EFFECTS OF FEEDING NUCLEOTIDES WITH CORN GERM MEAL OR DRIED CORN DISTILLERS GRAINS ON RECEIVING AND GROWING CALVES

by

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Abstract

Effects of nucleotides (NA) (PSB Complex; DSS Global, Chicago, IL) with corn germ meal (CGM) or dried corn distillers grains (DDG) on growth performance, digestibility, *in vitro* ruminal gas production, and mucosal immunity were analyzed in 4 experiments. In Exp. 1, 213 crossbred heifers (BW= 262 ± 67.4 kg) were used in a complete block design with a 3 x 2 factorial arrangement of treatments to determine the net energy values of CGM in comparison to DDG and the effects of NA at three inclusion levels (0, 2, and 4 g) during an 84-d receiving period. Pens were randomly assigned to one of six treatments: 1) CGM with no NA (CGM0), 2) CGM with 2 g/heifer daily NA (CGM2), 3) CGM with 4 g/heifer daily NA (CGM4), 4) DDG with no NA (DDG0), 5) DDG with 2 g/heifer daily NA (DDG2), and 6) DDG with 4 g/heifer daily NA (DDG4). There were no significant effects of NA or the type of corn byproduct on growth performance ($P \geq 0.15$). Exp. 2, was conducted to determine the performance and mucosal immunity effects of NA using 240 crossbred heifers (BW= 268 ± 34.1 kg). Pens were randomly assigned to three treatments which consisted of diets 4, 5 and 6 from Exp. 1. Calves were blocked by weight and assigned to a pen for 56-d. There were no significant effects of NA on growth performance results ($P \geq 0.18$). On d 28, fecal samples were collected from approximately 5 calves from each pen and analyzed for secretory IgA concentration. NA inclusion did not affect fecal IgA concentration ($P = 0.15$). Exp. 3, utilized 4 ruminally cannulated Holstein heifers in a 4 x 4 Latin square design. The four treatments included diets 1 and 4 from Exp. 1 along with those two diets supplemented with 3 g/heifer daily NA. Ruminal pH increased as NA was included ($P < 0.05$). Ammonia concentrations were greater for DDG than for CGM ($P < 0.01$). Ruminal propionate concentration was less in diets that contained NA ($P < 0.05$). DDG diets led to greater concentrations of butyrate, isobutyrate, isovalerate, and

valerate in ruminal fluid than CGM diets ($P < 0.01$). Valerate concentrations were decreased by NA when included in DDG diets, but not when added to CGM diets (interaction, $P < 0.01$). Isovalerate concentrations were increased by NA when included in CGM diets, but not when added to DDG diets (interaction, $P = 0.01$). An *in vitro* study, Exp. 4, evaluated 24-h gas production effects of the 6 treatments in Exp. 1. Gas production was decreased linearly by the inclusion of NA in DDG diets, but it was unaffected by NA in CGM diets (interaction, $P < 0.01$). CGM can be included in receiving and growing diets at 24.5% on a DM basis in place of DDG while maintaining growth performance, digestibility, and gas production. There was no effect of NA on growth performance, digestibility, or mucosal immunity, but there was an effect on ruminal gas production and ruminal parameters. Further research is needed to determine the effects of NA on receiving and growing cattle.

Key words: receiving cattle, corn germ meal, nucleotide

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Chapter 1 - Review of Literature

Introduction

Minimizing cattle health issues and attaining desired performance targets during the receiving and growing phase are constant challenges of the United States cattle feeding industry. During the receiving and growing phase, calves typically are recently weaned and experience various physical and psychological stressors which can create health issues and depress feed intake (Galyean and Hubbert, 1995), generating a challenge for the cattle industry. To reduce stress and thereby maximize the outcome of newly arrived calves, proper receiving management is critical. Feed intake typically is low in stressed, newly received calves (Lofgreen, 1983; Lofgreen, 1988; Hutcheson and Cole, 1986; Galyean and Hubbert, 1995). To encourage feed intake, formulating a diet that is palatable and meets nutritional requirements, while being economically feasible is imperative. Byproducts are commonly used as an ingredient in growing and receiving rations because of their availability, nutrient value, and cost (Leupp, 2008). Feed additives containing antibiotics, coccidiostats, and ionophores have been developed to minimize the effects of stress and enhance calf health, thereby increasing growth performance. Ingredient selection when formulating a diet for receiving and growing calves is a critical first step towards proper receiving period nutrition management.

Status of Receiving and Growing Calves

Upon weaning, a calf can experience many different outcomes. Most calves go through some sort of post weaning program which varies widely in growing structure and type (Peel, 2003). The weaning program can be termed as a stocker/backgrounding operation that is located

at the original birthplace of the calf or at a separate entity for a period of time before entering the finishing stage of the calf's life. The program can also be termed as the receiving stage in which the calf is shipped after weaning to the finishing phase. The overall goal of the receiving and growing stage of the calf's life is to maintain health, improve nutrition, and increase body weight in preparation for the finishing stage where the animal's performance can be optimized.

Stress, whether it be psychological (restraint, handling, or novelty) or physical stress (hunger, thirst, fatigue, injury, or temperature extremes) is often subjected to the newly weaned calf as it is entering a feedlot environment (Grandin, 1997). Loerch and Fluharty (1999) suggested that the greatest stress imposed by marketing calves is the weaning period. This is a big change for the calf, as it is denied its dam's milk and social contact with its dam and other cattle (Stookey et al., 1997). After weaning, the calf is marketed, transported, and comingled with other calves to then proceed to the feedlot. Calves are often commingled during this time with calves of different backgrounds, and even more so, calves with different immunological statuses. Factors that may contribute to stress during the period of transportation includes feed and water deprivation, overcrowding, poor air quality, poor sanitation, and unexpected noise (Loerch and Fluharty, 1999). Once the animal arrives at the feedlot, it is then subjected to processing, further commingling, a new environment, and potentially, a new feed. Processing (dehorning, castration, vaccination, etc.) is an obvious physical stress that may overwhelm an animal, followed by the stress of commingling (Loerch and Fluharty, 1999). During this time, the animal's social rank within a pen is tested and established, adding further stress to the animal (Grandin, 1997). Loerch and Fluharty (1999) defined feedlot environment stress as acclimating to mud, manure, poor air quality, and exposure to a new social dominance order and new pathogens. The newly received calf if recently weaned is accustomed to the dam's milk, not the

typical mixed diet presented to the animal upon arrival at the feedlot. This can cause stress, as well as have an impact upon digestibility of a new diet.

The multitude of stressors inflicted upon the animal at weaning, marketing, transportation, commingling, and the arrival at the feedlot has an impact on the health status of the animal. Stress negatively affects the immune system at a time when the animal is more likely to be exposed to infectious agents as a result of commingling (Blecha et al., 1984). The most common infectious agent available to receiving calves is bovine respiratory disease (BRD), a viral/bacterial disease that causes morbidity and mortality, and continues to be the most significant health problem facing the U.S. beef cattle industry (Duff and Galyean, 2007). In a survey conducted by Loneragan et al. (2001a), from 1994 to 1999 and averaged over time, the mortality ratio was 12.6 deaths for every 1,000 calves entering the feedlot. Of those deaths, 57.1% were attributed to a respiratory tract infection (Loneragan et al., 2001a).

The causative agents of BRD are a combination of bacterial and viral pathogens. The bacterial agents include *Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni* (Duff and Galyean, 2007) with *Mannheimia haemolytica* being the most common organism associated with the disease (Pandher et al., 1998). In combination with these bacterial pathogens are viral agents. These are identified as infectious bovine rhinotraceitis (IBR), parainfluenza-3 (PI3), bovine viral diarrhea virus (BVDV), and bovine enteric coronavirus (Plummer et al., 2004). Of the viral pathogens, BVDV is often focused on; BVDV can be transmitted either horizontally (postnatal transmission) or vertically (fetal infection). A calf can become persistently infected (PI) via fetal infection, and continues to shed the virus over its lifetime (McClurkin et al., 1984). PI animals offer a threat of transmission to healthy calves free of the

infection (Duff and Galyean, 2007), and when paired with stress and commingling, transmission is a likely outcome.

During the first 27 days of the receiving period, some animals might be removed from their pens for examination and potential treatment of BRD (Buhman et al., 2000). Correct diagnosis of these animals is critical during this time that could harm or help the animal's health status. Calves suffering from BRD display symptoms that include nasal or ocular discharge, depression, lethargy, emaciated body condition, labored breathing, or any combination of these. Usually calves are considered morbid when rectal temperature reaches ≥ 39.7˚C (Duff and Galyean, 2007). Observation of animals by humans is the primary method of BRD recognition (Hanzlicek, 2010). Because of the subjective nature of observation, and the observational skill and experience variability between observers, diagnosis is not always accurate (Duff and Galyean, 2007). Previous research has confirmed the lack of sensitivity of observational methods. In one study, 5,976 calves in a Midwestern feedlot revealed a BRD morbidity incidence of 8.17%, but at harvest 61.9% of the animals had lung lesions, suggesting a previous BRD challenge (Schneider et al., 2009). This proves that daily animal checks and accurate diagnosis is vital to ensure a healthy population and reduce chronicity. If an animal is properly diagnosed and treated, there is a lesser risk of having negative effects on subsequent performance and health.

Stresses associated with weaning, marketing, and transport of beef cattle have marked effects on health, but effects on feed intake are also important (Galyean and Hubbert, 1995). Calves that arrive at the feedlot may be unaccustomed to waterers and feed bunks due to prior environment surroundings. If calves are unaccustomed to new methods of feed and water provision, feed intake can be jeopardized. Adequate feed intake is important because it provides

essential nutrients to the animal that have an impact on health and growth performance. Typically, feed intake is low in stressed, newly received calves as shown by Hutcheson and Cole (1986) in a study comparing feed intake of healthy and morbid calves. For healthy calves, during the first 7 days of the arrival period, feed intake averaged 1.55% of BW daily, and when averaged for the first 28 days, intake was 2.71% of BW daily. Feed intake was even lower for morbid calves with an average of 0.9% of BW daily for the first 7 days after arrival and averaged 1.84% of BW daily for the first 28 days. Within the stress of the receiving and arriving process, several factors are involved in the ability of newly weaned calves to adapt to their new diet (Fluharty, 2003). Calves that are transported by truck undergo periods of feed and water deprivation that can alter rumen environment and function (Loerch and Fluharty, 1999), which in turn can contribute to low feed intake. The rumen status of the newly arrived calf has been an ongoing subject of research. Baldwin (1967) published that the total number of bacteria in the rumen is reduced by 10 to 25% of normal after a 48-h period of feed and water deprivation. The total number of bacteria in the rumen was based on *in vitro* experiments calculating rumen fermentative capacity and rumen fermentative activity. Twenty-seven years later, Fluharty et al. (1994) conducted a more applicable study that contradicted Baldwin (1967). Newly weaned, fistulated steers were used to determine the effects of energy density and protein source in receiving diets on *in situ* dry matter (DM) disappearance, neutral detergent fiber (NDF) and nitrogen disappearance, concentrations of ruminal bacteria, protozoa, ammonia and pH. Fluharty et al. (1994) suggested that the viable total and cellulolytic ruminal bacteria concentrations are not drastically reduced by weaning and 24-h stresses and, furthermore, that the ruminal microbial population is able to effectively digest available substrate following feed and water deprivation. Fluharty et al. (1996) subsequently studied the effects of the duration of feed and water

deprivation on ruminal microbes and ruminal characteristics of newly weaned and feedlot-adapted calves. Conclusions from this study indicate that ruminal volume, DM, total weight of ruminal contents, and protozoal numbers decrease as duration of the fasting period increases, and this decrease is related to a reduction in DM intake (DMI). Prior research shows that poor performance and low DMI of newly arrived feedlot calves are not a result of reduced ruminal bacterial numbers and digestive capacity (Loerch and Fluharty, 1999).

Management personnel have the ability to implement several strategies that can positively affect a newly arrived calf's feed intake to ensure adequate nutrient uptake. Selecting preconditioned calves that have been offered creep fed, exposed to a feedbunk, and/or preweaned (Loerch and Fluharty, 1999) can have a positive impact on an animal's performance including increased feed intakes. Another strategy to mitigate low feed intakes is to reduce stress-associated behavior (Loerch and Fluharty, 1999). How an animal is handled early in life will have an effect on its physiological response to stressor later in life (Grandin, 1997). Also, how an animal is handled when loading, unloading, sorting, and processing can be correlated with feed intake. The goal of increased feed intake is to increase nutrient intake respectively. A management strategy that can be implemented to increase nutrient intake is to increase the nutrient density of the diet to offset the low feed intakes of newly arrived cattle (Loerch and Fluharty, 1999). The series of events a newly received calf undergoes, and thereby the effects, emphasize the importance of a well-balanced receiving and growing diet which presents a solid starting place for calves that are transitioning from the weaning to the growing phase.

Role of Energy

Growing calves have specific energy needs to perform biochemical, physiological, and nutritional processes that account for their maintenance and growth. As the high stressed calf first enters the feedlot environment, feed intake is negatively affected for the first few days to weeks after arrival (Lofgreen, 1988). As feed intake is negatively affected, components of the diet, including energy, are compromised and later can lead to health issues. One strategy to increase energy intake that has been previously evaluated to improve calf health is to add artificial sweeteners or other flavoring agents (Rivera et al., 2004). The most commonly used approach is to increase the energy concentration in the diet by increasing the level of concentrate in respect to roughages (Rivera et al., 2005). Unstressed cattle have opposite feeding behaviors compared with newly arrived stressed cattle (Lofgreen, 1983). Typically, unstressed calves will consume enough feed to properly maintain their energy requirements, and to fulfill their other intake requirements, they dilute the diet by ingesting lower energy dietary ingredients. In contrast, stressed calves consume less low energy ingredients (roughages) and more high energy ingredients without any regards to energy requirements (Lofgreen, 1983).

Although it seems that stressed calves have a higher feed intake on high-energy diets, increasing energy levels in the receiving diets usually results in higher morbidity but better performance (Lofgreen, 1983). Lofgreen et al. (1980) fed flaked-milo at 25, 50, and 75% of the diet and found that with increasing levels of concentrate there was an increase of morbidity. The percent of calves treated for BRD was 47, 49, and 57% respectively. The following year, Lofgreen et al. (1981) compared millet hay alone with millet hay plus 75% concentrate milled feed and found that animals on hay alone tended to have fewer sick days although they had lower gains. Fluharty et al. (1996) compared 70, 75, 80 and 85% concentrate diets in their first

experiment and found that DMI increased as percent concentrate increased. In contrast to Lofgreen et al. (1980), they did not report negative effects of concentrate level on morbidity of newly received steer calves. Energy concentrations did not influence performance or overall morbidity in a study by Berry et al. (2004) where differing dietary energy and starch concentrations were evaluated for effects on performance and health of newly received feedlot calves in a 42-d receiving period. These authors noted that cattle fed high energy diets had a lesser incidence of shedding *Pasteurella multiocida* and *Histophilus somni* pathogens in calves that received one or more antimicrobial treatments. The results between the three studies mentioned are quite different. Differences between the three can be attributed to source of cattle, time of year, nature of the diet, management practices, and other unknown factors that could likely confound the relationship between concentrate level and BRD morbidity (Galyean et al., 1999). Whether the effects of immunity are associated with energy is still open to question, but adequate energy intake and body energy stores are important for all bodily functions (Duff and Galyean, 2007).

Role of Protein

Protein requirements for beef cattle are represented in grams per day and are based on weight and production targets. The amounts required per day are then presented as a percentage of DMI. Oftentimes, for newly received calves, DMI is low during the initial weeks, as previously discussed. To offset the low DMI, higher concentrations of dietary crude protein (CP) are required (Fluharty and Loerch, 1995). Eck et al. (1988) reported that incoming steers should receive a 12.5% CP diet, however, even a 12.5% CP receiving diet might not meet the CP requirements if DMI is low. Cole and Hutcheson (1990) pointed out that it is nearly impossible

to formulate a diet that will enable cattle to gain weight when they are consuming 1% or less of their BW daily. They calculated that the required CP percentage would range from 21% at an intake of 1% of BW daily to approximately 10% at an intake of 3% of BW daily. This presents the challenge of how much protein to formulate for based on the variation of feed intake.

Galyean et al. (1993) conducted an experiment to determine the effects of different CP concentrations in the receiving diet on health and performance of newly received calves. Calves had been in transit for 19.5 h and were assigned to one of three CP concentrations (12, 14, or 16%) for 42 days. Average daily gain and daily DMI increased with increasing levels of CP. However, more calves were treated for symptoms of BRD on the 16% CP diet and the 12% CP diet than on the 14% CP diet. To determine if receiving diet CP concentration would affect subsequent performance, calves were held in respective pens for 42 days (post receiving phase) following the previous 42 day receiving period and were fed a common 14% CP diet. Concentrate level of the 14% CP diet was 75% for the first week, after which calves were stepped up to an 85% concentrate diet. Results of the post-receiving period indicated that calves fed the 12% CP diet during the 42-day receiving phase, compensated during the post-receiving phase indicating that the CP concentration fed during the receiving period did not affect the overall performance. Fluharty and Loerch (1995) conducted three trials to determine the effects of CP concentrations and source on receiving cattle performance. In the first trial, they used a 2 x 4 factorial experiment, with one factor being CP concentration (12, 14, 16, or 18%) and the other factor being protein source (soybean meal vs. spray-dried blood meal). Soybean meal and blood meal differ as protein sources in the sense that 35% of protein from soybean meal is rumen undegraded protein (RUP) (NRC, 2000), and 82% of protein from spray-dried blood meal is RUP (Beef, 2015). Veira et al. (1980) concluded that to achieve efficient protein utilization, the

diet should provide adequate N for optimum microbial growth, and if extra protein is required by the host, protein should be supplied to bypass ruminal fermentation. For the entire 42-day trial, calves assigned to blood meal diets resulted in 7.4% greater gains compared to soybean meal diets. Gain:feed increased with increasing CP concentration over the entire trial. Blood meal diets improved gain:feed by 11% for the 42 days. Morbidity increased with increasing CP concentration, but source did not influence morbidity. In the second trial, calves were assigned to six different CP concentrations (11, 14, 17, 20, 23, or 26%). No differences in DMI were observed, but maximum gain and feed:gain were observed with the 20% CP diet. Morbidity did not differ among treatments. In the third trial, the treatments were 1) 12.5% CP diet based on soybean meal, 2), phase-feeding of 23% CP in wk 1, 17% CP in wk 2, and 12.5% CP in wk 3 and 4. The percentage of morbidity was low across all treatments.

As dietary CP levels increase, morbidity rate tends to increase as observed by Galyean et al. (1993) and Fluharty and Loerch (1995) in trial 2. Metabolizable protein (MP), is a system that accounts for rumen degradation of protein and separates requirements into the needs of the microorganisms and the needs of the animal (NRC, 2000). Nissen et al. (1989) fed diet containing 5.2, 6.4, 7.4, or 9.5% MP to newly received calves. They reported a linear increase in ADG and improved feed: gain with increasing MP levels. However, he also found that the percentage of untreated calves decreased linearly with increasing MP. To better determine protein requirements of newly received calves, equations and tables based on the NRC (2000), previous research, and management experiences should be considered to come to a conclusion.

Concentrate Selection

Growing and receiving cattle are typically fed diets that contain approximately 50 to 75% concentrate due to specific nutrient requirements and economics. Concentrates are higher in energy value than roughages, and they are typically cereal grains and their byproducts. The most common cereal grains that are used in growing and receiving cattle diets are corn, sorghum, barley, wheat, and oats. Corn and its byproducts are commonly used as the concentrate portion in feeder cattle rations. The common use of corn and its byproducts as a concentrate source can be explained by its feed value and high availability.

Before 1920, increased corn production was attributed to increased land area, whereas after 1935, land area devoted to corn production declined and the increase in corn production was a result of increased yield per unit of land area (Farnham et al., 2003). Since then, U.S. corn production has dramatically increased; corn production went from 2.0 billion bushels yearly during the 1930s to 12.6 billion bushels yearly today, on the same amount of acreage (81.99 million acres) (Karlen et al., 2012). This dramatic increase in corn production can be attributed to new technologies and advancement in genetics. According to the USDA-ERS, corn is responsible for 93.8% of the United States feed grain production compared with barley, oats, and sorghum (ERS, 2015). In 2007, researchers at Texas Tech University surveyed 29 feedlot nutritionists and found that the primary grain used in beef cattle feedlots was corn (Vasconcelos and Galyean, 2007). Wheat, sorghum and barley followed the top grain choice of corn as the second, third, and fourth most used grain (Vasconcelos and Galyean, 2007). Corn is a clear choice for newly received cattle diets in comparison with the other common cereal grains because of its consistent composition and flexibility for further processing. Owens et al. (1997)

investigated performance of cattle fed different grains processed by various methods and found that the mean G:F for cattle fed dry rolled corn was 5.1% greater than the mean G:F for cattle fed dry rolled barley, and Zinn (1993) found similar results with steam rolled barley and corn. Zinn (1993) reported that steam rolled corn improved G:F of cattle 6.2% compared with cattle fed steam rolled barley. Likewise, Loe et al. (2006) compared dry rolled barley and dry rolled corn and found that corn fed steers were 23% more efficient than barley fed steers. Steers fed corn gained faster, consumed less, and had heavier final BW. The consistent performance of cattle that are fed corn can be attributed to the minimal variation in starch content when compared to other cereal grains like wheat, sorghum, barley, and oats. With minimal variation in starch content, diets are more consistent and there is then less risk of ruminal or digestive upset (Herrera-Saldana et al., 1990).

Value of Corn Fed to Ruminants

Corn is of great value as a feed ingredient because of its high energy, and low fiber content. The high energy content is a result of the high starch content; corn grain contains 72% of DM as starch (Huntington, 1997). Behind the rich nutrient profile of corn is the kernel structure which consists of the hull, endosperm, and the germ. The fibrous hull, about 6% of the kernel, surrounds the entire structure (Blanchard, 1992). The majority of the hull is the pericarp, a dense outer layer of dead cells that help to protect the seed. Underneath the hull, is the endosperm that makes up 82% of the kernel (Blanchard, 1992). The endosperm is comprised mostly of starch, in fact making up 86.6% of the total starch of the corn kernel. There are two regions of the endosperm, the soft and the hard. The soft, or floury region contains large and round starch granules in a thin protein matrix, providing much of starch (Blanchard, 1992) and is

the most susceptible to later processing and digestion (Kotarski et al., 1992). In the hard, or horny area the protein matrix is much thicker to hold the starch granules more firmly (Blanchard, 1992). The last component of the corn kernel is the embryo, or germ, constituting 12% of the kernel (Blanchard, 1992). Oil and protein are the main components of the germ, where energy and protein provided to ruminants are found.

Light-weight, younger cattle are able to efficiently “process” whole corn kernels through mastication that damages the pericarp to allow bacterial attachment (Lofgreen, 1988). Beauchemin et al. (1994) observed that most kernels were broken during consumption of whole-shelled corn by cows, suggesting that corn processing might not be necessary to optimize digestion. A study by Siverson et al. (2014) concluded that whole shelled corn can be fed to receiving and growing cattle as an energy source with responses similar to those of dry rolled corn. Although whole corn grain is fed to ruminants, many methods of processing have been employed in an attempt to improve its utilization by livestock (Scott et al., 2003). The underlying goal is to increase the amount of energy (starch) available to the animal, thereby increasing gain efficiency (Scott et al., 2003). The most common type of processing method used in large-scale beef cattle feedlots is steam-flaking, followed by dry-rolling and high-moisture harvesting and storage (Vasconcelos and Galyean, 2007). Nutrient content and performance results vary across corn processing methods, but ultimately the cost and effectiveness is the driving force for which is utilized.

Ethanol Industry

In addition to an ingredient source for the cattle feeding industry, corn also serves the purpose of being further developed into ethanol for fuel. Corn is the most important and

economical source of starch in the United States. The starch derived from the kernel can be readily converted into glucose and fermented into ethanol (Bothast and Schlicher, 2005). Ethanol production has dramatically increased since the 1970's world oil crisis, the clean air legislation in the 1990's and the passing of the 2005 energy bill (Bothast and Schlicher, 2005). Ethanol production from corn is an alternative fuel source for the United States. The United States has a dependence on foreign oil and to decrease this reliance and promote local economies, domestic substitutes for energy sources are needed (Murthy et al., 2006). In 2014, ethanol plants in 29 states produced a record of 14.3 billion gallons proving that the United States is indeed the leading ethanol producer with 60% of the global output (RFA, 2015). During the process of manufacturing ethanol from corn, byproducts are created and can be used as concentrate sources in receiving and growing rations. As ethanol production reached record levels in 2014, so did the output of animal feed coproducts with production of approximately 39 million metric tons of feed (RFA, 2015). Utilizing byproducts as a feedstuff for ruminants is economically practical because of their availability, nutrient value, and cost (Leupp, 2008).

Dry Milling Byproducts

Ethanol can be manufactured from corn by two processes; dry or wet milling. 67% of ethanol is produced by dry milling and this can be attributed to the fact that the focus is maximizing the capital return per gallon of ethanol (Bothast and Schlicher, 2005). This means that the focus is not on producing other products, but on producing the most amount of ethanol. This system requires the lowest amount of investment and operational requirements in comparison with the alternative, wet milling.

The dry milling process utilizes as much of the corn kernel as possible. There are five steps to the dry mill ethanol process: grinding, cooking, liquefaction, saccharification, and fermentation (Bothast and Schlicher, 2005). During the grinding step, corn is screened then hammer-milled to a medium-coarse to fine-grind meal. The flour-like substance is then combined with water and alpha-amylase enzymes to form a slurry. The cooking step entails the resulting mash being cooked, sterilized to kill non-desirable bacteria, and further liquefied. The mash is cooled and a glucoamylase enzyme is added (saccharification) along with yeast to convert glucose to ethanol and carbon dioxide (fermentation) (Kalscheur et al., 2008).

After fermenting for 48 to 72 hours, the mash is then distilled to form ethanol and whole stillage (remaining water and solids). Whole stillage is then centrifuged to separate the coarse solids from the liquids. The liquid is then evaporated to become condensed distillers solubles or syrup. The coarse solids are considered the major byproducts of ethanol production by dry milling. They include wet distillers grains, which can be combined with condensed distillers solubles to form wet distillers grains with solubles, or dried to form dried distillers grains (Kalscheur et al., 2008). For every bushel of corn that is manufactured, 2.8 gallons of ethanol, 8.16 kg of carbon dioxide, and 8.16 kg of distiller grains are produced. In other terms, each bushel or kernel that is processed, one-third becomes ethanol, carbon dioxide or distillers grains (Kalscheur et al., 2008).

Dried Distillers Grains-Overview

Dried distillers grains are the most predominant byproduct produced by the dry milling process; 60% of the distillers produced are dried, 27% is wet distillers, and 13% modified distillers (RFA, 2015). Dried distillers grains are developed by combining wet distiller grains

with condensed distiller solubles and drying the mixture. Drying the mixture is preferable for ethanol plants because wet distiller grains have a shelf life of only one to two weeks which can make transportation, handling, and storage challenging (Bothast and Schlicher, 2005). It is certainly energy-intensive to dry wet distiller grains into dried distiller grains, but the production of a uniform, stable, and high-quality feed product is essential to the profitability of the plant, resulting in a domination of dried distiller grains (Bothast and Schlicher, 2005).

Feeding Value of Dried Distillers Grains

During the dry milling procedure, after fermentation, the starch is removed from the mixture and the other nutrients, mainly protein, fat, fiber and P found within corn grain become more concentrated (Stock et al., 2000). For example, crude protein increases from 10% in the original corn grain to 30% in dried distiller grains plus solubles, fat from 4 to 12%, NDF from 12 to 36%, and P from 0.3 to 0.9% all on a DM basis (Klopfenstein et al., 2008). During the 1990s, for reasons previously stated, the production of ethanol increased and the feeding value of dried distillers grains shifted. Previously, dried distillers grains was used as a protein source, but after the dramatic increase of ethanol production, there was a major paradigm shift; distillers grains being used as an energy source rather than a protein source (Klopfenstein et al., 2008). Dried distillers grains are an excellent source of protein with 65% of the ~30% CP as RUP (Erickson et al., 2012).

An issue that can arise when feeding distillers grains is the increase in concentrations of minerals, specifically P and S. Traditionally, distillers grains contain between 0.65 and 0.95% P, which when balanced for proper Ca:P ratio is not a concern (Klopfenstein et al., 2008). Whilst processing corn into ethanol, sulfuric acid is used for pH control and cleaning, resulting in S

levels of 0.6 to 1.0% or greater (Klopfenstein et al., 2008). Elevated levels of dietary S are problematic, posing a health concern (Loneragan et al., 2001b). High levels may lead to polioencephalomalacia, reduced DMI and ADG, and reduced Cu stores (Klopfenstein et al., 2008).

Acidosis is a metabolic disease that occurs when the pH of the rumen falls below 5.5 as a result of ingesting increasing amounts of rapidly fermentable carbohydrates. Distillers grains can decrease acidosis related challenges by diluting dietary starch, i.e., fermentable carbohydrates (Erickson et al., 2012). Distillers grains are relatively low in starch, high in fiber, protein and fat due to ethanol production. During the dry milling procedure, the starch in corn grain is converted to glucose which is later converted to ethanol. As a result of the low starch content, feeding distillers grains can dilute dietary starch and influence rumen metabolism (Erickson et al., 2012). Feeding wet corn gluten feed helps prevent the risk of acidosis with high-grain diets, as observed by greater rumen pH in steers (Krehbiel et al., 1995). Wet corn gluten feed has amounts of fiber similar to that of distillers grains, indicating that prevention of acidosis is linked with feeding of distillers grains (Klopfenstein et al., 2008).

New technologies that alter the dry milling process make it challenging for nutritionists to formulate diets. These new technologies alter the nutrient content of distillers grains, and can vary from plant to plant.

Wet Milling Byproducts

Unlike, the dry milling process, the wet milling focuses on investing in a technology that will separate and produce valuable byproducts, making it much more capital and energy intensive (Bothast and Schlicher, 2005). As a result, only 33% of ethanol is produced by means

of wet milling (Bothast and Schlicher, 2005). However, the dry milling procedure accounts for only 10% of total corn oil production (Watson, 1988). Both methods fulfill different purposes and produce different byproducts for cattle use.

The overall goal of wet milling is to separate the kernel into distinct products, thereby obtaining highly purified individual components of corn (Herold, 1999). As outlined by (Blanchard, 1992) the wet milling procedure begins with the whole cleaned corn grain being steeped by soaking for 40 hours in warm water with added sulfur dioxide. This softens the kernel for further grinding, loosens the protein matrix, and removes soluble material which is then evaporated. After the 40-hour steeping process, the corn is then passed through degerminating mills. The purpose of the mill is to tear the kernels apart to free the germ fraction of the grain. The germ is separated due to it being lower in density, because it contains 85% of the corn's oil (Blasi et al., 2001). It can be separated in hydrocyclones because of its density measurements, after which it is washed free of starch and gluten, dewatered, and dried.

The remaining material, which consists of starch, gluten, fiber, and kernel fragments is put through fine-grinding mills which releases the remaining starch and gluten. Because the fiber fraction is not easily ground, it is separated, washed on a series of screens, and later dewatered and dried. The starch-gluten slurry screened free of fiber is centrifuged to separate the gluten. The gluten is then thickened in another set of centrifuges, dewatered, and dried. The remaining starch slurry is purified with fresh water in hydrocyclones and can be further processed for ethanol or high fructose corn syrup production (Blanchard, 1992).

The wet milling industry produces an array of high value byproducts. There are several byproducts that are utilized in ruminant diets. Corn bran, corn gluten feed, corn gluten meal, corn germ meal, and condensed fermented corn extractives or corn steep liquor are all byproducts that

are regularly used in the feed industry (Loy and Wright, 2003). There are no absolute byproduct yields in corn wet milling. The yields depend on the range of byproducts being made, the equipment available, and the composition of the corn (Blanchard, 1992). In general terms, for every bushel of corn, the wet milling process will yield 0.78 kg corn oil, 1.55 kg condensed fermented corn extractives, 0.83 kg corn germ meal, 2.67 kg corn gluten feed, 1.16 kg gluten meal, and 14.63 kg starch (Blasi et al., 2001).

Corn Germ Meal-Overview

Germ is separated from the kernel virtually completely to manufacture corn oil because it contains on a DM basis, 45 to 50% oil, 13% protein, and 12% starch (Blanchard, 1992). The oil extraction process is a sector in its own. Not every wet corn mill has the equipment to process corn germ into oil and meal (Johnson and May, 2003). The process for oil recovery and germ meal production is outlined by Johnson and May (2003). Germ is first softened by heat and steam before pressure is applied to rupture the oil cells. Oil can be extracted via a conventional expeller or solvent extraction. An expeller essentially applies pressure and squeezes the germ, decreasing the oil content from 45 to 6%. In solvent extraction, germ is pressed to an oil content of 13 to 20% (Bredeson, 1983), flaked with roller mills, and solvent extracted with hexane to lower the oil content to approximately 1.5%. Solvent extracted germ meal, the remaining residue, is desolventized with heat by means of direct and indirect steam. The oil rich solvent, called miscella, is heated, steamed and vacuumed to be further refined.

Corn germ meal can be used as a component of livestock feeding systems. The amino acid profile is better than corn as a whole (Blanchard, 1992), and it can be used as a carrier of nutrient supplements, such as vitamins, minerals, and medicants in animal feeds (Johnson and

May, 2003). Solvent extracted corn germ meal can be combined with corn steep liquor and corn bran to produce corn gluten feed (Johnson and May, 2003).

Corn Germ Meal in Diets

As stated previously, corn germ meal has a much better nutritional balance than whole corn grain, making it a desirable feed ingredient. Corn germ meal has medium protein and energy content and typically is used as a component in swine and poultry rations (Blanchard, 1992). On a DM basis, corn germ meal contains 25% protein, and 1.5% oil (Blanchard, 1992). Very little literature exists on solvent extracted germ meal as an ingredient for ruminants, and less for receiving and growing cattle.

Kelzer et al. (2009) investigated the effects of feeding three corn milling coproducts on intake, milk production, ruminal fermentation, and digestibility of lactating Holstein cows. The three coproducts that were examined were dried distiller grains, dehydrated corn germ meal, and high protein dried distillers grains. Cows that were fed the dehydrated corn germ meal in the first experiment tended to consume more feed and tended to produce more milk. The authors suggested that it is possible that the higher fat content of this diet resulted in a greater supply of energy and thus allowed animals to produce more milk. In the second experiment, the digestibilities of the diet were not different. Kelzer et al. (2009) concluded that dairy diets can be successfully formulated to include 15% of diet DM as corn-milling coproducts while maintaining yields of milk and milk components. Although full fat corn germ meal and lactating dairy animals are quite different from corn germ meal and receiving and growing cattle, this information still is useful.

Similarly, Miller et al. (2010) evaluated full fat germ as a replacement for whole cottonseed and tallow in total mixed rations for lactating dairy cows. DMI, milk yield, energy corrected milk, lactose percentage, protein and lactose yield and somatic cell count did not differ among diets. Milk fat percentage and fat yield were lower with the full fat germ diets. The authors concluded that additional studies need to be conducted to determine the amount of full-fat corn germ to feed to lactating dairy cows. Sulpizio et al. (2010) compared tallow and dried full fat corn germ as supplemental energy sources to finishing beef heifers. ADG was 1.36 kg for the tallow fed heifers; this compares with the heifers that were fed corn germ with an ADG of 1.34 kg. This posed no significant differences, but DMI was greater for the heifers fed the corn germ than for the heifers fed tallow. Thus, heifers fed the corn germ as a supplemental energy source were 3.4% less efficient. The authors concluded that when priced appropriately corn germ is a suitable substitute as a supplemental energy source in finishing diets.

The previous literature summarized involves full fat germ meal as an energy source in ruminant diets. Solvent extracted germ meal has little oil content, making it hard to compare to the previous literature on full fat germ meal. Herold (1999) conducted a few studies more relevant to solvent extracted germ meal in order to assess the energy value. When evaluating the effect of solvent extracted germ meal with and without steep liquor on performance of finishing steers and lambs, Herold (1999) found that the energy value of corn germ meal was similar to or greater than dry rolled corn in finishing cattle. In lambs, solvent extracted corn germ meal diminished dietary energy density and digestibility in lambs when compared to dry rolled corn. Herold (1999) also conducted a receiving trial and two finishing trials to evaluate corn germ as an ingredient in wet corn gluten feed by combining steep liquor with corn germ or dry corn bran. In the receiving trial, DMI and ADG did not differ among treatments, but feed efficiency tended

to be greater for the corn germ diet. He concluded that corn bran promotes greater DMI, whereas corn germ and corn steep liquor possess higher energy content than bran. Overall, more research on the energy value of solvent extracted corn germ meal is needed regarding the effects of corn germ meal on the growth performance of receiving and growing cattle.

Nucleotides as a Feed Additive

Newly arrived calves that are recently weaned undergo changes in the gastrointestinal tract which, in turn, can lead to health problems. Calves, when recently weaned, are more susceptible to diseases such as BRD. Viral pathogens can destroy villus structure and decrease absorptive surface area of the small intestine (Bridger et al., 1978; Saif et al., 1986; Holland et al., 1992). Feed additives have been developed to assist in the transition for calves in ways typical ingredients cannot. Nucleotides may serve as an immune boosting feed additive that can minimize the effects of stress and enhance calf health by accelerating the immune response, thereby increasing health and growth performance.

Structure of Nucleotides

Nucleotides are subunits of nucleic acids such as DNA or RNA that are composed of a phosphate group, a five-carbon sugar, and a nitrogenous base. Nucleotides participate in many biochemical pathways and are constantly synthesized and degraded in all tissues, especially in tissues with a rapid turnover rate such as cells in the immune system, intestinal mucosa, skin, and progenitors of leukocytes or erythrocytes (Uauy, 1989). Besides their role in biochemical pathways, studies have suggested that dietary nucleotides may promote the development of the gastrointestinal structure (Uauy et al., 1990), control intestinal microorganisms (Gil et al., 1986),

and influence immune function (Lee et al., 2007). There are three potential sources of nucleotides: *de novo* synthesis, salvage pathways, and the diet (Cosgrove, 1998; Boza, 1998). The *de novo* synthesis of nucleotides is a metabolically costly process that requires considerable amounts of energy in the form of ATP (Carver and Allan Walker, 1995). The salvage pathway requires less energy than the *de novo* synthesis because it recycles 90% or more of the purine bases under fed conditions, suggesting that the pathway is dependent on the availability of free purine and pyrimidine bases (Uauy, 1989). Some tissues have limitations for *de novo* synthesis and therefore require nucleotides via the salvage pathway (Savaiano and Clifford, 1981; LeLeiko et al., 1983; Gil and Uauy, 1995). An example is the intestinal mucosa, the haematopoietic cells of bone marrow, leukocytes, erythrocytes and lymphocytes which are all incapable of *de novo* synthesis (Sanderson and He, 1994) and are reliant on a supply of nucleotides via the diet (Cosgrove, 1998).

Nucleotide Requirements of Animals

Nucleotides are naturally present in all feedstuffs (Clifford and Story, 1976) but are much lower in concentration than mammalian milk (Mateo et al., 2004). The nucleotide concentration in milk of mammals is species specific and the concentration of most nucleotides changes during the lactation period (Gil and Sanchez-Medina, 1982). Feedstuffs are not typically analyzed for nucleotide concentration and content, but the little data that there is suggests that in comparison with colostrum and milk, there are low concentrations of individual nucleotides in feedstuffs such as barley, soy protein concentrate, and soybean meal (Mateo and Stein, 2004). Soy protein concentrate is prepared from defatted soybean meal by extraction of the soluble carbohydrates and typically contain 70% CP (Berk, 1992). Low concentrations of nucleotides in feedstuffs may

prove a potential problem for young animals that are recently weaned and stressed. The diet the young animals are leaving behind is rich in nucleotides and the weaning diet contains a low concentration of nucleotides that is insufficient to cope with potential stressors that compromise health. Supplemental nucleotides are required in growing young animals because they are more susceptible to disease, infection, or inflammation of the intestinal tissue (Uauy et al., 1990). It is even more important to supplement with nucleotides due to the fact that *de novo* nucleotide synthesis is absent (Savaiano and Clifford, 1981) or limited (LeLeiko et al., 1983) in the intestine.

Feeding Nucleotides to Monogastrics

In rats and pigs, supplementation of nucleotides has improved intestinal morphology, and reduced diarrhea (Kulkarni et al., 1986; Uauy et al., 1990). Uauy et al. (1990) evaluated the relative need for exogenous nucleosides in the small intestine at a time of rapid growth in the developing rat. They found that the amount of mucosal protein and mucosal DNA of the gut was significantly affected by exogenous nucleosides; the nucleoside-fed group had 50% more protein and 77% more DNA than those fed the nucleoside-free diet. Villi in the proximal segment of the intestine were longer in the nucleoside-fed animals which could reflect greater amount of protein and DNA. Larger villi allow more surface area, which can increase the small intestinal absorption of nutrients. They found evidence showing accelerated maturation of the intestine in response to dietary nucleosides with increases in both sucrase and maltase activity in the middle and distal segments of the intestine. They concluded that nucleosides may be considered semi-essential nutrients for optimal gut growth and development in the weanling rat.

In piglets, Sauer et al. (2012) determined the effects of a mixture of free nucleotides supplemented post weaning from d 20 to 39 or 40 on growth performance, immunological status, small intestinal morphology, and bacterial numbers in digesta of jejunum and cecum in feces. Average daily feed intake increased with the inclusion of nucleotides, whereas ADG, G:F, and final BW did not differ between treatments. Erythrocyte volume fraction did not differ between treatments. The nucleotide fed piglets had a greater plasma concentration of IgA than the control piglets, although IgG and IgM concentrations did not differ between groups. No differences in small intestine length, duodenal, jejunal and ileal villi height and crypt depth were observed between the treatments. Also, bacterial numbers of *Enterococcus* spp*., Clostridium,* and total bacteria were not different between the control and the nucleotide fed diet. Sauer et al. (2012) concluded that supplementing the diet of weaning piglets with pure nucleotides resulted in an increase in plasma IgA concentrations without altering gut morphology, bacterial numbers, and growth performance.

Feeding Nucleotides to Ruminants

Overall, there is contrasting literature published on supplementing nucleotides to pigs and rats. There is very little research on supplementing nucleotides to ruminants, and specifically no research on the addition of nucleotides in receiving and growing diets of beef cattle. In two experiments, Mashiko et al. (2009) investigated the effects of feeding a milk replacer supplemented with uridine 5'-monophosphate (UMP) at 2 g/d on the immune status of newborn calves. In Exp. 1, newborn Holstein bull calves were fed milk replacer with or without the UMP supplementation from d 4 to 10 after birth. They found that IgA concentration of the ileal mucosa was greater in the UMP group than in the control group but there was no difference in

jejunal mucosa. Also, there tended to be an increase in interferon-γ concentration by peripheral blood mononuclear cells on d 24 in the calves supplemented with UMP. In Exp. 2, treatments were the same as Exp. 1, but calves were fed from d 4 to 56 after birth. The proliferation of peripheral blood mononuclear cells was greater in the UMP treatment than in the control on d 14, 28, and 42. There also was an increase in interferon-γ concentration by peripheral blood mononuclear cells on d 28 and 42 in the UMP treated group compared to the control. From these results they concluded that dietary UMP can affect the immune response of newborn calves because it could stimulate a humoral or mucosal immunity.

Kehoe et al. (2008) evaluated supplementation of milk replacer with nucleotides on intestinal absorptive function and animal health in pre-weaned dairy calves. Three treatments consisting of 23 calves each were fed milk replacer supplemented with no nucleotides, purified nucleotides, or nucleotides from an extract of *Saccharomyces cerevisiae*. Average daily gain, health scores, fecal DM, and fecal bacteria were monitored. Blood samples were analyzed for packed cell volume, glucose, blood urea nitrogen, and creatinine. Calves were monitored for fecal scores and fecal fluidity, and intestinal function was evaluated by measuring absorption of orally administered xylose. Four calves per treatment were harvested to evaluate intestinal morphology, enzyme activities, and nucleoside transporter mRNA expression. Calves that were fed the milk replacer with nucleotides from an extract of *Saccharomyces cerevisiae* had increased nucleoside transporters mRNA, numerically longer villi, and lower alkaline phosphatase than the other two treatments. Calves that were fed the milk replacer with purified nucleotides had the highest detrimental and lowest beneficial bacteria overall, indicating an unfavorable intestinal environment. Growth measurements, fecal DM, fecal bacteria population, and plasma concentrations of glucose, blood urea nitrogen, creatinine, and IgG were not different

among the three treatments. They concluded that calves supplemented with nucleotides from an extract of yeast tended to increase calf intestinal function, provide a more beneficial intestinal environment, and improve intestinal morphology.

Nucleotide Metabolism in Ruminants

Supplementing nucleotides to ruminants is a fairly new concept, but the metabolism of nucleotides in ruminants is an ongoing process as ruminants derive nucleotides naturally from feedstuffs, mucosal secretions, and lysed microbial cells in the form of nucleic acids (McAllan, 1982). Variable amounts of nucleic acids are present in the most commonly used ruminant feedstuffs ranging from 1 to 50 g/kg dietary DM (McAllan, 1982). In forages, nucleic acids comprise 5.2 to 9.5% of the total nitrogen.

Nucleic acids (DNA or RNA) fed as plant material or as pure compounds are rapidly hydrolyzed in the rumen. Nucleic acids are degraded into oligonucleotides (polynucleotides that contain a small number of nucleotides) or nucleotides by nucleases, as described by (McAllan and Smith, 1973b). Oligonucleotides are then degraded into nucleotides by nuclease enzymes. From there, phosphate groups are cleaved by nucleoside phosphorylases to result in nucleosides (nitrogen base + sugar). Nucleosides are broken down by glycohydrolase enzymes that cleave the sugar off of the nitrogen base. The nitrogen base is then left for further absorption and excretion in the urine, nucleic acid synthesis, or converted into ammonia for further amino acid synthesis (McAllan and Smith, 1973b). When free RNA or DNA were fed to ruminating lambs, little to none survived to the abomasum (Razzaque and Topps, 1972) and when these compounds were introduced into the rumens of young steers they were rapidly degraded with the transient appearance of small amounts of oligonucleotides, nucleosides and bases (McAllan and Smith,

1973a). These derivatives disappeared from the rumen much more rapidly than could be accounted for by transfer to the lower digestive tract.

Little net changes occur in the amounts of nucleic acids between the rumen and duodenum (Ellis and Bleichner, 1969; Smith and McAllan, 1971). About 15 to 35 g/kg dietary DM entering the duodenum are nucleic acids of which approximately 60 to 70% is RNA (Smith and McAllan, 1971; McMeniman, 1975). About 85% of nucleic acids entering the duodenum in young calves receiving hay and concentrate diets are of microbial origin. As far as supplementing nucleotides, more research is needed on the effects on metabolism since these nucleotides are not of feedstuff origin. Moreover, research is needed on the effects of nucleotides on the immune function and growth performance of cattle, especially newly arrived receiving and growing beef calves.

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Chapter 2 - Effects of feeding nucleotides with corn germ meal or dried corn distillers grains on receiving and growing calves

Introduction

To improve profitability for newly arrived stressed cattle, it is essential to minimize feed costs and health issues while optimizing growth performance. During the receiving and growing phase, calves typically are recently weaned and experience various physical and psychological stressors which can create health issues and depress feed intake (Galyean and Hubbert, 1995). Selecting ingredients low in cost while providing adequate nutrients is especially important during this phase. Byproducts are commonly used in growing and receiving diets because of availability, nutrient value, and cost (Leupp, 2008). Corn germ meal (CGM) is a byproduct manufactured by the corn wet milling industry, has medium protein and energy content, and is often used in swine and poultry diets (Blanchard, 1992). Very little information exists on the feeding value of CGM (solvent extracted) for beef cattle.

Feeding nucleotides (NA), a natural, immune boosting feed additive, may improve the gastrointestinal health of an animal. NA are subunits of nucleic acids such as DNA or RNA that are composed of a phosphate group, a five-carbon sugar, and a nitrogenous base. NA are naturally found in all feedstuffs (Clifford and Story, 1976), but most feedstuffs contain a lower concentration than milk (Mateo et al., 2004). Supplemental nucleotides are required in growing young animals because they are susceptible to disease, infection, or inflammation of the intestinal tissue (Uauy et al., 1990). Overall, there is contrasting literature published on supplementing nucleotides to pigs and rats (Uauy et al., 1990; Sauer et al., 2012). There is very little research on supplementing nucleotides to ruminants (Kehoe et al., 2008; Mashiko et al.,

2009), and specifically there is no research on the addition of nucleotides to receiving and growing diets for beef cattle.

The objectives of these experiments were to determine: 1) the effects of CGM in comparison to dried corn distillers grains (DDG) on growth performance, digestibility, and *in vitro* gas production, and 2) the effects of NA on growth performance, digestibility, gas production, and mucosal immunity, of receiving and growing cattle.

Materials and Methods

Animal care practices were approved by the Kansas State University Institutional Animal Care and Use Committee protocols 3299.8 and 3574.

Experiment 1. Receiving and Growing Cattle Performance Study I

Two hundred thirteen crossbred heifers (BW= 262 ± 67.4 kg) were shipped from three separate sources (Searcy, AR; Snook, TX; and Melbourne, AR) to the Kansas State University Beef Stocker Unit over a 5-d period from June 15 to June 19, 2015. The heifers were used in a complete block design with a 3 x 2 factorial arrangement of treatments to determine the energy value of corn germ meal (CGM) in comparison to dried corn distillers grains (DDG) and the effect of adding a nucleotide (NA) (PSB Complex; DSS Global, Chicago, IL) at three inclusion levels (0, 2 and 4 g/d). Calves were blocked by source (n = 3), stratified by arrival weight within each block, and assembled into pens containing 11 or 12 heifers. Pens were randomly assigned one of the six treatments, which allowed three pens per treatment with a total of 18 pens filled for the experiment. Each of the 18 pens was of equal size (9.1 x 15.2 m) and soil surfaced with a concrete fenceline bunk (9.1 m) that was attached to a 3.6-m concrete apron.

The six treatment diets were: 1) CGM with no NA (CGM0), 2) CGM with 2 g/heifer daily NA (CGM2), 3) CGM with 4 g/heifer daily NA (CGM4), 4) DDG with no NA (DDG0), 5) DDG with 2 g/heifer daily NA (DDG2), and 6) DDG with 4 g/heifer daily NA (DDG4) (Table 2.1). Diets within CGM or DDG were formulated to include the same amount of each ingredient with the exception of the NA. There were three different NA supplements mixed and pelleted, and they were formulated to provide 0, 2 or 4 g NA/heifer daily when DMI was 8.2 kg/d. At this inclusion level, the NA treatments provide 0, 0.242, and 0.489 g NA/kg dietary DM. Diets were formulated to be isocaloric and isonitrogenous. As a result, CGM and DDG diets included different amounts of specific ingredients where CGM diets had larger inclusions of prairie hay, and DDG diets had larger inclusions of cracked corn and alfalfa hay.

At the time of arrival, calves were weighed individually, and ear tagged with an individual identification number. Calves were held in six pens overnight with free choice access to prairie hay and water. The day after arrival (d 0), heifers were weighed, tagged with a pen number, and vaccinated for respiratory and clostridial diseases. For protection against clostridial bacteria, Vision 7 Somnus with Spur (7-way clostridial modified-live vaccine; Merck Animal Health, Madison, NJ) was used, and Pyramid 5 (Boehringer Ingelheim, St. Joseph, MO) a modified-live vaccine for protection against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) was administered. Calves were dewormed with 5 mL 1% ivermectin wt/vol and 10% wt/vol clorsulon (Ivomec Plus; Merial Animal Health, Duluth, GA) and given a subcutaneous injection of 1.5 mL of Excede (200 mg ceftiofur equivalents (CE) per mL; Zoetis, Florham Park, NJ) for protection against bovine respiratory pathogens *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni.* On d 14, cattle were revaccinated with Bovi-Shield Gold 5 (Zoetis, Florham

Park, NJ), a modified-live virus vaccine with strains of IBR, BVD, PI3, and BRSV. To combat bovine respiratory disease (BRD), 4.5 mg/kg BW daily of Aureomycin (22.7 g chlortetracycline hydrochloride/kg; Zoetis, Florham Park, NJ) was added to all diets for five consecutive days, two times during the trial; d 31 to 35 and d 51 to 55. Heifers were individually weighed on d 0 (initiation of study), 28, 56, 84 and 85 (completion of trial).

Calves were fed once daily using a Roto-Mix (Model 414-14B, Dodge City, Kansas) wagon at approximately 0730 h. The amount of feed delivered and the amount that disappeared from each pen was recorded daily. The disappearance of diets from each bunk was measured daily by weighing the feed delivered to each bunk and subtracting the refusals left the next morning. Refusals were measured at approximately 0600 h to calculate dry matter intake (DMI) and to determine the amount to be fed that day. Feed samples of each diet were collected weekly from each bunk and stored frozen (-20˚C) for later analysis. Feed samples were dried in a 55˚C oven, air equilibrated, and ground through a 1-mm screen using a Wiley mill. Ground feed samples were composited by period and shipped to a commercial lab (SDK Laboratories, Hutchinson, KS) for analysis of DM (105˚C), N (AOAC, 1997), NDF and ADF (Van Soest et al., 1991), Ca (Bower and Rains, 1988), P (AOAC, 1997), starch (Richards et al., 1995), ash (Undersander, 1993), and fat content (Sukhija and Palmquist, 1988) (Table 2.2).

Health of heifers was evaluated daily and any heifers that appeared to have signs of sickness or bloat were removed from their pen. Once restrained in a chute, rectal temperature was measured. Treatment was based on rectal temperature and number of previous treatments. Treatments for calves with temperature of < 40.6˚C were: first treatment, 19.8 mg/kg BW Biomycin (200 mg/mL oxytetracycline; Boehringer Ingelheim, St. Joseph, MO), second treatment, 40 mg/kg BW and 2.2 mg/kg BW Resflor Gold (30 mg/mL florfenicol and 16.5

mg/mL flunixin; Merck Animal Health, Madison, NJ), third treatment, 2.5 mg/kg BW Draxxin (100 mg/mL tulathromycin; Zoetis, Florham Park, NJ), and fourth treatment, 1.1 to 2.2 mg/kg BW Excenel (50 mg/mL ceftiofur hydrochloride; Zoetis, Florham Park, NJ). Treatment for calves with temperature > 40.6˚C were: first treatment, 40 mg/kg BW and 2.2 mg/kg BW Resflor Gold (30 mg/mL florfenicol and 16.5 mg/mL flunixin; Merck Animal Health, Madison, NJ), second treatment, 2.5 mg/kg BW Draxxin (100 mg/mL tulathromycin; Zoetis, Florham Park, NJ), third treatment, 1.1 to 2.2 mg/kg BW Excenel (50 mg/mL ceftiofur hydrochloride; Zoetis, Florham Park, NJ), and fourth treatment, 19.8 mg/kg BW Noromycin 300 LA (300 mg/mL oxytetracycline; Norbrook Inc., Lenexa, KS). Following treatment heifers were returned to their pen.

Experiment 2. Receiving and Growing Cattle Performance Study II

Three hundred Brahman x Hereford crossbred heifers (BW= 268 ± 34.1 kg) were shipped from one source in Council Grove, KS to the Kansas State University Beef Stocker Unit on January 6, 2016. Calves had been purchased via online live auctions from Huntsville, TX and shipped to a backgrounding lot in Council Grove, KS on December 8, 2015. On December 19, 2015 heifers were vaccinated with Express 5 (Boehringer Ingelheim, St. Joseph, MO), a modified live virus vaccine with strains of IBR, BVD, PI3, and BRSV. For protection against clostridial bacteria, Vision 7 Somnus with Spur (7-way clostridial modified-live vaccine; Merck Animal Health, Madison, NJ) was used, and for protection against respiratory pathogens, *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni,* Super Poly-Bac (Texas Vet Lab Inc., San Angelo, TX) was administered. Calves were dewormed with 0.5 mg/kg BW Cydectin (5 mg/mL moxidectin; Boehringer Ingelheim, St. Joseph, MO), given Fusogard

(Elanco, Indianapolis, IN) a foot rot vaccine for protection against *Fusobacterium necrophorum,* and implanted with SolidBac (Zoetis, Florham Park, NJ) for aid against pink eye.

Upon arrival at the Kansas State University Beef Stocker Unit, heifers were immediately weighed, and each was tagged with an individual identification number. Calves were held in 24 pens and fed a common diet until January 13, 2016. In this experiment, heifers were used in a complete block design to evaluate the effects of NA (PSB Complex; DSS Global, Chicago, IL) in three inclusion levels (0, 2, and 4 g/heifer daily) on growth performance and mucosal immunity. Calves were allotted to 10 blocks of 30 heifers, stratified to pens by arrival weight within each block, and assembled into pens containing 10 heifers. Pens were randomly assigned to one of three treatments, which allowed 10 pens per treatment with a total of 30 pens used for the experiment. During the duration of the study, it was observed that 6 of the 30 pens were not performing to their potential due to the fact that they were in newly constructed pens. It was then decided, for the growth performance objective, to only include 24 of the 30 pens in the statistical analysis. Therefore, only 24 pens (n = 8) of 10 heifers per pen, and 8 pens per treatment were used in the growth statistical analysis of growth performance in this study. The three treatment diets were DDG0, DDG2, and DDG4 from Exp. 1, and the diets and NA inclusions were formulated to be identical to Exp. 1 (Table 2.1). On January 13, 2016 (d 0) calves were weighed, tagged with a pen number, and revaccinated with Pyramid 5 (Boehringer Ingelheim, St. Joseph, MO), a modified live virus vaccine with strains of IBR, BVD, PI3, and BRSV. Heifers were weighed on d 0 (initiation of study), 28, 56, and 57 (completion of trial).

Calves were fed once daily using a Roto-Mix feed wagon (Model 414-14B, Dodge City, KS) at approximately 0730 h. The amount of feed delivered and the amount that disappeared from each pen was recorded daily. The disappearance of diets from each bunk was measured

daily by weighing the feed delivered to each bunk and subtracting the refusals left the next morning. Refusals were measured at approximately 0700 h to calculate DMI and to determine the amount to be fed that day. Feed samples of each diet were collected weekly from each bunk and stored frozen (-20˚C) for later analysis. Feed samples were dried in a 55˚C oven, air equilibrated, and ground through a 1-mm screen using a Wiley mill. Ground feed samples were composited by period and shipped to a commercial lab (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, ADF, Ca, P, starch, ash, and fat content as described in Exp. 1 (Table 2.2). Fecal samples were collected from the rectum on d 28 as they came through the chute/scale to be weighed and were frozen at -20˚C for further analysis of IgA concentration. Samples were thawed and analyzed for total IgA by ELISA (sIgA ELISA analysis, Bethyl Laboratories, Montgomery, TX).

Health of heifers was monitored daily and any heifers that appeared to have signs of sickness or bloat were treated and treatment protocols followed that of Exp. 1 and once appropriate treatment was determined calves were treated and returned to their pen.

Experiment 3. Digestibility Study

Four ruminally cannulated Holstein heifers were used in a 4 x 4 Latin square design in an experiment to evaluate diet digestibility and ruminal parameters. Treatment periods consisted of four consecutive 15-d periods; each period included 10 d of treatment adaptation, 4 d of fecal collection, and 1 d for sampling of rumen fluid. The four treatments were: 1) CGM0, 2) CGM0 with 3 g/heifer daily NA top-dressed to the diet, 3) DDG0, and 4) DDG with 3 g/heifer daily NA top-dressed to the diet. The CGM and DDG diets were the same as Exp. 1. Heifers were housed in individual stalls (3.7 x 3.7 m) with 2 cm thick rubber mats surfaced with 0.75 m^3 pine

shavings inside a temperature-controlled barn (10 to 21˚C). Heifers were allowed free movement in individual stalls and only restrained during sample collection. Before feeding, heifers were moved to a larger pen and allowed approximately 1 h of exercise while feed pans and stalls were cleaned, and feed was distributed.

Heifers were fed once daily at 0800 h. Heifers were provided feed in amounts allowing for ad libitum intake and amounts of feed provided were designed to allow for at least 10% feed refusals. Feed samples were collected on d 10 through 14 and composited for each heifer for each period. Feed refusals were collected at approximately 0700 h on d 11 through 15 and composited for each heifer for each period. Ingredient samples of each diet were collected on d 10 through 14 and were composited for each period. Feed samples, refusals, and ingredients were dried at 55˚C, air equilibrated and ground through a 1-mm screen using a Wiley mill. Feed samples, refusals, and ingredients were shipped to a commercial lab (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, ADF, Ca, P, starch, ash, and fat content as described for Exp. 1. Chromic oxide (10 g/d) was top dressed and mixed by hand into each diet for each heifer on d 4 through 14. On d 11 through 14, fecal samples were collected from the rectum 3 times daily (every 8 h) with fecal sampling beginning 2 h later than the previous day so that samples represented each 2-h interval after feeding. Fecal samples were stored and frozen (-20˚C) for later analysis. Fecal samples were thawed, dried at 55˚C, air equilibrated, and ground through a 1-mm screen using a Wiley mill. Fecal samples were composited within each period for each heifer and shipped to a commercial lab (SDK Laboratories, Hutchinson, KS) for analysis of DM, CP, NDF, ADF, Ca, P, starch, ash, and fat content as described for Exp. 1. The Cr concentrations in feed refusals and fecal samples were analyzed by atomic absorption spectrophotometry (Williams et al., 1962).

On d 15 of each period, rumen fluid samples were collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding. Subsequent to the 0 h sampling, Co-EDTA (0.4 g Co) dissolved in 200 mL of water, was mixed into the rumen (Udén et al., 1980). Rumen samples were immediately analyzed for pH with a portable pH meter (Orion, Beverly, MA) then strained through 8 layers of cheesecloth. Once strained, rumen fluid was pipetted into 2-mL micro-centrifuge tubes containing 0.25 mL of m-phosphoric acid and were frozen at -20˚C for later analysis of ammonia (Broderick and Kang, 1980), lactate (Barker and Summerson, 1941), and VFA. In addition, 20 mL of fluid was collected and frozen at -20˚C for Co analysis to determine ruminal liquid dilution rate. Liquid passage rates were calculated from ruminal cobalt concentrations at 2 to 18 h after dosing of Co-EDTA. Passage rate was calculated using the nonlinear procedure of SAS (SAS Inst., Inc., Cary, NC) by regressing the natural logarithm of Co concentration against time for each heifer within each period.

Experiment 4. In Vitro Study: Gas Production

To determine the effect of a NA (PSB Complex; DSS Global, Chicago, IL) with either CGM or DDG on gas production, a randomized complete block design with a 3 x 2 factorial arrangement of treatments was used. The experiment had four replicates and the six treatments 1) CGM with no NA (CGM0), 2) CGM with 2 g/heifer daily NA (CGM2), 3) CGM with 4 g/heifer daily NA (CGM4), 4) DDG with no NA (DDG0), 5) DDG with 2 g/heifer daily NA (DDG2), and 6) DDG with 4 g/heifer daily NA (DDG4). Ruminal digesta was obtained from two of the four ruminally cannulated Holstein heifers from Exp. 3 where one was fed CGM0 and one was fed DDG0. Ruminal contents were acquired at approximately 0730 h, prior to feeding and strained through four layers of cheese cloth into pre-warmed insulated thermoses. From there,

the thermoses were transported to the Kansas State University Pre-Harvest Food Safety Laboratory.

In the laboratory, ruminal fluid was decanted into separatory funnels, gassed with nitrogen for several minutes, and incubated at 39˚C for approximately 1 h to allow fluid to stratify. The bottom sediment layer was discarded and the bacteria rich layer was kept for use as inoculum. Batch cultures were prepared in 250 mL screw-top bottles and filled with 100 mL buffer solution, 50 mL ruminal fluid, and 2.0 g of substrate. The buffer solution was prepared as described by McDougall (1948) for synthetic saliva. The substrate was one of the six treatments and was prepared by taking a representative sample of DDG0 and CGM0 diets from Exp. 1 and 2. The feed sample was then dried at 55˚C, air equilibrated and ground through a 1-mm screen using a Wiley mill. From there, NA was added in each of the two diets at three different doses (0, 2 or 4 g). For each treatment, NA was added at 0 g per 2 g of substrate, 0.000488 g per 2 g of substrate, and 0.000978 g per 2 g of substrate. The rumen inoculum used for each treatment correlated with the substrate being fermented. For example, the heifer fed DDG0 provided the inoculum for treatments DDG0, DDG2, and DDG4. In each replication, there was one bottle used as a blank that contained only buffer and rumen inoculum. Initial pH was measured using a benchtop pH meter (Thermo Orion model 230 A; Thermo Fisher Scientific Inc., Waltham, MA) after combining of contents. After measuring pH, culture bottles were gassed with nitrogen, capped with Ankom^{RF1} modules (Ankom^{RF} Gas Production System; Ankom Technology, Macedon, NY), and placed into a shaking incubator (New Brunswick Scientific Co., Inc., New Brunswick, NJ) for 24 h. The incubator maintained a 39˚C temperature with gentle agitation. Gas pressure was recorded at 15 min intervals. After 24 h, bottles were removed from the shaking incubator and final pH was measured.

This experiment was repeated to increase power. All procedures followed the above description, except the rumen inoculum was derived from the remaining two ruminally cannulated Holstein heifers.

Statistical Analyses

Exp. 1 data were analyzed in SAS (SAS Inst., Inc., Cary, NC) using the MIXED procedure with byproduct, NA, byproduct x NA as fixed effects and block as a random effect. Initial body weight was used as a fixed covariate. Contrast statements were used to assess the overall effect as well as the linear and quadratic effects. Generalized quadratic solutions were used to determine dietary NE_m and NE_g values based on DM intake and cattle growth performance using the NRC (1996) equations for each pen of cattle during the 84-d feeding period.

Data for Exp. 2 were analyzed in SAS using the MIXED procedure with NA as a fixed effect and block as a random effect. Initial body weight was used as a fixed covariate. Contrast statements were used to assess the overall effect as well as linear and quadratic effects of treatment.

The pooled analysis of Exp. 1 and 2 were analyzed in SAS using the MIXED procedure with NA as a fixed effect. Initial body weight was used as a fixed covariate. A blocking variable was created that combined experiment and block together. Contrast statements were used to assess the overall effect of treatment as well as the linear and quadratic effects of treatment. Only data from d 0 to 56 were used for Exp. 1 to coincide with data from Exp. 2.

For Exp. 3, data were analyzed as a Latin square with a factorial arrangement of treatments using the MIXED procedure in SAS. Fixed effects included byproduct (CGM or

DDG), NA, NA x byproduct, and period. Heifer was a random effect. Treatment means were calculated using the LSMEANS option. Ruminal fermentation parameters were analyzed as repeated measures with the model containing byproduct, NA, NA x byproduct, sampling time, time x NA, time x byproduct, time x byproduct x NA, and period. Heifer was included as a random term. The repeated term was time, and heifer x period served as the subject; the covariance structure was spatial power.

Exp. 4 gas production data were analyzed using the MIXED procedure of SAS. Fixed effects included byproduct, NA, byproduct x NA, time, byproduct x time, NA x time, and byproduct x NA x time. Block was used as a random effect. Contrast statements were used to assess the overall effect as well as the linear and quadratic effects of treatment.

Results and Discussion

Experiment 1

Low morbidity was observed in this study with a total of twelve heifers treated for illness (Table 2.4). Five calves were treated for pinkeye, one for foot rot, one for bloat, and the remaining five for bovine respiratory disease. Only two out of the five heifers that were treated for bovine respiratory disease were treated more than once. Three heifers were removed from this study due to death; two animals died of bacterial infections, and one of chronic bovine respiratory disease (Table 2.5). All data from the three animals were removed from this study.

There were no significant effects of the type of byproduct, either CGM or DDG, on dry matter intake from d 0 to 28 (P = 0.76), d 28 to 56 (P = 0.96), d 56 to 84 (P = 0.19), and for the complete feeding period, d 0 to 84 (P = 0.55) (Table 2.3). No difference in DMI was observed

when lactating Holstein cows were fed corn-milling coproducts, comparing dried corn distillers grains plus solubles, dehydrated corn germ meal, and high protein dried distiller grains (Kelzer et al., 2009). Although full fat germ meal was used in the Kelzer et al. (2009) study, this information is still useful. For the entire 84 d feeding period, the calves averaged an intake of 9.16 kg dry matter per day across all treatments. Average daily gain was also not significantly different between CGM and DDG treatments. On d 0 to 28, d 28 to 56, d 56 to 84, d 0 to 84, heifers respectively averaged 1.42 kg ADG (P = 0.57), 1.22 kg ADG (P = 0.65), 0.64 kg ADG (P = 0.83), 1.11 kg ADG (P = 0.88). Similar to DMI and ADG, feed efficiency was not significant due to the two different byproducts; calves that were fed CGM were just as efficient as the calves fed DDG ($P \geq 0.34$). Kelzer et al. (2009) found that milk production response to an inclusion of corn germ meal, dried distillers grains plus solubles, and high protein dried distillers grains was not different. Growth performance is different from milk production, as is the fat level in this study's corn germ meal, but, it is still useful information that can be translated to our experiment.

One of the objectives of this experiment was to determine the NE_m and NE_g of CGM in comparison to DDG because of the lack of literature found on quantifying the energy value of CGM. Our hypothesis was that CGM would be similar to DDG in energy content, and with the performance results of this study and the calculated NE_m and NE_g, it proved correct. There was no significant effect of CGM or DDG on NE_m (P = 0.25) or NE_g (P = 0.31). This proves that when corn germ meal is included in a diet at 24.5% on DM basis offers the same NE_m and NE_g values as a diet with 22.0% dried corn distillers grains on DM basis when both diets are formulated to be isocaloric and isonitrogenous.

No linear effect ($P \geq 0.58$) or quadratic effect ($P \geq 0.28$) of a nucleotide additive fed at rates of 0, 2, or 4 g/ heifer daily were observed on dry matter intake. Likewise, there was no linear effect ($P \geq 0.15$) or quadratic effect ($P \geq 0.70$) of NA on ADG. Feed efficiency also was not different across NA treatments ($P \geq 0.15$). These findings are similar to that of Kehoe et al. (2008), where they supplemented nucleotides in milk replacer to preweaned dairy calves. All growth measurements were similar for calves that were not supplemented with nucleotides, supplemented with purified nucleotides, and supplemented with nucleotides from an extract of *Saccharomyces cerevisiae* (Kehoe et al., 2008). Over the entire 6-wk period and post-weaning, feed intake was not different between treatments (Kehoe et al., 2008).

Experiment 2

No morbidity and mortality was observed in this study for the entire 56-d feeding period. Intake of dry matter was not different across treatments (Table 2.6). Average DMI for d 0 to 28, d 28 to 56, and d 0 to 56 was 8.59 kg/d ($P \geq 0.46$), 8.77 kg/d ($P \geq 0.44$), and 8.68 kg/d ($P \geq 0.43$) respectively. Average daily gain of these heifers was also not different across three different treatments. Over the entire feeding period, from d 0 to 56, calves gained on average 0.98 kg per day ($P \geq 0.23$). The nonsignificant effects of NA on DMI and ADG translates to the nonsignificant effects on feed efficiency in this experiment ($P \geq 0.26$). The results of this experiment are in agreement with Exp. 1 in that there were no significant differences of feeding a nucleotide additive to receiving calves on performance.

On d 28 of this experiment, approximately 5 random fecal samples were taken from each pen to determine if feeding NA would alter total IgA concentration. Fecal IgA concentration was not different among treatments with the treatment DDG0 having a IgA concentration of 847

ng/mL, DDG2 with 625 ng/mL, and DDG4 with 718 ng/mL ($P \geq 0.15$) (Figure 2.1). Although there was no difference in IgA concentration in fecal samples across the three treatments, there did tend to be a quadratic dose effect ($P = 0.11$) with DDG2 having the lowest fecal IgA concentration. NA did not provide a linear dose effect across treatments ($P = 0.24$). Dietary nucleotides have been reported to have many biological activities in the digestive system, including the growth and development of the small intestine as well as the intestinal repair after chronic diarrhea (Bueno et al., 1994). Overall, very little literature is available on the effects of feeding nucleotides on mucosal immunity. Immunoglobulin A plays important roles in mucosal immunity by preventing pathogenic microbes from adhering to the mucosal epithelium and by neutralizing toxins and viruses (Mashiko et al., 2009). Secreted IgA are therefore critical for maintaining a stable gut microbiota (Suzuki et al., 2004). Our experiment, though much different, differed in results with that of Mashiko et al. (2009). They investigated the effects of feeding a milk replacer supplemented with uridine 5' –monophosphate (UMP) at 2 g per day on the immune status of newborn calves. They found that IgA concentration of the ileal mucosa was greater in the UMP group than in the control group, but no difference in jejunal mucosa. Nagafuchi et al. (2000) observed that fecal IgA levels significantly increased in mice that were fed a nucleotide-supplemented diet than those fed a nucleotide free diet. More research is warranted on the effects of feeding NA to animals, and even more specifically, receiving and growing cattle.

Experiment 1 & 2 pooled analysis

Pooled effects of the addition of a nucleotide feed additive on gain, intake, and efficiency of Exp. 1 and 2 are listed in Table 2.7. No effects of the addition of NA were

observed on DMI throughout this analysis. Heifers in both Exp. 1 and 2 averaged a DM intake of 8.65 kg/d for the 56 d feeding period ($P = 0.72$). Therefore, there were no linear ($P = 0.58$) or quadratic effects ($P = 0.57$) of NA on DM intake. ADG was also not affected by the NA ($P \geq 0.40$), and thus there were no linear ($P \geq 0.64$) or quadratic effects ($P \geq 0.20$) of treatment.

Experiment 3

Diet composition, intake, and nutrient digestibility of the diets fed in Exp. 3 are listed in Table 2.8. DM intake was not affected by the type of corn byproduct included in the diet, CGM and DDG ($P \geq 0.46$), or NA ($P \geq 0.40$) and averaged across treatments was 10.04 ± 0.46 kg. The DM intake results from the digestibility experiment agrees with the fact that there were no DM intake differences in Exp. 1 and 2. Likewise, starch, non-starch, and ADF intake were not affected by CGM and DDG ($P \geq 0.14$) or NA ($P \geq 0.40$). DM digestibility was not different across treatments, averaging 69.5 ± 1.50% from CGM and DDG ($P = 0.60$) or NA ($P = 0.84$). Similarly, digestibility of starch, non-starch, and ADF were not affected by CGM and DDG ($P \geq 0.18$) or NA ($P \geq 0.12$). Within the diet, starch ($P = 0.04$) and ADF ($P \leq 0.01$) composition was higher for CGM compared to DDG diets. As discussed, there was no differences in starch and ADF digestibility between CGM and DDG treatments, even though starch and ADF content differed significantly. These results support that the byproduct CGM can replace DDG with no differences in intake, or digestibility and agrees with research by Kelzer et al. (2009). Kelzer et al. (2009) observed that there were no digestibility differences when comparing full fat corn germ meal, dried distiller grains plus solubles, and high protein dried distiller grains.

The effects of CGM and DDG, and the addition of NA on ruminal fermentation characteristics are presented in Table 2.9. Ruminal pH was not affected by the two different

byproducts, CGM and DDG ($P \geq 0.34$). The NA when present at 3 g/heifer daily increased ruminal pH significantly ($P = 0.03$), thereby making the ruminal pH less acidic, compared to the diets without NA. A less acidic ruminal environment aids in fiber digestion and can increase the productivity of cellulolytic bacteria. Although NA had a significant impact on rumen pH, there was no byproduct x NA interaction ($P = 0.93$).

Lactate concentration in ruminal fluid was not affected by either CGM or DDG ($P \geq 0.85$) or by NA ($P \geq 0.76$). Interestingly, ammonia concentration was lowest when animals were fed the CGM diet compared to animals fed the DDG diet ($P < 0.01$) which reflects the ruminal degradability of the diets. The NA had no effect ($P = 0.85$) on ammonia concentrations in the ruminal fluid, and no byproduct x NA interaction was found ($P = 0.37$).

The DDG diets had more total VFA concentration compared to the CGM diets ($P = 0.04$). This is reflected not only by the differing byproducts, but also by the different diets. Both diets, CGM and DDG, were formulated to be isocaloric and isonitrogenous, and as a result there were different amounts of prairie hay, alfalfa hay, cracked corn, and corn gluten meal between the two. The higher amount of total VFA concentration in DDG diets translates to the amount of acetate where the DDG diets tended to have higher concentration of acetate than the CGM diets ($P = 0.08$). Although there were no significant effects of NA on total VFA ($P = 0.19$) or acetate concentration ($P = 0.60$), there was a significant decrease in propionate concentration ($P = 0.03$). Butyrate, another VFA measured in this experiment, had higher concentrations in DDG diets compared to CGM diets ($P < 0.01$). There was no NA effect ($P = 0.15$), but there tended to be a byproduct x NA interaction ($P = 0.06$) within the DDG diets. As NA was present in DDG diets, there was a numerical decrease in butyrate concentration. There was a significant byproduct effect on isobutyrate concentration where DDG diets had higher concentrations ($P <$

0.01) of isobutyrate in ruminal fluid than CGM diets. There was no effect of NA on isobutyrate concentration ($P = 0.13$). Valerate and isovalerate was higher in DDG diets compared to CGM diets ($P < 0.01$). For isovalerate specifically, there was no NA effect but there was a byproduct x NA interaction ($P \leq 0.01$) detected within the CGM diets. Within the CGM diets, as NA was present, it increased isovalerate concentrations ($P < 0.01$). NA had a significant effect on valerate concentration which was decreased when present ($P = 0.02$), especially when fed within the DDG diets ($P < 0.01$) demonstrating a byproduct x NA interaction. Fluid passage rate was not affected ($P \geq 0.44$) by the two different byproducts, CGM or DDG, and whether or not a NA was present.

Experiment 4

The effect of the addition of NA with either corn germ meal or dried corn distillers on 24-h gas production is represented in Figure 2.2. Gas production, expressed in mL, was affected by time during the 24-h incubation ($P < 0.001$), NA ($P < 0.001$), and NA x byproduct ($P < 0.001$) during the 24-h incubation. There was no effect of byproduct (CGM and DDG) on gas production ($P = 0.77$). As increasing amounts of NA was added to the DDG diets there was a linear decrease in the total ruminal gas volume ($P < 0.001$). However, there was no interaction between CGM and DDG, NA and time ($P = 1.00$).

Conclusions

There were no significant effects of CGM and DDG on growth performance, digestibility, or gas production in Exp. 1, 3, and 4. Results of these experiments indicate that CGM diets can be fed to receiving and growing cattle in place of DDG diets while maintaining

DMI, ADG, feed efficiency, digestibility and ruminal gas production. When CGM is included in a diet at 24.5% on DM basis, it offers the same NE_m and NE_g values as a diet with 22.0% DDG on DM basis when both diets are formulated to be isocaloric and isonitrogenous. This can be useful information because very little is known regarding the feed value of CGM in receiving and growing diets. This might be useful at times when CGM has greater availability and lower cost than DDG.

There was no significant effect of NA on growth performance, digestibility, or mucosal immunity in Exp. 1, 2, 3, and 4. However, there was an effect on ruminal gas production and ruminal parameters. More research is warranted on the addition of NA in receiving and growing cattle. Further research could entail testing a larger dose than what was used in this study, a rumen protected nucleotide, and specific nucleotide subunit such as uridine 5'-monophosphate on receiving and growing cattle.

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Tables

Table 2.1. Composition of diets (% of DM) containing corn germ meal, dried corn distillers grains and a nucleotide additive fed during Exp. 1, 2, 3, and 4.

	Corn germ meal			Dried distillers grains		
	Nucleotide additive, g/d					
Item	0	2	4	0	2	4
Ingredient						
Cracked corn	25.5	25.5	25.5	29.0	29.0	29.0
Corn germ meal	24.5	24.5	24.5	-	-	-
Dried distillers grains	-	-	-	22.0	22.0	22.0
Prairie hay	18.0	18.0	18.0	10.7	10.7	10.7
Alfalfa hay	13.0	13.0	13.0	22.8	22.8	22.8
Corn steep liquor	7.0	7.0	7.0	7.0	7.0	7.0
Corn gluten meal	4.0	4.0	4.0	0.5	0.5	0.5
Limestone	1.5	1.5	1.5	1.5	1.5	1.5
Mineral supplement[1]	1.0	1.0	1.0	1.0	1.0	1.0
Nucleotide additive[2]	5.5	5.5	5.5	5.5	5.5	5.5

[1] Mineral supplement was formulated to contain (DM basis) 18.7% Ca, 4.14% P, 0.24% Mg, 0.43% K, 26.88% NaCl, 10.62% Na, 16.38% Cl, 1.43% S, 399.41 ppm Fl, 35.66 ppm Co, 177.79 ppm I, 775.26 ppm Fe, 6516.67 ppm Mn, and 4018.94 ppm Zn.

[2] Nucleotide additive was formulated to provide 0, 2 or 4 g/heifer daily when DMI was 8.2 kg/d. At this inclusion level, the NA provides 0, 0.242, or 0.489 g/kg dietary DM.

Table 2.2. Analyzed composition of diets (% of DM) fed during Exp. 1, and 2.

	Corn germ meal			Dried distillers grains		
	Nucleotide additive, g/d					
Item	0	2	4	0	2	4
Composition, analyzed (Exp. 1)[1]						
DM, %	77.4	77.5	77.6	76.4	77.0	76.5
CP, % of DM	18.3	18.9	18.1	18.9	18.7	19.4
Starch, % of DM	19.5	18.7	19.0	18.0	17.3	16.1
Ether extract, % of DM	2.6	2.7	2.6	4.1	4.1	4.4
ADF, % of DM	18.4	17.8	18.1	16.5	16.6	17.2
Ca, % of DM	0.96	0.94	1.00	1.12	1.08	0.99
P, % of DM	0.51	0.53	0.50	0.55	0.54	0.57
NE_m, Mcal/kg	1.79	1.79	1.77	1.81	1.80	1.81
NE_g, Mcal/kg	1.07	1.07	1.07	1.09	1.09	1.08
Composition, analyzed (Exp. 2)[1]						
DM, %				75.0	74.7	74.9
CP, % of DM				20.2	19.5	20.0
Starch, % of DM				16.0	16.3	16.8
Ether extract, % of DM				3.9	3.9	3.8
ADF, % of DM				20.2	21.3	21.3
Ca, % of DM				1.22	1.27	1.23
P, % of DM				0.58	0.56	0.56
NE_m, Mcal/kg				1.75	1.73	1.72
NE_g, Mcal/kg				1.02	1.00	1.01

[1] Feed samples were analyzed by a commercial laboratory (SDK Laboratories, Hutchinson, KS).

Table 2.3. Effects of corn germ meal and dried corn distillers grains and the addition of a nucleotide additive on beef heifer gain, intake, and efficiency (Exp. 1).

	Corn germ meal			Dried distillers grains								
	Nucleotide additive, g/d							*P*-value				
Item	0	2	4	0	2	4	SEM	BP[1]	NA-L[2]	NA-Q[3]	BPxNA-L[4]	BPxNA-Q[5]
No. of pens	3	3	3	3	3	3						
No. of animals	35	35	36	36	35	36						
Days on feed	84	84	84	84	84	84						
Calculated NE_m, Mcal/kg[6]	1.59	1.63	1.62	1.63	1.70	1.64	0.07	0.25	0.62	0.27	0.89	0.66
Calculated NE_g, Mcal/kg[6]	0.98	1.02	1.01	1.01	1.08	1.03	0.06	0.31	0.63	0.30	0.87	0.61
Initial BW, kg	262.3	261.6	261.1	261.8	261.4	261.5	15.9	0.78	0.20	0.72	0.41	0.91
Final BW, kg	355.8	352.7	355.7	349.2	358.8	357.3	6.80	0.88	0.33	0.72	0.32	0.23
DMI, kg/d												
d 0 to 28	7.78	7.43	7.83	7.67	7.72	7.80	0.92	0.76	0.66	0.28	0.83	0.31
d 28 to 56	9.51	8.96	9.29	9.00	9.44	9.36	0.71	0.96	0.84	0.77	0.42	0.26
d 56 to 84	11.0	10.7	10.9	10.0	10.2	10.7	0.97	0.19	0.58	0.65	0.44	0.94
d 0 to 84	9.40	8.99	9.30	8.88	9.10	9.26	0.83	0.55	0.65	0.56	0.45	0.48
ADG, kg												
d 0 to 28	1.36	1.38	1.46	1.33	1.49	1.50	0.16	0.57	0.15	0.80	0.71	0.48
d 28 to 56	1.30	1.20	1.23	1.13	1.25	1.23	0.10	0.65	0.90	0.97	0.38	0.43
d 56 to 84	0.63	0.64	0.64	0.62	0.69	0.64	0.09	0.83	0.85	0.72	0.99	0.75
d 0 to 84	1.11	1.08	1.13	1.04	1.16	1.14	0.08	0.88	0.28	0.70	0.39	0.25
G:F												
d 0 to 28	0.171	0.187	0.189	0.177	0.196	0.194	0.015	0.47	0.15	0.41	0.99	0.87
d 28 to 56	0.137	0.134	0.132	0.126	0.134	0.132	0.010	0.64	0.96	0.78	0.56	0.72
d 56 to 84	0.055	0.059	0.059	0.063	0.070	0.060	0.010	0.59	0.96	0.55	0.72	0.66
d 0 to 84	0.118	0.121	0.121	0.119	0.130	0.124	0.007	0.34	0.41	0.28	0.93	0.45

[1] BP indicates byproduct effect.

[2] NA-L indicates nucleotide additive linear effect.

[3] NA-Q indicates nucleotide additive quadratic effect.

[4] BPxNA-L indicates byproduct x nucleotide additive linear effect.

[5] BPxNA-Q indicates byproduct x nucleotide additive quadratic.

[6] NE_m and NE_g calculated based on equations from the 1996 NRC.

Table 2.4. Effects of corn germ meal and dried corn distillers grains and the addition of a nucleotide additive on beef heifer morbidity (Exp. 1).

Disease	Treatment
BRD	DDG0
BRD	DDG0
BRD	DDG0
BRD	CGM2
BRD	DDG4
Pinkeye	CGM0
Pinkeye	CGM2
Pinkeye	CGM4
Pinkeye	DDG2
Pinkeye	DDG4
Footrot	DDG4
Bloat	CGM2

Table 2.5. Effects of corn germ meal and dried corn distillers grains and the addition of a nucleotide additive on beef heifer mortality (Exp. 1).

Death Type	Treatment
Bacterial infection	DDG2
Bacterial infection	DDG4
Chronic BRD	CGM0

Table 2.6. Effects of the addition of a nucleotide feed additive to diets containing dried corn distillers grains on beef heifer gain, intake, and efficiency (Exp. 2).

	Nucleotide additive g/d				*P*-value	
Item	0	2	4	SEM	Linear	Quadratic
No. of pens	8	8	8			
No. of animals	80	80	80			
Days on feed	56	56	56			
Initial BW, kg	267.1	268.1	267.9	6.97	0.49	0.54
Final BW, kg	323.5	324.0	320.4	1.73	0.42	0.29
DMI, kg/d						
d 0 to 28	8.66	8.72	8.39	0.21	0.46	0.51
d 28 to 56	8.94	8.78	8.60	0.26	0.44	0.96
d 0 to 56	8.80	8.75	8.49	0.22	0.43	0.73
ADG, kg						
d 0 to 28	1.43	1.35	1.31	0.06	0.18	0.85
d 28 to 56	0.57	0.65	0.57	0.06	0.96	0.23
d 0 to 56	1.00	1.00	0.94	0.03	0.23	0.36
G:F						
d 0 to 28	0.165	0.155	0.157	0.005	0.26	0.40
d 28 to 56	0.084	0.112	0.093	0.017	0.72	0.28
d 0 to 56	0.114	0.115	0.111	0.003	0.54	0.58

Table 2.7. Effects of the addition of a nucleotide feed additive to diets containing dried corn distiller grains on beef heifer gain, intake, and efficiency-pooled analysis (Exp. 1 & 2).

	Nucleotide additive, g/d				*P*-value	
Item	0	2	4	SEM	Linear	Quadratic
No. of pens	11	11	11			
No. of animals	116	115	116			
Days on feed	56	56	56			
Initial BW, kg	265.7	266.3	266.2	6.31	0.55	0.59
Final BW, kg	322.0	325.2	322.4	6.75	0.90	0.19
DMI, kg/d						
d 0 to 28	8.40	8.44	8.23	0.22	0.51	0.57
d 28 to 56	8.96	8.96	8.81	0.25	0.64	0.80
d 0 to 56	8.69	8.75	8.52	0.21	0.58	0.57
ADG, kg						
d 0 to 28	1.40	1.39	1.36	0.06	0.64	0.87
d 28 to 56	0.60	0.69	0.63	0.05	0.69	0.20
d 0 to 56	1.00	1.04	0.99	0.03	0.92	0.25
G:F						
d 0 to 28	0.169	0.166	0.167	0.007	0.78	0.82
d 28 to 56	0.082	0.105	0.090	0.013	0.64	0.25
d 0 to 56	0.116	0.120	0.117	0.003	0.78	0.39

Table 2.8. Effects of corn germ meal and dried corn distillers grains and the addition of a nucleotide additive on intake and digestibility of DM, starch, and ADF (Exp. 3).

	Corn germ meal		Dried distillers grains					
	Nucleotide additive, g/d					*P*-value		
Item	0	3	0	3	SEM	Byproduct	NA[1]	Byproduct x NA
No. of observations	4	4	4	4				
Diet composition, % DM								
Starch	23.8	23.8	22.5	22.5	0.005	0.04	1.0	1.0
ADF	16.0	16.0	15.4	15.4	0.002	0.01	1.0	1.0
Intake, kg/d								
DM	9.92	9.98	10.35	9.90	0.46	0.46	0.40	0.28
Starch	2.39	2.40	2.34	2.24	0.13	0.27	0.61	0.57
Non-starch	7.53	7.59	8.02	7.66	0.34	0.14	0.40	0.26
ADF	1.58	1.60	1.60	1.53	0.06	0.42	0.50	0.19
Digestibility, %								
DM	68.7	69.4	70.5	69.2	1.50	0.60	0.84	0.53
Starch	85.6	88.2	88.4	88.2	1.84	0.35	0.39	0.34
Non-starch	63.4	63.4	65.3	63.7	1.73	0.55	0.66	0.67
ADF	62.6	61.6	67.6	62.1	2.28	0.18	0.12	0.26

[1] NA indicates nucleotide additive.

Table 2.9. Effects of corn germ meal and dried corn distillers grains and the addition of a nucleotide additive on ruminal fermentation characteristics (Exp. 3).

	Corn germ meal		Dried distillers grains					
	Nucleotide additive, g/d					*P*-value		
Item	0	3	0	3	SEM[1]	Byproduct	NA[4]	Byproduct x NA
No. of observations	4	4	4	4				
Ruminal								
pH[2]	5.61	5.73	5.67	5.78	0.07	0.34	0.03	0.93
Lactate[2], m*M*	0.08	0.07	0.07	0.08	0.01	0.85	0.76	0.43
Ammonia[2], m*M*	2.6	2.8	4.5	4.2	0.46	< 0.01	0.85	0.37
Total VFA[2], m*M*	104.5	102.8	113.6	106.9	3.62	0.04	0.19	0.43
Acetate[2], m*M*	63.4	64.1	68.3	65.7	2.81	0.08	0.60	0.39
Propionate[2], m*M*	27.2	24.1	26.5	24.6	1.39	0.92	0.03	0.61
Butyrate[2], m*M*	10.7[a]	10.9[a]	14.1[b]	12.4[c]	1.03	< 0.01	0.15	0.06
Isobutyrate[2], m*M*	0.69	0.76	0.96	0.98	0.06	< 0.01	0.13	0.34
Isovalerate[2], m*M*	1.00[e]	1.36[d]	1.51[d]	1.38[d]	0.13	< 0.01	0.23	0.01
Valerate[2], m*M*	1.47[f]	1.58[f]	2.28[g]	1.86[h]	0.07	< 0.01	0.02	< 0.01
Fluid passage rate, %/h[3]	14.6	15.2	13.4	14.9	0.01	0.59	0.44	0.74

[1] Largest value among treatments is reported.

[2] Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding.

[3] Calculated values from samples collected at 2, 4, 6, 8, 12, and 18 h after feeding.

[4] NA indicates nucleotide additive.

[a,b,c] means within a row not bearing a common letter differ ($P \leq 0.05$).

[d,e] means within a row not bearing a common letter differ ($P \leq 0.01$).

[f,g,h] means within a row not bearing a common letter differ ($P < 0.01$).

Table 2.10. Effects of corn germ meal and dried corn distillers grains and the addition of a nucleotide additive on ruminal volatile fatty acid profile (Exp. 3).

	Corn germ meal		Dried distillers grains					
	Nucleotide additive, g/d					*P*-value		
Item	0	3	0	3	SEM[1]	Byproduct	NA[3]	Byproduct x NA
No. of observations	4	4	4	4				
Ruminal VFA, % of total								
Acetate[2]	60.9	62.5	60.3	61.6	0.83	0.07	< 0.01	0.70
Propionate[2]	25.9	23.3	23.2	23.0	1.45	≤ 0.01	0.03	0.07
Butyrate[2]	10.2[c]	10.5[d]	12.3[c,d]	11.4[c,d]	0.72	< 0.01	0.40	0.05
Isobutyrate[2]	0.67	0.75	0.85	0.92	0.05	< 0.01	0.04	0.90
Isovalerate[2]	0.97[b]	1.35[a]	1.34[a]	1.31[a]	0.12	0.12	0.09	0.05
Valerate[2]	1.41[a]	1.52[b]	1.98[c]	1.73[d]	0.06	< 0.01	0.05	< 0.01

[1] Largest value among treatments is reported.

[2] Average of values collected at 0, 2, 4, 6, 8, 12, 18, and 24 h after feeding expressed as a percent of total VFA.

[3] NA indicates nucleotide additive.

[a, b, c, d] means within a row not bearing a common letter differ ($P \leq 0.04$).

[a, b] means within a row not bearing a common letter differ ($P \leq 0.02$).

[c,d] means within a row not bearing a common letter differ ($P \leq 0.05$).

Figures

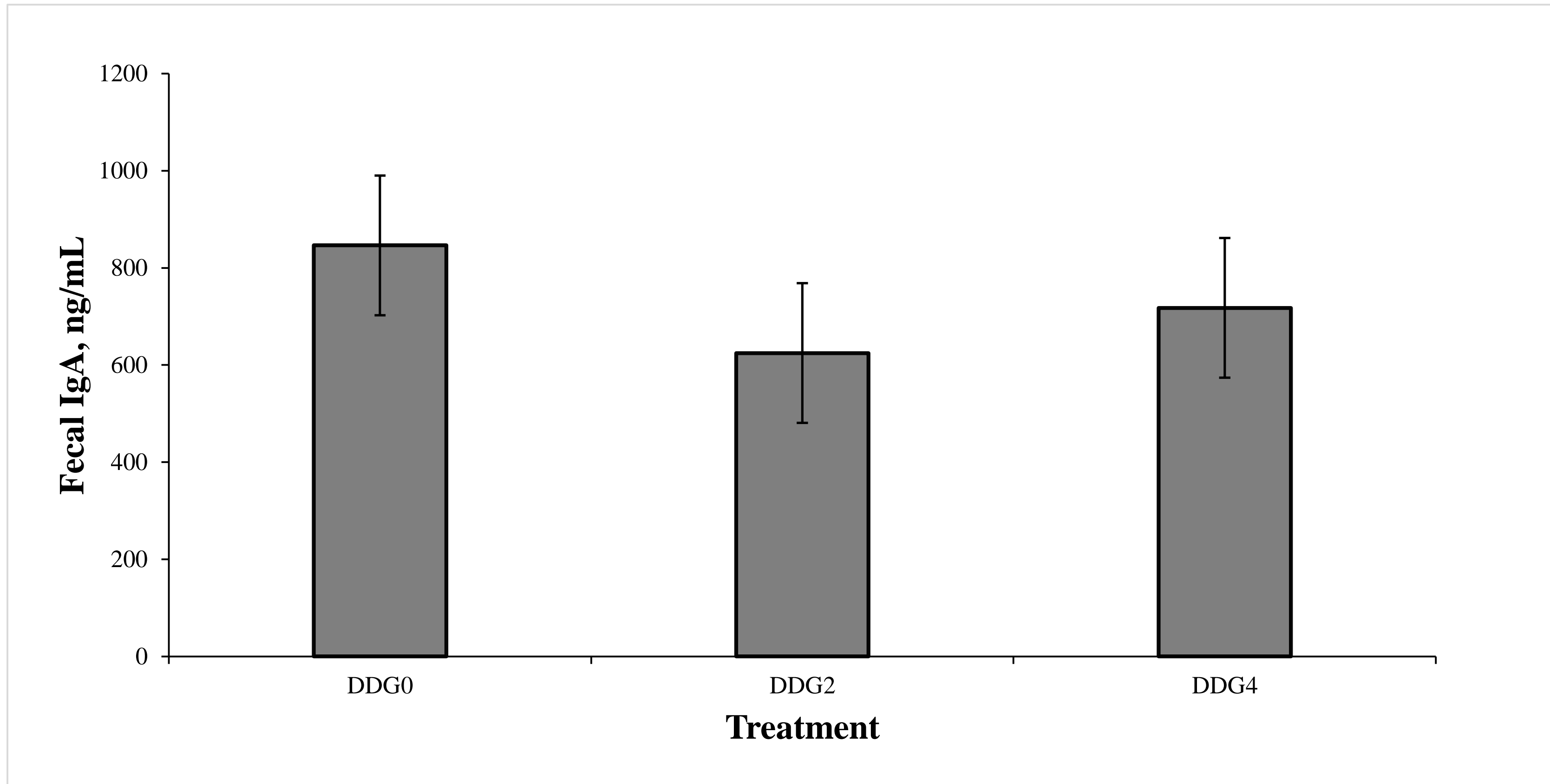

Figure 2.1. Concentration of secretory IgA in fecal samples collected on d 28 (Exp. 2). There was no linear dose (P = 0.24), or quadratic (P = 0.11) effects of nucleotides. Values are least square means ± SEM, where SEM = 144 and n = 147. Forty-nine fecal samples were taken from DDG0, 48 from DDG2, and 50 from DDG4.

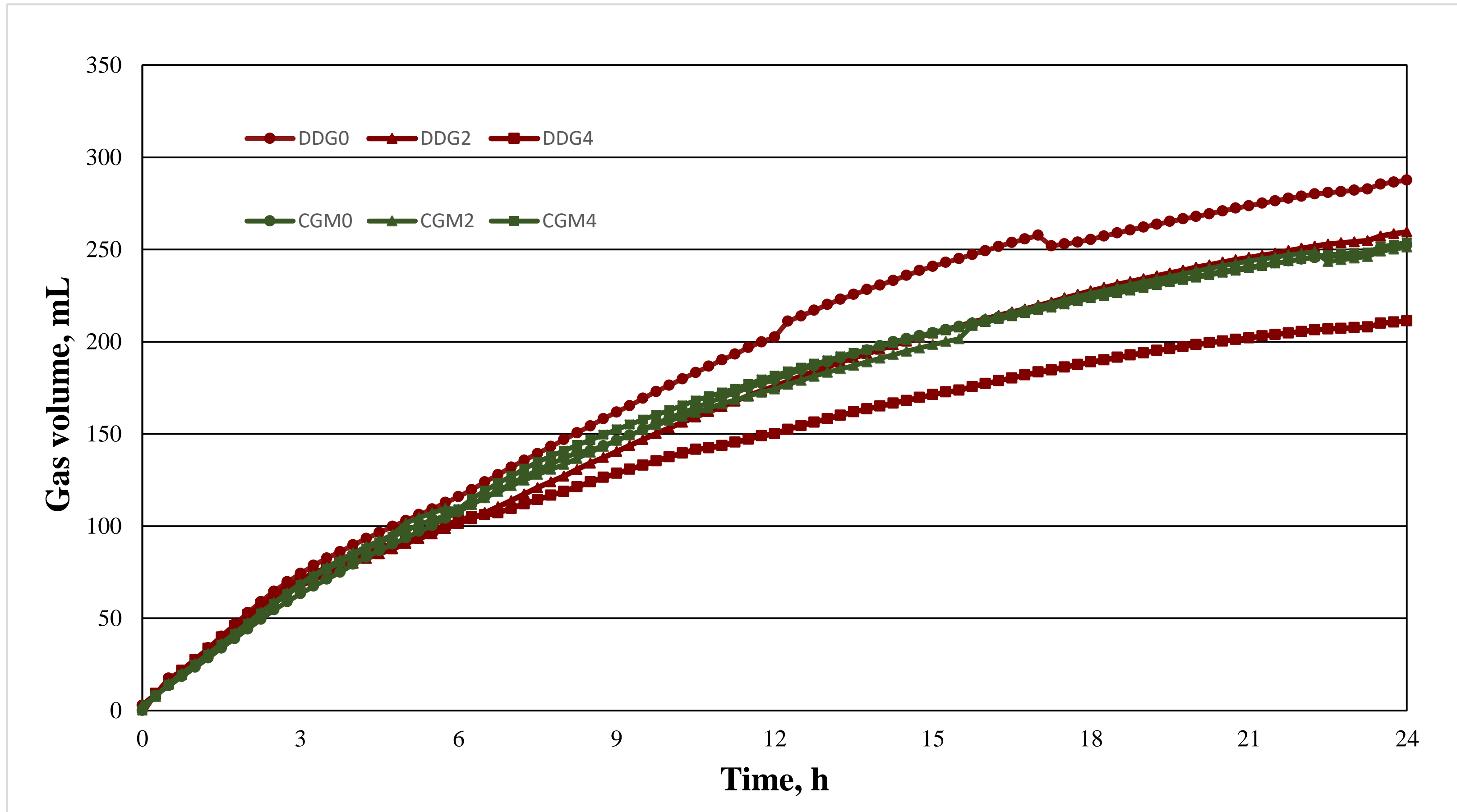

Figure 2.2. Effect of the addition of a nucleotide feed additive with either corn germ meal or dried corn distillers grains on 24-h gas production (mL) (Exp. 4). No interaction between byproduct, nucleotide feed additive and time, $P = 1.00$. SEM[1] = Standard error of the mean of the combination of byproduct, nucleotide feed additive and time. Effect of time, $P < 0.001$, effect of byproduct, $P = 0.77$, linear effect of NA, $P < 0.001$, quadratic effect of NA, $P = 0.66$, linear effect NA x byproduct, $P < 0.001$, and quadratic effect of NA x byproduct, $P = 0.89$.