% DM.

Implications

Supplementing bypass protein to growing cattle on a corn silage diet linearly increased ADG and ending BW by meeting MP and lysine requirements. Once MP requirements of the calves are met, excess MP provided from RUP could be used as energy. Corn silage RUP values vary depending on method of analysis. Using the an *in vitro* technique, values average 24% RUP as a % of CP but when using the *in vitro* technique we need to consider the variability in the purine:nitrogen ratio due to the microbes in the rumen fluid inoculum. If the purine:nitrogen ratio is low, estimates of RUP content would be overestimated using *in vitro* procedures. *In situ* RUP values averaged 9% RUP as a % of CP with no NDS and 26.5% RUP as a % of CP when incubated in NDS. RUP could be underestimated using the *in situ* method due to washout of feed particles that were not degraded. Rumen undegradable protein could also be underestimated when using NDS to correct for microbial contamination.

Moisture content of corn silage at the time of harvest and the amount of time corn silage is ensiled continually impacts protein availability. Based off these studies, the value that is recommended for corn silage RUP digestibility is 32%.

Corn silage has very low RUP and increased moisture coupled with increased ensiling time can further decrease RUP of corn silage. Therefore, in order to meet MP requirements of growing cattle on a corn silage diet, there needs to be a supplemental source of RUP.

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Table 1. Diets fed to individually fed growing steers

Ingredient	Treatment ¹				
	0.0%	3.25%	6.5%	9.75%	13%
<i>Diet composition, % of diet DM</i>					
Corn Silage	85.0	85.0	85.0	85.0	85.0
RDP supplement ²	15	11.25	7.5	3.75	0
RUP supplement ³	0	3.75	7.5	11.25	15.0
Supplemented RUP ⁴	0.4	1.7	3.0	4.2	5.5
<i>Protein sources, % of diet DM</i>					
Soypass	0	2.0	3.9	5.9	7.8
Empyreal	0	1.3	2.6	3.9	5.2
Urea	1.5	1.2	0.9	0.6	0.3

¹ Supplement levels consisted of 0, 3.25, 6.5, 9.75 and 13% Soypass + Empyreal (RUP sources as a % of diet DM).

² RDP supplement consisted of 79.2% corn, 2.9% limestone, 2.5% tallow, 9.7% urea, 2.0% salt, 3.2% dicalcium phosphate, trace minerals, vitamin A-D-E, and Rumension-90.

³ RUP supplement consisted of 52% Soypass, 34.7% Empyreal, 1.9% corn, 3.2% limestone, 2.5% tallow, 1.7% urea, 2.0% salt, 1.5% dicalcium phosphate, trace minerals, vitamin A-D-E, and Rumension-90.

⁴ % of RUP provided in the Supplement

Table 2. Effects of increasing supplemental rumen undegradable protein in corn silage based growing diets on steer performance

Variable	Treatments ¹					SEM	<i>P</i> - Value	
	0%	3.25%	6.5%	9.75%	13%		Lin.	Quad.
Initial BW, kg	274	275	274	276	274	11.8	0.99	0.93
Ending BW, kg	367	378	376	392	389	16.6	0.01	0.68
Day 1-37								
Interim BW, kg	314	321	323	331	331	14.7	0.03	0.69
DMI, kg/d	7.0	7.2	7.3	7.4	7.1	0.56	0.68	0.33
ADG, kg	1.06	1.24	1.34	1.49	1.53	0.21	< 0.01	0.52
G:F	0.150	0.172	0.185	0.201	0.216	0.010	< 0.01	0.76
Day 38-83								
DMI, kg/d	7.7	8.5	8.3	8.4	8.3	0.70	0.22	0.16
ADG, kg	1.07	1.06	1.18	1.18	1.28	0.14	0.11	0.87
G:F	0.142	0.138	0.128	0.148	0.143	0.010	0.60	0.32
Day 1-83								
DMI, kg/d	7.3	7.8	7.8	7.9	7.7	0.59	0.34	0.19
ADG, kg	1.07	1.20	1.18	1.36	1.34	0.12	< 0.01	0.55
G:F	0.148	0.154	0.154	0.172	0.174	0.010	< 0.01	0.61

¹All cattle were fed 85% corn silage with a combination of RDP and RUP supplements to achieve either 0, 3.25, 6.5, 9.75, or 13% supplemental RUP (% of diet DM). The RUP source was a blend of SoyPass + Emyreal in the final diet.

Table 3. Rumen undegradable protein of 37 and 42% DM corn silage using *in vitro* techniques

	Treatments				SEM	<i>P</i> -value
	37% CS	42% CS	SoyPass	SBM		
CP, %	7.2	6.5	48.1	48.5	-	-
RUP, % CP	22.7	23.9	47.1	16.9	7.58	0.02
RUP, % DM	2.2	1.2	22.7	8.0	1.59	< 0.01

¹Treatments consisted of each feed sample, incubated for either 16 or 24 hours
There was no interaction of feed sample and incubation time ($P \geq 0.70$); therefore, only main effects are present

Table 4. Dry matter digestibility, Rumen undegradable protein, and rumen undegradable protein digestibility of 37 and 42% DM corn silage using in situ techniques

	Treatments				SEM	<i>P</i> -value	
	37 % CS		42 % CS			DM	Incubation
	20	30	20	30			
Incubation, h	20	30	20	30			
CP, %	7.2	7.2	6.5	6.5	-	-	-
DM Digestibility, %	66.1	68.9	64.6	69.8	0.85	0.84	0.19
RUP, % DM without (NDS)	2.1	1.8	1.7	1.6	0.19	0.15	0.32
RUP, % DM with (NDS)	0.67	0.64	0.51	0.53	0.02	0.12	0.87
RUP, % CP without (NDS)	29.2	25.1	26.8	24.7	2.81	0.64	0.73
RUP, % CP with (NDS)	9.3	8.9	8.9	8.4	0.04	0.07	0.07

RUP Dig. Without (NDS), % ¹	45.0	34.8	35.4	3.48	3.39	0.50	0.46
RUP Dig. With (NDS), % ²	32.2	32.3	32.6	31.9	0.59	0.94	0.56

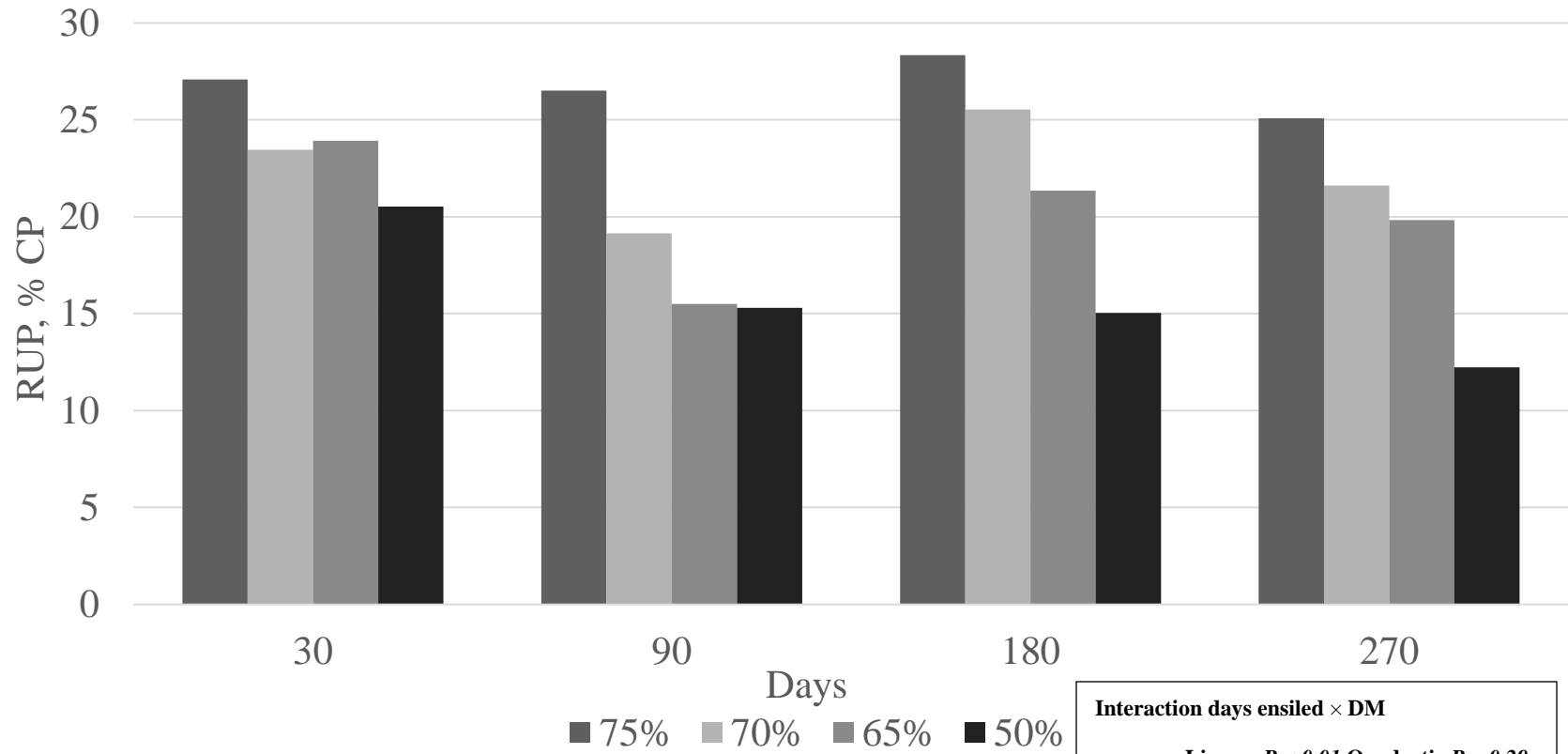
¹Treatments consisted of either 37 or 42% corn silage incubated for either 20 or 30 hours

²RUP digestibility without refluxing in Neutral Detergent Fiber Solution to correct for microbial contamination after rumen incubation

³RUP digestibility corrected for microbial contamination by refluxing in Neutral Detergent Fiber Solution

There was no interactions of feed sample and incubation time ($P \geq 0.30$); therefore, only main effects are presented

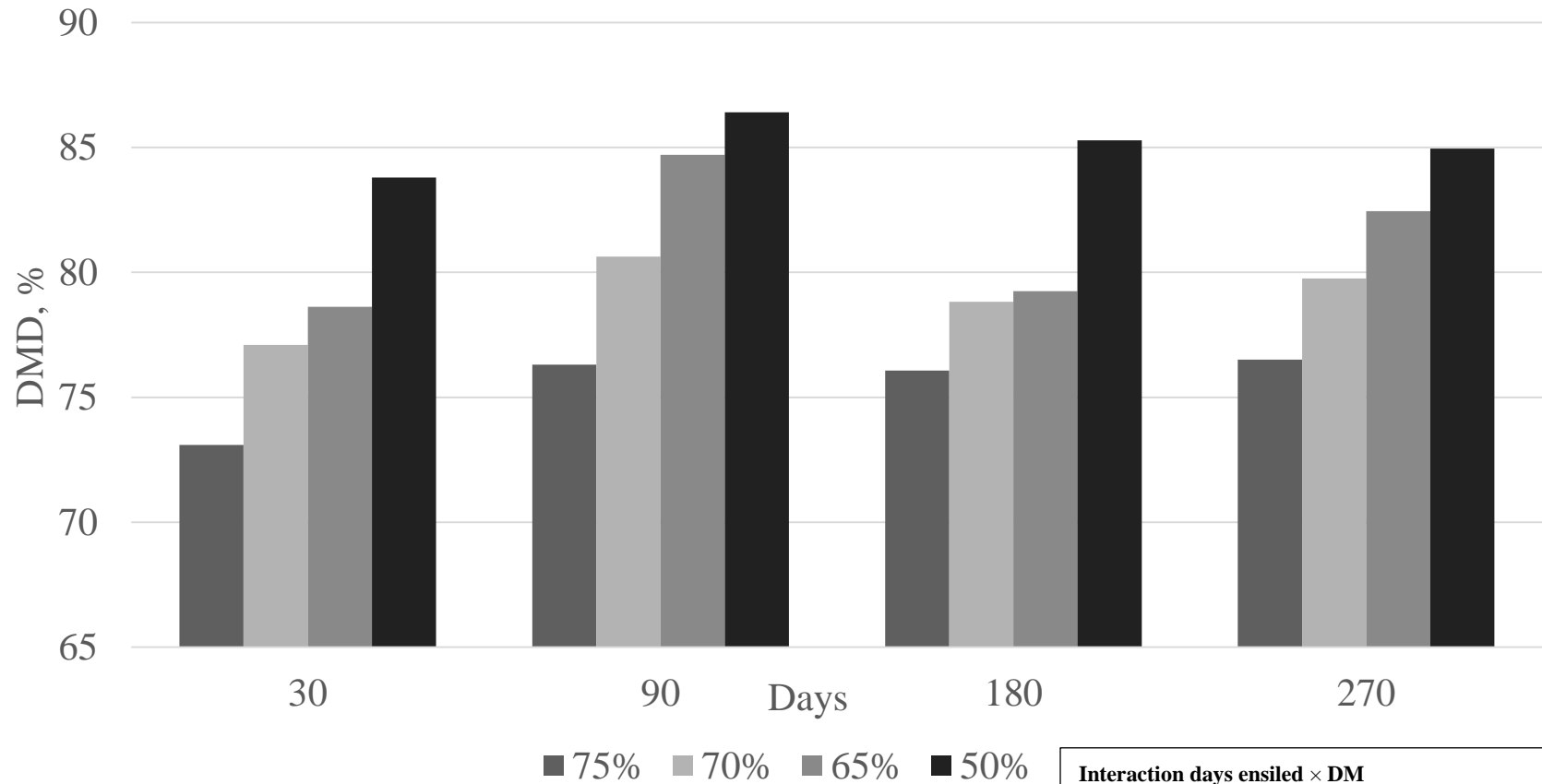
Figure 1. Effect of dry matter and days ensiled on percent rumen undegradable protein content of high moisture corn



DRC was reconstituted to 75, 70, 65 and 50% DM and ensiled for 30, 90, 180 and 270 days to determine effect on RUP content.

Interaction days ensiled × DM
 Linear: $P < 0.01$ Quadratic $P = 0.29$
 75% DM: Linear $P = 0.50$ Quadratic $P = 0.23$
 70% DM: Linear $P = 0.74$ Quadratic $P = 0.89$
 65% DM: Linear $P = 0.28$ Quadratic $P < 0.01$
 50% DM: Linear $P < 0.01$ Quadratic $P = 0.05$

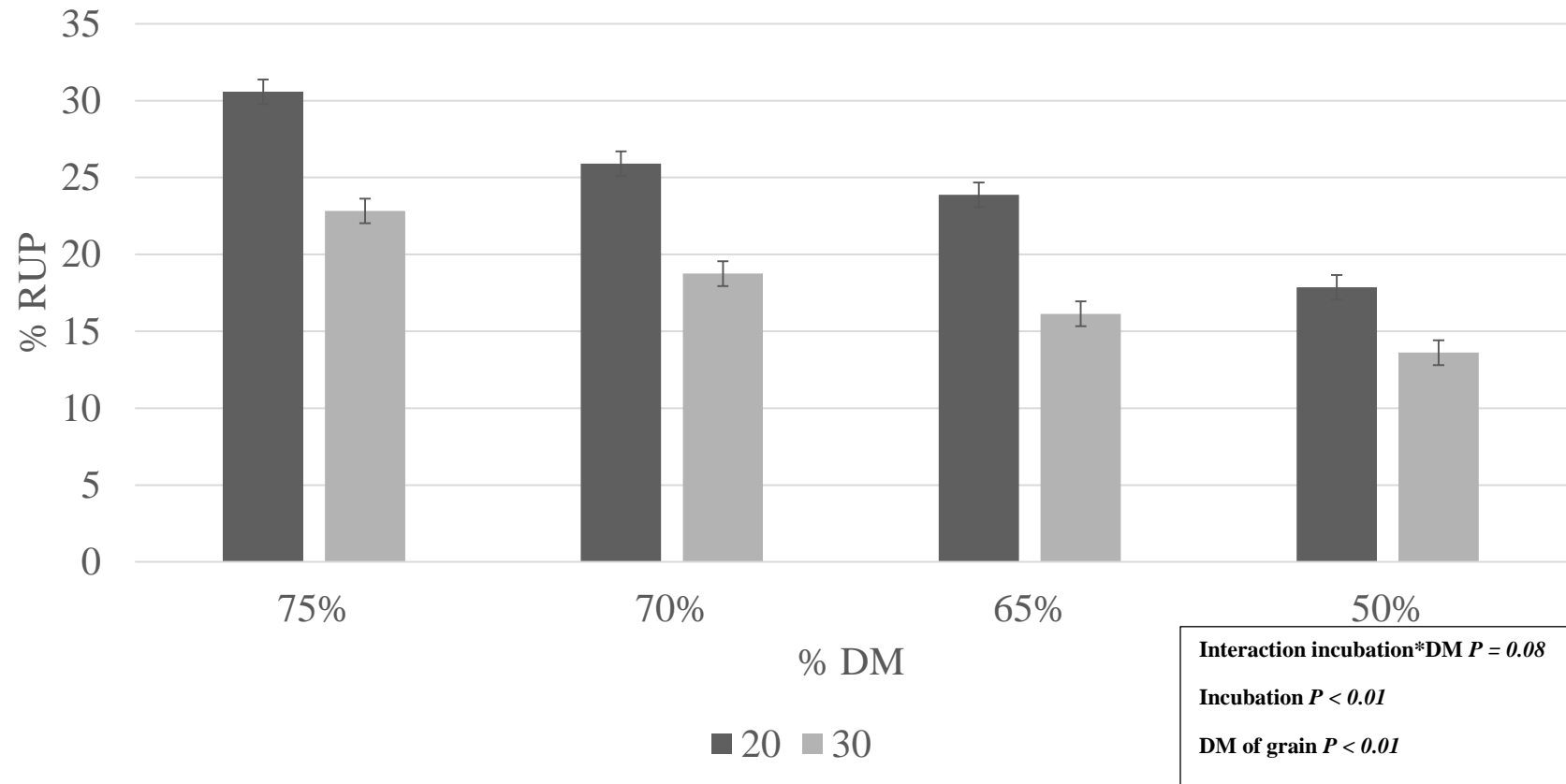
Figure 2. Effect of dry matter and days ensiled on dry matter digestibility of high moisture corn



DRC was reconstituted to 75, 70, 65 and 50% DM and ensiled for 30, 90, 180 and 270 days then ruminally incubated to determine effect on DMD.

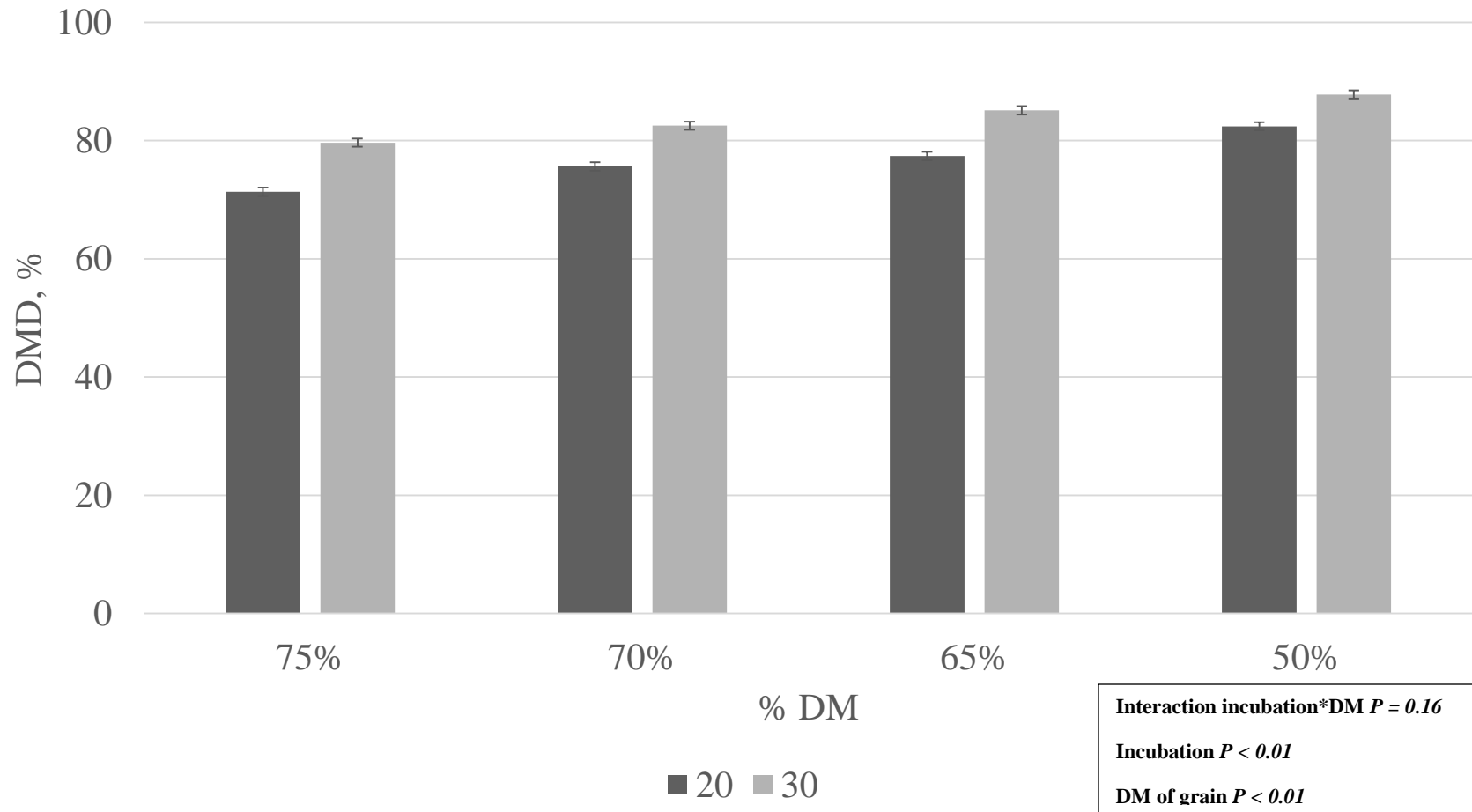
Interaction days ensiled × DM
 Linear: $P < 0.01$ Quadratic $P = 0.48$
 75% DM: Linear $P = 0.01$ Quadratic $P = 0.13$
 70% DM: Linear $P = 0.13$ Quadratic $P = 0.15$
 65% DM: Linear $P = 0.24$ Quadratic $P = 0.21$
 50% DM: Linear $P = 0.39$ Quadratic $P = 0.01$

Figure 3. Effect of rumen incubation time and dry matter of corn on percent rumen undegradable protein



DRC was reconstituted to 75, 70, 65 and 50% DM and then ruminally incubated for 20 or 30 hours to determine effect on RUP.

Figure 4. Effect of rumen incubation time and dry matter of corn on dry matter digestibility



DRC was reconstituted to 75, 70, 65 and 50% DM and then ruminally incubated for 20 or 30 hours to determine effect on DMD.

Chapter IV. The Effect of Inoculants on Nutrient Losses of Corn Silage and High-moisture Corn Stored in PVC Mini Silos¹

C. R. Oney *, J. L Gramkow *, A. K. Watson *², G. E. Erickson *², J. C. MacDonald *,
M. K. Biddlecome †

*Department of Animal Science, University of Nebraska, Lincoln 68583; †Schaumann
Inc. Mendota Heights, MN 55120

¹Funding provided by Schaumann Inc. Mendota Heights, MN.

²Corresponding authors: gerickson4@unl.edu and awatson3@unl.edu

Abstract

Two experiments were conducted to determine the effects of BONSILAGE CORN 200G (Bon-Silage) and BONSILAGE HMC 200G (Bon-HMC) on nutrient losses and aerobic stability of corn silage and high moisture corn (HMC), respectively, while stored in PVC mini silos. In Exp. 1, corn silage and HMC were harvested at 35 and 70% DM, respectively. Corn silage was inoculated with Bon-Silage (*L. buchneri* and *Lactobacillus plantarum*) at either 0, 200,000, or 400,000 CFU/g and ensiled in mini PVC silos for 30 or 90 days. High moisture corn was inoculated with BONSILAGE HMC 200G (*L. buchneri*, *Pediococcus acidilactici*, and *Lactobacillus plantarum*) at either 0, 300,000, or 600,000 CFU/g and ensiled in mini PVC silos for 30 or 90 days. In Exp. 2, HMC was harvested at 68% DM and inoculated at either 0 or 600,000 CFU/g with BONSILAGE HMC 200G and ensiled for either 90 or 120 days. There were four replicates of silage and HMC in each treatment for both Exp. 1 and 2. Nutrient and fermentation analysis along with an aerobic stability test were conducted after the designated ensiling period. In Exp. 1, pH of corn silage linearly ($P < 0.01$) increased as inoculant application level increased. Corn silage ensiled for 90 days had greater NDS than corn silage ensiled for 30 days ($P = 0.02$), but not for ADF content ($P \geq 0.16$). Starch content of corn silage linearly increased ($P = 0.02$) as level of inoculant increased. Lactic acid production decreased linearly ($P < 0.01$) as level of inoculant increased and acetic acid production increased linearly as level of inoculant increased and was greatest for silage ensiled for 90 days ($P < 0.01$). In Exp. 1, pH of HMC increased linearly as level of inoculant increased ($P = 0.03$). There was a linear decrease in NDS ($P < 0.05$) as inoculant level increased, and was further decreased with increased ensiling time.

Neither inoculation nor ensiling time affected ADF of HMC ($P \geq 0.14$). Starch content of HMC decreased as inoculant level increased ($P = 0.02$). Lactic acid decreased linearly as inoculant level increased ($P = 0.01$) and acetic acid linearly increased as level of inoculant increased ($P < 0.01$), with the greatest increase in HMC ensiled for 90 days. In Exp. 2, pH of HMC was greatest for HMC that had been inoculated ($P < 0.01$). Starch, NDS, and ADF content were all unaffected by both inoculant and time ensiled ($P \geq 0.09$). Lactic acid production was less for inoculated HMC, and acetic acid production was greatest for inoculated HMC, and was further increased for HMC ensiled for 120 days ($P < 0.01$). The addition of *L. buchneri* to corn silage and HMC impacts the proportion of acids produced and therefore possibly can impact the overall nutrient losses.

Key Words: Corn Silage, High Moisture Corn, Inoculant

Introduction

Lactic acid bacteria (LAB) containing inoculants have been developed to enhance fermentation and mitigate aerobic spoilage of ensiled feeds. Homofermentative LAB have the ability to convert one molecule of glucose directly into two molecules of lactic acid, decreasing pH and allowing for better DM and energy recovery in silages (Muck and Kung, 1997). However, utilization of these LAB inoculants has not always been reported to positively affect aerobic stability (Muck and King, 2007) and has even been reported to enhance the growth of yeasts, therefore decreasing aerobic stability (Moon et al., 1980).

Lactobacillus buchneri, a heterofermentative LAB possess a unique pathway that allows it to degrade two molecules of lactic acid to form 1 molecule of acetic acid and 1 molecule of 1,2-propanediol, in addition to CO₂ and traces of ethanol, while yielding 1 molecule of ATP (Oude Elferink et al., 2001). Acetic acid inhibits the growth of yeasts, which are the leading cause of spoilage in silage and high-moisture corn (HMC) that have been exposed to oxygen. Moon (1983) reported that acetic acid has good antifungal properties which are strengthened with low pH; therefore, increases in acetic acid in the silage environment, which contains lactic acid, can effectively inhibit the growth of yeasts. Kleinschmit and Kung (2006a) attributed improvements in aerobic stability of silages to increased concentrations of acetic acid produced by *L. buchneri*.

Inoculating with a mixture of *L. buchneri* and homofermentative LAB has been shown to increase lactic acid production, rapidly drop pH, and improve DM recovery (Kung et al., 2003). However, results of aerobic stability have been variable when inoculating with this combination (Kleinschmit and Kung 2006a; Arriola et al., 2011).

Thus, the objectives of these experiments were to determine the effects of inoculant on nutrient losses and aerobic stability of corn silage and HMC, while stored in PVC mini silos.

Material and Methods

Exp. 1

Corn Silage. Corn silage was harvested at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE on September 14, 2015 at 35% DM. Prior to bunker packing, 54.4 kg of fresh green chopped corn silage was brought to the University of Nebraska-Lincoln's metabolism area in a 50 gallon barrel. Silage was inoculated with a Leland Food Mixer, Model L-1000, by mixing 27.2 kg of sample with *L. buchneri* and *Lactobacillus plantarum* (BONSILAGE CORN 200G; Schaumann Inc. Mendota Heights, MN.) at 400,000 colony forming units (CFU)/g of silage by using a hand held spray bottle and mixed for 7 minutes as inoculate was applied. 9.1 kg of this inoculated sample was then added to 9.1 kg of fresh non-inoculated silage and mixed in the feed mixer for 7 minutes to obtain 18.1 kg of silage inoculated at 200,000 CFU/g. This yielded 18.1 kg of silage at each inoculate level: 0 CFU/g, 200,000 CFU/g, and 400,000 CFU/g.

Corn silage was packed into mini PVC silos (0.002 m³; 0.08 ft³) at 232.4 kg DM/m³ (14.5 lbs DM/ft³; which is representative of the corn silage packing density used in the cattle industry) allowing for 0.51 kg of DM or 1.36 kg as is silage per silo. Silos were sealed using covers fitted with gas release valves to ensure an anaerobic environment. Silos were stored for 30 or 90 days in a temperature controlled room. A total of 36 mini PVC silos of corn silage were made with 4 silos at each time point for

each inoculate level. On the designated opening day (30 or 90 days), silos were weighed, emptied, sub-sampled and frozen.

All lab analysis, except for yeast and mold counts, was conducted by Dairy One (Ithaca, New York) while yeast and mold counts were analyzed by Midwest Laboratories (Omaha, NE). Water soluble carbohydrates (WSC) was conducted using the West Virginia Procedure (Hall et al., 1999), dry matter was determined using the Goering and Van Soest (1970) method, ADF was determined using the ANKOM, and NDS was also determined using the ANKOM (ANKOM Technology Method 5,6; Van Soest et al., 1991). Crude protein was analyzed using the AOAC 990.03 method (Leco Corporation, St. Joseph, MI), pH was analyzed using thermo orion combination sure-flow pH electrode and thermos orion 410 meter (Thermo fisher Scientific, Waltham, MA), and Ammonium-N was extracted using a reciprocating shaker (Kalra, 1998). Organic matter was calculated by taking the total N minus ammonium-N, Starch was analyzed in a YSI 2700 SELECT biochemistry analyzer (YSI Incorporated Life Sciences), acetic, propionic, butyric, and iso-butyric acids were analyzed using gas chromatography, and lactic acid was analyzed in a biochemistry analyzer (YSI Incorporated Life Sciences). Yeast and mold counts were analyzed using the FDA/BAM method (Elliot et al. 1995).

Following the ensiling process, half of the silage sample that had been removed from the mini silos was evaluated for aerobic stability. Silage was removed from the freezer, allowed to thaw, and mixed by hand for thirty seconds. After mixing, silage was added to 1000 mL plastic bottle. Bottles were filled to 1 inch from the top, and then weighed. After getting an initial weight, bottles were temperature probed using a ThermoWorks digital temperature probe to get an initial temperature. After getting initial

weight and temperature, bottles were stored in a temperature controlled room (21°C) for two weeks. To determine aerobic stability bottles were weighed and temperature probed twice per day (0800 and 1500) for two weeks. Temperature and DM weight lost over time were evaluated to determine aerobic stability after ensiling.

High-moisture Corn. High-moisture corn was harvested at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE on September 26, 2015 at 70% DM. The same procedure as described above (corn silage procedure) was used for HMC, with the exception of inoculant used, packing density, and level of inoculant applied. High moisture corn was inoculated with *L. buchneri*, *Pediococcus acidilactici*, and *Lactobacillus plantarum* (BONSILAGE HMC 200G; Schaumann Inc.) A packing density of 720.8 kg DM/m³ (45 lbs DM/ft³) was used which allowed for 1.6 kg of DM or 2.3 kg as is corn per mini silo. The three inoculant levels for HMC were 0, 300,000 and 600,000 CFU/g. Lab analyses and aerobic stability were also conducted the same as above.

Exp. 2

High-Moisture Corn. High-moisture corn was harvested at the Eastern Nebraska Research and Extension Center (ENREC) near Mead, NE on Septemeber 24, 2016 at 68% DM. The same procedure as described in Exp. 1 was used to inoculate and mix the HMC sample. The same HMC inoculant used in Exp. 1, was utilized in Exp. 2 (BONSILAGE HMC 200G). High moisture corn was again packed at a 720.8 kg DM/m³ (45 lbs DM/ft³), allowing for 1.6 kg DM or 2.3 kg as is corn per mini silo. In Exp. 2, 0 CFU/g and 600,000 CFU/g were used as inoculant treatments. High moisture corn was ensiled for either 90 or 120 days with 4 silos per treatment at each time point (16 silos

total). All lab analysis was conducted by the same laboratories that completed the lab analysis for Exp. 1 and aerobic stability was again tested by recording weight and temperature change over a three week period.

Statistics. In Exp. 1, data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Data were analyzed as a 2×3 factorial using the 30 and 90 day samples. Exp. 2 was analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), and data were analyzed as a 2×2 factorial.

Results and Discussion

Exp. 1 – Corn Silage

No interaction of days ensiled and level of inoculant ($P \geq 0.20$) was observed for pH. Silage pH increased linearly ($P = 0.05$) as CFU level increased from 0 to 400,000 (Table 1). Corn silage pH also increased as days ensiled increased ($P < 0.01$). This agrees with data presented by Kleinschmit and Kung (2006a) who reported that corn silage treated with *L. buchneri* had greater ($P < 0.01$) pH values compared to untreated silage and that pH increased as the level of *L. buchneri* in the silage increased. Total acids, as a % of DM, showed a tendency ($P = 0.06$) for a quadratic interaction between CFUs and days ensiled. Total acids had a tendency to decrease linearly ($P = 0.07$) as CFUs increased and was greater ($P < 0.01$) for corn silage ensiled for 90 days (7.6, 7.87, 7.26) compared to corn silage ensiled for 30 days (7.12, 6.92, 6.93).

There was no interaction observed ($P \geq 0.28$) for % DM. Neither level of inoculant or time ensiled affected the % DM of the corn silage ($P \geq 0.24$). Organic

matter of corn silage was not affected by inoculant level or time ensiled ($P \geq 0.28$) and averaged 94.3% across all treatments (Table 1). There was no interaction of days ensiled by inoculant level for NDS content ($P \geq 0.45$). There was a tendency for NDS to decrease linearly ($P = 0.07$) as CFU level increased from 0 to 400,000 CFU/g. Days ensiled also affected NDS level ($P = 0.02$), with corn silage ensiled for 30 days having less NDS (averaging 37.66) than corn silage ensiled for 90 days (averaging 40.75). Contrary to the current study, Der Bedrosian et al. (2012) conducted a study that compared different corn hybrids and length of ensiling on the composition and nutritive value of corn silage and reported no effect of time ensiled on the concentrations of NDS. However, it is important to note that no inoculant was used in the study conducted by Der Bedrosian et al. (2012). Sanderson (1993) also reported no differences in the concentrations of NDS in corn silage ensiled for either 40 or 186 days, and they did use inoculants in this study. What is important to note is that *L. buchneri* was not used. There was no interaction observed for ADF concentration ($P \geq 0.39$). No differences in ADF concentrations were observed between the three inoculant levels or the two ensiling times ($P \geq 0.16$). There was no interaction for starch content ($P \geq 0.83$). As CFU level increased from 0 to 400,000 starch content linearly increased ($P = 0.02$). Out of three different inoculants used on corn silage in a study by Arriola et al. (2011) two of them had numerically greater starch values as a % of DM; however, they were not significantly different than the control.

There was no interaction observed for days ensiled with level of inoculant for lactic acid or acetic acid concentration as a % of DM ($P \geq 0.14$). As level of inoculant increased from 0 to 400,000 CFU/g lactic acid linearly decreased ($P < 0.01$). Acetic acid

concentration linearly increased ($P < 0.01$) as CFU level increased from 0 to 400,000 CFU/g and silage ensiled for 90 days had greater acetic acid concentrations as a % of DM ($P < 0.01$) compared to silage ensiled for 30 days. The lactic:acetic acid ratio linearly decreased ($P < 0.01$) as CFU level increased from 0 to 400,000 and was greater for silage ensiled for 30 days compared to silage ensiled for 90 days ($P < 0.01$). This agrees with data from Kleinschmit and Kung (2006a) who reported that corn silage treated with *L. buchneri* had lower concentrations of lactic acid and lactic acid concentration was decreased further with higher levels of application. In another study by Kleinschmit and Kung (2006b) the authors reported that as days ensiled increased, acetic acid concentration (DM basis) increased for silage treated with *L. buchneri*.

During aerobic analysis, there was no interaction of inoculant level and days ensiled ($P = 0.31$) for % DM weight lost in silage following 30 and 90 day storage (Figure 1 and 2). Inoculate and ensiling time both had no effect on % DM lost ($P \geq 0.68$). There was no interaction ($P = 0.12$) for temperature change in silage samples collected on day 30. Temperature was not different between the three inoculant levels ($P = 0.83$) in silage stored for 30 days and increased quadratically ($P < 0.01$) over the 13 day aerobic stability test (Figure 3). There was an interaction ($P < 0.01$) for temperature of corn silage ensiled for 90 days. Silage inoculated with 400,000 CFU/g was 1.8°C cooler ($P < 0.01$) than the non-inoculated treatment and temperature of all three treatments increased quadratically ($P < 0.01$) over the 13 day aerobic stability test (Figure 4).

Exp. 1 – High Moisture Corn

There were no days ensiled by level of inoculant interactions for pH, total acids, or organic matter of HMC ($P \geq 0.09$). There was a tendency for an interaction ($P = 0.06$) observed for % DM. The pH increased linearly ($P = 0.03$) as level of inoculant increased from 0 to 600,000 CFU/g and time ensiled had no effect ($P = 0.52$) on pH (Table 2). Contrary to the current study, Hoffman et al. (2011) reported that pH of HMC decreased with longer ensiling time but was less significant for HMC inoculated with *L. buchneri*. As inoculant level increased from 0 to 600,000 CFU/g total acids decreased ($P = 0.05$) and the HMC ensiled for 30 days had less total acids compared to HMC ensiled for 90 days ($P < 0.01$). Organic matter as a % of DM was not affected by level of inoculant nor time ensiled. Dry matter was not affected by level of inoculant or time ensiled ($P \geq 0.35$) which agrees with data from Taylor and Kung (2002) who reported no differences in DM of HMC inoculated and ensiled for 92 days.

There was no interaction observed between days ensiled and inoculant for NDS ($P \geq 0.63$). As level of inoculant increased from 0 to 600,000 CFU/g, NDS linearly decreased ($P = 0.05$). Corn ensiled for 30 days had significantly greater NDS levels compared to HMC ensiled for 90 days ($P < 0.01$). Contrary to the current study, Kung et al. (2007) reported no differences in NDS when comparing HMC without an inoculant to HMC samples inoculated with a variety of inoculants and stored for 90 days. There was a quadratic interaction ($P = 0.05$) of inoculant and days ensiled for ADF. For corn ensiled 30 days, ADF was greatest for HMC inoculated at 300,000 CFU/g followed by the control (0 CFU/g) and the 600,000 CFU/g had the lowest ADF. Within 90 day HMC, no effect of inoculation; however, numerically decreased as inoculant level increased from 0 to 600,000 CFU/g. There was a tendency for an ensiling time and inoculant

interaction for starch content ($P \geq 0.07$). The starch content of the 30 and 90 day HMC decreased linearly ($P = 0.04$) as inoculant level increased and the corn stored for 90 days had less starch compared to the 30 day.

There was no interaction observed for lactic acid or acetic acid concentrations ($P \geq 0.28$). Lactic acid concentration as % of DM decreased linearly ($P = 0.01$) as level of inoculant increased from 0 to 600,000 CFU/g. Concentration of acetic acid increased linearly ($P < 0.01$) as level of inoculant increased from 0 to 600,000 CFU/g and was greater ($P < 0.01$) for HMC ensiled for 90 days compared to HMC ensiled for 30 days. A quadratic interaction ($P = 0.02$) was observed for the lactic:acetic acid ratio. The lactic:acetic acid ratio was significantly less for HMC inoculated with *L. buchneri* and ensiled for 90 days. The ratio linearly decreased ($P < 0.01$) as level of inoculant increased from 0 to 600,000 CFU/g and was significantly less ($P < 0.01$) for HMC ensiled for 90 days. High moisture corn inoculated with *L. buchneri* and ensiled for 90 days produced significantly more acetic acid and less lactic acid compared to the non-inoculated HMC. This agrees with data presented by Taylor and Kung (2002) who reported when compared to a control (non-inoculated HMC), HMC that was inoculated with *L. buchneri*, either by itself or in combination with another lactic acid bacteria, had decreased levels of lactic acid and increased levels of acetic acid. Kung et al. (2007) also reported that when compared to a control, HMC inoculated with *L. buchneri* had decreased levels of lactic acid and increased levels of acetic acid which agrees with the current study.

There was an interaction of days ensiled and inoculant ($P < 0.01$) on % DM lost during the aerobic stability test. The HMC stored for 30 days % DM lost increased with

inoculant level and the % DM lost in corn stored for 90 days decreased as level of inoculant increased. As inoculant level increased DM lost in the 30 day HMC increased and DM lost in the 90 days decreased as inoculant increased ($P = 0.03$) (figure 5 and 6). When considering the HMC ensiled for 90 days the non-inoculated HMC had greater DM losses than that of HMC for both the 300,000 and 600,000 CFU/g levels of inoculant. There was an interaction ($P < 0.01$) for temperature change in HMC samples collected on day 30. Non-inoculated corn had a lower temperature ($P < 0.01$) compared to the inoculated corn and temperature of all three treatments quadratically increased ($P < 0.01$) over the 13 day aerobic stability test (Figure 7). There was an interaction ($P < 0.01$) for temperature of HMC ensiled for 90 days (Figure 8). Corn inoculated at 600,000 CFU/g was 1.8°C cooler than corn inoculated at 300,000 CFU/g and 5.2°C cooler than the non-inoculated corn.

Exp. 2 – High Moisture Corn

There were no interactions of days ensiled and inoculant on pH, total acids, DM, DM lost during ensiling, or organic matter ($P \geq 0.11$). The pH of HMC was greatest ($P < 0.01$) for the inoculated HMC in both the 90 and 120 day ensiling periods. There was no effect of length of ensiling ($P = 0.57$) on pH (Table 3). This agrees with data presented by both Taylor and Kung (2002) and Kung et al. (2007) who reported that inoculated HMC, when compared to a control, had greater pH. Total acids decreased when HMC was inoculated ($P < 0.01$) and the HMC ensiled for 120 days had a greater amount of total acids ($P < 0.01$) compared to the HMC ensiled for 90 days. Dry matter was lower for HMC that was inoculated ($P = 0.01$). Percent dry matter lost during ensiling was less

for HMC that was inoculated ($P < 0.01$) compared to the non-inoculated sample. Corn that was ensiled for 120 days had a greater % of DM loss ($P < 0.01$) compared to HMC that was ensiled for 30 days. Contrary to the current study, Taylor and Kung (2002) reported no differences in DM recovery when comparing a control (no inoculant) to HMC inoculated with either *L. buchneri* by itself or in combination with other lactic acid bacteria and ensiled for 92 days.

Organic matter (%) was not affected by inoculation ($P = 0.55$); however, HMC ensiled for 90 days had less organic matter ($P = 0.04$) compared to HMC ensiled for 120 days. Both NDS and ADF of HMC were not affected by inoculation or time ensiled ($P \geq 0.28$) which agrees with data from Kung et al. (2007) who reported no differences in NDS and ADF content when comparing inoculated HMC to non-inoculated HMC ensiled for 90 days. Starch content was also not affected by inoculation or ensiling time and averaged 76.3% across all treatments ($P \geq 0.16$). When considering the HMC starch content from Exp. 1, starch content linearly decreased as level of inoculant increased; however, in Exp. 2 starch content of HMC was not affected by inoculant.

An inoculant by ensiling time interaction ($P < 0.01$) was observed for lactic acid concentration of HMC. Inoculated HMC in both ensiling periods had less lactic acid than the non-inoculated HMC and the 120 day non-inoculated HMC produced the most lactic acid while the 120 day inoculated HMC produced the least lactic acid. There was also an inoculant by ensiling time interaction ($P = 0.01$) for acetic acid production. Acetic acid increased when HMC was inoculated and was greatest for the HMC ensiled for 120 and inoculated followed by the 90 day inoculated HMC. The lactic:acetic acid ratio was significantly less ($P < 0.01$) for inoculated HMC compared to non-inoculated HMC.

Similar to Exp. 1, these data agree with data presented by both Taylor and Kung (2002) and Kung et al. (2007).

There was no interaction of days ensiled and inoculant ($P = 0.52$) on % DM lost during the aerobic stability test following the 90 and 120 day storage (Figure 9 and 10). The % DM loss of HMC that was inoculated was lower ($P = 0.01$) than the HMC that was not inoculated. There was no effect of days ensiled ($P = 0.46$) on % DM loss of HMC during the 21 day aerobic stability test. There was an interaction ($P < 0.01$) for temperature change in HMC samples during the aerobic stability test following the 90 and 120 day storage. The inoculated HMC was 1.1°C cooler ($P < 0.01$) than the non-inoculated corn and temperature of both the inoculated and non-inoculated corn increased quadratically ($P < 0.01$) over the 21 day aerobic stability test (Figure 11). HMC inoculated and ensiled for 120 days was 3.1°C cooler ($P < 0.01$) than the non-inoculated corn and temperature of both the inoculated and non-inoculated corn quadratically increased ($P < 0.01$) over the 21 day aerobic stability test (Figure 12).

Implication

Overall, the current studies demonstrated that treating corn silage and high moisture corn with *L. buchneri* combined with other lactic acid bacteria affects the fermentation process and nutrient losses by decreasing the amount of lactic acid production and increasing the amount of acetic acid production. Ensiling time and level of inoculant applied both play a role in the proportion of lactic acid and acetic acid produced, total acids and pH. The increase in acetic acid in the later stages of ensiling

could be partially responsible for the increased aerobic stability observed when using *L. buchneri*.

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Table 1. Effect of *L. buchneri* and *Lactobacillus plantarum* (BONSILAGE CORN 200G; Schaumann Inc.) on nutrient recovery of corn silage after ensiling for 30 or 90 days (Exp. 1)

Item							SEM	<i>P</i> – Values ²				
	30 Days			90 Days				Days	CFU		Interaction	
	0 ¹	200,000 ¹	400,000 ¹	0 ¹	200,000 ¹	400,000 ¹			L	Q	L	Q
Total Acids, % DM	7.12	6.92	6.93	7.6	7.87	7.26	0.24	<0.01	0.07	0.46	0.95	0.06
pH	3.45	3.93	3.95	3.95	3.93	4.03	0.09	<0.01	0.05	0.60	0.20	0.21
DM, %	31.9	32.8	32.0	32.1	31.5	32.0	0.08	0.25	0.81	0.24	0.90	0.28
OM, % DM	94.4	94.6	94.2	94.4	94.0	94.2	0.16	0.28	0.55	0.72	0.56	0.05
TDN	70.8	70.8	70.5	69.5	69.8	69.8	0.39	0.01	1.0	0.72	0.66	0.38
NDS	38.83	36.83	37.33	41.9	40.6	39.75	1.40	0.02	0.07	0.34	0.83	0.45
ADF	26.70	24.08	24.90	25.88	25.1	24.85	1.32	0.57	0.16	0.29	0.50	0.39
Starch, % DM	33.73	34.33	36.60	32.20	33.08	35.70	1.35	0.07	0.02	0.60	0.90	0.83
Sugars, % DM	2.08	1.95	1.88	2.40	2.00	1.90	0.17	0.32	0.12	0.66	0.39	0.67
CP, % DM	9.15	9.15	9.28	9.1	9.25	9.18	0.10	0.85	0.35	0.79	0.81	0.34
Yeast, CFU/g	0	0	0	0	0	0	--	--	--	--	--	--
Mold, CFU/g	0	0	0	0	0	50	48391	<0.01	0.27	0.51	0.25	0.51
NFC, % DM	45.15	45.3	44.38	40.13	41.65	41.98	1.12	<0.01	0.64	0.57	0.97	0.58
WSC, % DM	2.08	1.95	1.90	2.40	2.00	1.90	0.19	0.43	0.09	0.57	0.48	0.67
Lactic Acid, % DM	5.39	5.26	4.83	5.36	5.06	4.03	0.22	0.08	<0.01	0.19	0.14	0.75
Acetic Acid, % DM	1.72	1.66	2.09	2.01	2.76	3.19	0.18	<0.01	<0.01	0.78	0.15	0.40
Lactic:Acetic, % DM	3.15	3.22	2.34	2.68	1.94	1.27	0.19	<0.01	<0.01	0.20	0.11	0.33
Propionic Acid, % DM	0.005	0	0.0075	0.005	0	0.038	0.007	0.10	0.02	0.04	0.63	0.15
Butyric Acid, % DM	0	0	0	0	0	0	-	-	-	-	-	-
Iso-Butyric Acid, % DM	0	0	0	0	0	0	-	-	-	-	-	-
Ammonia, % DM	0.33	0.31	0.30	0.39	0.39	0.41	0.02	<0.01	0.82	0.65	0.93	0.75
Ammonia-N, % DM	3.5	3.25	3.25	4.25	4.25	4.5	0.31	<0.01	1.0	0.65	1.0	0.49

¹Level of inoculant applied to sample CFU/g; CFU = colony forming unit

²*P* – values were considered significant $\alpha \leq 0.05$; Days represents the effect of ensiling time on corn silage; CFU represents the linear or quadratic effect of inoculant level on corn silage; Interaction represents the linear or quadratic interaction of days and CFU on corn silage

Table 2. Effect of *L. buchneri*, *Pediococcus acidilactici*, and *Lactobacillus plantarum* (BONSILAGE HMC 200G; Schaumann Inc.) on nutrient recovery of HMC after ensiling for 30 or 90 days (Exp. 1)

Item							SEM	<i>P</i> – Values ²				
	30 Days			90 Days				Days	CFU		Interaction	
	0 ¹	300,000 ¹	600,000 ¹	0 ¹	300,000 ¹	600,000 ¹			L	Q	L	Q
Total Acids, % DM	1.52	1.43	1.45	1.76	1.64	1.55	0.06	<0.01	0.05	0.58	0.71	0.29
pH	4.13	4.17	4.18	4.08	4.23	4.35	0.07	0.52	0.03	0.81	0.09	0.97
Dry Matter, %	68.4	68.3	68.7	68.9	68.7	68.5	0.18	0.35	0.85	0.42	0.06	0.59
Organic Matter, % DM	98.6	98.5	98.6	98.6	98.6	98.7	0.04	0.52	0.63	0.05	0.65	0.27
TDN	89.0	89.0	89.0	89.0	89.0	89.3	0.11	0.36	0.25	0.52	0.76	0.63
NDS	8.05	7.93	7.56	7.28	7.23	6.53	0.30	<0.01	0.05	0.42	0.63	0.71
ADF	2.65 ^b	3.13 ^a	2.53 ^b	3.28 ^a	2.98 ^a	2.95 ^{ab}	0.18	0.14	0.23	0.24	0.62	0.05
Starch, % DM	73.63 ^a	71.63 ^b	72.00 ^b	72.95 ^{ab}	73.30 ^a	71.93 ^b	0.66	0.02	0.04	0.77	0.63	0.07
Sugars, % DM	0.90 ^a	0.87 ^a	0.63 ^b	0.78 ^{ab}	0.85 ^a	0.73 ^b	0.12	0.03	0.21	0.34	0.07	0.73
CP, % DM	9.28	9.33	9.23	8.95	8.98	8.8	0.07	<0.01	0.19	0.19	0.95	0.39
Yeast, CFU/g	140175	660767	13230000	1062500	468200	622500	4389720	0.51	0.24	0.51	0.20	0.56
Mold, CFU/g	0	0	0	0	0	0	--	--	--	--	--	--
NFC, % DM	77.3	77.23	77.83	78.43	77.83	79.35	0.34	<0.01	0.05	0.23	0.99	0.46
WSC, % DM	0.9	0.87	0.63	0.78	0.85	0.83	0.09	0.79	0.21	0.34	0.87	0.61
Lactic Acid, % DM	1.37	1.22	1.25	1.56	1.15	0.91	0.13	0.51	0.01	0.49	0.60	0.78
Acetic Acid, % DM	0.15	0.20	0.20	0.20	0.47	0.60	0.07	<0.01	<0.01	0.44	0.28	0.65
Lactic:Acetic, DM	9.17 ^a	6.10 ^c	6.46 ^c	7.95 ^b	2.46 ^d	2.40 ^d	0.79	<0.01	<0.01	0.01	0.27	0.02
Propionic Acid, % DM	0	0	0	0	0.02	0.04	0.01	0.01	0.02	1	0.49	1
Butyric Acid, % DM	0	0	0	0	0	0	-	-	-	-	-	-
Iso-Butyric Acid, % DM	0	0	0	0	0	0	-	-	-	-	-	-
Ammonia, % DM	0.16	0.17	0.17	0.26	0.31	0.31	0.01	<0.01	0.03	0.06	0.27	0.12
Ammonia-N, % CP	2.0	2.0	2.0	3.0	3.5	3.5	0.17	<0.01	0.16	0.44	0.26	0.51

¹Level of inoculant applied to sample CFU/g; CFU = colony forming unit

³*P* – values were considered significant at ≤ 0.05 ; Days represents the effect of ensiling time on corn; CFU represents the linear or quadratic effect of inoculant level on corn; Interaction represents the linear or quadratic interaction of days and CFU on corn

Table 3. Table 3. Effect of *L. buchneri*, *Pediococcus acidilactici*, and *Lactobacillus plantarum* (BONSILAGE HMC 200G; Schaumann Inc.) on nutrient recovery of HMC after ensiling for 90 or 120 days (**Exp. 2**)

Item	90 Days		120 Days		SEM	Days	<i>P-Value</i> ²	
	0 ¹	600,000 ¹	0 ¹	600,000 ¹			CFU	Days*CFU
Total Acids, % DM	2.19	1.98	2.41	2.16	0.051	<0.01	<0.01	0.65
pH	4.00	4.23	3.93	4.28	0.021	0.57	<0.01	0.11
Dry Matter, %	68.4	67.4	67.7	67.2	0.003	0.14	0.01	0.34
DM lost during ensiling, g	60.5	39.6	77.4	63.0	4.66	<0.01	<0.01	0.50
DM lost during ensiling, %	4.49	3.15	5.98	4.93	0.003	<0.01	<0.01	0.68
Organic Matter, % DM	0.133	0.110	0.168	0.233	0.035	0.04	0.55	0.23
TDN	88.5	88.25	88.75	88.75	0.26	0.18	0.64	0.64
NDS	8.40	8.15	8.05	7.23	0.476	0.21	0.28	0.56
ADF	2.48	2.53	2.28	1.85	0.24	0.09	0.45	0.34
Starch, % DM	76.68	76.73	76.83	78.08	0.51	0.16	0.22	0.26
Sugars, % DM	1.05	1.00	0.65	1.20	0.496	0.84	0.62	0.56
CP, % DM	8.60 ^b	8.78 ^a	8.75 ^a	8.60 ^b	0.08	0.88	0.88	0.06
Yeast	1622705	9755	3191000	19265	1390796	0.58	0.11	0.59
Mold	0	0	0	7.5	3.75	0.34	0.34	0.34
NFC, % DM	77.4	77.48	77.63	78.58	0.518	0.23	0.34	0.42
WSC, % DM	0.80	0.63	0.65	0.55	0.132	0.41	0.32	0.78
Lactic Acid, % DM	1.89 ^a	0.95 ^b	2.07 ^a	0.83 ^b	0.04	0.44	<0.01	<0.01
Acetic Acid, % DM	0.29 ^c	1.03 ^b	0.32 ^c	1.33 ^a	0.045	<0.01	<0.01	0.01
Lactic:Acetic, % DM	6.92	0.93	6.97	0.62	0.75	0.87	<0.01	0.81
Propionic Acid, % DM	0	0.003	0.008	0.003	0.003	0.23	0.68	0.23
Butyric Acid, % DM	0.01	0	0.04	0	0.011	0.21	0.04	0.21
Iso-Butyric Acid, % DM	0	0	0	0	-	-	-	-
Ammonia, % DM	0.29	0.39	0.32	0.44	0.004	<0.01	<0.01	0.08
Ammonia-N, % CP	3.25	4.25	4.00	5.00	0.176	<0.01	<0.01	1.0

¹Level of inoculant applied to sample CFU/g; CFU = colony forming unit

²*P* – values were considered significant at ≤ 0.05 ; Days represented the effect of ensiling time on corn; CFU represented the effect of inoculant on corn; Days*CFU represents the interaction of ensiling time and inoculant on corn

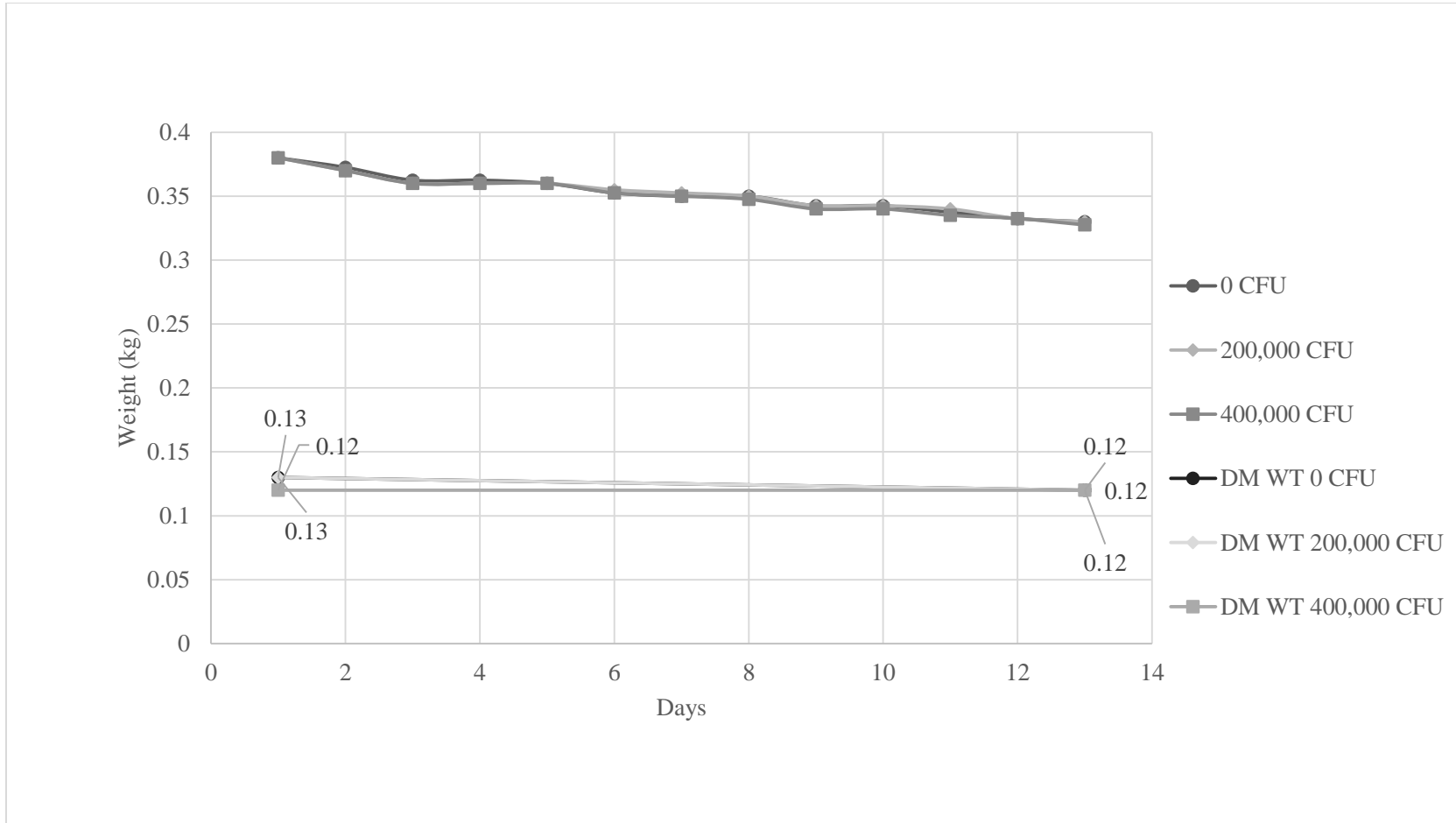


Figure 1. As is and DM weight change over a 13 day aerobic stability test of corn silage ensiled for 30 days (Exp. 1) There was no interaction of inoculant and days ensiled for silage ($P = 0.31$); level of inoculant ($P = 0.84$) and days ensiled ($P = 0.68$) did not affect % DM loss (kg) of silage

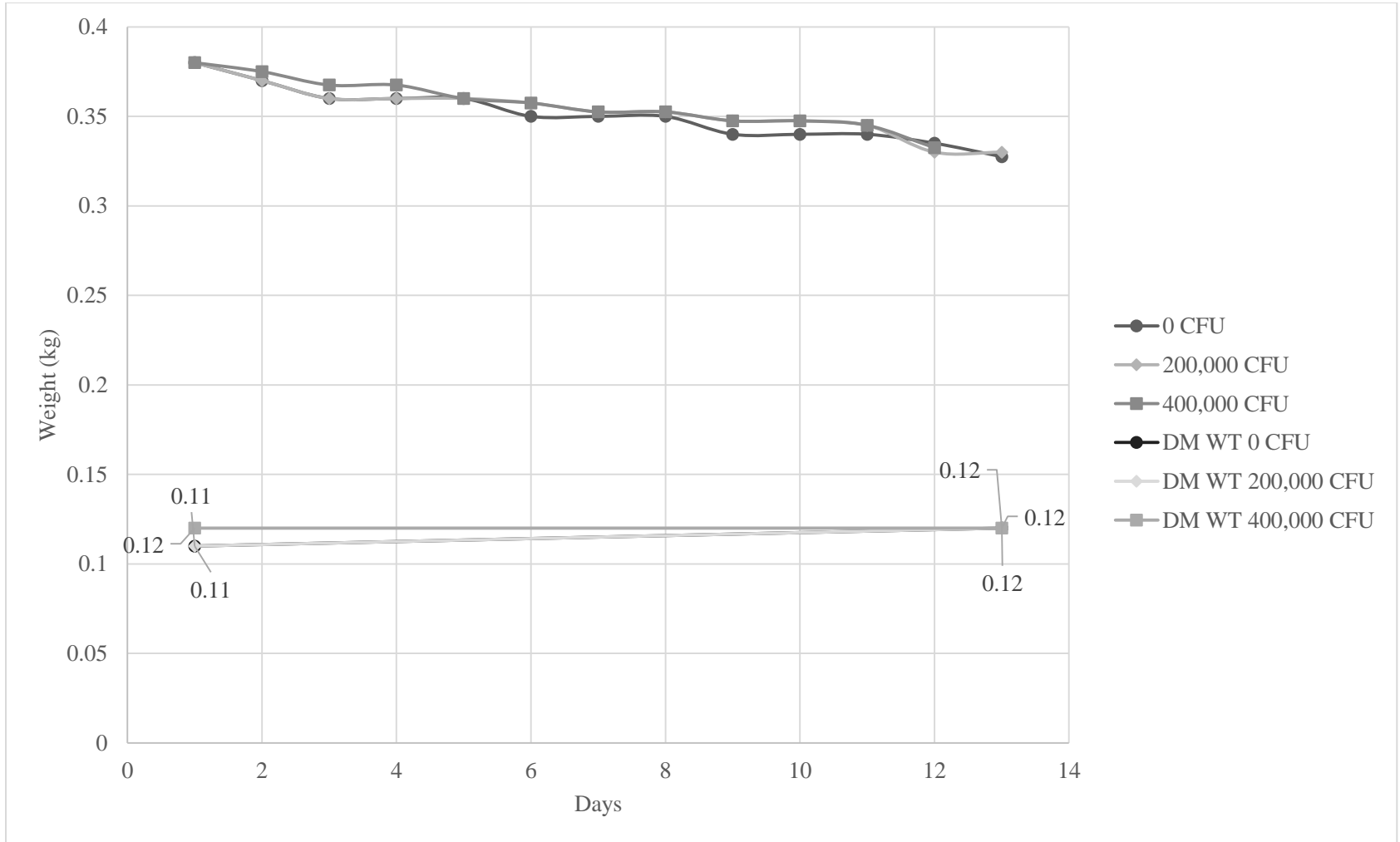


Figure 2. As is and DM weight change over a 13 day aerobic stability test of corn silage ensiled for 90 days (Exp. 1) There was no interaction of inoculant and days ensiled for silage ($P = 0.31$); level of inoculant ($P = 0.84$) and days ensiled ($P = 0.68$) did not affect % DM loss (kg) of silage

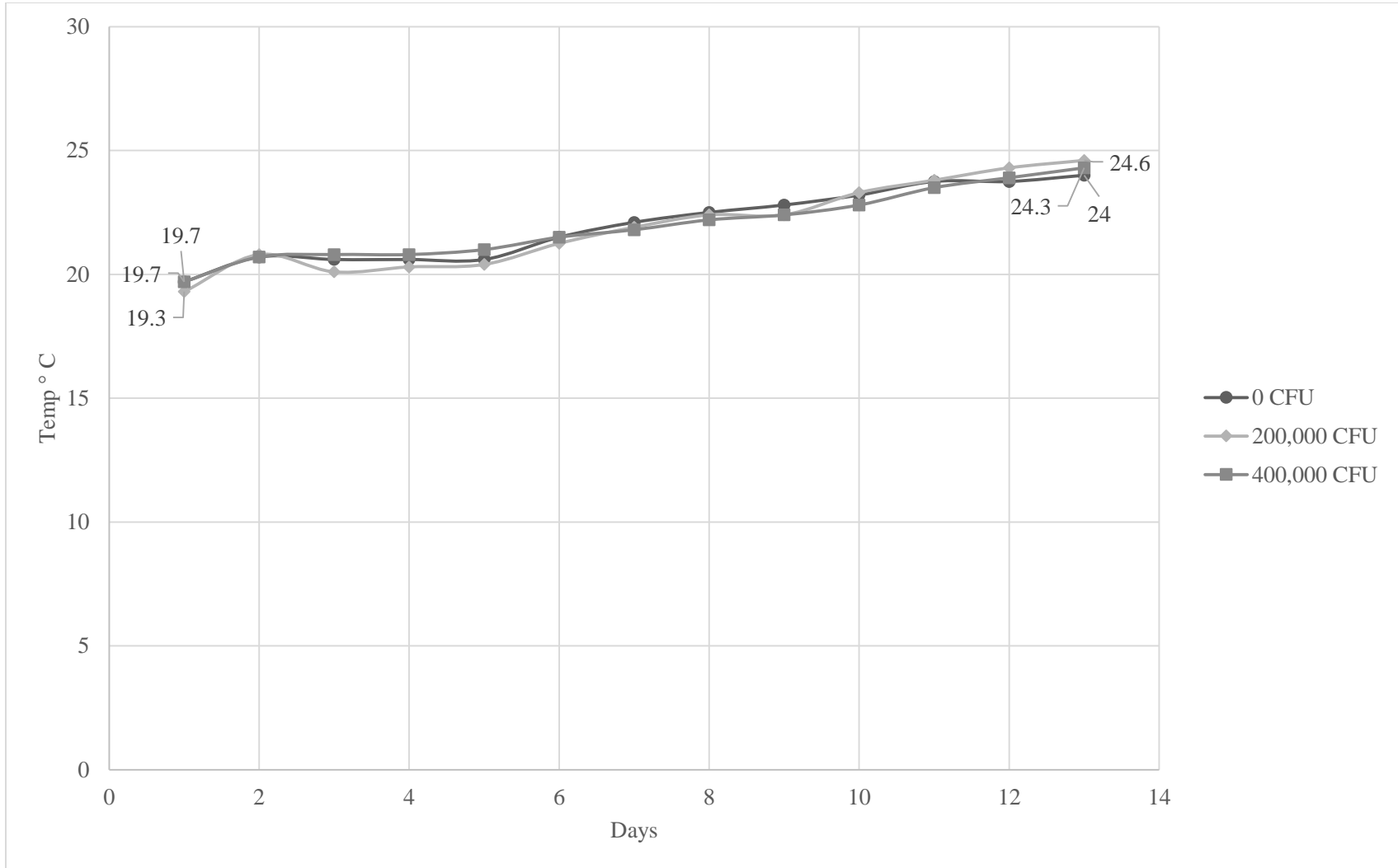


Figure 3. Temperature change over a 13 day aerobic stability test of corn silage ensiled for 30 days (Exp. 1) There was no interaction ($P = 0.12$) for temperature change in silage samples collected on day 30. Temperature was not different between the three inoculant levels ($P = 0.83$) and increased quadratically ($P < 0.01$) over the 13 day aerobic stability test

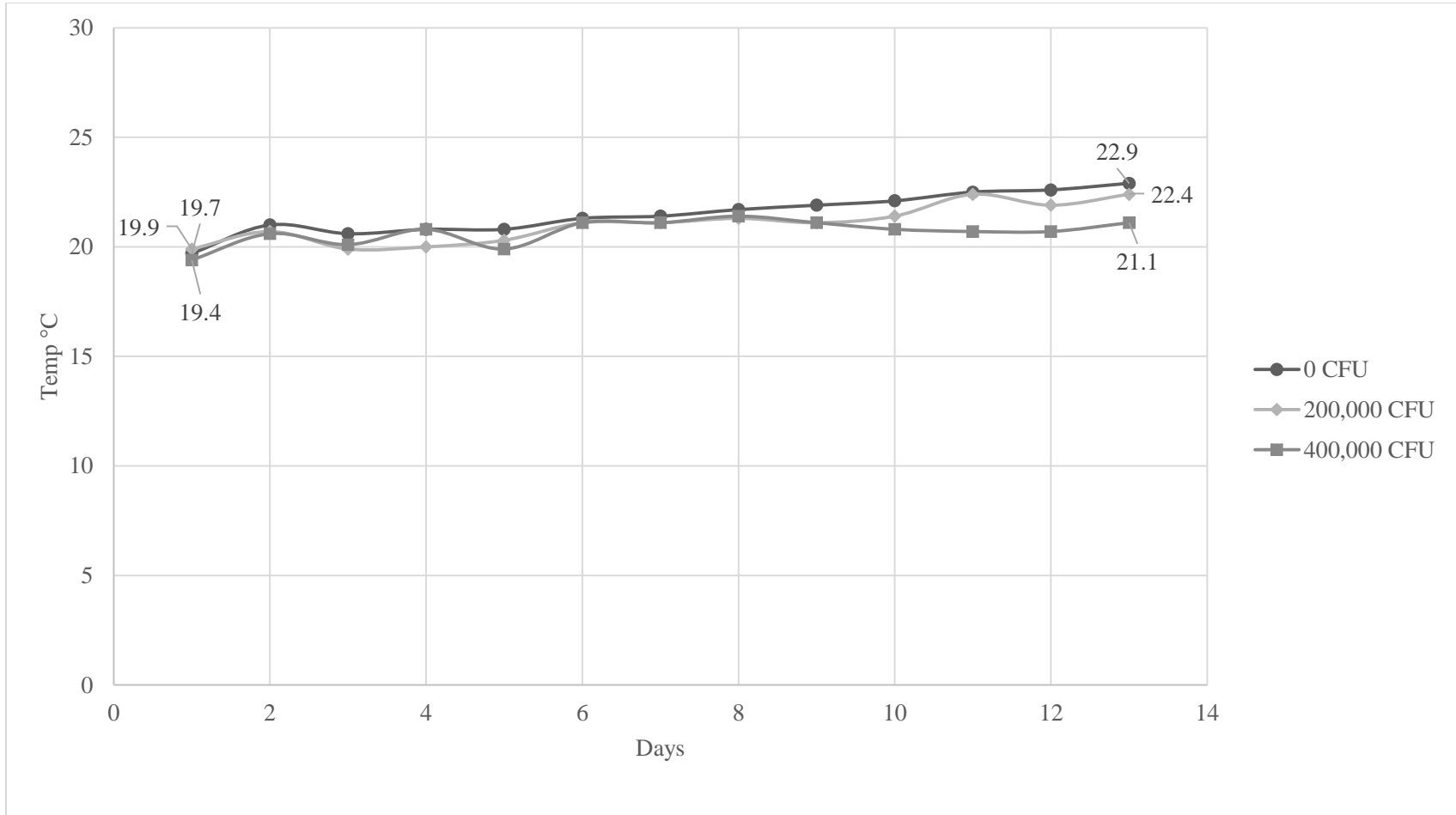


Figure 4. Temperature change over a 13 day aerobic stability test of corn silage ensiled for 90 days (Exp. 1) There was an interaction ($P < 0.01$) for temperature of corn silage ensiled for 90 days. Silage inoculated with 400,000 CFU/g was 1.8°C cooler ($P <$

0.01) than the non-inoculated treatment and temperature of all three treatments increased quadratically ($P < 0.01$) over the 13 day aerobic stability test

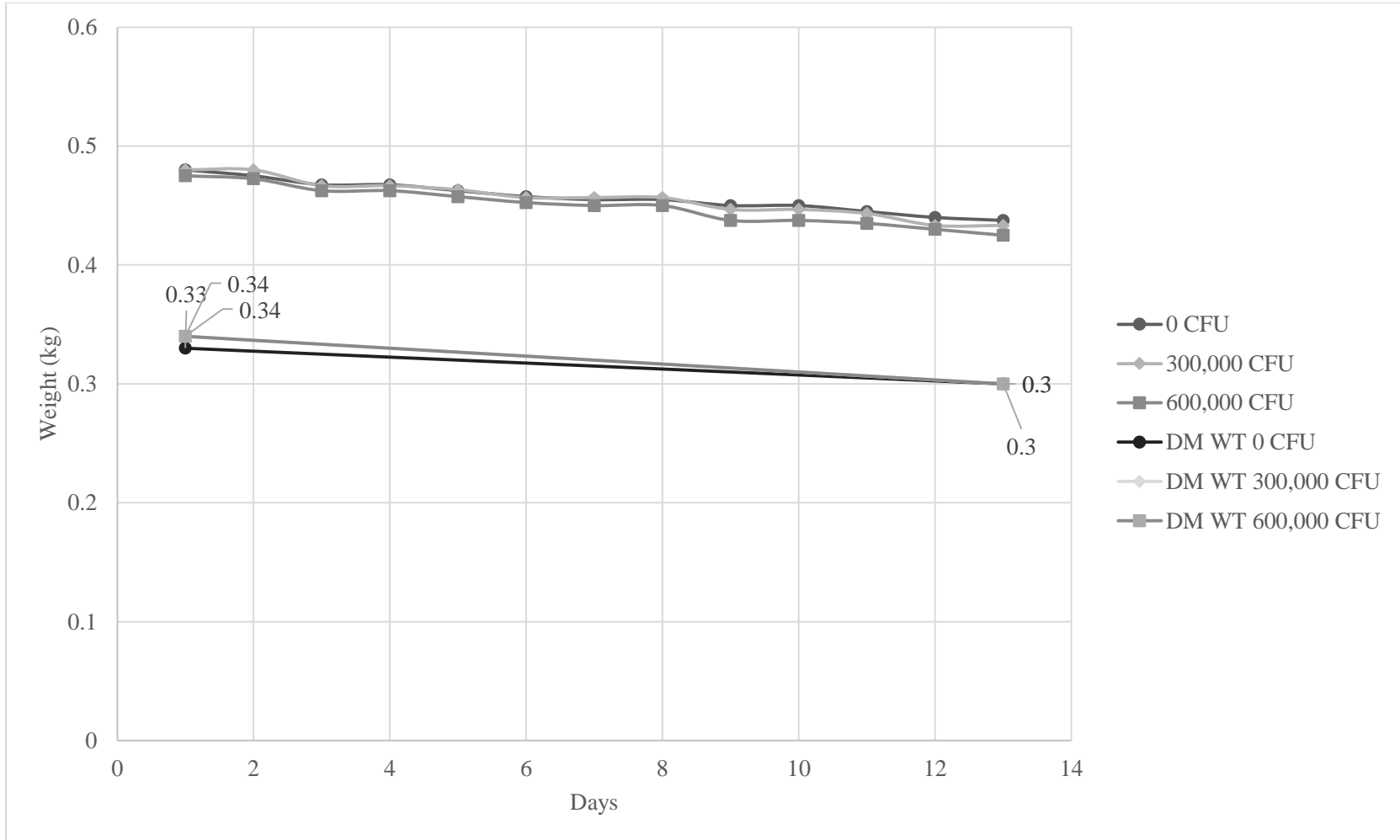


Figure 5. As is and DM weight change over a 13 day aerobic stability test of HMC ensiled for 30 days (Exp. 1) There was an interaction of days ensiled and inoculant ($P < 0.01$) on % DM lost. The HMC stored for 30 days % DM lost increased with inoculant level and the % DM lost in corn stored for 90 days decreased as level of inoculant increased.

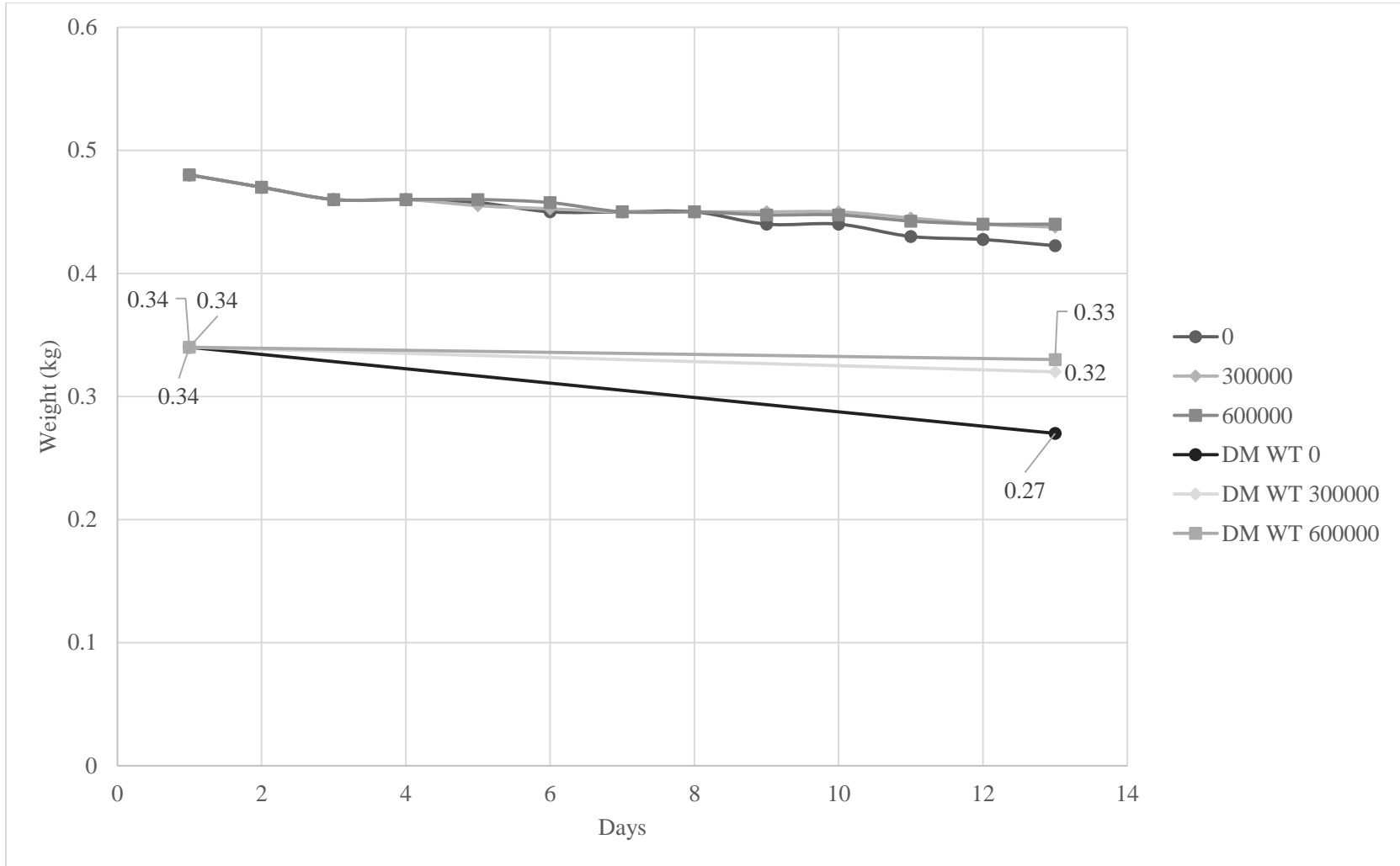


Figure 6. As is and DM weight change over a 13 day aerobic stability test of HMC ensiled for 90 days (Exp. 1) There was an interaction of days ensiled and inoculant ($P < 0.01$) on % DM lost. The HMC stored for 30 days % DM lost increased with inoculant level and the % DM lost in corn stored for 90 days decreased as level of inoculant increased.

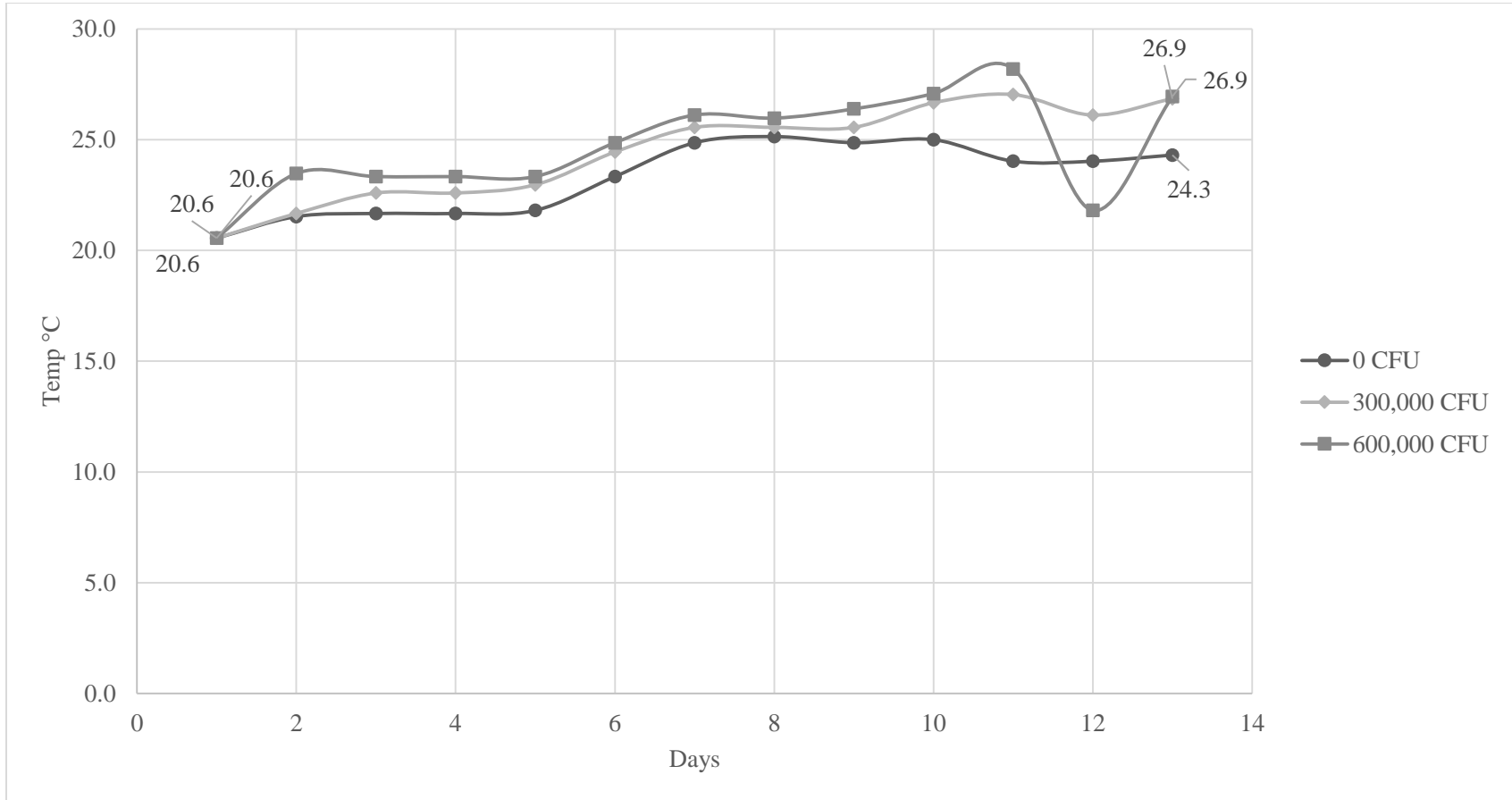


Figure 7. Temperature change over a 13 day aerobic stability test of HMC ensiled for 30 days (Exp. 1) There was an interaction ($P < 0.01$) for temperature change in HMC. Non-inoculated corn had a lower temperature ($P < 0.01$) compared to the inoculated corn and temperature of all three treatments quadratically increased ($P < 0.01$) over the 13 day aerobic stability test.

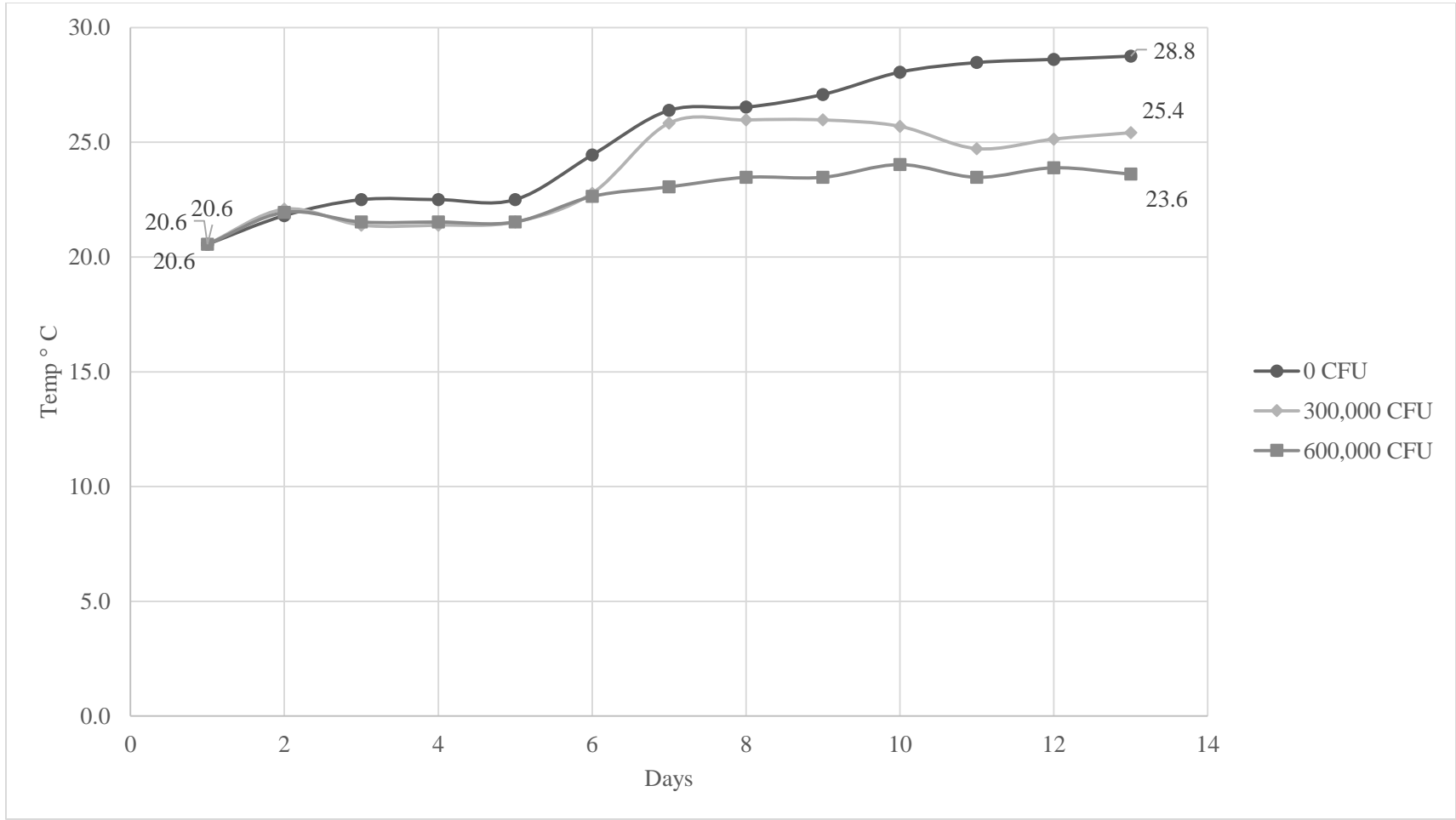


Figure 8. Temperature change over a 13 day aerobic stability test of HMC ensiled for 90 days (Exp. 1) There was an interaction ($P < 0.01$) for temperature of HMC. Corn inoculated at 600,000 CFU/g was 1.8°C cooler than corn inoculated at 300,000 CFU/g and 5.2°C cooler than the non-inoculated corn.

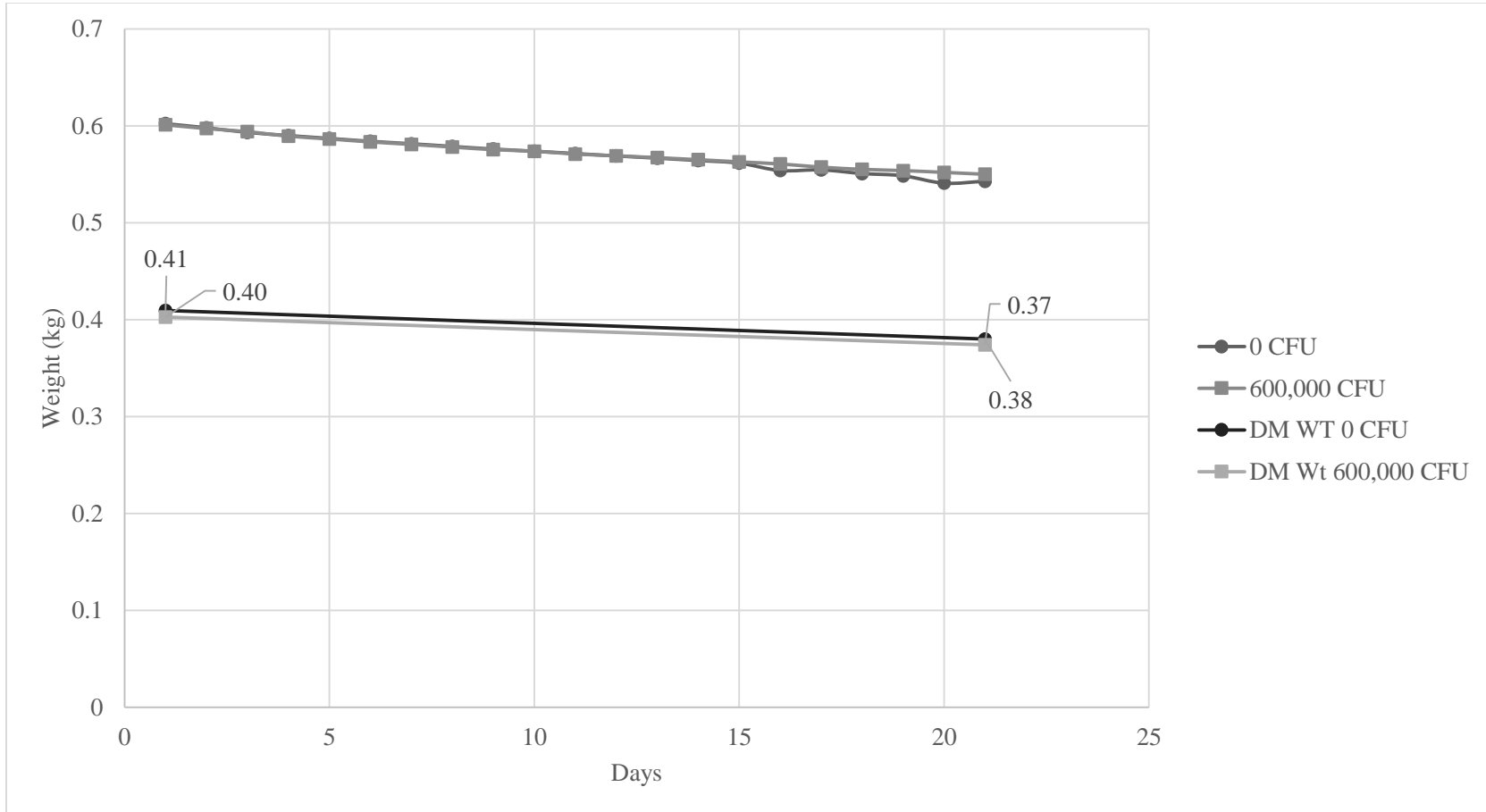


Figure 9. As is and DM weight change over a 21 day aerobic stability test of HMC ensiled for 90 days (Exp. 2) There was no interaction of days ensiled and inoculant ($P = 0.52$) on % DM lost during the aerobic stability test following the 90 and 120 day

storage. The % DM loss of HMC that was inoculated was lower ($P = 0.01$) than the HMC that was not inoculated. There was no effect of days ensiled ($P = 0.46$) on % DM loss of HMC during the 21 day aerobic stability test.

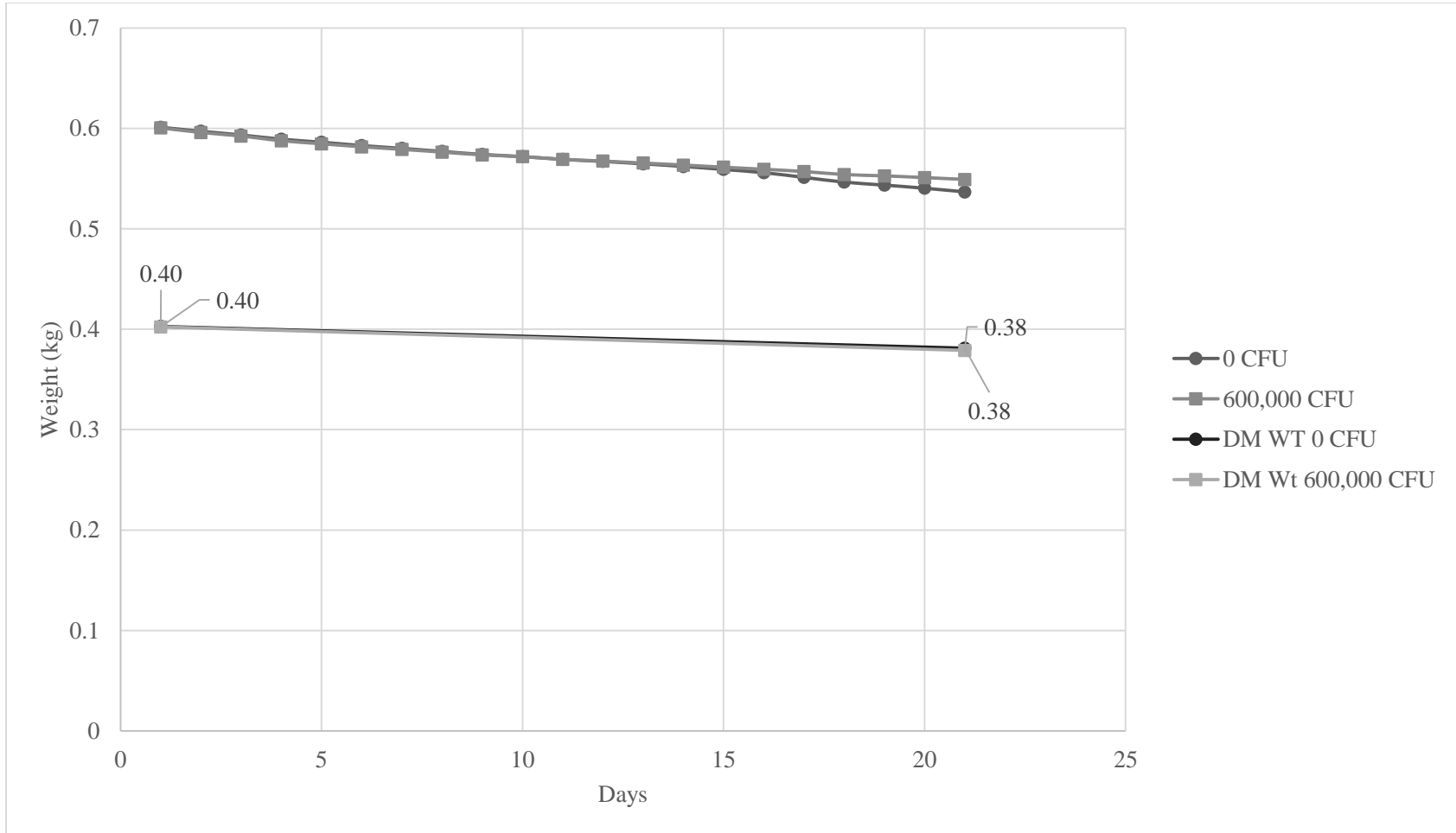


Figure 10. As is and DM weight change over a 21 day aerobic stability test of HMC ensiled for 120 days (Exp. 2) There was no interaction of days ensiled and inoculant ($P = 0.52$) on % DM lost during the aerobic stability test following the 90 and 120 day

storage. The % DM loss of HMC that was inoculated was lower ($P = 0.01$) than the HMC that was not inoculated. There was no effect of days ensiled ($P = 0.46$) on % DM loss of HMC during the 21 day aerobic stability test.

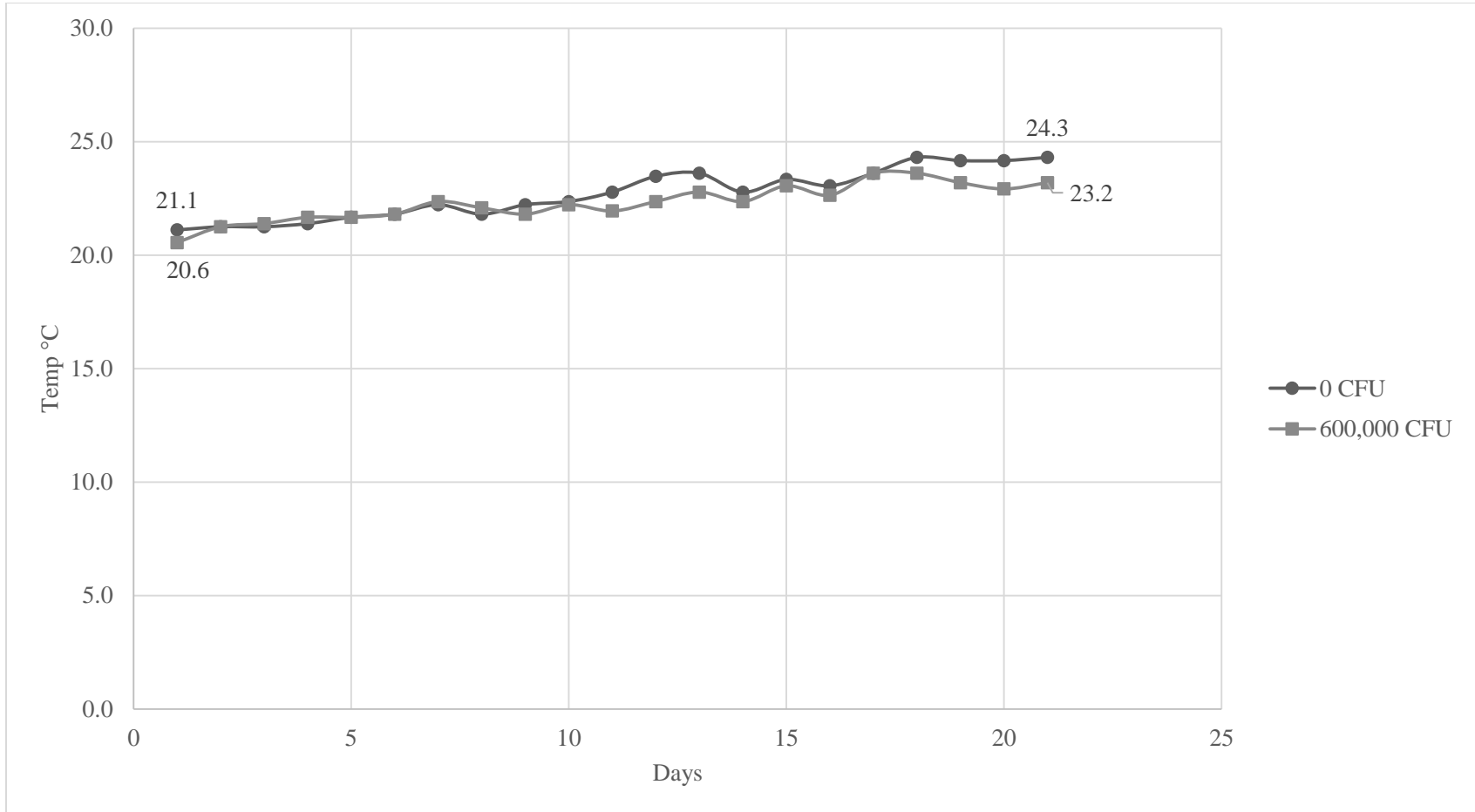


Figure 11. Temperature change over a 21 day aerobic stability test of HMC ensiled for 120 days (Exp. 2) There was an interaction ($P < 0.01$) for temperature change in HMC. The inoculated HMC was 1.1°C cooler ($P < 0.01$) than the non-inoculated corn and temperature of both the inoculated and non-inoculated corn increased quadratically ($P < 0.01$) over the 21 day aerobic stability test

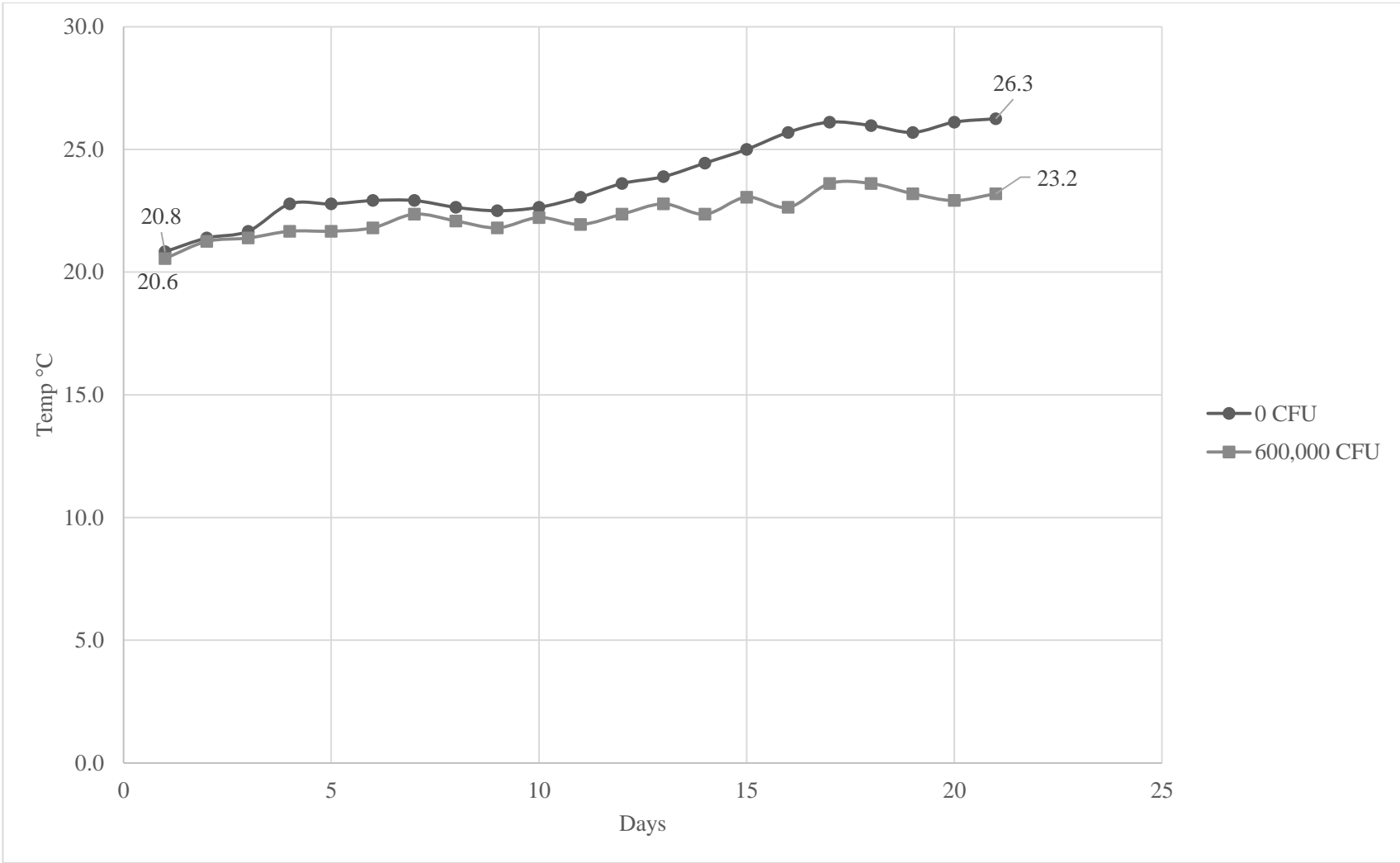


Figure 12. Temperature change over a 21 day aerobic stability test of HMC ensiled for 120 days (Exp. 2) There was an interaction ($P < 0.01$) for temperature change in HMC. Corn was 3.1°C cooler ($P < 0.01$) than the non-inoculated corn and temperature of both the inoculated and non-inoculated corn quadratically increased ($P < 0.01$) over the 21 day aerobic stability test.